UNIVERSIDADE D
COIMBRA

Carla Sofia da Palma Grosso

# Synthesis of Novel Indoles and Bis(indolyl)methanes: Approaches to Structures with Biological Activity

**Tese no âmbito do Doutoramento em Química, ramo de Síntese Orgânica, orientada pela Professora Doutora Teresa Margarida Vasconcelos Dias de Pinho e Melo, coorientada pelo Professor Doutor Américo Lemos e apresentada ao Departamento de Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.**

Fevereiro de 2023

UNIVERSIDADE D
COIMBRA

Carla Sofia da Palma Grosso

# Synthesis of Novel Indoles and Bis(indolyl)methanes: Approaches to Structures with Biological Activity

**Tese no âmbito do Doutoramento em Química, ramo de Síntese Orgânica, orientada pela Professora Doutora Teresa Margarida Vasconcelos Dias de Pinho e Melo, coorientada pelo Professor Doutor Américo Lemos e apresentada ao Departamento de Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.**

Fevereiro de 2023

*Aos meus pais que tanto lutaram e sonharam com este dia!*

*Aquilo que vou dizer*
*Não é uma despedida*
*Não creio que vá morrer*
*Mas sinto-me acabado para a vida*

*Pela família fiz tudo*
*O quanto que se podia*
*E fi-lo sempre a meu modo*
*Fiz tudo como sabia*
*Fi-lo tudo com denodo*

*Sou amigo de toda a gente*
*Conto com alguns amigos*
*Não consegui ser diferente*
*Não sei se tenho inimigos*

*Inimigos, se os tiver,*
*Por mim não foram contados*
*Mas digo-lhes: Podem crer,*
*Estão todos equivocados*

*Meus pais desejaram para os filhos*
*Uma formação superior*
*Porque viram com bons olhos*
*Seu grande e real valor (...)*

*José Grosso em "Grosso modo se fala"*

# Agradecimentos

O desenvolvimento de um projeto de Doutoramento é algo muito complexo e trabalhoso. É um caminho repleto de grandes vitórias e muitas derrotas e até desespero. Porém é um caminho que se faz caminhado com muita persistência, resiliência, adaptação e uma vontade imensa de atingir os objetivos a que nos propomos. No fim, a alegria de materializar este caminho numa tese é sem dúvida algo que não consigo explicar por palavras.

Este objetivo, que por vezes se torna solitário, não foi feito sem a cooperação, companheirismo e amizade de muitos, o que faz que o saldo final seja extremamente positivo e compensador quer a nível científico quer humano. Desta forma, as palavras que se seguem, são o meu mais profundo agradecimento a todos aqueles que, de forma direta ou indireta, me ajudaram nesta caminhada.

À Professora Doutora Teresa Pinho e Melo, agradeço a orientação científica, a transmissão de conhecimentos, e a constante capacidade para me manter otimista, mesmo nos momentos mais complicados. Agradeço-lhe a confiança que sempre depositou em mim, e por sempre me ter dado espaço para dar as minhas opiniões e contributos para melhorar substancialmente este projeto.

Ao Professor Doutor Américo Lemos, agradeço por me ter aberto as portas para a realização deste projeto, a transmissão de conhecimentos, e a total disponibilidade durante esta caminhada.

À Doutora Ana Lúcia, com quem partilhei frustrações e sucessos, por me ter acompanhado (aturado) ao longo desta jornada, pelo seu otimismo, por todo o seu companheirismo, amizade e solidariedade, estando sempre presente e disposta a ajudar no necessário sem nunca pedir nada em troca.

À Doutora Elisa Serra, Doutora Dina Murtinho, à Doutora Marta Piñero por toda a ajuda, colaboração e disponibilidade que me proporcionaram durante esta caminhada.

À Doutora Susana Lopes, à Doutora Isabel Soares e ao Doutor Nelson Pereira, pela partilha de conhecimentos, ajuda e disponibilidade.

Ao Nuno Alves, Pedro Brandão, Américo Alves, à Cláudia Alves, à Carla Gomes, à Nélia Tavares, ao Tever John Sase e a todos os restantes membros do grupo de Química Orgânica da Universidade de Coimbra, pela forma como me receberam e acolheram.

À Dona Lurdes, pela sua imensa paciência, disponibilidade, solidariedade, amizade, boa disposição, pelas suas mãos de fada e pelos docinhos que tive o privilégio de saborear.

A todos os funcionários do Departamento de Química e do Centro de química por toda ajuda prestada durante este projeto.

À Zaida Almeida e ao Pedro Cruz, pela sua amizade e pelos agradáveis momentos que passamos juntos. Ao Pedro Cruz em particular pela colaboração e paciência nos estudos de ressonância magnética nuclear.

À Rosa Assunção, amiga de coração, pela sua amizade sem fim, por todo o apoio que me deu e continua a dar, por toda a força que me transmite, por acreditar nas minhas capacidades e por me fazer sentir em casa.

Ao Francisco Geraldes, meu amigo do coração, pela sua amizade, por todo o apoio que me deu ao longo dos anos, pela boa disposição e pela sua disponibilidade.

Ao Ricardo Jorge, o meu amigo de sempre e para sempre, por todos os bons momentos que passamos.

Ao Paulo e à Gabi por fazerem parte da minha vida, pela amizade, apoio, disponibilidade durante esta caminhada.

Ao Pedro e Catarina por tanto que me têm dado, pela amizade, pela paciência, pela boa disposição e por me terem "abraçado" nos maus momentos e festejado nos bons.

À Teresa Sousa por toda a sua amizade e por não me ter deixado ir abaixo nos momentos mais difíceis e por vibrar com os momentos felizes.

A todos os meus amigos do Trianon, Viriato, Bruno Sousa, Paulo Anjos, Isabel Neto, Teresa Amaral, Hélder Araújo, José Eusébio, Eugénia, Paulo Correia, JP e Guida por todos os momentos que passámos juntos.

À Cecília Castro, minha irmã, minha amiga, minha confidente que acompanhou o meu percurso e que nunca duvidou das minhas capacidades.

À Susana Nobre, outra minha irmã e amiga que me tem acompanhado e com quem posso contar sempre. Apesar da distância está sempre cá.

Ao meu querido maninho Rui Inglês, uma das pessoas mais bonitas que conheço e que tenho a enorme sorte de a ter na minha vida. O rui é uma luz na minha vida, é o que me mostra permanentemente que a vida é linda e divertida, e quando não o é mostra-me sempre o caminho de volta. Obrigada por tanto carinho e amizade.

Aos meus amigos de sempre e para sempre que me acompanharam, Débora Mendes, Patrícia Correia, Filipa Andrade, Marta Moreira e família.

Às minhas queridas amigas Ana Fernandes e Elizabete Fernandes por toda a amizade, apoio e boa disposição.

Por fim, tenho de agradecer aqueles que fazem de mim quem sou, aos meus maiores admiradores, aos que sempre me apoiaram, a minha maior base de apoio, os que tanto se sacrificaram para me dar as bases para nunca deixar de lutar e principalmente de sonhar e que independentemente das minhas vitórias ou derrotas estão sempre de braços abertos para me receber, a minha família. Ao meu Pai, José Gonçalves Grosso, à minha Mãe Dorina Grosso, agradeço todo o amor, dedicação, formação, educação, e apoio que me deram.

À minha Irmã Teresa, pela amizade, companheirismo, lealdade, amor e por me ter dado uma das pessoas mais importantes da minha vida, o meu Sobrinho Tiago.

À minha, Tia Maria, Madrinha, segunda Mãe, que tanto me apoiou e ajudou em todas a etapas da minha vida, sempre com amor, paciência e disponibilidade

# Institutional and Financial Support

The work presented in this thesis was carried out at Organic Chemistry Laboratory of the Department of Chemistry (DQ) from the University of Coimbra. The biological assessments were carried out at CIQA from Unviversity of Algarve and at Research Institute for Medicines (iMed.ULisboa) - Cellular Function and Therapeutic Targeting Group from University of Lisboa.

This work was financially supported by SFRH/BD/130198/2017 and COVID/BD/151903/2021 grants from Fundação para a Ciência e Tecnologia (FCT), whom I gratefully thank.

# Table of Contents

# Abstract

Indoles are present in several biologically active natural and synthetic compounds, displaying activities such as antimicrobial, anti-inflammatory, and antitumour, making the indole core a relevant scaffold for drug discovery. Thus, the development of efficient synthetic routes for the assembly of functionalised indoles has been widely explored and continues to attract great attention. Our contribution involved the development of an unprecedented approach to bis(indolyl)methanes (BIMs) via bis-hetero-Diels-Alder reaction of nitroso- and azoalkenes with indoles using dichloromethane (DCM) or the $H_2O$:DCM system as solvents. The methodology proved to be versatile and with broad scope, leading to a library of new BIMs bearing oximes and hydrazones substituted at the methylene bridge. Furthermore, the novel BIMs showed relevant *in vitro* anticancer activity and a promising scaffold has been identified.

In fact, the exciting biological activity of our lead molecule, [(*E*)-1-((4-bromophenyl)-1-hydroxyiminomethyl)bis(1*H*-indol-3-yl)methane], particularly against leukaemia and lymphoma cell lines, led us to build a library of new derivatives in order to establish struture-activity relationships and eventually to find an even better anticancer agent.

The synthesis of BIMs was also explored using Natural Deep Eutectic Solvents (N**A**DESs), an emerging class of nonconventional and "green" solvents, in order to develop a more sustainable approach.

After testing several NADES, the best results were obtained when a NADES based on choline chloride:glycerol was used. The results were very exciting, since, besides achieving the initial goal, the BIMs were obtained in much shorter reaction times, an improvement of the yields was also observed when arylhydrazones were used. Furthermore, we were also pleased to develop a novel strategy for the preparation of carbonyl-BIMs via one pot procedure using natural deep eutectic solvents.

Several methodologies using organic solvents were also tested in order to obtain carbonyl-BIMs via hydrolysis of the isolated BIM oximes or hydrazones. However, these compounds were only achieved by methodologies consisting in the treatment with sodium bisulfite or paraformaldehyde in the presence of amberlyst-15 in acetone/$H_2O$.

The synthetic strategy approach to bis(heterocycle)methanes was extended to the synthesis of bis- and tris(pyrazolyl)methanes (BPMs and TPMs) through the bis-1,4-

conjugated addition reaction of pyrazoles to nitroso- and azoalkenes. This reaction was carried out under mild and simple reaction conditions, giving bis(pyrazolyl)methane derivatives which are difficult to obtain by other methods. The ready access to a wide diversity of the starting oximes, hydrazones, and pyrazoles allowed simple modulation of the bis(pyrazolyl)methane's structure. The possibility of producing tris(pyrazolyl)methanes following the same methodology was also disclosed.

One strategy to find new compounds with biological activity is to explore the carboxylic acid/tetrazole bioisosterism. Therefore, a synthetic approach to 3-(1*H*-tetrazol-5-yl)-indole derivatives, bioisosters of 1*H*-indole-3-carboxylic acids, was developed. Arynes, generated *in situ* from 2-(trimethylsilyl)aryl triflates and KF, reacted smoothly with 2-(4-nitrobenzyltetrazolyl)-2*H*-azirines to give 3-(4-nitrobenzyltetrazolyl)-indole derivatives with high selectivity and in good yields. Deprotection of the tetrazole moiety of indole gave access to a new class of indole derivatives bearing a tetrazole moiety.

In summary, the work developed during this PhD thesis led to the development of new synthetic methodologies for an extensive library of new indole derivatives.

*Keywords*: Nitrosoalkenes, Azoalkenes, Bis(indolyl)methanes, Bis(pyrazolyl)methanes, Tetrazolyl-2*H*-azirines, 3-Tetrazolyl-indoles, Natural Deep Eutectic Solvents.

# Resumo

Os indóis estão presentes em vários compostos naturais e sintéticos biologicamente ativos, exibindo atividade antimicrobiana, anti-inflamatória e antitumoral, o que torna o núcleo indólico um elemento estrutural relevante em química medicinal. Assim, o desenvolvimento de vias sintéticas eficientes para a construção de indóis funcionalizados tem sido amplamente explorado e continua a atrair grande atenção. Desta forma, a presente tese de Doutoramento centrou-se no desenvolvimento de uma nova estratégia sintética para a obtenção de bis(indolil)metanos (BIMs) através da reação bis-hetero-Diels-Alder de nitroso- e azo-alquenos e indóis utilizando diclorometano (DCM) ou o sistema $H_2O$:DCM como solventes. A metodologia provou ser versátil tendo permitido a obtenção de uma biblioteca de BIMs *meso*-substituídos com grupos oxima e hidrazona. Os novos BIMs apresentaram atividade anticancerígena relevante *in vitro*, tendo sido identificado um composto com propriedades particularmente promissoras.

De facto, a surpreendente atividade biológica do composto líder, [(*E*)-1-((4-bromofenil)-1-hidroximinometil)bis(1*H*-indol-3-il)metano], em particular contra linhas celulares de leucemia e linfoma, levou-nos à construção de uma biblioteca de novos derivados com o objetivo de estabelecer relações de estrutura-atividade e eventualmente encontrar um potencial agente anticancerígeno ainda mais eficaz.

A síntese de BIMs foi também explorada utilizando Solventes Eutéticos Naturais Profundos (NADES), uma classe emergente de solventes *verdes* não convencionais, com o objetivo de desenvolver uma abordagem mais sustentável. Após o estudo de vários NADESs, os melhores resultados foram obtidos aquando da utilização de um NADES à base de cloreto de colina:glicerol. Os resultados foram extremamente satisfatórios, uma vez que, além de se atingir o objetivo inicial, os BIMs foram obtidos com tempos de reação muito mais curtos e observou-se também uma melhoria dos rendimentos quando hidrazonas arílicas foram utilizadas como precursores. Além disso, o uso destes solventes permitiu o desenvolvimento de uma nova estratégia para a obtenção de BIMs carbonílicos.

Várias metodologias convencionais, usando solventes orgânicos, foram estudadas a fim de se obterem BIMs carbonílicos via hidrólise das correspondentes oximas ou hidrazonas. No entanto, estes compostos só foram sintetizados a partir de metodologias

que consistiram no tratamento com bissulfito de sódio ou com paraformaldeído na presença de amberlyst-15 em acetona/$H_2O$.

A estratégia sintética para a obtenção de bis(heterociclo)metanos foi alargada à síntese de bis- e tris(pirazolil)metanos (BPMs e TPMs) via bis-adição conjugada-1,4 de pirazóis e nitroso- e azo-alquenos. Esta transformação foi realizada em condições de reação suaves e simples, tendo-se obtido derivados de bis(pirazolil)metano difíceis de obter por outros métodos. A facilidade de acesso a uma grande diversidade de oximas, hidrazonas e pirazóis, permitiu a modulação da estrutura do bis(pirazolil)metano. A síntese de um tris(pirazolil)metano seguindo a mesma metodologia foi também conseguida com sucesso.

Uma estratégia para encontrar novos compostos com atividade biológica consiste em explorar o bioisosterismo do ácido carboxílico/tetrazol. Neste contexto, foi desenvolvida uma estratégia sintética para a obtenção de derivados de 3-(1*H*-tetrazol-5-il)-indol, bioisósteros de ácidos 1*H*-indol-3-carboxílicos. Assim, arinos gerados *in situ* a partir de 2-(trimetilsilil)aril triflatos e KF, reagiram em condições suaves com 2-(4-nitrobenziltetrazolil)-2*H*-azirinas originando derivados de 3-(4-nitrobenziltetrazolil)-indol, com elevada seletividade e bons rendimentos. A desproteção do grupo tetrazol presente nestes compostos levou à obtenção de uma nova classe de derivados de indol contendo um grupo de tetrazol como substituinte.

Em resumo, o trabalho desenvolvido durante esta tese de doutoramento levou ao desenvolvimento de novas metodologias sintéticas para obtenção de uma extensa biblioteca de novos derivados do indol.

*Palavras-Chave*: Nitrosoalquenos, Azoalquenos, Bis(indolil)metanos, Bis(pirazolil)metanos, Tetrazolil-2*H*-azirinas, 3-Tetrazolil-indóis, Solventes Eutéticos Naturais Profundos.

# Abbreviations, Acronyms and Symbols

| | |
|---|---|
| Ac | Acetyl |
| aq. | Aqueous |
| Ar | Aryl |
| BIM | Bis(Indolyl)Methane |
| Bn | Benzyl |
| brs | broad singlet |
| Bu | Butyl |
| Boc | *tert*-Butoxycarbonyl |
| Cat | Catalyst |
| CC | Choline Chloride |
| $^{13}$C NMR | Carbon Nuclear Magnetic Resonance |
| COSY | COrrelated SpectroscopY |
| Cpr | Cyclopropyl |
| d | doublet |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| DCE | 1,2-Dichloroethane |
| DCM | Dichloromethane |
| DES | Deep Eutectic Solvents |
| dd | double duplet |
| ddd | double double duplet |
| DEPT | Distortionless Enhancement by Polarization Transfer |
| DFT | Density Functional Density |
| DIPA | DiIsoPropylAmine |
| DMA | DiMethylAcetamide |
| DMF | *N*, *N*-DiMethylFormamide |
| DMSO | Dimethylsulphoxide |
| DMSO-$d_6$ | hexadeuterodimethylsulfoxide |
| E | Electrophile |

| | |
|---|---|
| equiv. | equivalent |
| ESI | ElectroSpraY |
| Et | Ethyl |
| Gly | Glycerol |
| h | hours |
| Het | Heterocycle |
| HMBC | Heteronuclear Multiple-Bond Correlation |
| HMQC | Heteronuclear Multiple-Quantum Correlation |
| $^{1}$H NMR | Proton Nuclear Magnetic Resonance |
| HOMO | Highest Occupied Molecular Orbital |
| HRMS | High-Resolution Mass Spectra |
| $IC_{50}$ | half maximal Inhibitory Concentration |
| IUPAC | Union of Pure and Applied Chemistry |
| IR | Infra-Red spectroscopy |
| *J* | coupling constant |
| LDA | Lithium DiisopropylAmide |
| LUMO | Lowest Unoccupied Molecular Orbital |
| m | multiplet |
| M | Molecular ion |
| Me | Methyl |
| $MePh_3PCl$ | MethyltriPhenylphosphonium Chloride |
| min | minutes |
| m.p | meting point |
| NADES | Natural Deep Eutectic Solvents |
| NBS | *N*-bromosuccinimide |
| NCS | *N*-Chlorosuccinimide |
| NMR | Nuclear Magnetic Resonance |
| NOESY | Nuclear Overhauser Effect SpectroscopY |
| PTSA | *para*-Toluenesulfonic acid |
| PCC | Pyridinium chlorochromate |

| | |
|---|---|
| PE | PolyEthylene glycol |
| Ph | Phenyl |
| PhAA | Phenylacetic acid |
| PNB | *p*-Nitrobenzyl |
| ppm | parts per million |
| Pr | Propyl |
| Py | Pyridine |
| q | quartet |
| s | singlet |
| t | triplet |
| TBAB | Tetra- *n*-ButylAmmonium Bromide |
| TBAC | Tetra-*n*-Butyllammonium chloride |
| TBPCl | Tetra-*n*-Butylphosphonium chloride |
| Tf | Triflate |
| THF | Tetrahydrofuran |
| TLC | Thin Layer Chromatography |
| TMS | Tetramethylilsilane |
| TPABr | TetraPropylAmmonium Bromide |
| Ts | Tosyl |
| U | Urea |

# List of Publications Related to the Scientific Topic of the Thesis

**Manuscripts published in international journals with peer review process**

**1.** Ana L. Cardoso, Susana M. M. Lopes, Carla Grosso, Marta Pineiro, Américo Lemos, and Teresa M. V. D. Pinho e Melo "*One-Pot Synthetic Approach to Dipyrromethanes and Bis(indolyl)methanes via Nitrosoalkene Chemistry*", Journal of Chemical Education, **2021**, 98, 2661-2666, (DOI: 10.1021/acs.jchemed.1c00184).

**2.** Cláudia Alves, Carla Grosso, Pedro Barrulas, José A. Paixão, Ana L. Cardoso, Anthony J. Burke, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Asymmetric Neber Reaction in the Synthesis of Chiral 2-(Tetrazol-5-yl)-2H-Azirines*", Synlett, **2020**, 31, 553-558, (DOI: 10.1055/s-0039-1691533).

**3.** Carla Grosso, Amadeu Brigas, Jesús M. de los Santos, Francisco Palacios, Américo Lemos and Teresa M. V. D. Pinho e Melo "*Natural deep eutectic solvents in the hetero-Diels–Alder approach to bis(indolyl)methanes*", Monatshefte für Chemie - Chemical Monthly, **2019**, 150, 1275–1288, (DOI: 10.1007/s00706-019-02421-7).

**4.** Carla Grosso, Marta Liber, Amadeu F. Brigas, Teresa M. V. D. Pinho e Melo, and Américo Lemos "*Regioselectivity in Hetero Diels-Alder Reactions*", Journal of Chemical Education, **2019**, 96, 148-152, (DOI: 10.1021/acs.jchemed.7b00933).

**5.** Carla Grosso, Ana L. Cardoso, Cátia Marques, Luísa Barreira, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Hetero-Diels-Alder approach to Bis(indolyl)methanes*", Bioorganic & Medicinal Chemistry, **2017**, 25, 1122-1131, (DOI: 10.1016/j.bmc.2016.12.028).

***6.*** Carla Grosso, Ana Lúcia Cardoso, Américo Lemos, João Varela, Luísa Custódio, Luísa Barreira, Teresa M. V. D. Pinho e Melo "*Novel approach to bis(indolyl)methanes: De novo synthesis of 1-hydroxyiminomethyl derivatives with anti-cancer properties*", European Journal of Medicinal Chemistry, **2015**, 93, 9-15, (DOI: 10.1016/j.ejmech.2015.01.050).

**7.** Carla Grosso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Conjugate Addition of Pyrazoles to Halogenated Nitroso- and Azoalkenes: A New Entry to Novel Bis(pyrazol-1-yl)methanes*", Synlett, **2014**, 25, 2868-2872, (DOI: 10.1055/s-0034-1379306).

**Oral Communications**

**1.** Carla Grosso, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Synthesis of new bis(indolyl)methanes with anti-cancer properties*", 11º Encontro Nacional de Química Orgânica e 4º Encontro Nacional de Química Terapêutica, **2015**, Porto, Portugal.

**2.** Carla Grosso, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Novel Synthesis of Bis(indolyl)methanes with Anticancer Properties*", Coimbra Chemistry Centre Day, **2014**, Coimbra, Portugal.

**Conference posters and awards**

**1.** Carla Grosso, Terver John Sase, Nuno G. Alves, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo " *Exploring the reactivity of tetrazolyl-2H-azirines towards arynes: selective synthesis of indole derivatives*", 14th national organic chemistry meeting & 7th national medicinal chemistry meeting, **2022**, Caparica, Portugal. Award: **Best poster in organic chemistry.**

**2.** Carla Grosso, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Synthesis of Novel Indoles and Bis(indolyl)methanes: Approaches to Structures with Biological Activity*" Coimbra Chemistry Centre Day, **2021**, Coimbra, Portugal.

**3.** Carla Grosso, Nuno G. Alves, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*Selective Synthesis of 3-Tetrazolyl-indoles from 2-(Tetrazolyl-5-yl)-2H-azirines and Arynes*", International symposium on synthesis and catalysis ISYSYCAT **2021**, Évora, Portugal.

**4.** Carla Grosso, Cláudia C. Alves, Ana L. Cardoso, Anthony Burke, Américo Lemos, Teresa M. V. D. Pinho e Melo, "*Asymmetric Neber Reaction in the Synthesis of Tetrazolyl-Azirine Assemblies*", International symposium on synthesis and catalysis ISYSYCAT, **2019**, Évora, Portugal.

**5.** Carla Grosso, Amadeu Brigas, Teresa M. V. D. Pinho e Melo, Américo Lemos "*Natural Deep Eutectic Solvents in the Synthesis of Bis(indoly)methanes*", 12th National Organic Chemistry Meeting and 5th National Medicinal Chemistry Meeting, **2018**, Coimbra, Portugal.

**6.** Carla Grosso, Amadeu Brigas, Teresa M. V. D. Pinho e Melo, Américo Lemos "*Natural Deep Eutectic Solvents in the Synthesis of Bis(indoly)methanes*", Italian-

Spanish-Portuguese Joint Meeting in Medicinal Chemistry MedChemSicily, **2018**, Sicily, Italy.

**7.** Carla Grosso, Ana L. Cardoso, Américo Lemos, Teresa M. V. D. Pinho e Melo "*New Bis(indolyl)methane hydrazones with potencial biological activity*", 7th Spanish-Portuguese-Japonese Organic Chemistry Symposium, **2015**, Sevilha, Spain.

***8.*** Carla Grosso, Américo Lemos, João Varela, Luísa Barreira, Luísa Custódio, Maria João Rodrigues, Teresa M. V. D. Pinho e Melo "*Novel Synthesis of Bis(indolyl)methanes with Anticancer Properties*", XXII International Symposium on Medicinal Chemistry EFMC/ISMC, **2014**, Lisboa, Portugal.

# Chapter 1

# General Introduction


## Abstract

Indole ring system is the most important heterocycle available in natural compounds. Owing to great structural diversity, the indole ring system has become an important structural requirement in many pharmaceutical agents. Due to their wide range applications, BIMs and their derivatives have become interesting targets for organic chemists. As a result, many synthetic methods for their preparation have been reported. However, given the extent of the studies conducted, it was decided to present the most relevant, covering their great diversity.

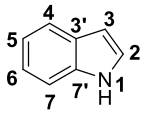
# Chapter 1. General introduction

# 1. Introduction

The chemistry of heterocyclic compounds plays a particularly important role in the progress and development of modern organic chemistry. Heterocycles can be found in a very large proportion of natural compounds. The study of these compounds occupies a notorious place in chemistry with an enormous range of applications in the fields of pharmaceutical science, agrochemicals, photochemistry, dyes and so on. Heterocyclic systems are also very important building-blocks for new materials having interesting electronic, mechanical, or biological properties.[1] Within this important group of compounds, are the nitrogen-based heterocycles. Nitrogen-based heterocyclic chemistry is an important and unique area among the applied fields of organic chemistry, with a considerable amount of research contributing to the development of novel molecules. Numerous N-heterocyclic compounds are widely distributed in nature, having also important physiological and pharmacological properties. These nitrogen-containing heterocyclic molecules have shown diverse applications gaining notoriety in the fields of organic chemistry as well as in the pharmaceutical industry.[2] Moreover, FDA databases reveal that almost 75 per cent of single small molecule drugs contain a nitrogen heterocycle.[3] Due to the ability of the nitrogen atom to easily form hydrogen bonding with biological targets, N-heterocyclic cores present numerous therapeutic applications and are used as building blocks of several of new drug candidates.[1, 2]

## 1.1. Indole scaffold

Indole **1.1** or benzo[*b*]pyrrole (figure 1.1) is a planar heteroaromatic bicyclic molecule in which a six-membered benzene ring is fused to a five-membered pyrrole ring.




1.1

**Figure 1.1** Chemical structure of Indole.

The origin of the name “Indole”, derived from the word India, as the heterocycle was first isolated from a blue “Indigo” produced in India. Since its first isolation by Adolf Von Baeyer in 1866[4], indole is often considered to be the most important heterocyclic molecular scaffold.

Indole appears in a significant number of natural products and across the entire chemical industry in fields such as agriculture, animal health, dyes and pigments, nutraceuticals and dietary supplements, flavours enhancers and perfumes. [1] However, it is in the pharmaceutical industry that its importance becomes crucial. Indole is probably the most important structure in drug discovery. Depending on substituents indole derivatives may have a range of biological activities such as anti-microbial, anti-inflammatory, anti-tumour, anti-diabetic, anti-parkinsonian, anti-viral, anti-histamines and anti-oxidants.[5-14] Some naturally occurring indoles derivatives are shown in Figure 1.2. One of the most known is Tryptophan (Trp) **1.2**, an amino acid used as building block in the biosynthesis of proteins. This indole derivative cannot be synthesised by animals, including humans, being only obtained through the diet, making it an essential amino acid. Trp **1.2** is also a biochemical precursor of other important compounds, such as the neurotransmitters serotonin **1.3** and tryptamine **1.4** as well as the neurohormone melatonin **1.5**.[15]

Other relevant naturally occurring indole derivative is indole-3-acetic acid **1.6**, the most common naturally occurring plant hormone of the auxin class.[16]

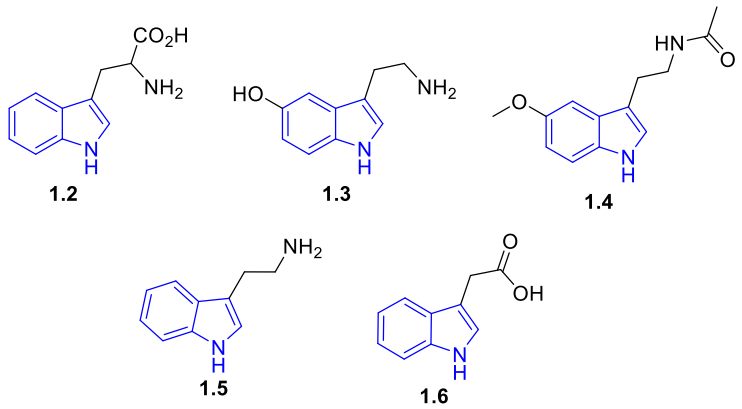



**Figure 1.2** Examples of naturally occurring indole derivatives.

Thus, it is expected that many marketed drugs also present the indole core in their chemical structure. Among others, Fluvastatin **1.7**, is a HMG-CoA reductase inhibitor, which is used to prevent cardiovascular diseases as well as cholesterol-lowering agent;[17] Indomethacin **1.8** (cyclooxygenase antagonists), is a non-steroidal anti-inflammatory drug[18]; Tadalafil **1.9**, is a selective inhibitor of phosphodiesterase type-5 used in the treatment of pulmonary arterial hypertension and also for erectile dysfunction[19]; Ondansetron **1.10**, is a serotonin (5HT3) receptor antagonist, a drug used to avoid nausea

and vomiting caused by cancer chemotherapy, radiation therapy, or surgery[20]; the hypertensive drug Pindelol **1.11**, is a nonselective beta blocker and also an antagonist of the serotonin 5-HT1A receptor and in the last few years has been receiving the attention of researchers for the potential treatment of depression[21, 22] and Vinblastine **1.12**, is a chemotherapy medication used in the treatment of a number of types of cancers such as Hodgkin´s lymphoma, lung, brain, melanoma and testicular cancer (Figure 1.3).[23]

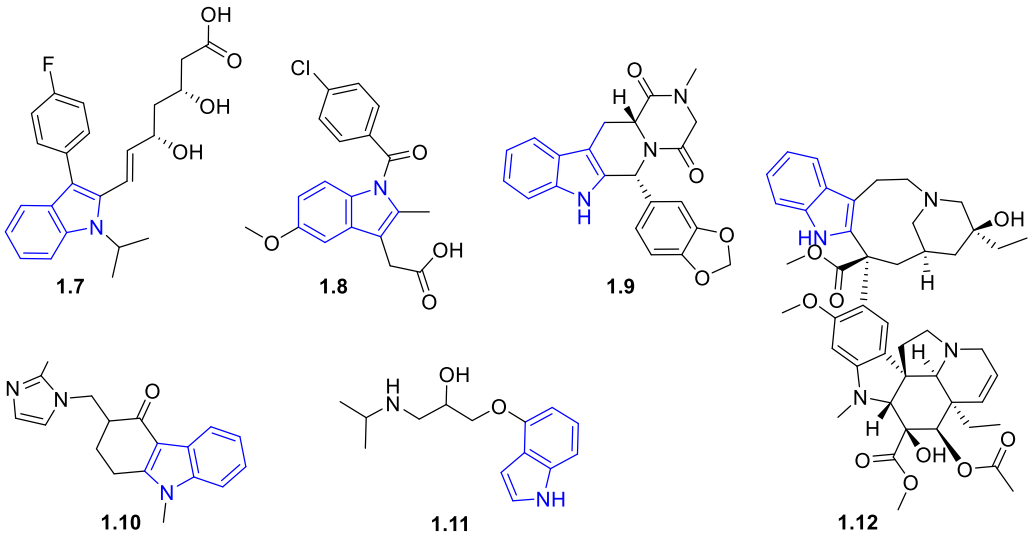



**Figure 1.3** Examples of marketed drugs containing the indole core.

## 1.2 Bis(indolyl)methanes

The constant search to establish easy and environmental benign strategies for the synthesis of indole derivatives, is one of the goals of the scientific community. Particularly interesting are the molecules carrying the bis-indolyl skeleton which have gained substantial attention due to their powerful biological activities. Bis(indolyl)methanes (BIMs) are an important group of alkaloids commonly found in both marine and terrestrial sources exhibiting a broad range of biological activities including anticancer against several types of human tumour cells (Figure 1.4).[24-26] There are distinct classes of BIMs according to the way the two indoles are connected. The most common structures are the 3,3'-BIMs, where the indoles are connected through their most reactive position, C-3, which will be the focus of this thesis.

Naturally occurring BIMs such as Streptindole **1.13**, a genotoxic metabolite of human intestinal bacteria *Streptococcus faecieum* IB 37, isolated by Osawa an Nakimi in 1983, was found to have good antiviral activity against tobacco mosaic virus (TMV) and

fungicidal activity against 14 kinds of phytopathogenic fungi; [26-28] Vibroindole **1.14**, isolated from the marine bacterium *Vibrio parahaemolycitus*, is useful in the treatment of chronic fatigue, fibromyalgia and irritate bowel syndrome;[26, 29-31] Arundine **1.15**, isolated by Khuzhaev and co-workers in 1994, from the roots of *Arunto donax*, present protective cancer activity, particularly against mammary and prostate tumour cells;[26, 32, 33] Arsindoline A **1.16** and B **1.17**, both isolated from a marine bacterial strain CB101 ( *Aeromonas sp.*), showed cytotoxicity against K562, a human erythroleukemic cell line ( Figure 1.4).[26]

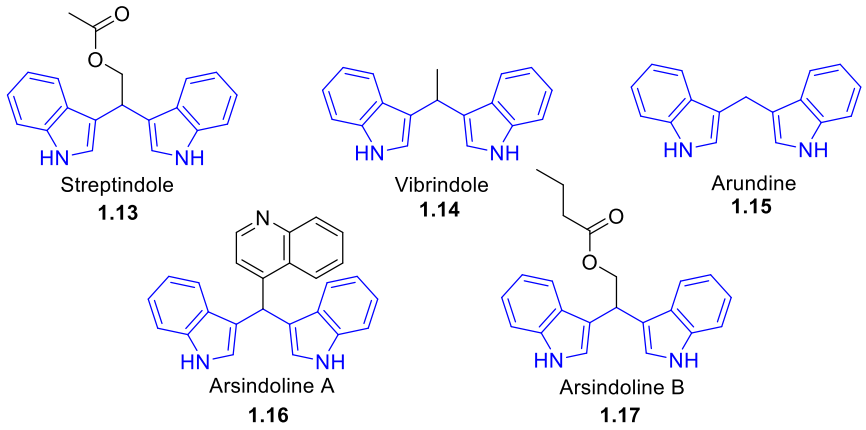



**Figure 1.4** Selected natural occurring BIMs.

Furthermore, synthetic BIM derivatives also exhibit a wide range of biological activities including anti-cancer (e.g. **1.18** and **1.19**),[34, 35] anti-bacterial (e.g.**1.20**),[36] anti-inflammatory (e.g. **1.21**),[37] anti-proliferative (e.g. **1.22**),[38] anti-antioxidant (e.g. **1.23**),[39] analgesic (e.g. **1.24**),[40] they can act as histone deacetylase inhibitors (e.g. **1.25**)[41]and carbonic anhydrase II inhibitor (e.g. **1.26**) (Figure 1.5). In addition to their wide biological applications, BIMs have also been used as dyes and colorimetric sensors.[42-46]

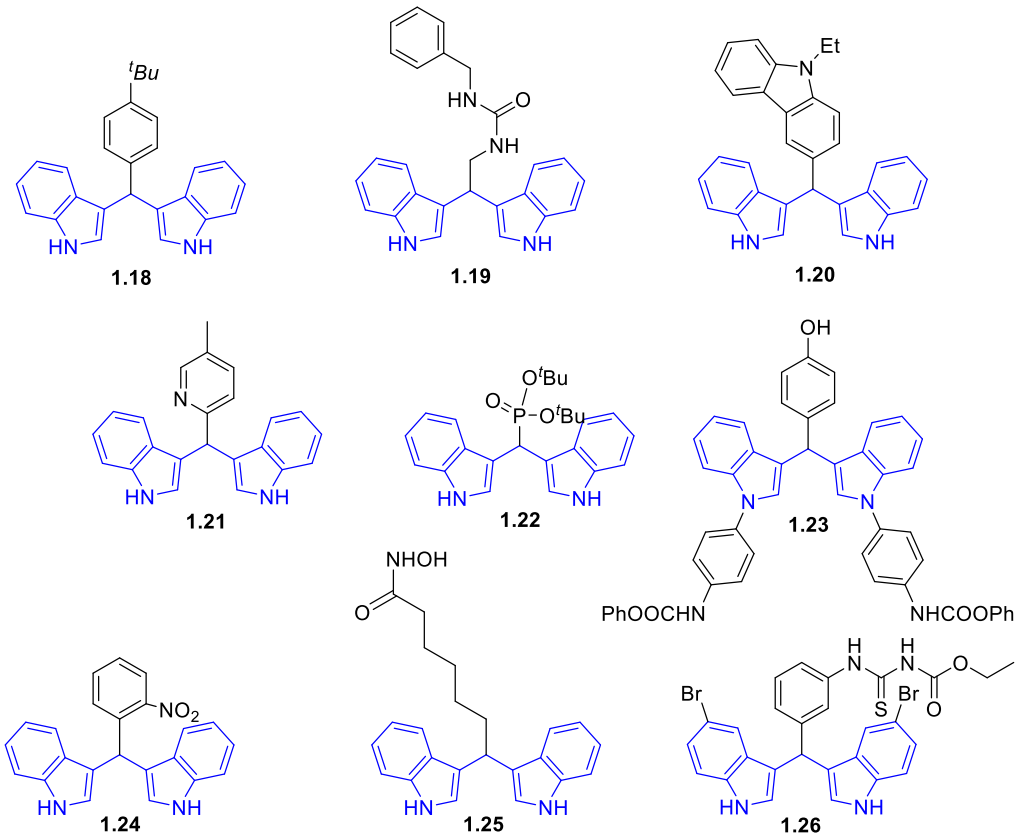



**Figure 1.5** Selected bioactive synthetic BIM derivatives.

### 1.2.1 Synthesis of bis(indolyl)methanes

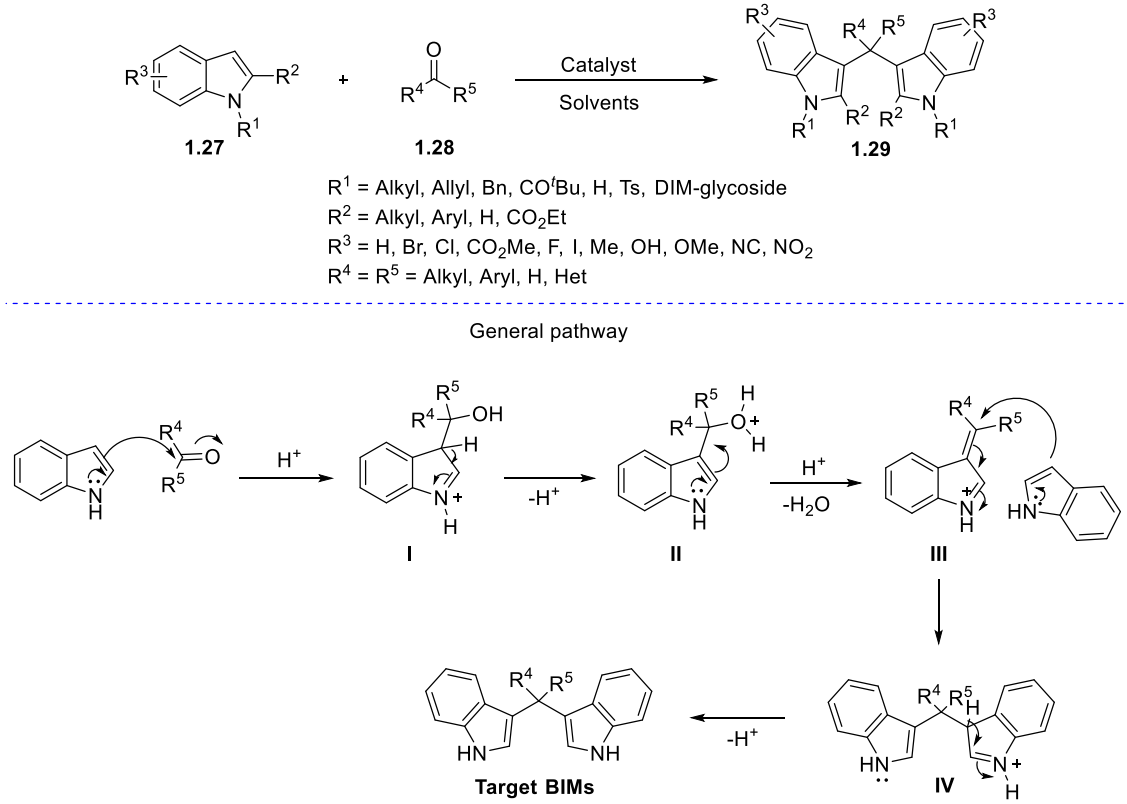
Due to their wide range applications, BIMs and their derivatives have become interesting targets for organic chemists. As a result, many synthetic methods for their preparation have been reported.[26, 47-54] However, given the extent of the studies conducted, it was decided to present the most relevant, covering their great diversity.

#### 1.2.1.1 Synthesis of bis(indolyl)methanes from indoles and carbonyl compounds

BIMs were first prepared by Fischer in 1886 from a substituted phenylhydrazine and an aldehyde or ketone under acidic conditions.[55] Later, Walther and Clemen reported the synthesis of BIMs by using formaldehyde and different indoles in an ethanol-water solution.[56] In the earlier of 60´s, Kamal and Qureshi synthesised several BIMs by condensation of several aliphatic, aromatic, and heterocycle aldehydes with indoles in water under various pH conditions.[57] Since then, the Friedel-Crafts alkylation of aldehydes or ketones under protic and/or Lewis acid catalysis become the most common route for the synthesis of BIMs. Factors such as the type of catalysts, the search for milder reaction

conditions, the reusability of catalysts, the efficiency of the reaction, easier workups or environmental benign procedures led to the development of new methods for the synthesis of BIMs. Lewis acids,[36, 58-69] protic acids,[70-78] solid acids[79-85] including nanocatalysts[86-101] and zeolites,[102, 103] resins,[104-106] transition metal-based catalysts,[107-115] heteropoly acids,[116-118], supramolecular catalysts,[119] biocatalysts,[120-125] natural juices,[126, 127] ionic liquids,[128-135] deep eutectic solvents,[136, 137] under non-conventional methods such as microwave radiation,[138-141] ultrasound, visible light radiation,[142] flow chemistry,[143, 144] mechanosynthesis,[145] have been widely used in the synthesis of BIMs. Additionally, in order to develop more environmentally safe processes, green solvents have also been commonly used in these reactions.[54, 146, 147]

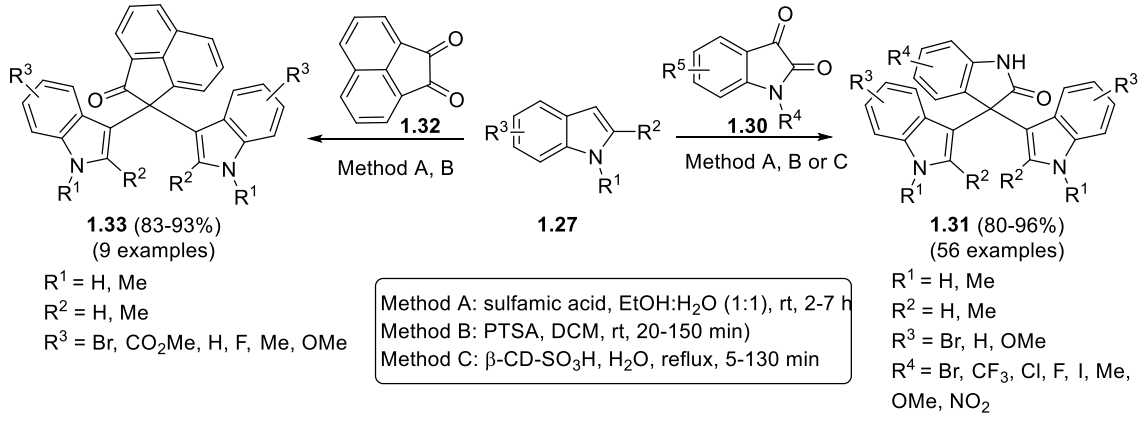
The general mechanism for the formation of 3,3'-BIMs in the presence of acids is described in Scheme 1.1. The Friedel-Crafts pathway leads to the formation of azafulvenium **III**, that undergo further addition on reacting with a second molecule of indole affording the intermediate **IV**, which after rearomatisation leads to the target BIMs.




**Scheme 1.1** The Friedel-Crafts alkylation of indole with aldehydes or ketones in the synthesis of BIMs.

An interesting example of this reactivity is the recently reported synthesis of BIMs starting from isatins **1.30** and acenaphthaquinone **1.32** (Scheme 1.2).[119, 148, 149]

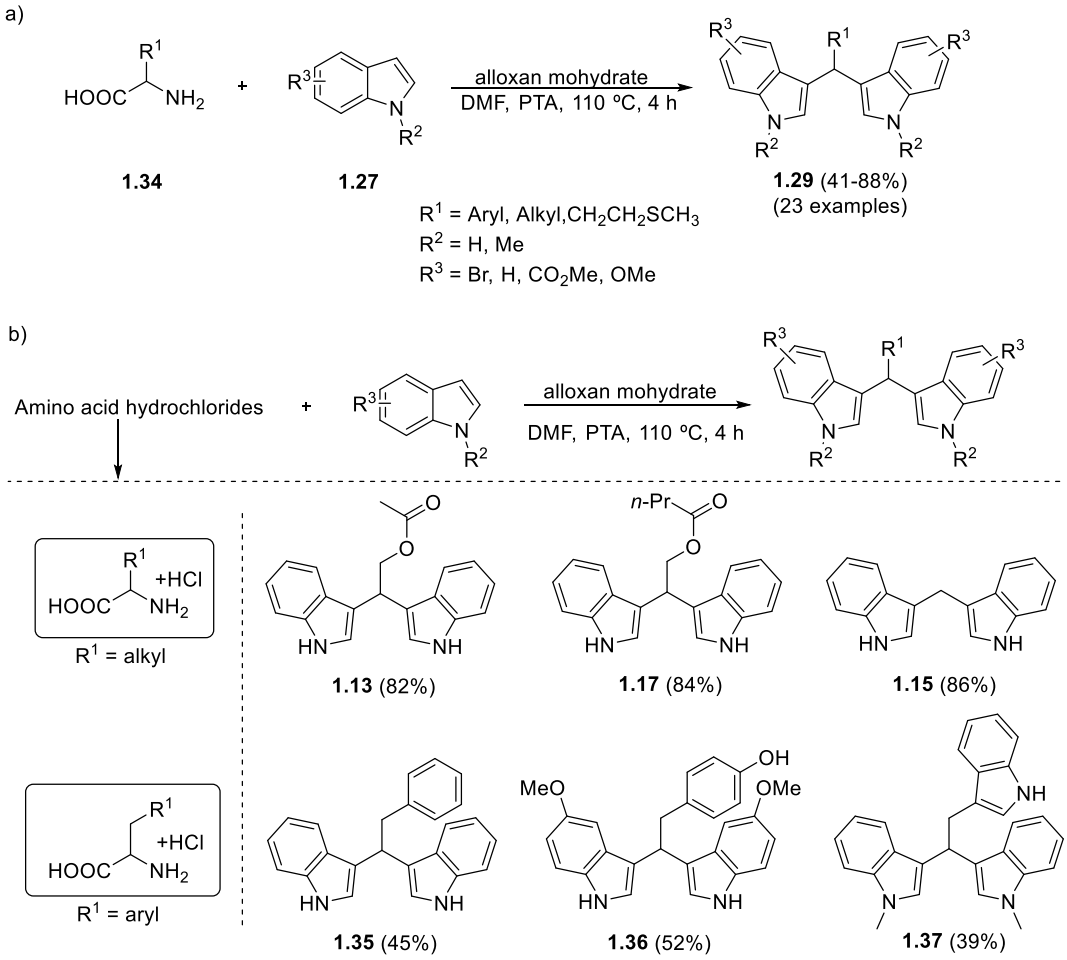
Sulfamic acid in EtOH:$H_2O$ (method A), *p*-toluene sulfonic acid (PTSA) in DCM (Method B), sulfonated β-cyclodextrin (β-CD) in $H_2O$ (Method C), at room temperature were used in the synthesis of BIMs **1.31**, while the BIMs **1.33** were synthesised using the method A and B. The reactions proved to be very efficient, as the target BIMS were obtained in very good yields regardless of the method used. However, under the optimal conditions, when 5-cyanoindole or 5-nitroindole were used, no products were isolated, probably due to the strong electron-withdrawing effect of these substituents (Scheme 1.2).




**Scheme 1.2** Synthesis of BIMS starting from isatins and acenaphthaquinone.

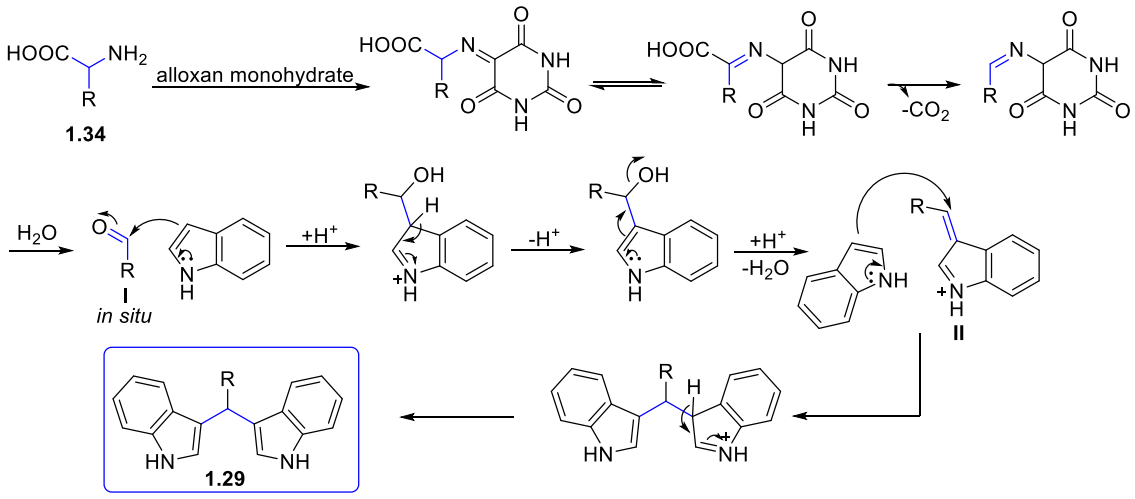
Some variations of these methods can be also found in the literature. For example, the synthesis of natural BIMs and its derivatives following a decarboxylative and deaminative dual coupling reaction of α-amino acids **1.34** with indoles **1.27**, promoted by alloxan monohydrate through a tandem reaction, was reported by Xiang *et al* (Scheme 1.3a).[150] The reactions were carried out using the strong solid phosphotungstic acid 44-hydrate (PTA) as catalyst, at 110 ºC during 4 h, affording the target BIMs **1.29** in good to excellent yields (41-88%). The efficiency of the reaction was shown to be dependent on the substituent pattern of the indole core, since the presence of neutral or electron-donating groups led to the desired products in excellent yields, whereas with electron-withdrawing groups the target compounds were obtained in good yields. Aromatic and aliphatic chain α-amino acids were also used, leading to lower conversions, suggesting that the electronic nature of the amino acids α-carbon has a considerable effect on the transformation.[150]

Natural occurring BIMs such as Streptindole **1.13** (82%) , Arsindoline B **1.17** (84%) and Arundine **1.15** (86%) could also be obtain in very good yields, starting from alkyl amino acid hydrochlorides. Amino acids such as phenylalanine, tyrosine, and tryptophan led to the desired BIMs **1.35** - **1.37** in yields ranging from 39 to 52% (Scheme 1.3b).[150]



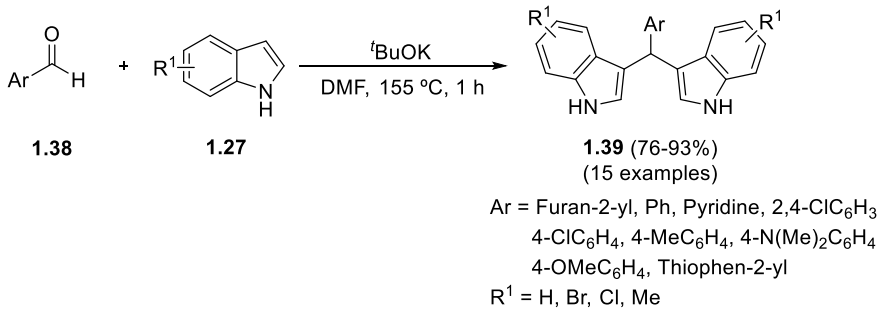

**Scheme 1.3** Synthesis of natural BIMs and its derivatives following a decarboxylative and deaminative dual coupling reaction of α-amino acids with indoles.

The proposed mechanism for this one-pot reaction is outlined in Scheme 1.4. The alloxan monohydrate promoted the *in situ* transformation of α-amino acids **1.34** into the corresponding aldehydes **I**, through a sequential condensation/hydrolysis process, which then reacted with the first molecule of indole **1.27** leading to the intermediate **II**. Finally, cation **II** alkylates another indole molecule affording the desired BIMs **1.29** (Scheme 1.4).[150]




**Scheme 1.4** Proposed mechanism for the synthesis of BIMs **1.29**.

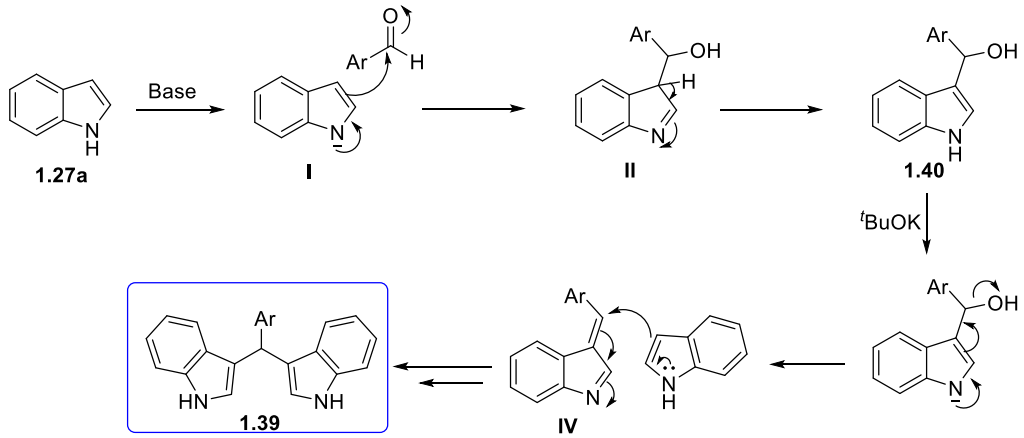
Recently, potassium *tert*-butanolate promoted synthesis of symmetrical BIMs from aromatic aldehydes **1.38** and indoles **1.27** was reported by Chong *et al.*.[151] Substrates bearing different functional groups, susceptible to acidic media, were well tolerated under the optimal conditions, and BIMs **1.39** were obtained in good to excellent yields (76-93%) (Scheme 1.5).




**Scheme 1.5** Synthesis of symmetrical BIMs from aromatic aldehydes and indoles mediated by alkaline reagent.

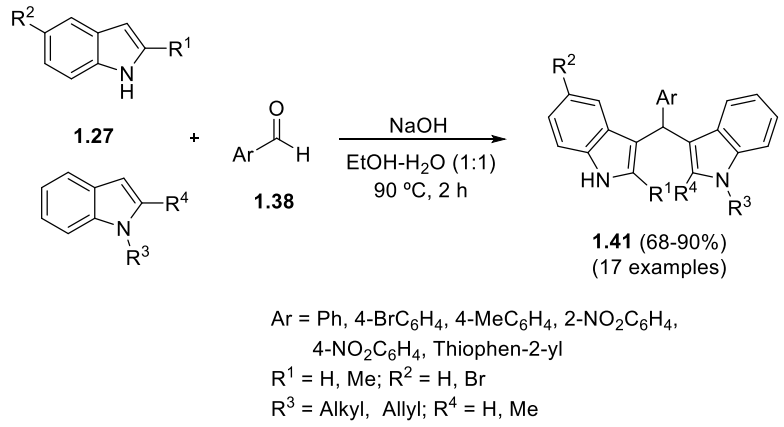
In order to try to shed some light on the mechanism for the formation of BIMs **1.39**, control experiments were carried out. First, the reaction between aldehyde and indole was performed in absence of base and the target BIM was not obtained, indicating that potassium *tert*-butanolate ($^t$BuOK) is essential for the reaction take place. On other hand, they found that the yield of BIMs **1.39** decreased significantly when the reaction was

performed at room temperature, and compound **1.40** become the major product. The result suggests that **1.40** might be an important intermediate in the overall transformation and, as expected, the target BIMs **1.39** were obtained from this intermediate under the optimal reaction conditions. Therefore, based on these results the proposed the mechanism by authors is outlined in Scheme 1.6. After treatment of indole with base, the intermediate **1.40** was generated and underwent dehydration to produce the intermediate **IV**. Addition of a second indole molecule to the latter led to the formation of BIM **1.39** (Scheme 1.6).



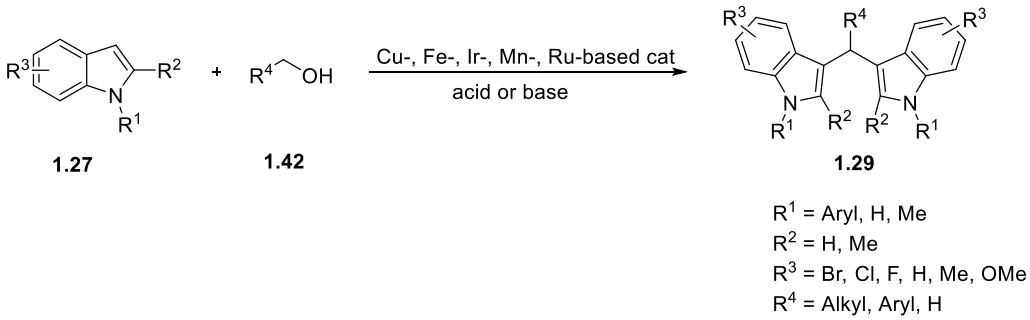

**Scheme 1.6** Proposed mechanism for the synthesis of indoles **1.39**.

Deb and co-workers[152] reported the first base-catalysed three-component cascade approach to unsymmetrical BIMs. In these transformations, mediated by NaOH in EtOH-$H_2O$ (1:1) at 90 °C, unsymmetrical BIMs **1.41** were obtained in yields ranging from 68% to 90% (Scheme 1.7). The efficiency of the reactions was dependent on the starting aldehyde since electron-rich aldehydes led to target BIMs in lower yield, producing also minor amount of symmetrical BIM, whereas electron-deficient aldehydes allowed for a more efficient synthesis of BIMs **1.41**. Both electron-donating and electron-withdrawing groups on the phenyl ring of the aldehydes such as –Me, –OMe, –$NO_2$, –Br, were well tolerated. The proposed mechanism is very similar to the one presented in Scheme 1.7. In fact, the reaction also proceeds via 3-indolylalcohol **1.40**, which was confirmed through isolation.




**Scheme 1.7** Base-catalysed three-component cascade approach to unsymmetrical BIMs.

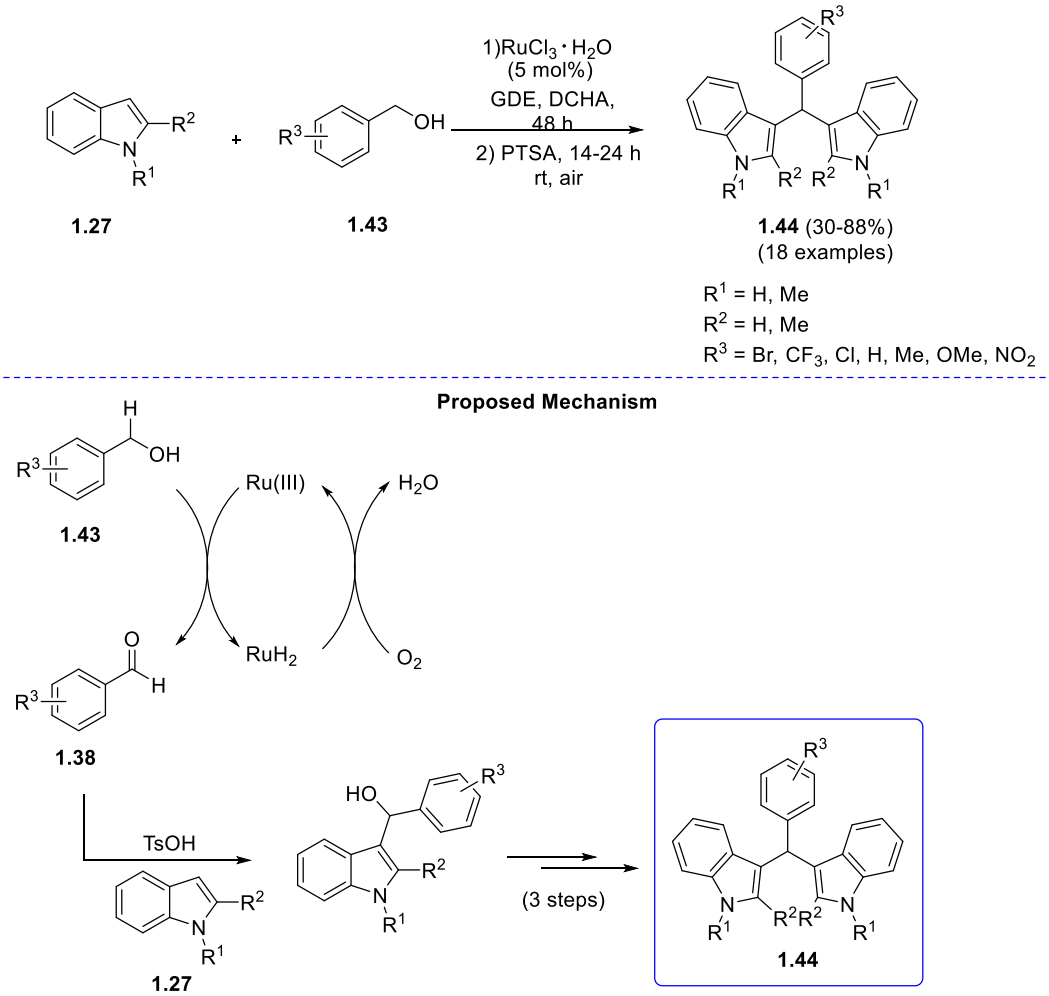
Another interesting strategy involves the *in situ* transformation of primary alcohols **1.42** into aldehydes or ketones. Cooper-,[153] iron-,[154] iridium-[155] manganese-[156] or ruthenium-based[157-159] catalysts in presence of acid or base have been used for this type of transformation (Scheme 1.8).




**Scheme 1.8** Overview of synthetic strategy towards BIMs starting from primary alcohols and indoles.

An example of this type of reactivity is outlined in Scheme 1.9. In this study, the reactions between indoles **1.27** (1.0 equiv.) and benzylic alcohols **1.43** (0.5 equiv.) were carried out in presence of $RuCl_2{\cdot}H_2O$ (5 mol%) and dicyclohexylamine (DCHA), in ethylene glycol dimethyl ether (GDE), at room temperature during 48 h. Then, after adding PTSA, the corresponding BIMs **1.44** were obtained in moderate to excellent yields (30-88%). The authors also disclosed that the use of 1.0 equiv. of DCHA had a significant impact on the efficiency of the reactions, since any decrease or increase in the amount of this amine led to a sharp decrease in yields. The substitution pattern at the benzyl alcohols also proved to have influence on the yield. Thus, benzylic alcohols with electron-donating

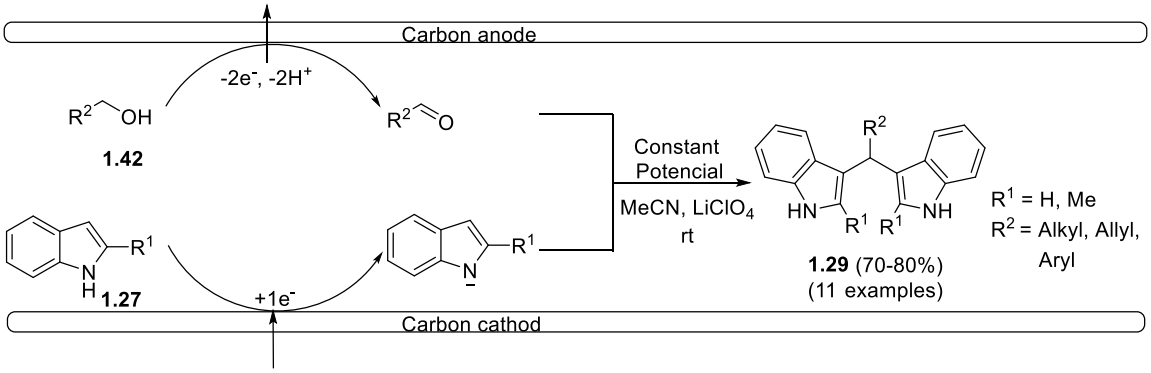
groups led to an improvement on the yield (65-83%), while the opposite was found when benzyl alcohols carrying electron-withdrawing groups were used (30-36%). The presence of halogens such as bromine or chlorine on the phenyl moiety of the alcohol, gave rise to the desired products in good to excellent yields (67-88%) (Scheme 1.9). According to the authors' proposal, the ruthenium-based catalyst promotes the *in situ* oxidation of the benzylic alcohols to aldehydes forming the ruthenium hydride which in turns reacts with molecular oxygen to regenerate the catalyst. After the formation of the aldehydes, the reaction follows the general mechanism shown in scheme 1.1 (Scheme 1.9).[157]




**Scheme 1.9** Reactions between indoles and benzylic alcohols towards BIMS.

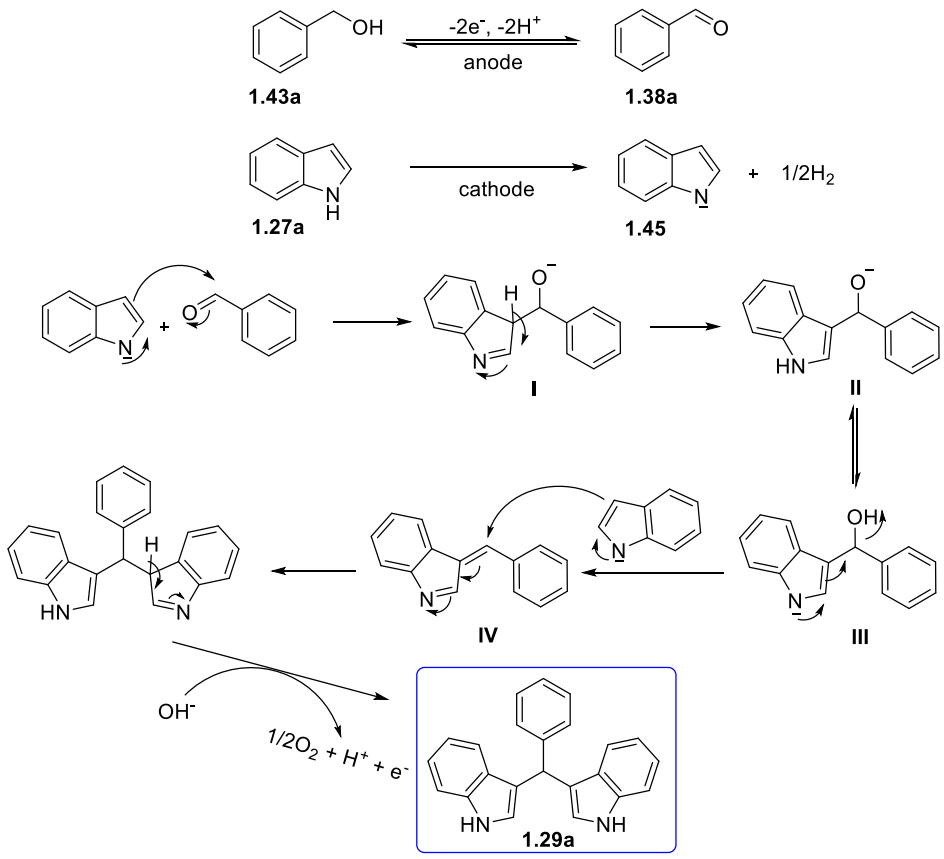
In addition to these methods, an interesting route for electro-oxidative synthesis of nanosized BIM nanoparticles, via controlled-potential coulometry in an undivided cell, using acetonitrile (MeCN) as solvent was described by Nikoofar and Ghambari.[160] (Scheme

1.10). The electro-chemical condensation of primary alcohols **1.42** and indoles **1.27**, in presence of $LiClO_4$ as electrolyte in MeCN at room temperature, gave rise to the desired products **1.29** in good to very good yield (70-80%). Experimental data showed that aryl alcohols and derivatives with electron-donating and electro-withdrawing substituents, reacted efficiently with indoles, since the corresponding BIMs were obtained in high yields. The results also showed that the reaction with aliphatic and allylic alcohols were well tolerated, but the condensation between indoles and aliphatic alcohols proceeded more slowly than the corresponding reaction with allylic alcohols (Scheme 1.10).[160]



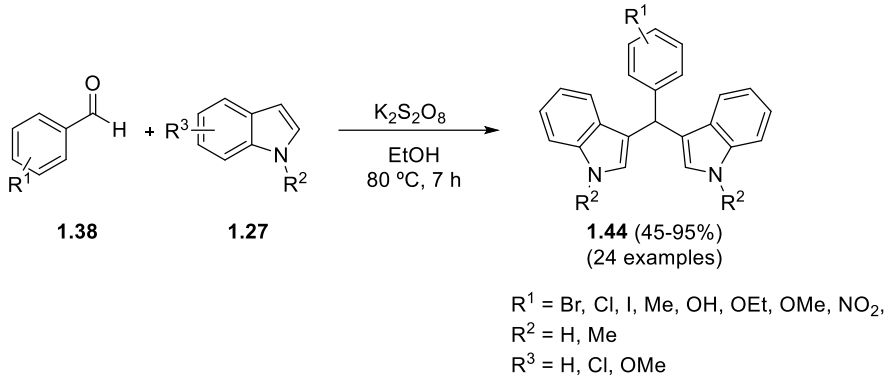

**Scheme 1.10** Electro-chemical condensation of terminal alcohols and indoles towards BIMs.

Although the mechanism has not been proven, the authors consider that the reaction follows the pathway shown in Scheme 1.11. First, the *in situ* generation of the aldehyde **1.38a** by oxidation of the benzylic alcohol **1.42a** and formation of the indole anion **1.45** occur at the anode and cathode, respectively. Then, the nucleophilic attack of the indole anion to the aldehyde leads to the formation of the intermediate **I**. This is then stabilised by an H-shift leading to intermediate **II** which is in equilibrium with **III**. After the release of the hydroxyl ion and a second nucleophilic attack of another indole anion **1.45**, intermediate **IV** is obtained, which in turn undergoes protonation by the *in situ* produced $H^+$, generated from the hydroxyl oxidation at the anode, leading to the desired BIM **1.29a**.



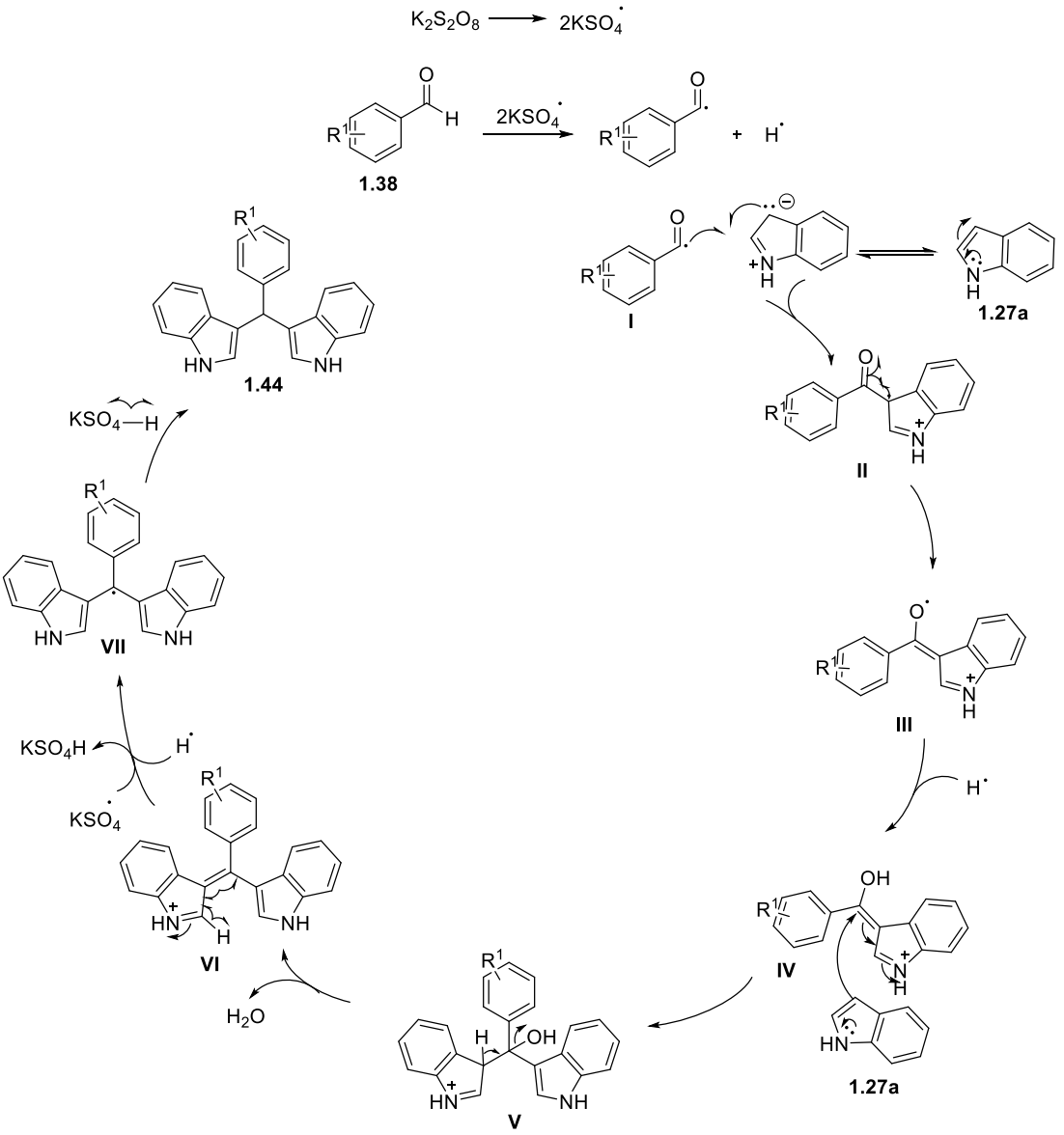

**Scheme 1.11** Proposed mechanism for the synthesis of BIMs **1.29**.

A radical pathway to synthesise BIMs, using potassium peroxodisulfate ($K_2S_2O_8$) as catalyst, was reported by Konwar and Bora.[161] Starting from a range of aromatic aldehydes **1.38** and indoles **1.27**, in ethanol at 80 ºC, during 7 h, BIMs **1.44** were obtained in moderate to excellent yields. Reactions between 1-methyl-1*H*-indole and electron deficient aldehydes ($R^1$ = Br, $R^1$ = Cl, $R^2$ = $NO_2$) were more efficient affording BIMs in higher yields (90%, 95% and 80%, respectively) than when electron-rich aldehydes were used. A moderate yield was observed when 2-iodobenzaldehyde was used, which can be rationalised considering the bulkiness of iodine (Scheme 1.12).




**Scheme 1.12 S**ynthesis of BIMs, using potassium peroxodisulfate as catalyst.

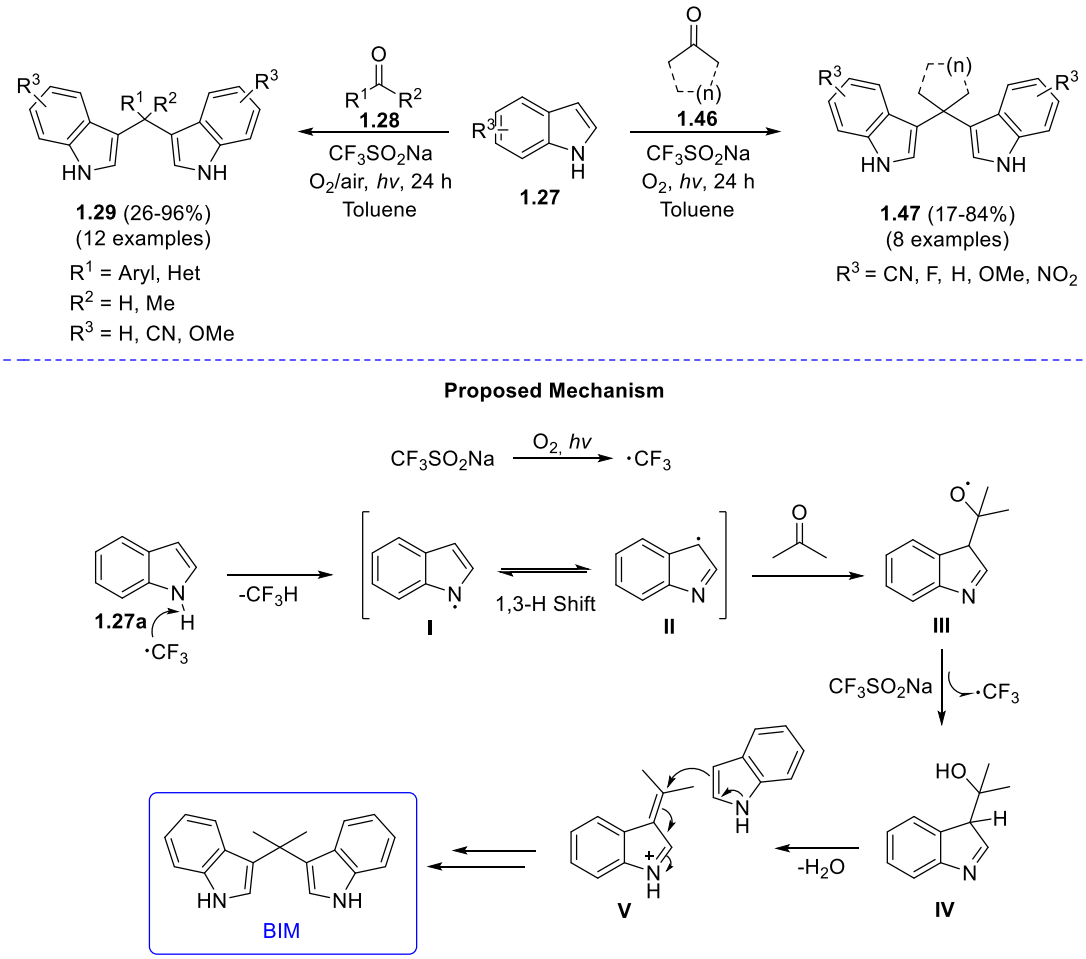
The experimental data suggested that the reaction follows a free radical pathway in presence of $K_2S_2O_8$, a known free radical initiator, as shown in the proposed catalytic cycle outlined in Scheme 1.13. In the first step, $K_2S_2O_8$ generates the $KSO_4$ radical which can react with the aldehyde **1.38** to form carbonyl radical **I**. This interacts with indole to form oxygen radical **III** via intermediate **II**. From the reaction of **III** with the species •H the intermediate **IV** is obtain. Subsequent attack by a second molecule of indole led to **V** which in turn loses a water molecule to form **VI**. Next, other $KSO_4$ radical removes a hydrogen from **VI** in order to stabilize the indole molecule and generating the tertiary radical **VII** along with $KSO_4H$. The abstraction of the hydrogen from $KSO_4H$ led to the desired BIM **1.44** regenerating the $KSO_4$ radical which again participates in another catalytic cycle (Scheme 1.13).




**Scheme 1.13** Proposed mechanism for the $K_2S_2O_8$-promoted synthesis of BIMs **1.44**.

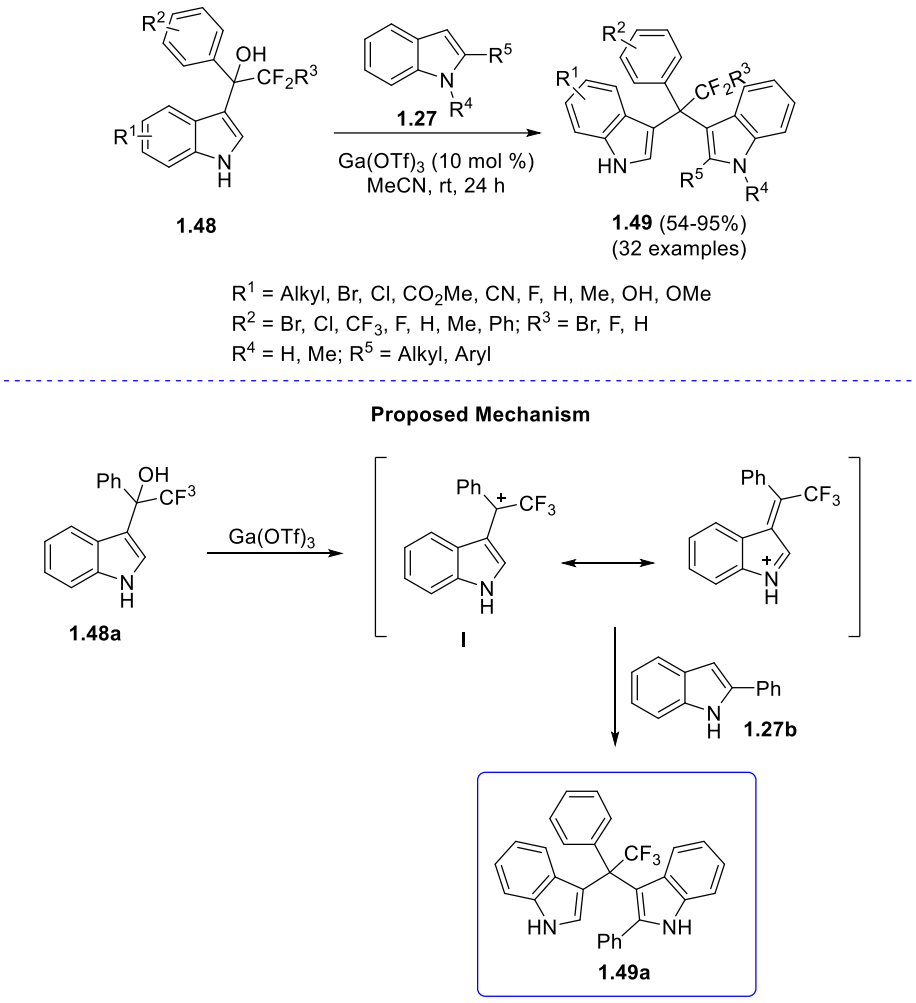
More recently, a one-step $CF_3SO_2Na$-mediated UV-light-induced Friedel−Crafts alkylation of indoles **1.27** with ketones (alkyl and aromatic) and aromatic aldehydes **1.28** was disclosed by Yang *et al.*.[162] The reactions were carried out in toluene at room temperature under UV radiation, during 24 h in an oxygen/air atmosphere (Scheme 1.14). Both electron-donating and electron-withdrawing groups on the indole moiety were screened, influencing the efficiency of the reaction with alkyl ketones **1.46**. For example, the presence of a cyano group at C-5 position resulted in the synthesis of the corresponding BIMs in very good yield (84%), whereas starting from 5-methoxy-indole, the efficiency of the reaction decreased, leading to the corresponding BIM **1.47** in 49% yield. More, starting

from 5-fluoroindole, the yield has fallen sharply with the corresponding BIM obtained in 17% yield . On the other hand, when aromatic ketones **1.28** ($R^1$ and $R^2 \neq H$) were tested in the reaction with 1*H*-indole, BIMs **1.29** were obtained in 26-62% yield. The highest yield was obtained when *p*-bromoacetophenone was used and the lowest yield using a ketone with a naphthyl group. Furthermore, compared to aromatic ketones, aromatic aldehydes **1.28** ($R^2 = H$) showed much higher efficiency, regardless of whether they had electron-donating or electron-withdrawing groups on the aromatic ring (43%-89%). The great advantage of the described method is the non-use of acid, base, transition metal or photocatalysts, showing a wide substrate range and scale-up capability. According to the mechanistic proposal, upon UV radiation and under $O_2$ atmosphere, the salt $CF_3SO_2Na$ is oxidised generating *in situ* the radical $\bullet CF_3$. These radical attacks the amine hydrogen of the first molecule of indole **1.27** to form the radical **I**, which easily isomerises to carbon radical **II** (1,3-H shift). The latter reacts with ketone (e.g. acetone) or an aldehyde generating an oxygen radical **III**, which in turns reacts with the salt and $H_2O$ to generate again the radical $\bullet CF_3$ and indole **IV**. Following a β-elimination and consequent generation of **V**, a second molecule of indole attacks the intermediate **V** which after rearomatization gives the target BIM (Scheme 1.14).[162]




**Scheme 1.14** One-step $CF_3SO_2Na$-mediated, UV-light-induced Friedel–Crafts alkylation of indoles.

A $Ga(OTf)_3$-catalysed Friedel-Crafts alkylation between di- or trifluoromethylated 3-indolylmethanols **1.48** and indoles **1.27** for the synthesis of unsymmetric BIMs **1.49** was also developed.[163] In this transformation, considering one representative example (reaction of **148a** with **1.27b**), after an initial activation of the alcohol substrate **1.48a** by the catalyst followed by elimination, carbocation **I** is generated. A subsequent Friedel-Crafts alkylation with indole **1.27**, led to the correspondent unsymmetric BIM (Scheme 1.15).

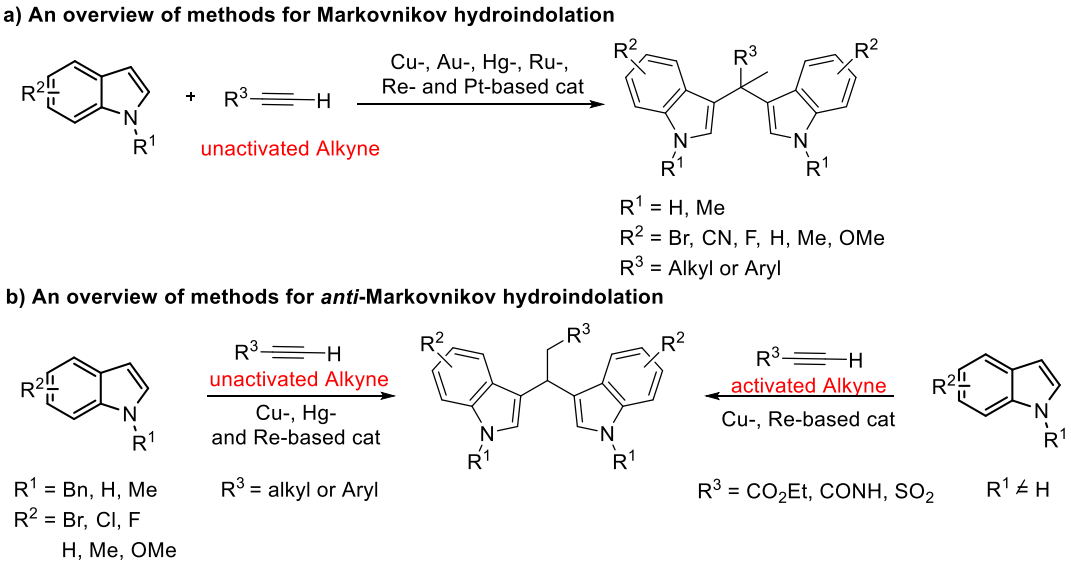



**Scheme 1.15** $Ga(OTf)_3$-catalysed Friedel-Crafts alkylation between di- or trifluoromethylated 3-indolylmethanols and indoles for the synthesis of unsymmetric BIMs.

### 1.2.1.2 Synthesis of bis(indolyl)methanes from indoles and alkynes, alkenes and allenes

Catalytic hydroindolation using alkynes as easily available substrates became a powerful approach for BIM synthesis. The great advantage of this method is that it does not require pre-functionalisation of the reaction partners, which makes it one of the most efficient synthetic routes for the synthesis of symmetrical and unsymmetrical BIMs. To date, various metal-based catalysts (cooper-, gold- , mercury-, ruthenium-, rhenium-, palladium- and platinum) for the hydroindolation reaction between indoles and terminal alkynes have been reported.[164-172] In contrast, metal-free catalytic hydroindolation is scarce, with only one single literature report.[173]

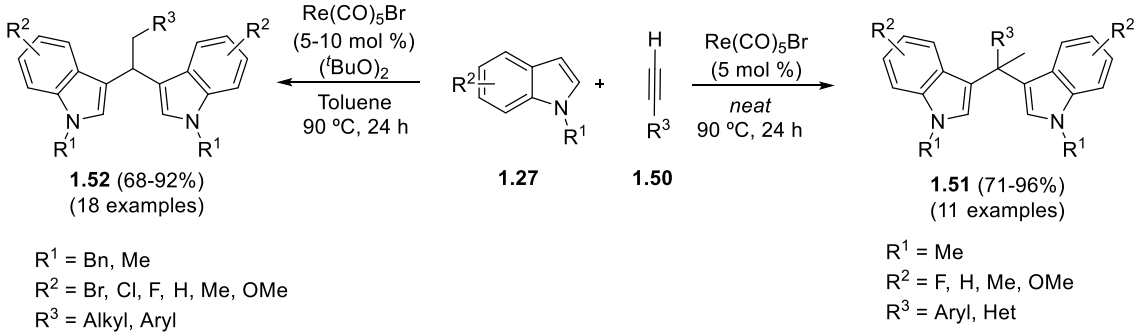
The control of the regioselectivity during the addition of indoles to terminal alkynes is still a challenge. The electronic imbalance of the triple bond in key transition metal-alkyne complex, results in the regioselective attack of indoles at the internal C atom of unactivated terminal alkynes, resulting in Markovnikov's adducts (Scheme 1.16a).[164-168, 173, 174] On the contrary, for the selective intermolecular *anti*-Markovnikov addition is necessary to create an electronic bias in the triple bond, in order to facilitate the indole addition at the terminal carbon of the alkyne. Therefore, activated alkynes containing electron-withdrawing groups such as sulfone, ester, and amide have been employed.[121, 175] However and despite being scarce, studies of a direct intermolecular *anti*-Markovnikov addition of indoles to unactivated terminal alkynes started to be carried out successfully (Scheme 1.16b).[168, 170]




**Scheme 1.16** a) Overview of method for Markovnikov and b) *anti*-Markovnikov hydroindolation.

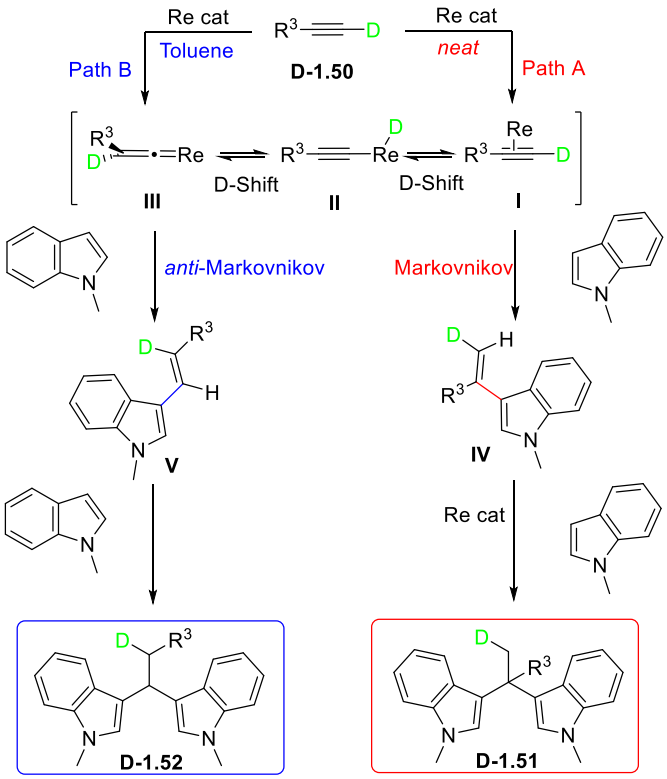
Among the various studies carried out on this topic, a particularly interesting one reported by Xia *et al.*, showed that by making slight changes to the method selectivity can be controlled.[168] According to the authors, the regioselectivity of rhenium-catalysed regiodivergent addition of indoles to unactivated terminal alkynes can be orientated towards a Markovnikov or *anti*-Markovnikov addition when the reaction is carried out under *neat* conditions or using toluene as solvent, respectively (Scheme 1.17). Therefore, the reactions between indoles **1.27** and terminal alkyl or aryl alkynes **1.50**, using $Re(CO)_5Br$ as catalyst, in *neat* conditions, during 24 h gave rise to the Markovnikov

adducts BIMs **1.51** in good to excellent yields (71-96%), whereas in toluene at 90 ºC for 24 h the *anti*-Markovnikov adducts BIM **1.52** were obtain in similar yields (68-92%). In both cases, *N*-substituted indoles proved to be suitable substrates for these transformations. In general, either electron-donating or electron-withdrawing substituent groups on the indole nuclei were well tolerated. However, reactions performed with phenylacetylene derivatives proved to be more complex, requiring the use of a di-*tert*-butyl peroxide additive (Scheme 1.17).[168]



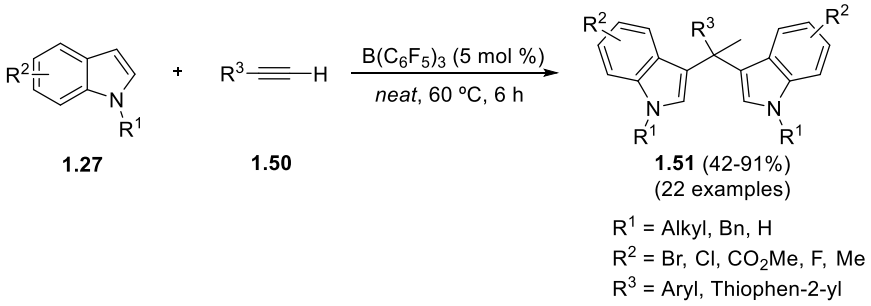

**Scheme 1.17** Rhenium-catalysed regiodivergent addition of indoles to terminal alkynes.

In order to investigate the reaction mechanism, reactions were carried out in absence of catalyst and no product was obtained, indicating that its use is essential in the hydrolindolation step. Reactions between deuterium-labeled 4-chlorophenylacetylene and indole were also performed. Based on the experimental data, the authors proposed a plausible mechanism shown in Scheme 1.18.[168] Thus, under neat conditions, coordination of the Re-based catalyst with the deuterated alkyne **D-1.50** afforded intermediate **I** (path A). Direct nucleophilic attack of the first molecule of *N*-substituted indole on the internal carbon of the C-C triple bond led to the 3-alkenylindole **IV**. Subsequently, the second hydroindolation gives rise to the Markovnikov product **D-1.51** The 1,2- or 1,3-D shift of intermediates **I** or **II**, respectively, could occur when the reaction was carried out in toluene leading preferentially to Re-vinylidene **III** (path B). Therefore, the nucleophilic attack of *N*-substituted indole on the carbon α to the Re center of intermediate **III** followed by protonolysis led to intermediate **V**. Similarly, the second Re-catalysed hydroindolation affords the *anti*-Markovnikov product **D-1.52** (Scheme 1.18).[168]



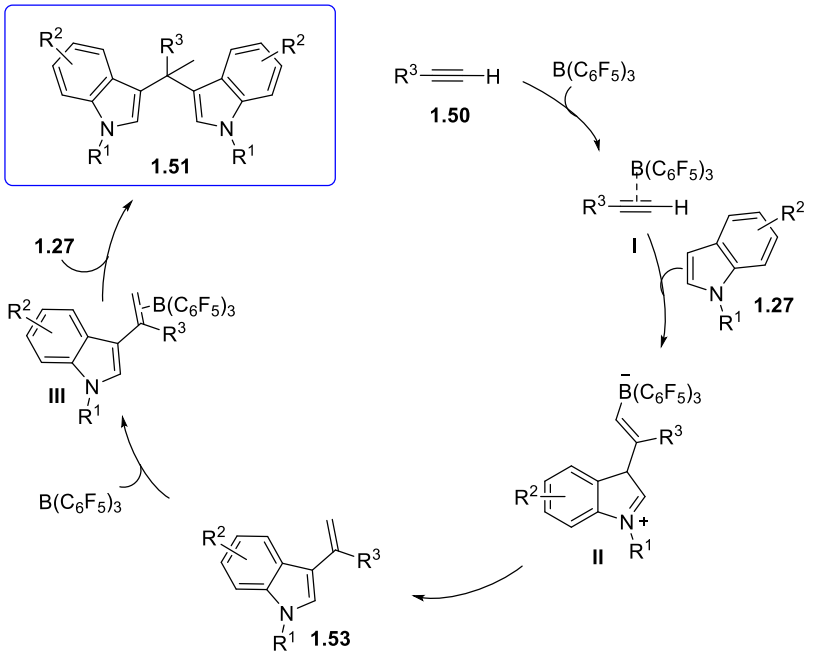

**Scheme 1.18** Proposed mechanism for the synthesis of BIMs **1.51** and **1.52**.

More recently, the first and only metal- and solvent-free catalytic intermolecular Markovnikov addition of indoles to aryl alkynes was reported by Ling and co-workers.[173] The reactions between indoles **1.27** and aryl terminal alkynes **1.50**, were carried out in the presence of $B(C_6F_5)_3$ as catalyst affording the target BIMs in moderate to good yields (42-89%). The study showed that the substitution pattern in the indole core affected the efficiency of the transformation. In fact, indoles bearing electron-donating or electron-withdrawing groups at C-5 and C-7 positions reacted smoothly with **1.50** giving the desired BIMs **1.51** in moderate to good yields (65-91%) whereas with indoles bearing those groups in C-6 position, lead to the target BIMs **1.51** in relatively higher yields. The methodology worked with both *N*-unsubstituted and *N*-substituted indoles, selectively providing a large library of BIMs (Scheme 1.19). [173]




**Scheme 1.19** Metal- and solvent-free catalytic intermolecular Markovnikov addition of indoles to aryl alkyne.

According to the mechanism proposed by the authors, the first step is the coordination of the catalyst to the terminal alkyne **1.50** affording the intermediate **I**. This follows a nucleophilic attack of indole on the internal carbon of the C-C triple bond to yield the zwitterionic boron derivative **II**, which after a subsequent protodeborylation and aromatization gives rise to the intermediate **1.53** with the regeneration of the catalyst. Next, the catalyst coordination with the latter affords the intermediate **III**. After the reaction with a second molecule of indole the target Markovnikov adduct **1.51** is obtained (Scheme 1.20).[173]

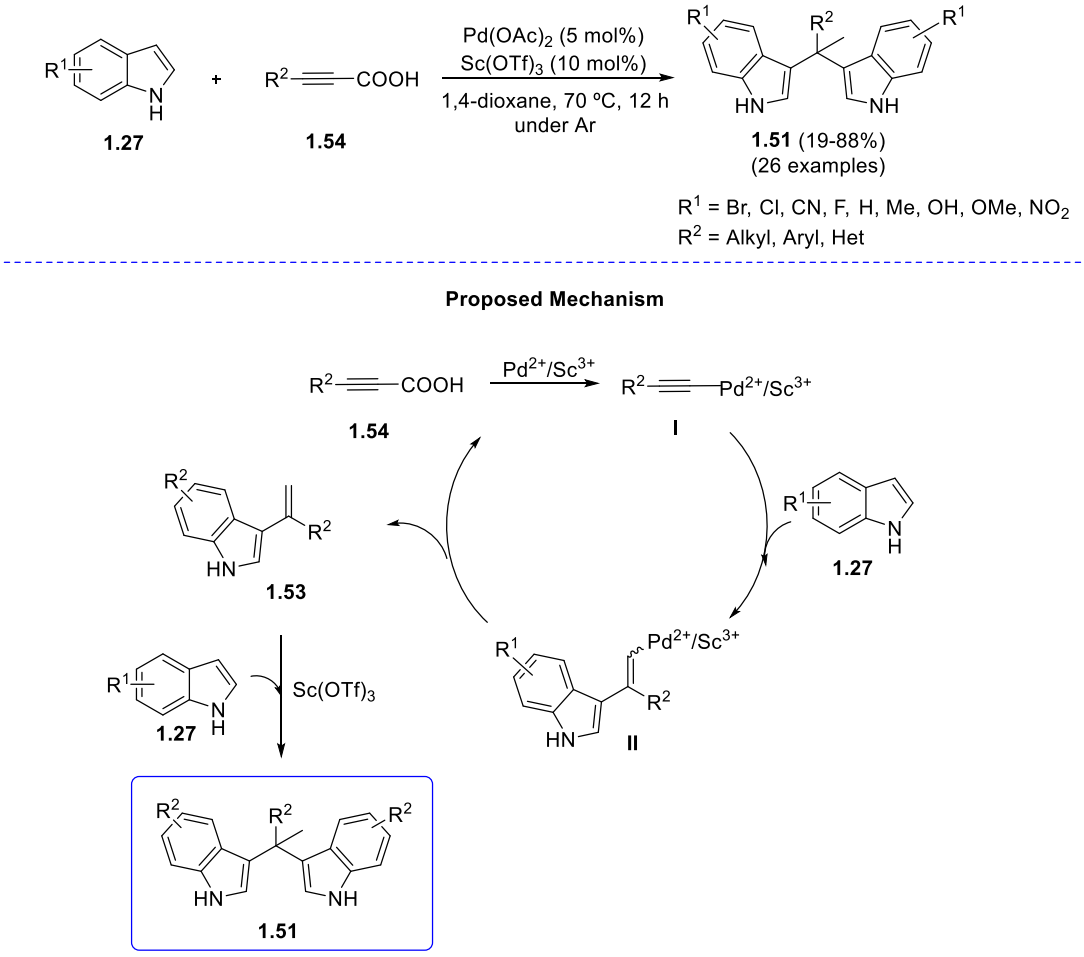



**Scheme 1.20** Proposed mechanism for the synthesis of BIMs **1.51**.

Another interesting example of this type of reactivity was described by Zeng e*t al*..[172] In this study, BIMs were synthesised through decarboxylative addition of propiolic acid

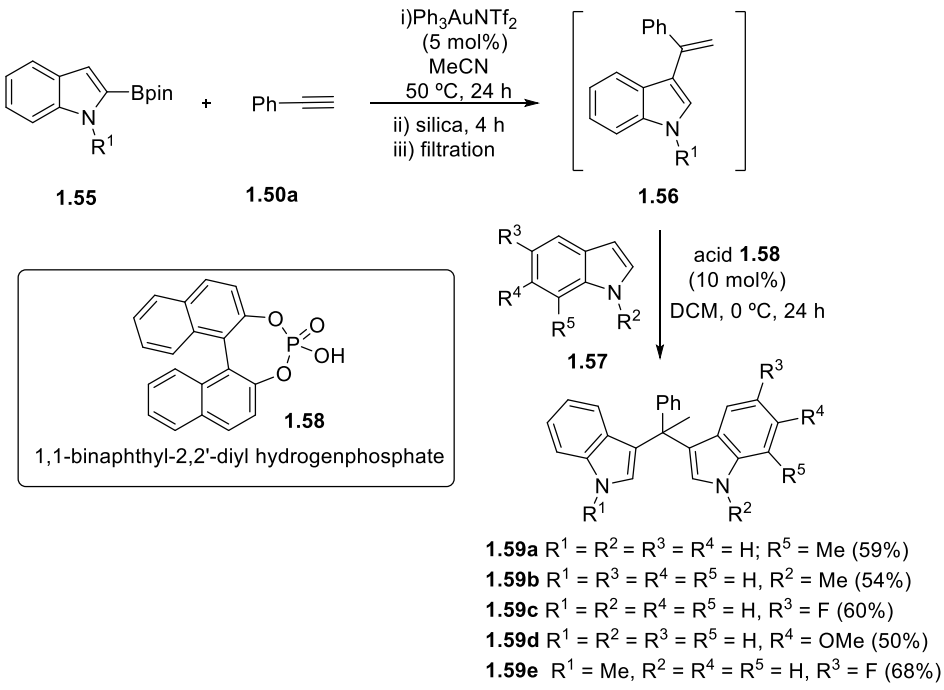
derivatives **1.54** to indoles **1.27** in presence of a Pd(II)/Lewis acid catalyst (Scheme 1.21). The use of a Lewis acid is required for the success of the transformation since no product was obtained when the reaction was carried out in the presence of the Pd-based catalyst. Within the Lewis acids screened, $Sc(OTf)_3$, was the one that led to BIMs **1.51** in higher yields. The reaction proved to be regioselective as the BIMs **1.51** resulting from the Markovnikov addition were obtained as major products. However, *anti*-Markovnikov adducts were also obtained in lower yield.

In view of these results, the proposed mechanism involves the *in situ* generation of hetero-bimetallic Pd(II)/Sc(III) **I** as the key species for the decarboxylative addition reaction. Subsequently, the nucleophilic attack of the indole molecule leads to the intermediate **II**, which is converted into intermediate **1.53** with the regeneration of the catalyst. The reaction of **1.53** with a second molecule of indole affords the target BIM **1.51** (Scheme 1.21).[172]




**Scheme 1.21** Pd(II)/Lewis acid-catalysed decarboxylative addition of propiolic acid derivatives to indoles for the synthesis of BIMs.

Although most of the methods reported for the reaction of indoles with unactivated alkynes produced symmetric BIMs, the development of methods to selectively obtain unymmetric BIMs has proved to be more challenging. Nevertheless, McLean *et al* described a selective one-pot gold-catalysed hydroindolation of indoles with terminal alkynes affording unsymmetrical BIMs via vinyl indoles.[171] In this study, 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indoles **1.55** reacted with phenylacetylene **1.50a**, using $PPh_3AuNTf_3$ as catalyst, to give the vinylindoles **1.56** which were used in the next step without further purification. Next, these intermediates reacted with indoles **1.57** via a Lewis acid-catalysed reaction leading to the target BIMs **1.59** in good yields (50-68%) (Scheme 1.18). Surprisingly, starting from 2-phenyl-1*H*-indole the unwanted less sterically bulky symmetrical BIM was obtained. However, the use of a labile bulky group such as Bpin at C-2 position of the first indole allowed the formation of vinylindoles **1.56** and consequently, the formation of the unsymmetrical BIMs (Scheme 1.22).

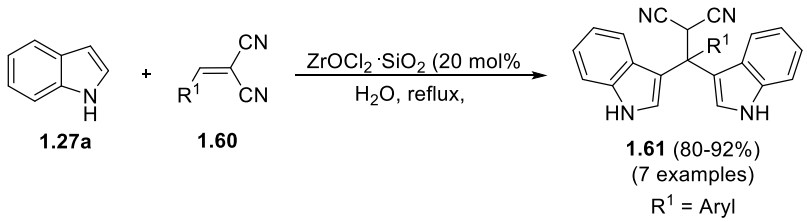



**Scheme 1.22** Selective one-pot gold-catalysed hydroindolation of indoles with terminal alkynes.

Reports on the synthesis between indoles and alkenes are scarce, with few studies been conducted.[176-178]

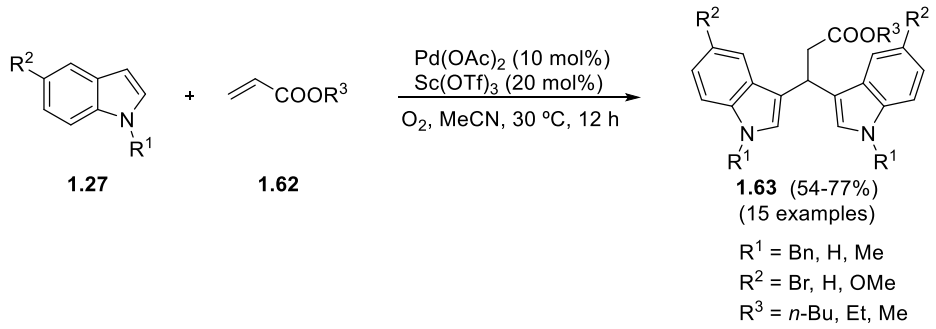
Jori and co-workers reported an efficient "on water" methodology for the synthesis of bis(indole) derivatives via Michael addition reaction of indole with electron-deficient

alkenes. The advantages of this approach to BIMs are the simplicity of the experimental procedure, high yields and the use of environmentally benign catalysts and solvents. Therefore, the reaction between 1*H*-indole (**1.27a**) and alkene **1.60** using $ZrOCl_2 \cdot SiO_2$ as catalyst, in water under reflux conditions, gave BIMs **1.61** in very good to excellent yields (80-92%). Both electron-donating and electron-withdrawing substituents in the phenyl moiety ($R^1$) were well tolerated (Scheme 1.23).[176]



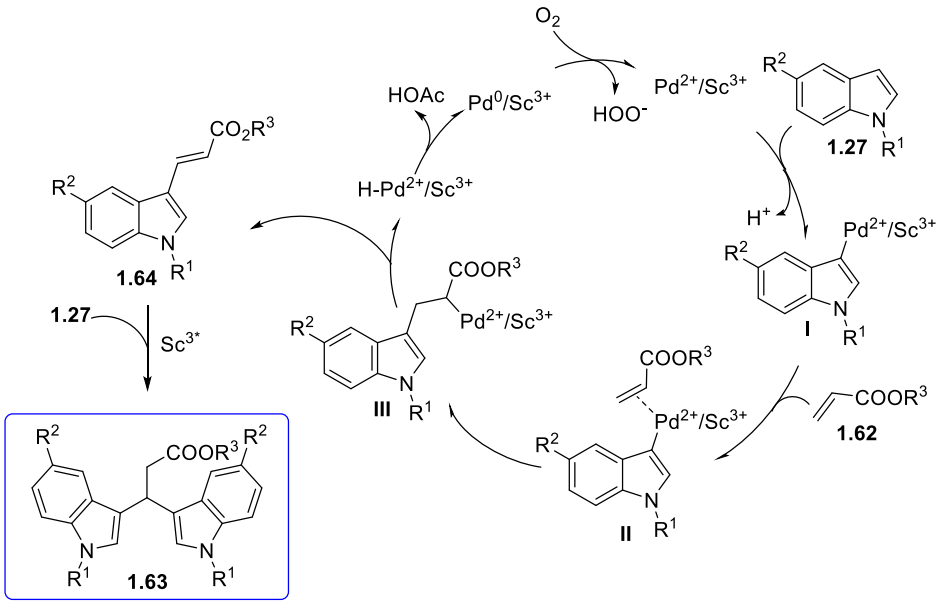

**Scheme 1.23** Michael addition reaction of indole with electron-deficient alkenes.

Zhang and co-workers described the first synthesis of BIMs via oxidative coupling of indoles with alkenes in the presence of a Pd(II)/Lewis acid-catalyst.[178] The optimal conditions were achieved by reacting indoles **1.27** with acrylates **1.62**, using 10 mol% of $Pd(OAc)_2$ and 20 mol% of $Sc(OTf)_3$, in acetonitrile at 30 ºC for 12 h and using $O_2$ as oxidant, leading to the target BIMs **1.63** in moderate to good yields (54-77%) (Scheme 1.24). Starting from *N*-unsubstituted indoles, the yield of the corresponding BIMs was higher starting from acrylates **1.62** with bulky alkyl groups ($R^3$) whereas with *N*-substituted indoles the efficiency decreased with the bulkiness of the protecting group. However, either electron-donating groups or bromine in the indole moiety were well tolerated (Scheme 1.24).



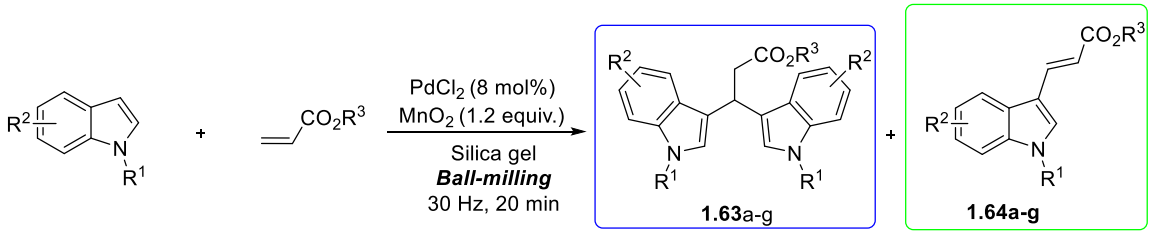

**Scheme 1.24** Synthesis of BIMs via oxidative coupling of indoles with alkenes in the presence of a Pd(II)/Lewis acid-catalyst.

Based on the experimental data, NMR studies and DFT calculations, the authors proposed the mechanism outlined in Scheme 1.25. First, the heterobimetallic $Pd^{2+}/Sc^{3+}$ catalyst was generated *in situ* acting as the key species for the oxidative coupling of indoles **1.27** with acrylates **1.62**. The activation of the C-3 of the indole core by $Pd^{2+}/Sc^{3+}$ generates intermediate **I**, which in turn coordinates with the acrylates to give the intermediate **II**. Next, **II** is converted into the coupling intermediate **III** following by a β-hydrogen elimination releasing indole **1.64**, which then undergoes indole addition catalysed by the cation $Sc^{3+}$ to afford the desired product **1.63** (Scheme 1.25).[178]




**Scheme 1.25** Proposed mechanism for the synthesis of BIMs **1.63**.

The search for non-conventional methods in the synthesis of BIMs, has also received great attention from the scientific community. An interesting example was the mechanochemically activated oxidative coupling of indoles with acrylates through C−H functionalization reported by Jia *et al.*[177] In this study, indoles **1.27** and acrylic esters, in presence of $PdCl_2$, using magnesium oxide ($MnO_2$) as oxidant agent and silica gel, were placed in a stainless-steel vessel with two stainless-steel balls at 25 Hz during 20 min. *N*-Methylindoles reacted with acrylates **1.62a** ($R^3$ = Et) and **1.62b** ($R^3$ = Bn) to afford BIMs **1.63a**,**b** along with the corresponding 3-vinylindoles **1.64a**,**b** in overall yields of 87% and 82%, respectively. Indoles bearing either electron-donating or electron-withdrawing groups on the indole core were well tolerated and their reaction with acrylate **1.62a** ($R^3$ = Et) led to the target BIMs **1.63c-g** as the main products in moderate to good yields (Scheme 1.26).[177]



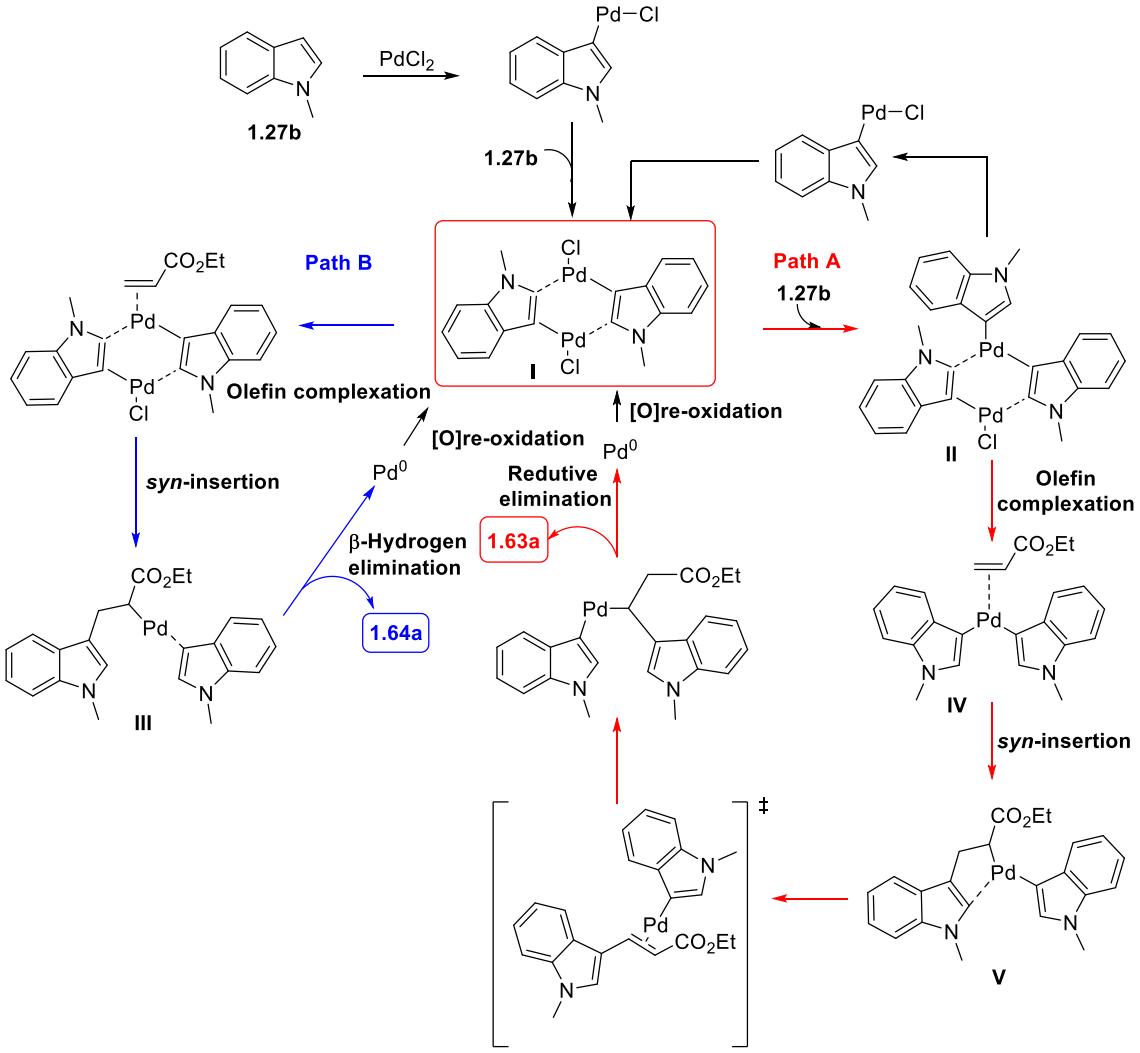

**1.27b** $R^1$ = Me, $R^2$ = H
**1.27c** $R^1$ = Me, $R^2$ = 7-Me
**1.27d** $R^1$ = Et, $R^2$ = 5-Cl
**1.27e** $R^1$ = Et, $R^2$ = 5-Br
**1.27f** $R^1$ = Et, $R^2$ = 7-Br
**1.27g** $R^1$ = Bn, $R^2$ = 5-OMe

**1.62a** $R^3$ = Et
**1.62b** $R^3$ = *n*-Bu

**1.63a/1.64a** $R^1$ = Me, $R^2$ = H, $R^3$ = Et [87% (90:10)]
**1.63b/1.64b** $R^1$ = Me, $R^2$ = H, $R^3$ = *n*-Bu [82% (88:12)]
**1.63c/1.64c** $R^1$ = Me, $R^2$ = 7-Me, $R^3$ = Et [79% (85:15)]
**1.63d/1.64d** $R^1$ = Et, $R^2$ = 5-Cl, $R^3$ = Et [82% (88:12)]
**1.63e/1.64e** $R^1$ = Et, $R^2$ = 5-Br, $R^3$ = Et [74% (85:15)]
**1.63f/1.64f** $R^1$ = Et, $R^2$ = 7-Br, $R^3$ = Et [76% (87:13)]
**1.63g/1.64g** $R^1$ = Bn, $R^2$ = 5-OMe, $R^3$ = Et [76% (87:13)]

**Scheme 1.26** Mechanochemically activated oxidative coupling of indoles with acrylates through C−H functionalization.

Based on an electrospray ionization (ESI-MS) spectrometry study the authors proposed a mechanism for the $PdCl_2$-catalysed reactions (Scheme 1.27). First, the transformation started with palladation at the C-3 position of the indole core, followed by intermolecular coordination to give the dimeric palladium complex **I**. This intermediate is common to both catalytic cycles. In path A (in red), another palladation occurs to give the intermediate **II** which is followed by olefin complexation and *syn*-insertion, producing intermediate **V**. Since the possibility of catalytic hydroarylation was ruled out after a control experiment, it was suggested that the intermediate **V** undergoes a palladium chain-walking/allylic cross-coupling pathway. A reductive elimination affords BIM **1.63a**, with the regeneration of the catalyst. In path B (in blue), an olefin complexation and *syn*-insertion affords intermediate **III** which after a β-hydrogen elimination gives the vinylindole **1.64a** concomitant with the regeneration of the palladium-based catalyst (Scheme 1.27).[177]

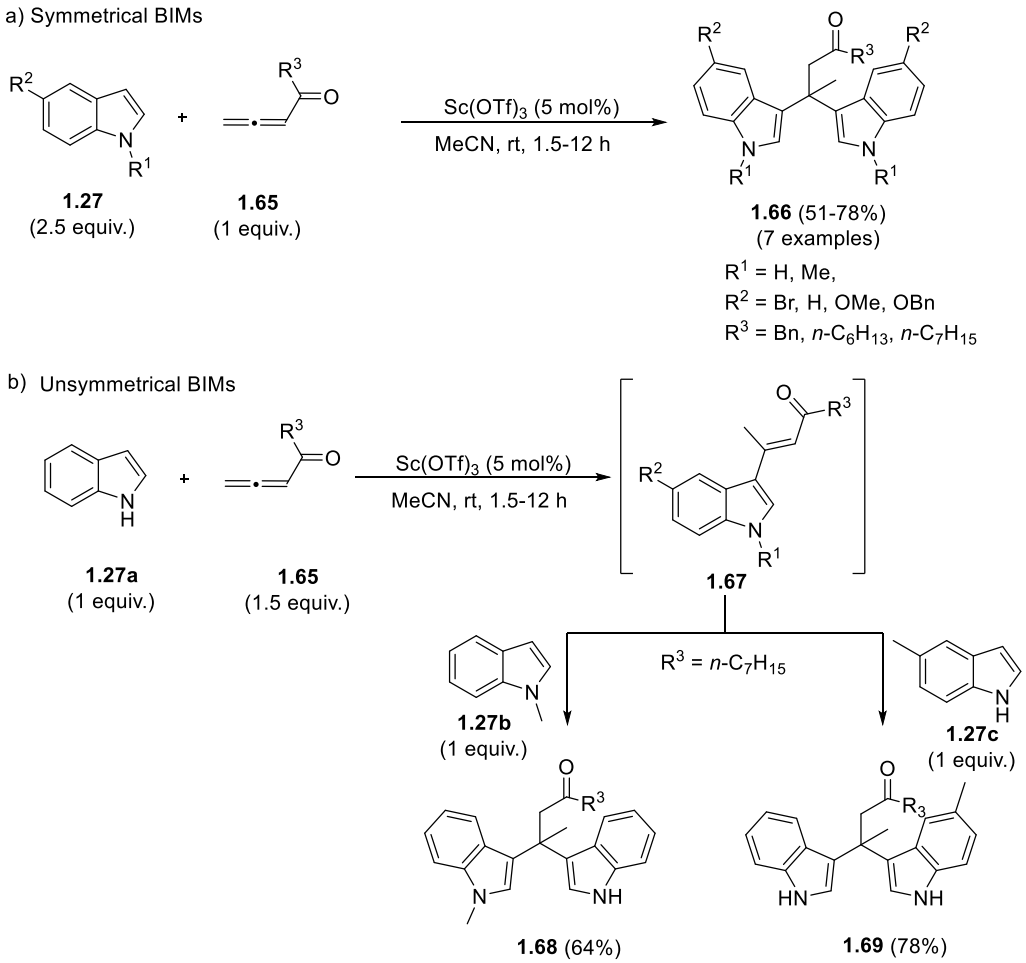



**Scheme 1.27** Proposed mechanism for the synthesis of BIMs via $PdCl_2$-catalysed reactions.

Allenes are singular structures in which one carbon atom has double bonds with each of its two adjacent carbon atoms. The synthetic utility of allenes is vast and new chemo-, regio- and stereo-selective transformations have been developed over the past years by exploring allenes chemistry. The reactivity of this class of compounds with indoles was also explored to obtain 3,3'-BIMs.[179-181]

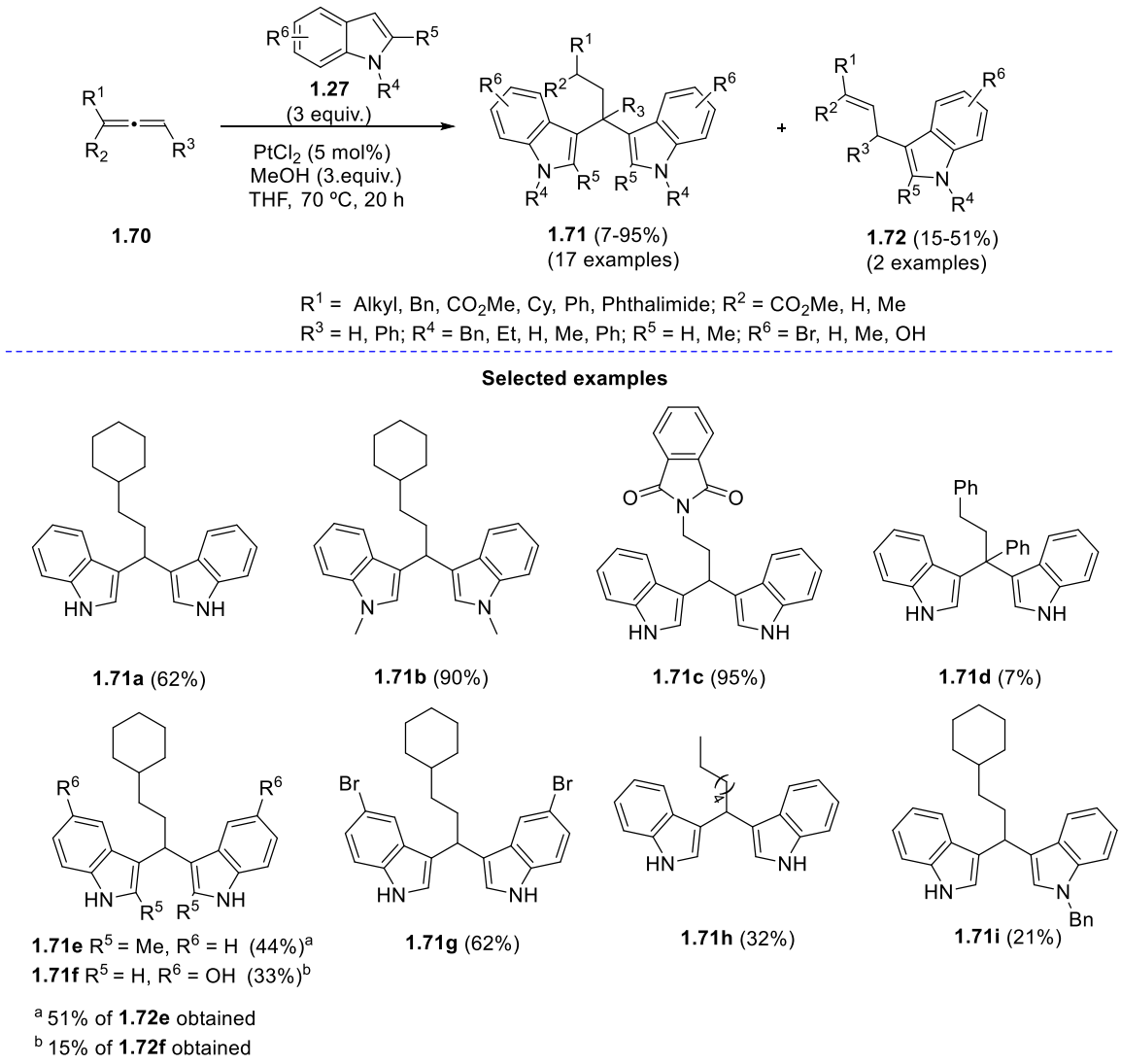
A $Sc(OTf)_3$-catalysed bisindolylation of 1,2-allenic ketone **1.65** was disclosed by Ma and co-workers.[179] The described method allowed the synthesis of symmetric and unsymmetric BIMs. Therefore, the one-pot reaction between indole **1.27** (2.5 equiv.) and 1,2-allenic ketones **1.65** (1 equiv.) in presence of catalytic $Sc(OTf)_3$ afforded symmetrical BIMs **1.66** in good yields. The substitution pattern in the indole core did not show to have great influence on the efficiency of the reaction (with the same 1,2-allenic ketone ($R^3$ = *n*-$C_7H_{15}$)), since very similar yields were obtained whether electron-donating or electron-

withdrawing groups were present (51-56%). The highest yield was obtained from the reaction between 1*H*-indole and allenic ketone with a benzyl substituent (78%) (Scheme 1.28a). Additionally, unsymmetrical BIMs could be effectively prepared when a stepwise double addition of indoles with 1,2-allenic ketone ($R^3$ = *n*-$C_7H_{15}$) was performed. Thus, the reaction between 1*H*-indole (**1.27a**) (1 equiv.) and 1,2-allenic ketone **1.65** ($R^3$ = *n*-$C_7H_{15}$) (1.5 equiv.), under the optimal conditions, led to the corresponding β-indolyl-α,δ-unsaturated(*E*)enone **1.67** which reacted with 1-methyl-indole (**1.27b**) (1 equiv.) or 5-methyl-indole (**1.27c)** (1 equiv.) to give the corresponding BIMs in 64% and 78% yield, respectively (Scheme 1.28).[179]



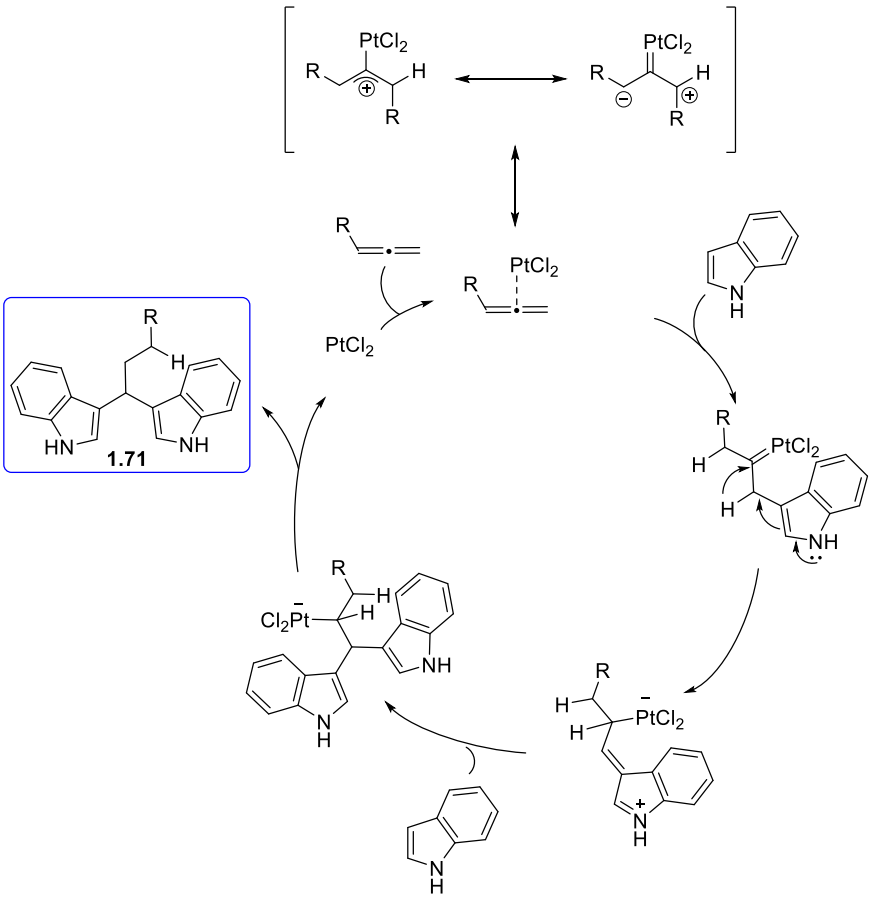

**Scheme 1.28** A $Sc(OTf)_3$-catalysed bisindolylation of 1,2-allenic ketone.

Munõz *et al*. described a platinum-catalysed bisindolylation of allenes. Under the optimal conditions (5 mol% of $PtCl_2$ as catalyst, methanol as additive, in THF, at 70 ºC, during 20 h), the reaction between allenes **1.70** and indoles **1.27** led to the desired BIMs in low to very good yield (Scheme 1.29).[180, 181] The use of methanol was a key feature for the effectiveness of these transformations. For example, an increase of yield from 23% to 62% of BIM **1.71a** was observed, when three equivalents of MeOH were added. The influence of the substitution on the indole core and the allene were also investigated. Experimental data showed that these transformations in general, under optimal conditions, work well whether the reaction is carried out with *N*-protected or *N*-unprotected indole, although the reaction with *N*-protected indoles were more efficient (synthesis of **1.71a** vs **1.71b**). When the reactions between 2-(propa-1,2-dien-1-yl)isoindoline-1,3-dione and 2-methyl-1*H*-indole and 5-hydroxy-1*H*- indole were carried out, in addition to the expected products **1.71e** (44%) and **1.71f** (33%), the corresponding 3-allyl-indoles **1.72** were also obtained. In one case, indole **1.72e** was even the major product (51%), showing that the substituent group on the indole nuclei has a significant influence on the reactivity. Additionally, this protocol also proved to be effective at unsymmetric BIMs synthesis. In fact, starting from (3-cyclohexylpropa-1,2-dien-1-yl)benzene by addition of 3 equivalents of 1*H*-indoles and 3 equivalents of 1-benzyl-indoles, the corresponding unsymmetric BIM **1.71i** was obtained in 21% yield. However, the corresponding symmetrical BIM was also isolated in similar yield (Scheme 1.29).[180, 181]




**Scheme 1.29** Platinum-catalysed bisindolylation of allenes.

According to the authors, deuteration studies supported the hypothesis of a zwitterionic Pt carbene intermediate stabilised by a polar environment as the key step (Scheme 1.30). However, other possible intermediate, namely insertion of platinum into the C3-H of the indole, a reversible reaction involving vinyl indole intermediates or a gold-type-catalysed reaction, in which allyl-indoles are obtained, could not be ruled out.[181]




**Scheme 1.30** Proposed mechanism for the synthesis of BIMs **1.71**.

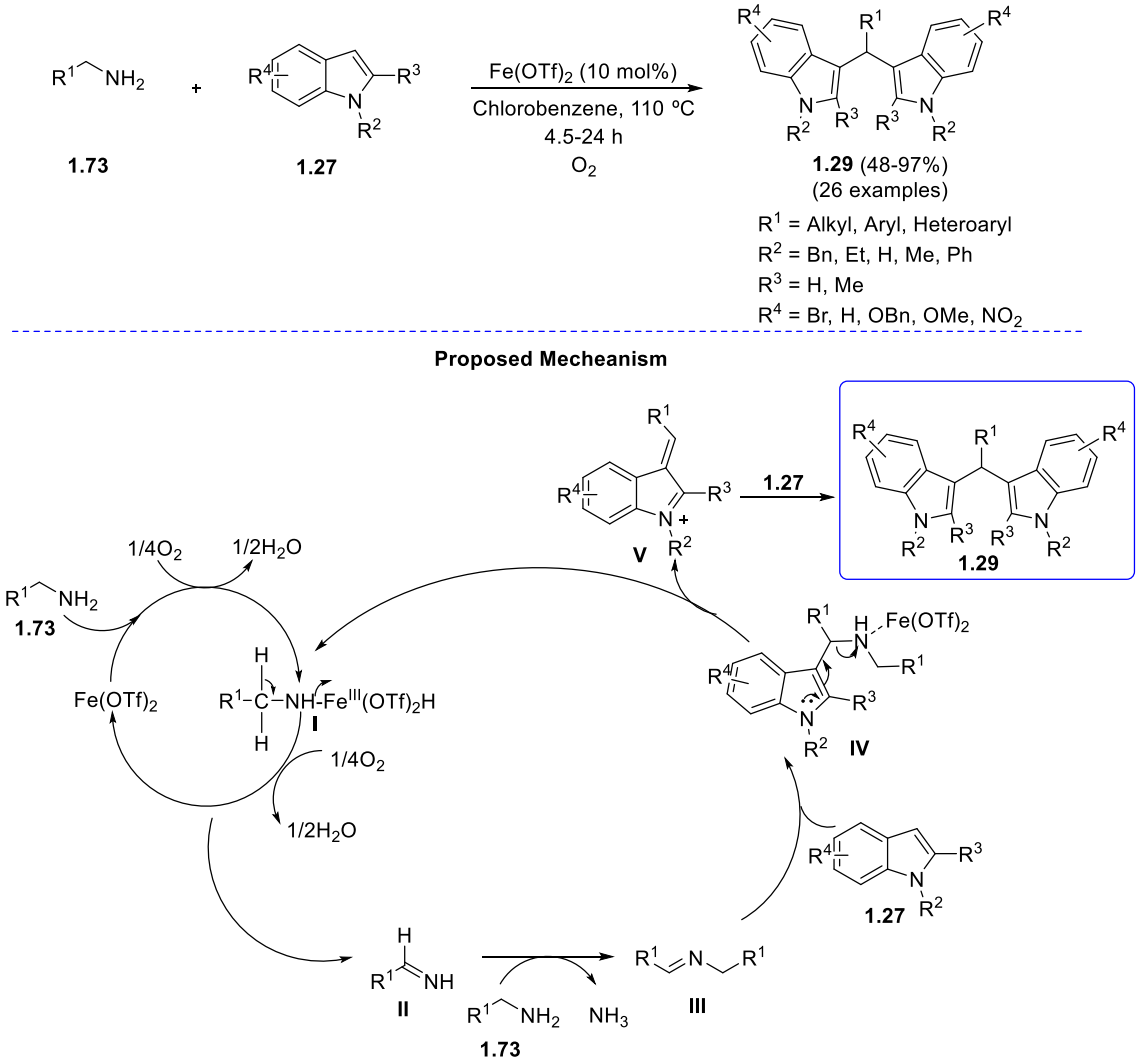
### 1.2.1.3 Synthesis of bis(indolyl)methanes from indoles and amines

Within the several approaches that have been investigated to achieve BIMs, the oxidative coupling of amines with indoles has also attracted the attention of researchers.

The first example of the synthesis of BIMs derived from this type of reactivity was described by Gopalaiah *et al*. in which an iron-catalysed oxidative coupling of several amines **1.73** (benzylamines, heteroarylmethylamines and aliphatic amines) and indoles **1.27**, in chlorobenzene at 110 ºC, under an atmosphere of molecular oxygen, led to the corresponding BIMs **1.29** in moderate to excellent yields (48-97%) (Scheme 1.31).[182] In general, benzylamines reacted smoothly with 1*H*-indole. The substituent group on benzylamines did not significantly influence the reactivity, both electron-donating and weakly electron-withdrawing substituents leading to the corresponding products in high yields (84-97%). Furthermore, the strategy was also extended to heteroarymethylamines such as pyridin-4-ylmethanamine and 2-thienyl-methylamine with 1*H*-indole. In both cases, the corresponding BIMs were obtained in very good yields (79% and 87%,

respectively). Moreover, aliphatic amines such as hexylamine and octylamine also reacted smoothly with 1*H*-indole, to give the desired coupling products in 48% and 55% yields, respectively. Different substituents groups in C-2 and C-5 of indole also showed no substantial impact on the efficiency of the process since the desired BIMs were formed in high yields (76-90%). Moreover, *N*-protected indoles were also employed affording the corresponding BIMs in excellent yields (75-81%) (Scheme 1.31).[182]

The proposed mechanism suggests as starting point the oxidative addition of the catalyst to the amines **1.73** leading to intermediate **I**. Further oxidation of **I** generates intermediates **II** which in turn react with a second molecule of **1.73** producing imines **III**. Then, the electrophilic substitution of indole with **III** occurs in the presence of the catalyst giving the 3-alkyl-indoles/iron complexes **IV** which leads to intermediate **V** with the concomitant regeneration of complex **I**. Finally, the desired BIMs **1.29** are obtained from intermediates **V** upon reaction with a second molecule of indole (Scheme 1.31).[182]




**Scheme 1.31** Iron-catalysed oxidative coupling of amines and indoles.

Similar studies were recently reported, namely the oxidative coupling of benzylamines and indoles catalysed by TEMPO/CuI disclosed by Yue and co-workers,[183] in which BIMs were obtained in good to excellent yields (75-93%). The 3,3'-BIMs' yield was influenced by electronic factors to some level, where the benzylamines having electron-donating groups furnished BIMs in slightly lower yields than benzylamines with electron-withdrawing groups. For the indole core, both *N*-unsubstituted and *N*-substituted indoles were well tolerated. However, the latter led to the target compounds in marginally higher yields.[183]

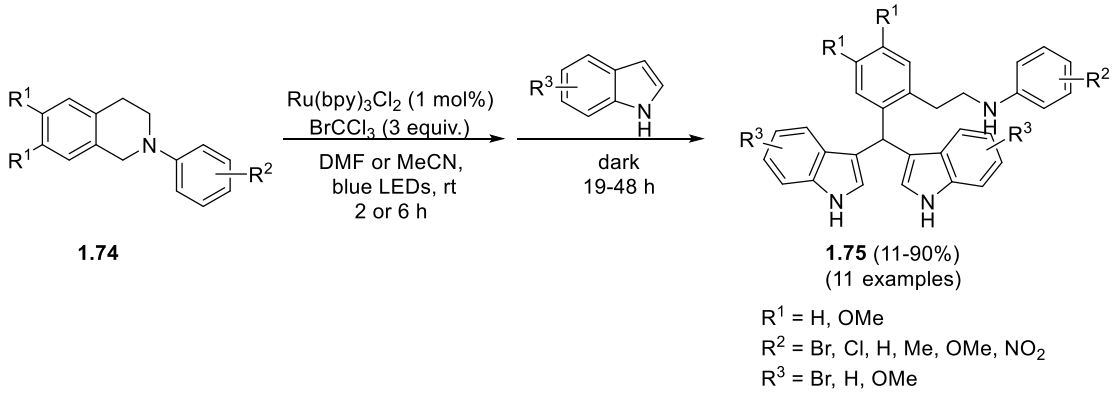
A water-soluble cobalt complex Co(bpb), was also found to be an efficient catalyst for the oxidative coupling of benzylamines with indoles to bis-(indolyl)methanes. [184]

Godage and co-workers also developed a straightforward iodine-catalysed C($sp^3$)-*H* bond functionalization of arylmethylamines leading to the synthesis of bis(indolyl)methanes.[185] In both studies, the arylamines were efficiently converted into the desired BIMs with yields up to 85%.

Additionally, BIMs were also synthesised by an efficient metal-free oxidative coupling of arylmethylamines with indoles, using molecular oxygen as oxidant and acetic acid as catalyst, providing the desired products in good to excellent yields (75-95%).[186]

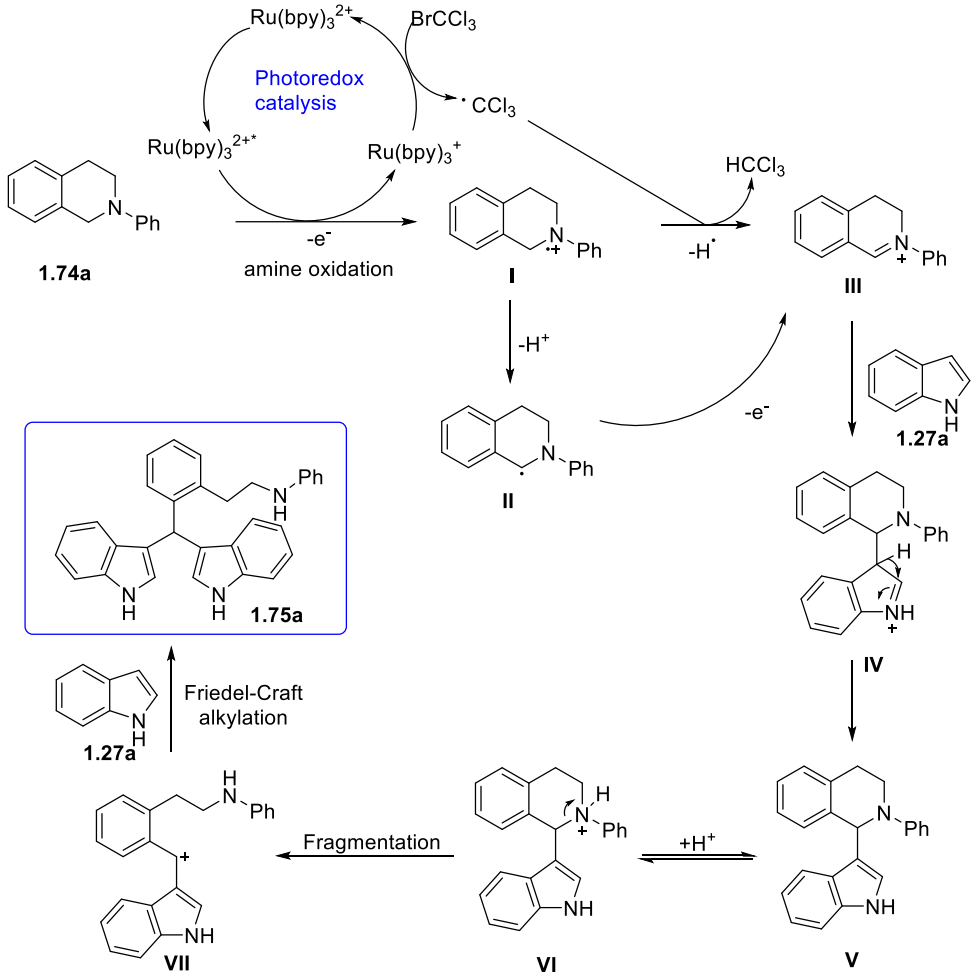
A different approach to 3,3'-BIMs was reported by Chen *et al*, in which a visible-light induced ring-opening bisindolation of tetrahydroisoquinolines **1.74** occurred (Scheme 1.32).[187] The catalytic protocol involved a mixture of tetrahydroisoquinoline, ruthenium-based catalyst [$Ru(bpy)_3Cl_2 \cdot H_2O$ (1 mol%)] and bromotrichloromethane (3 equiv, $BrCCl_3$), in *N*,*N*-dimethylformamide or acetonitrile, at room temperature, was irradiated with a strip of blue LEDs for 2 h or 6 h, depending on the solvent used (MeCN and DMF, respectively). Then, 5 equivalents of the appropriate indole were added, and the solution was stirred in the dark, during the appropriate time, to give the desired BIMs **1.75** in low to excellent yields (11-90%). Surprisingly, some reactions gave better results in MeCN and others in DMF. Which can be explained to differences in solubility of the reactants and intermediates. For example, the reaction carried out between 2-(4-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline and 6-methoxy-1*H*-indole in MeCN and DMF gave rise the corresponding BIM in 48 and 85% yield, respectively.

The highest yield, in both solvents, was achieved when 2-(2-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline reacted with 1*H*-indole, affording the desired BIM in 90% (MeCN) and 89% (DMF). On the other hand, the less efficient synthesis was observed starting from 6,7-dimethoxy-2-(2-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline and 1*H*-indole, leading to the product in 21% (MeCN) and 11% (DMF) yield.[187]



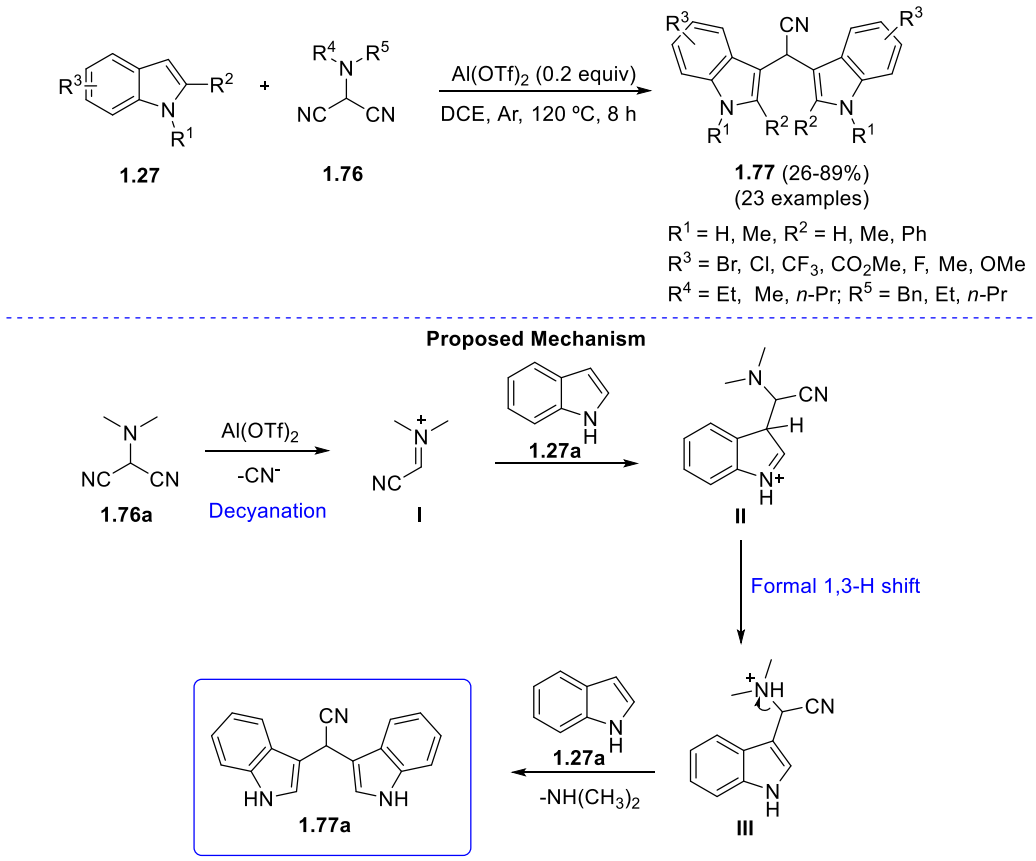

**Scheme 1.32** Oxidative coupling of benzylamines with indoles to BIMs, using a water-soluble cobalt complex.

The mechanistic proposal for this photocatalysed cascade reaction, using tetrahydroquinoline **1.74a** and 1*H*-indole, is outlined in Scheme 1.33. The $Ru^{2+}$ was excited to $Ru^{2+*}$, by blue LEDs irradiation and reductively quenched by tetrahydroisoquinoline to $Ru^{+}$, which then promotes the amine oxidation of **1.74a** affording intermediate **I**. Next, intermediate **II** can be obtained from **I**, following two different routes: by elimination of the hydrogen atom or by elimination of a proton, which after oxidation is transformed into iminium intermediate **III**. After the nucleophilic attack of indole **1.27a,** intermediate **IV** is formed which isomerises to **V**. After protonation, **V** is activated to undergo the C-N fragmentation leading to intermediate **VI**, which through Friedel-Craft alkylation with a second molecule of indole gives rise to the target BIM **1.75a** (Scheme 1.33).[187]




**Scheme 1.33** Proposed mechanism for the synthesis of BIMs **1.75**.

More recently, 1-cyano-BIMs were successfully synthesised from the tandem coupling reaction between indoles and *N*,*N*-disubstituted aminonitriles catalysed by $Al(OTf)_3$.[188] Using aminonitrile **1.76a** and indole **1.27a** as model substrates, the proposed mechanism for this transformation starts with a monodecyanation of the aminonitrile **1.76a** promoted by the catalyst affording intermediate **I**, which reacts with the first molecule of indole **1.27a** giving intermediate **II**. After a formal 1,3-H shift, **III** is formed and subsequent deamination/coupling reactions with a second molecule of indole leads to the target BIM **1.77a** (Scheme 1.34).




**Scheme 1.34** The tandem coupling reaction between indoles and *N*,*N*-disubstituted aminonitriles.

### 1.2.1.4 Synthesis of bis(indolyl)methanes from indoles and ethers

In recent decades, obtaining 3,3'-bis(indolyl)methanes through reactions of indoles with ethers has also been explored. Traditional chemistry, photoredox catalysis and electrochemical synthesis have been investigated for that propose (Figure 1.6).[189-191]

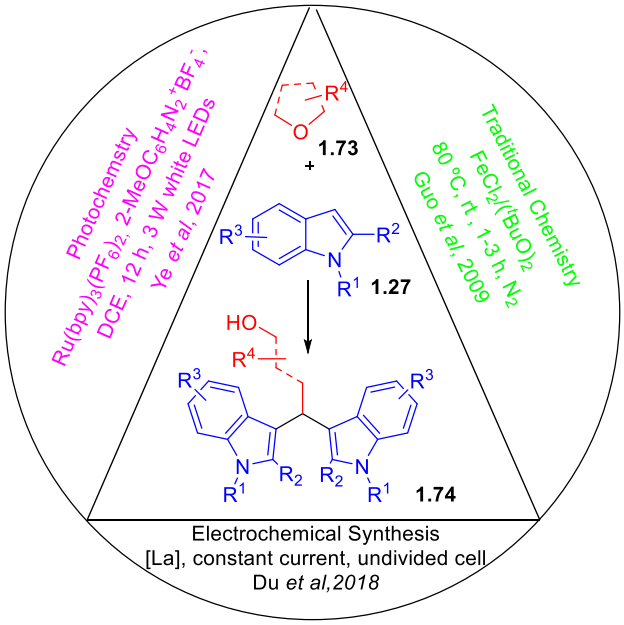
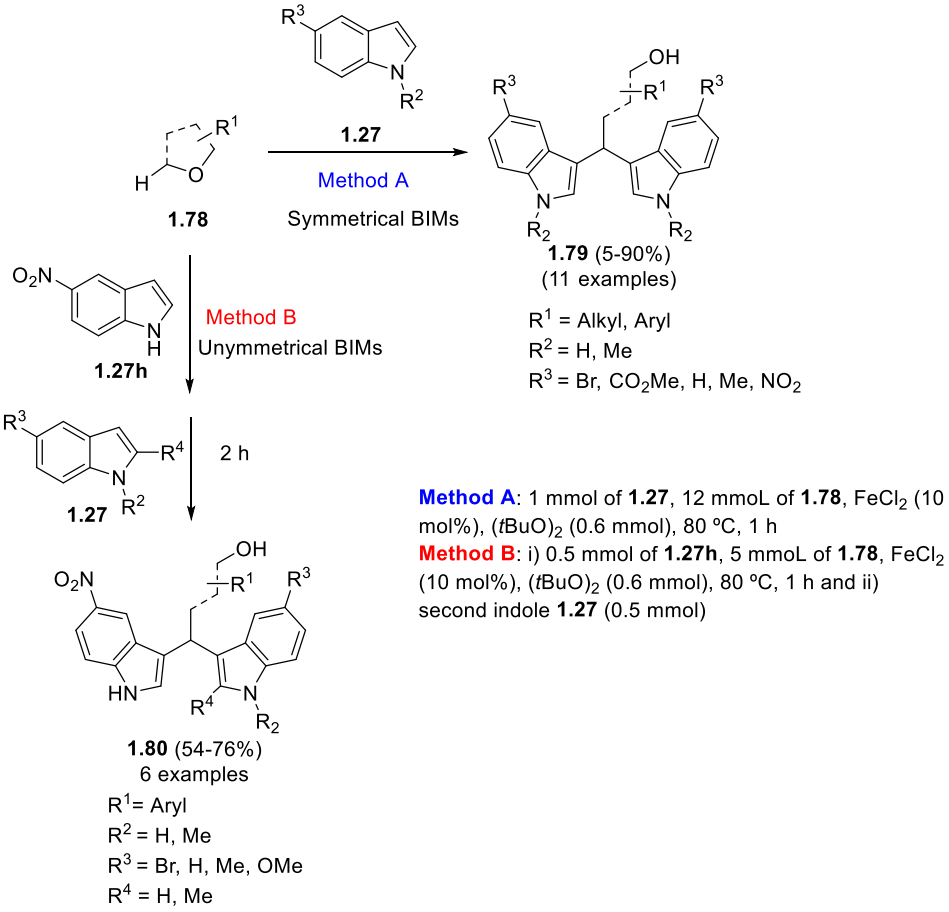


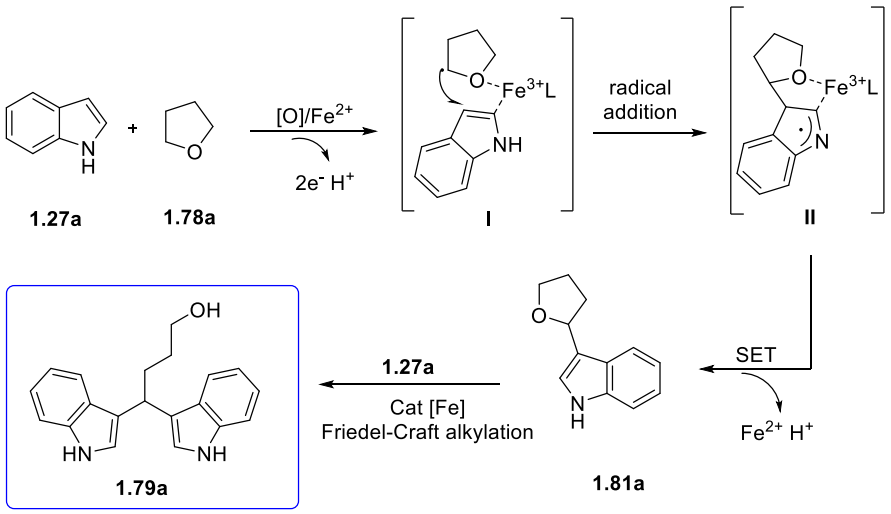

**Figure 1.6** Traditional chemistry, photoredox catalysis and electrochemical towards BIMs.

An efficient one-pot synthesis of either symmetrical or unsymmetrical BIMs via iron-catalysed C-H bond oxidation and C-O bond cleavage was reported by Guo *et al* (Scheme 1.35).[189] The reaction between ethers **1.78** and indoles **1.27**, under method A, gave the symmetrical BIMs **1.79** in good to excellent yields (62-90%). The highest yield was obtained between 1*H*-indole and 2,3-dihydrobenzofuran (90%) whereas the lowest one, was obtained in the reaction of 5-bromo-indole with isochromane (62%). Surprisingly, when the reaction between indoles such as methyl 1*H*-indole-5-carboxylate, 5-nitro-1*H*-indole or 1*H*-indole, and 1,3-dihydroisobenzofuran was carried out, the monoindolation product was also obtained together with the target BIMs **1.79**. Following method B, was possible to extend this strategy to the synthesis of unsymmetric BIMs **1.80** in 54-76% yield, by adding a different indole derivative into the final step of the reaction (Scheme 1.35).[189]



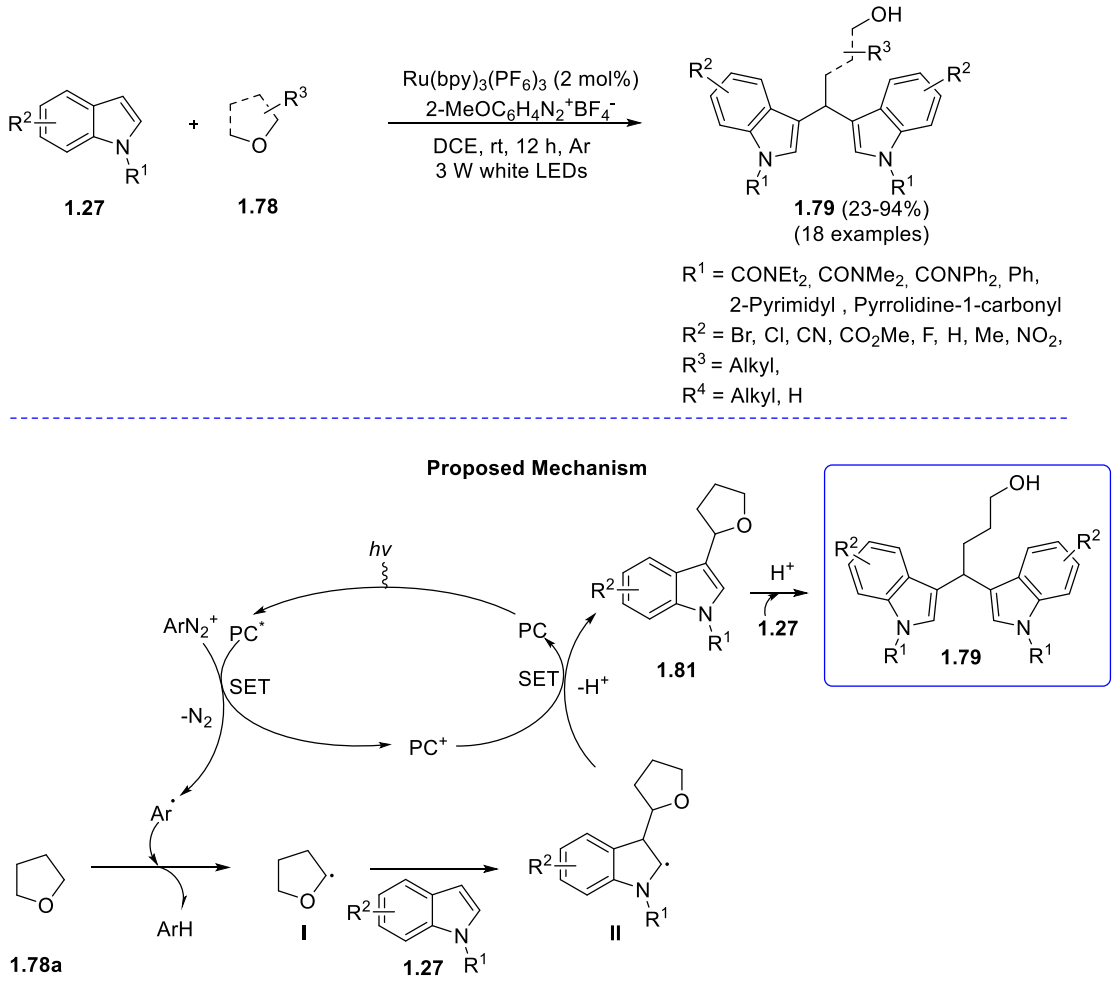

**Scheme 1.35** One-pot synthesis of either symmetrical or unsymmetrical BIMs via iron-catalysed C-H bond oxidation and C-O bond cleavage.

Based on the experimental data and some control experiments, namely reactions between deuterium-labeled indole and tetrahydrofuran, and competition experiments (in order to investigate the influence of the substitution pattern of the indoles), the authors proposed the mechanistic pathway (using 1*H*-indole and tetrahydrofuran as templates) depicted on Scheme 1.36. Thus, H-abstraction gave rise to intermediate **I** which, through a radical addition originated intermediate **II**. Further oxidation led to the oxidative coupling product **1.81** which following a Friedel-Craft alkylation produced **1.79**. The first indolation step of the reaction (C-C formation) was considered a radical process instead of an ionic one. In fact, this proposal was in line with the findings that the reaction was completely suppressed when 1.0 equiv. of TEMPO, a radical trapping reagent, was added (Scheme 1.36).[189]



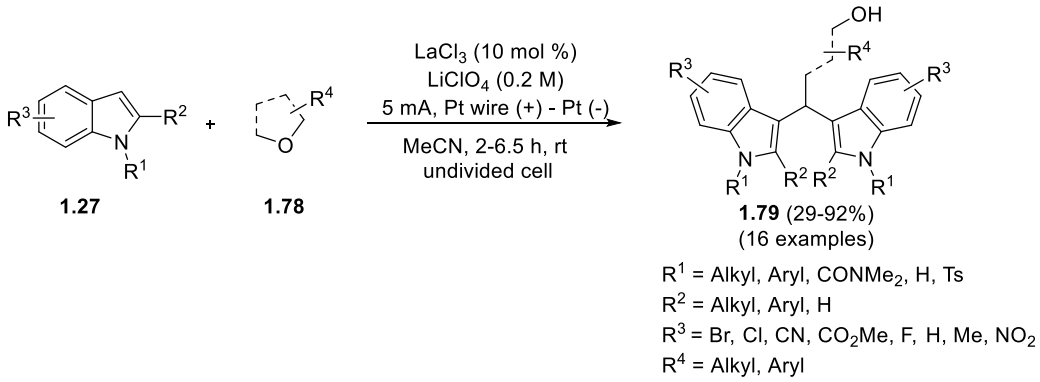

**Scheme 1.36** Proposed mechanism for the synthesis of BIMs **1.79** using 1*H*-indole and tetrahydrofuran as templates.

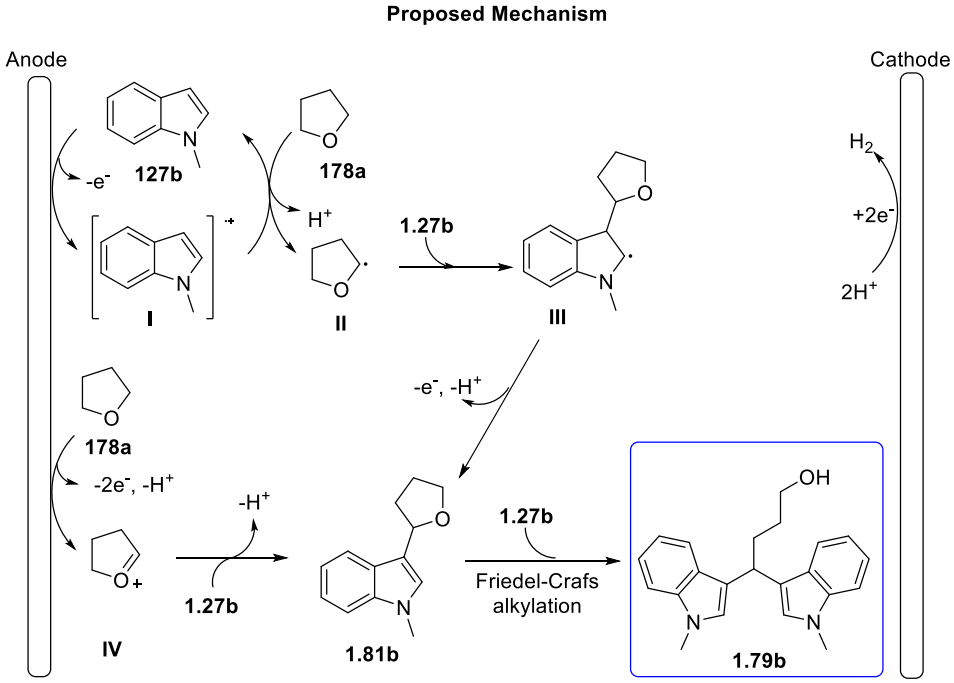
A similar study was disclosed by Ye *et al* in which a visible-light-induced synthesis of symmetric BIMs was reported.[190] In this investigation, reactions between several *N*-substituted indoles **1.27** and ethers **1.78** were carried out at room temperature, under argon atmosphere, using $Ru(bpy)_3(PF_6)_2$ as photocatalyst, methoxybenzenediazonium tetrafluoroborate as oxidant and 1,2-dichloroethane as solvent, furnishing the corresponding BIMs **1.79** in low to excellent yields (23-94%). The experimental results indicate a sequential photoredox catalysis induced radical addition and Friedel-Crafts proton-mediated alkylation mechanism as presented in the following Scheme 1.37.




**Scheme 1.37** Visible-light-induced synthesis of symmetric BIMs from indoles and ethers.

Although these procedures have proven to be quite efficient for the synthesis of BIMs, they showed several disadvantages, namely the use of an external oxidant, the need for high temperatures, the use of expensive catalysts and a stoichiometric amount of a single-electron-transfer reagent (SET). In attempt to overcome these disadvantages, Du and Hang developed an efficient synthesis of BIMs (up to > 92%), from indoles and ethers through an electrochemical method in presence of $LaCl_3$ catalyst. The great advantage of the method is that dangerous and toxic redox agents can be replaced by an electric current or can be generated *in situ* (Scheme 1.38).[191] In this study, the authors cctwo possible pathways for the reaction. In the main path, the formation of **1.81b** via intermediate **III** was considered. However, they don´t exclude the possibility of the monoindolylated product could be obtained through the reaction of indole with the alkoxycarbenium ion **IV**, produced by the anodic oxidation of the tetrahydrofuran **1.78a**.



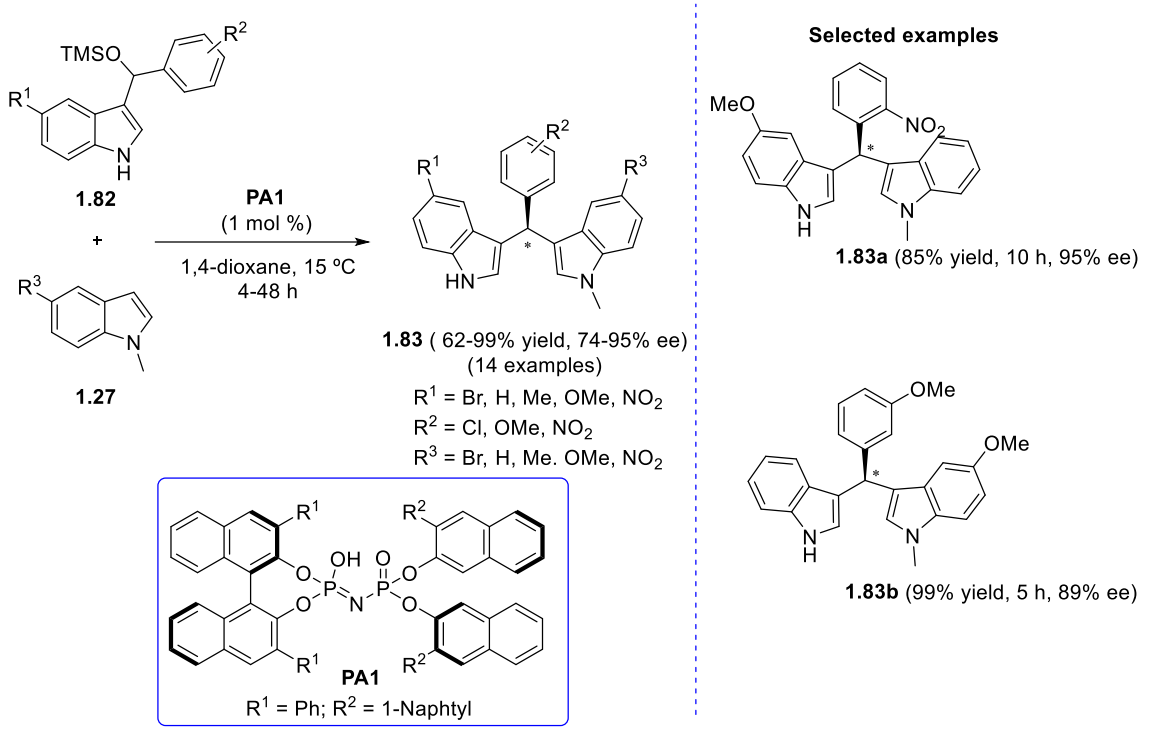

**Scheme 1.38** Synthesis of BIMs from indoles and ethers through an electrochemical method.

### 1.2.1.5 Enantioselective synthesis of bis(indolyl)methanes

Methodologies based on enantioselective Friedel-Crafts reaction of indolylmethanol,[192] 3-hydroxy-indolyloxindoles,[193] indolylmethanamine[194] or 3-indolylsulfanamide[195] derivatives with indoles have been also explored to the synthesis of unsymmetrical BIMs.

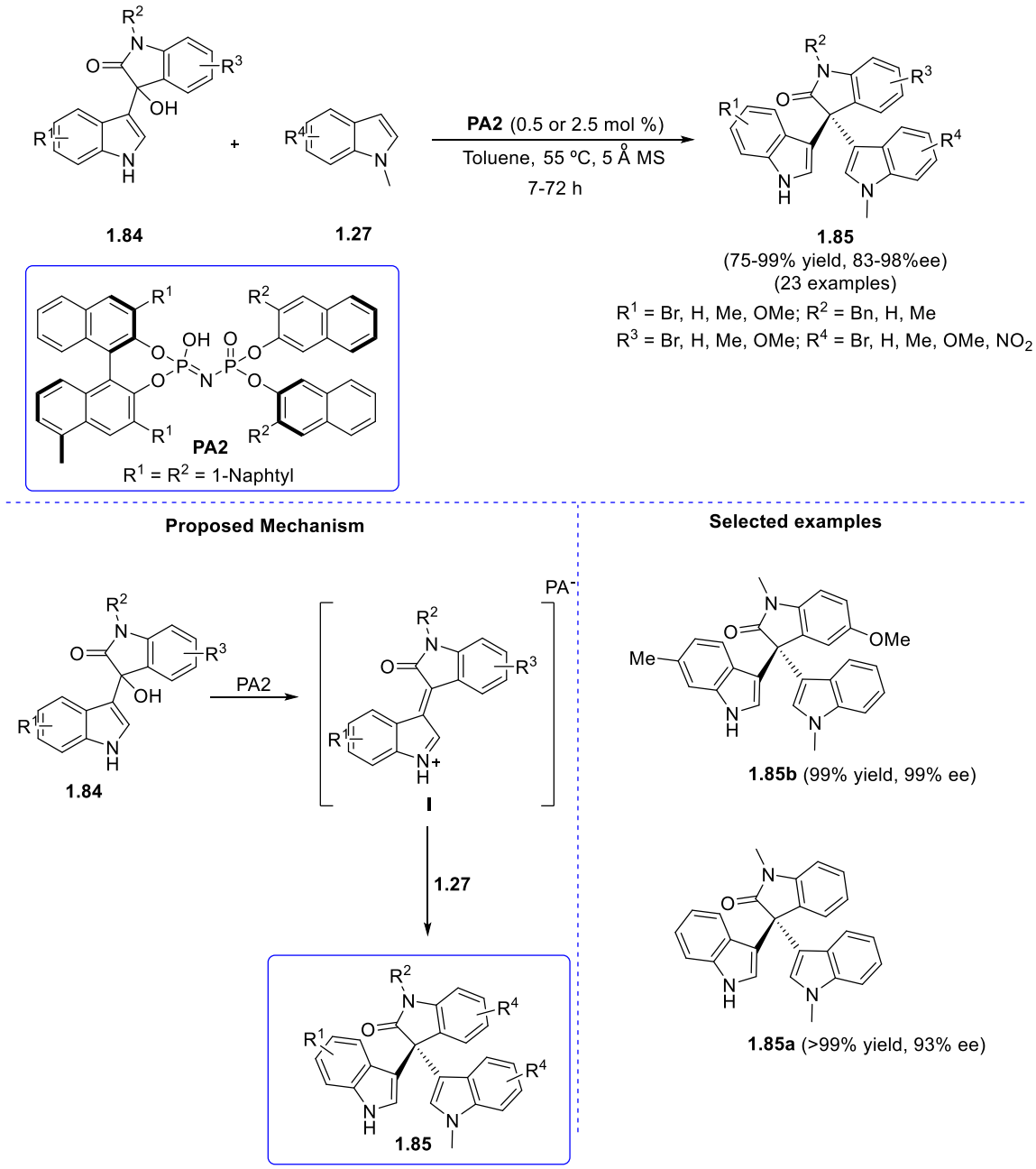
An enantioselective synthesis of unsymmetrical BIMs obtained from the reaction between indolylmethanol derivatives **1.82** with indoles **1.27**, catalysed by a chiral binol imidodiphosphoric acid **PA1** was disclosed by Zhuo and co-workers.[192] The substitution pattern at the phenyl group ($R^2$) of **1.82** or at the indole ($R^3$) **1.27** did not show to have great influence in the results, since very similar yields were obtained whether electron-donating

or electron-withdrawing groups were present. Therefore, this Friedel-Crafts alkylation process led to BIMs **1.83** in high yields (62-99%) and enantioselectivities (74-95% *ee*) (Scheme 1.39).[192]



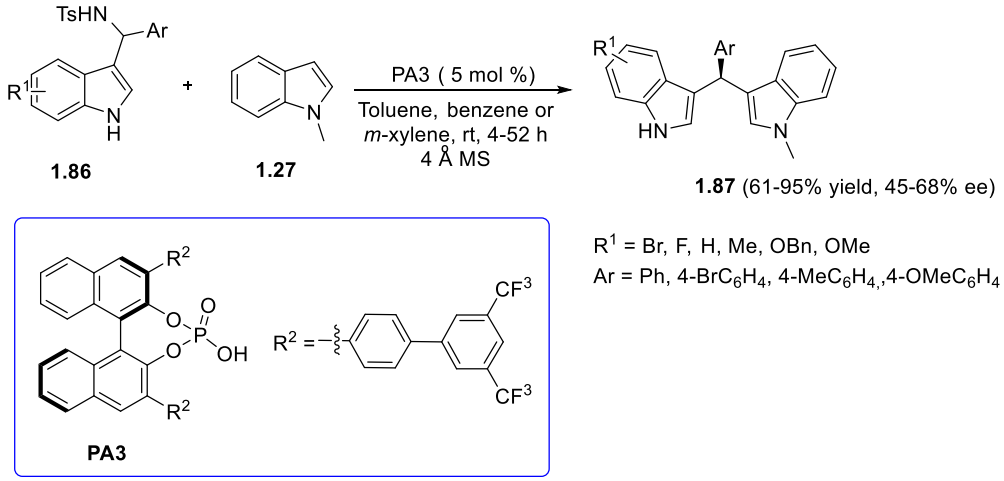

**Scheme 1.39** Enantioselective synthesis of unsymmetrical BIMs obtained from the reaction between indolylmethanol derivatives with indoles.

Zhuo and co-workers developed an enantioselective synthesis of 3,3-bis-(indol-3-yl)oxindoles using a similar strategy.[193] Thus, the Friedel-Crafts reaction between 3-hydroxy-3-indolyloxindoles and indoles, using a chiral imidodiphosphoric acid **PA2** (0.5 or 2.5 mol %), in toluene at 55 ºC, in presence of 5 Å molecular sieves (MS), gave rise to the target BIMs **1.85** in high yields (up to > 99%) with excellent enantioselectivity (up to 98% *ee*). In this transformation, the cationic vinyl intermediate **I** derived from 3-hydroxy-3-indolyloxindole **1.84** and the chiral Bronsted acid **PA2** produced a stable chiral ion pair, which suffers subsequent attack by an indole molecule **1.27** in a regio- and stereoselective fashion (Scheme 1.40).



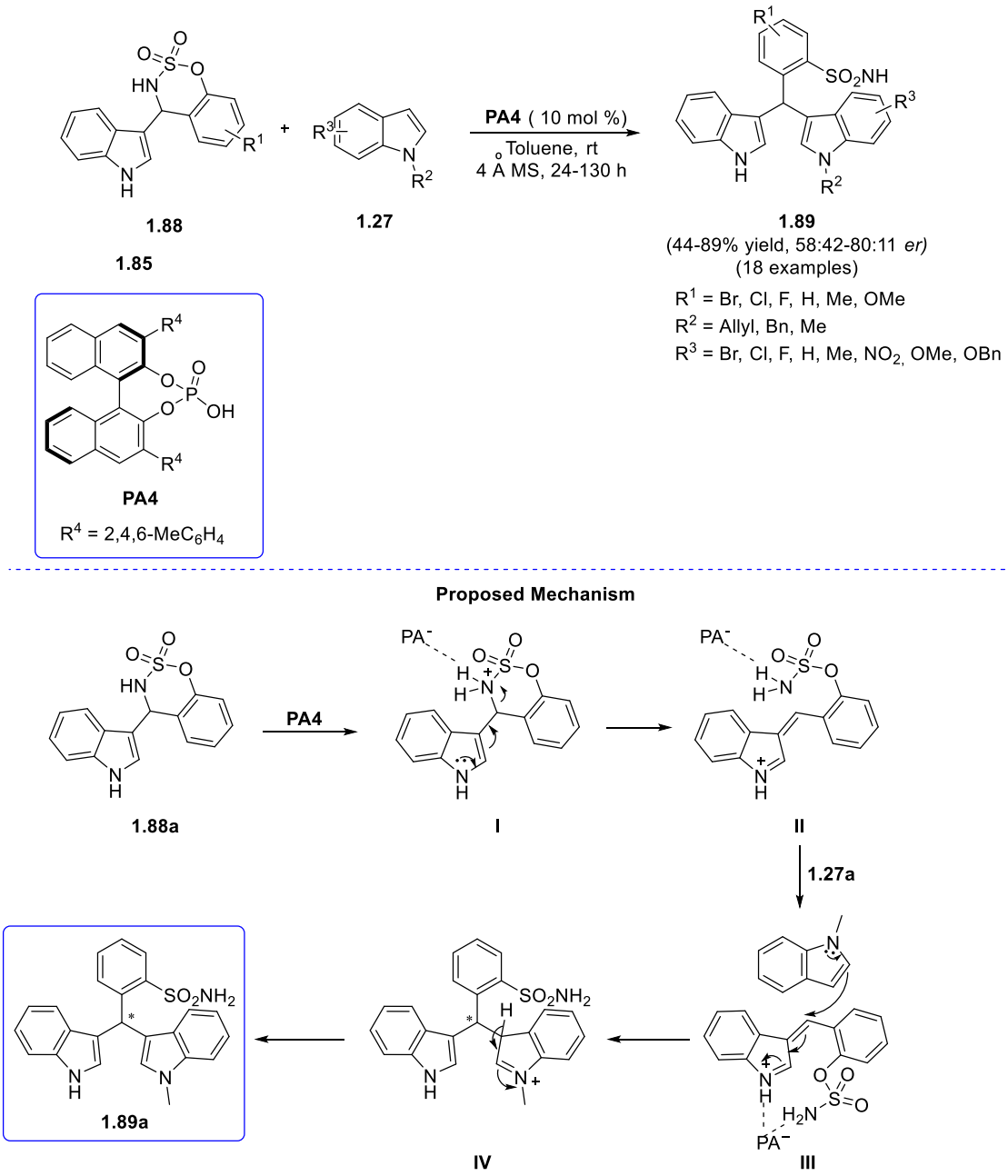

**Scheme 1.40** The enantioselective synthesis of 3,3-bis-(indol-3-yl)oxindoles.

Similar methodology was carried out by Sun and co-workers,[194] in which a enantioselective synthesis of BIMs catalysed by a chiral Bronsted acid was established. In these enantioselective processes, the Friedel-Craft alkylation between (3-indolyl)methamines **1.86** with indoles **1.27**, catalysed by the chiral phosphoric acid **PA3**, led to the target products **1.87** in good to excellent yields (61-95%) in moderate enantioselectivity (45-68% *ee)* as outlined in scheme 1.41.




**Scheme 1.41** The enantioselective synthesis of 3,3-bis-(indol-3-yl)oxindoles.

More recently, a chiral BINOL-phosphoric asymmetric acid-catalysed Friedel-Crafts alkylation starting from 3-indolylsulfamidates **1.88** was reported by Kim *et al.*[195] This methodology provided, for the first time, BIMs sulfamate derivatives **1.89** in moderate to good yields (44-89%) with moderate to excellent enantioselectivities (up to > 96:6 *er*). Based on experimental data the authors considered that the mechanism pathway initiated with a ring-opening of 3-indolylsulfamidates **1.88** promoted by the chiral **PA4**, generating intermediate **II** which forms a stable ion pair with BINOL-derived **PA4**. A subsequent nucleophilic attack of indole **1.27** leads to the corresponding sulfamate BIMs derivatives **1.89** (Scheme 1.42).

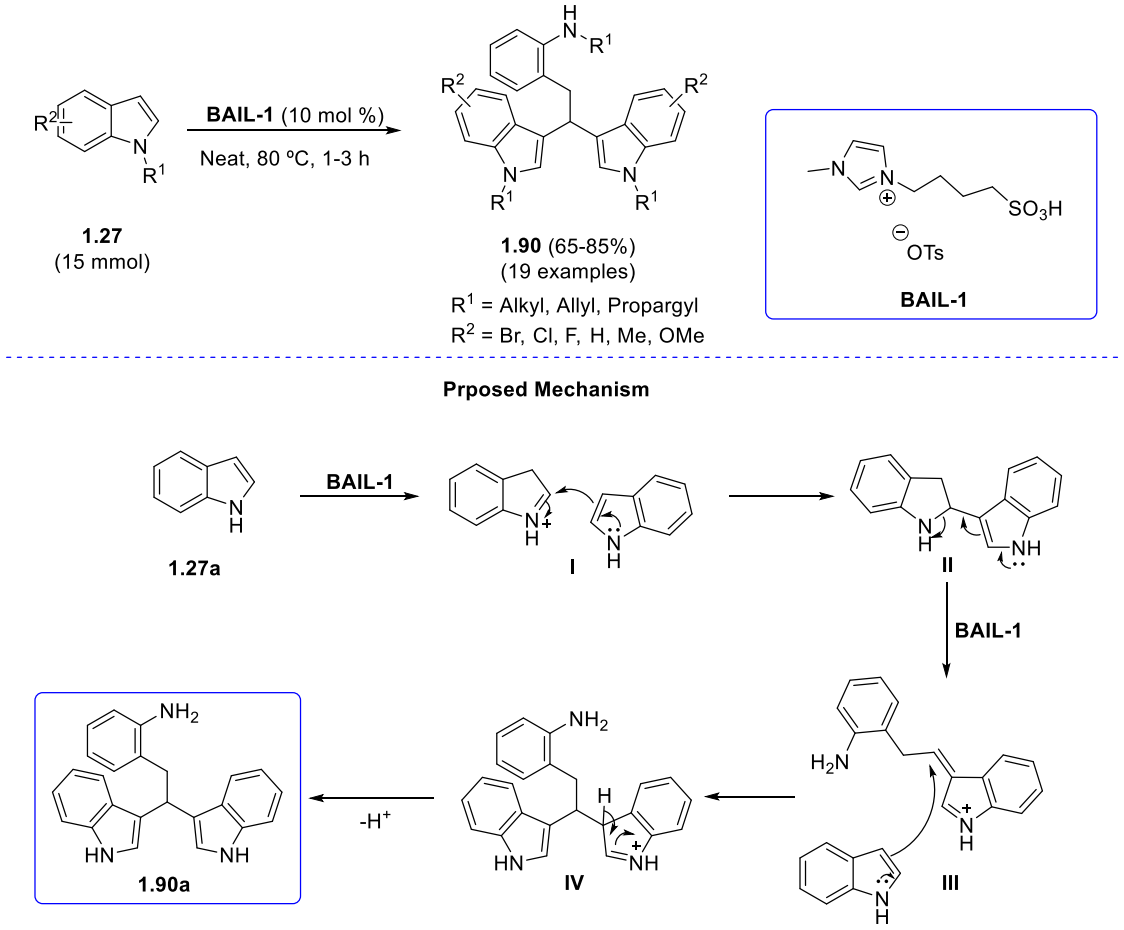



**Scheme 1.42** The chiral BINOL-phosphoric asymmetric acid-catalysed Friedel-Crafts alkylation between 3-indolylsulfamidates and indoles.

### 1.2.1.6 Miscellaneous methods

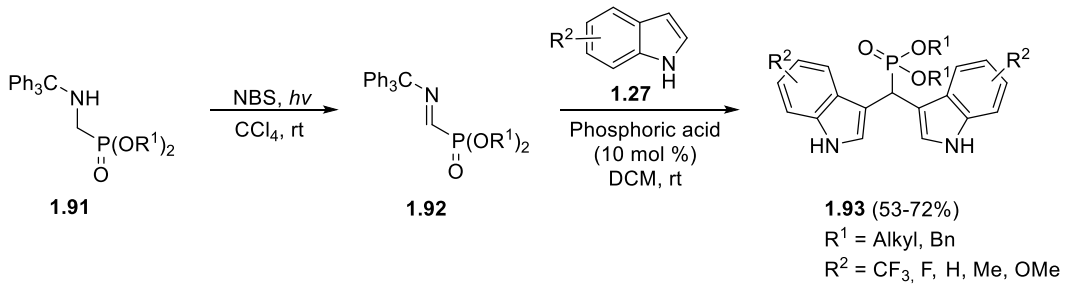
A efficient methodology to synthesise bis(indolyl)methanes derivatives by the self-coupling of indoles using Brønsted acidic ionic liquid (BAIL-1) as catalyst was recently disclosed by the Chatterjee research group.[196] This methodology presentes several advantages, namely short reaction times, easy isolation and purification process and being able to be carried out under neat conditions. BIMs **1.90** were obtained through the

generation of indolinium cation **I** followed by the reaction with another indole molecule to form intermediate **II**. Ring-opening of indole moiety of **II** leads to intermediate **III** which by addition of a third molecule of indole and subsequent rearomatisation affords the corresponding BIMs **1.90** ( Scheme 1.43).



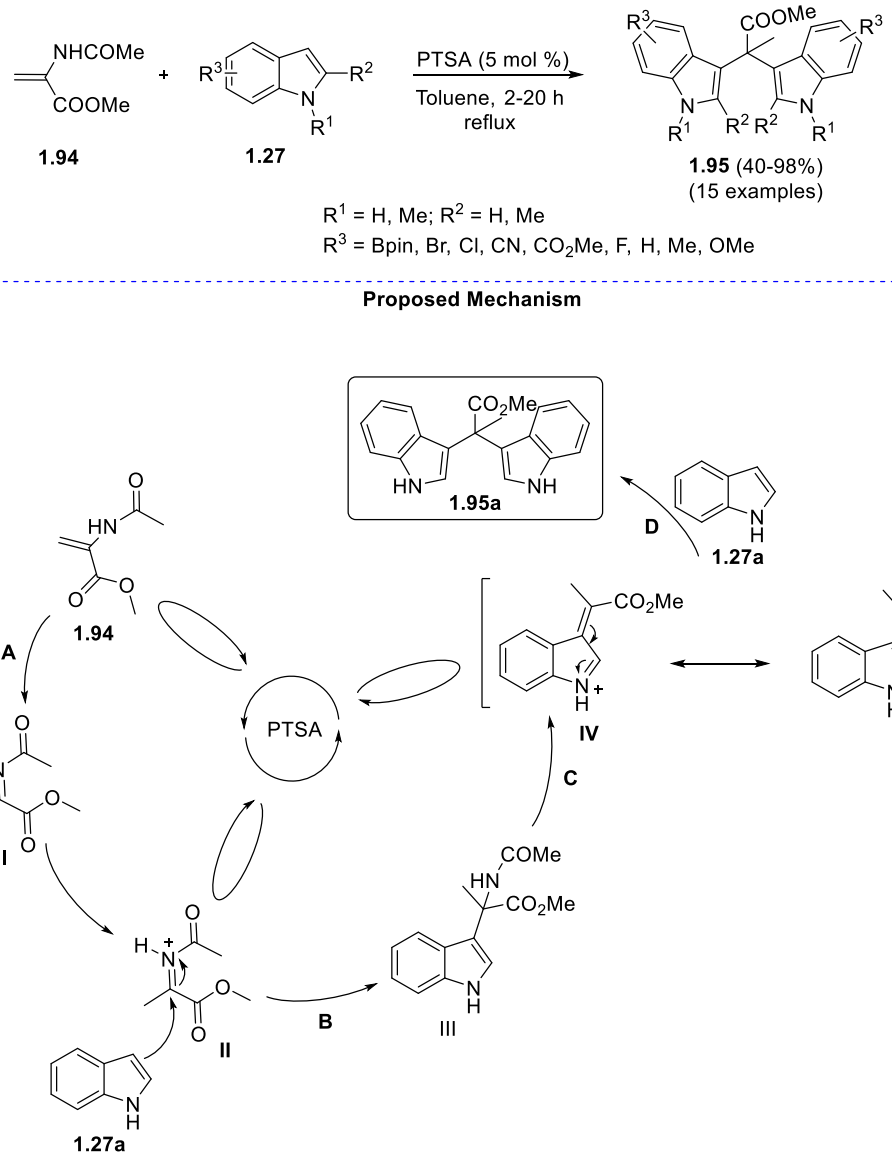

**Scheme 1.43** The self-coupling of indoles using Brönsted acidic ionic liquid (BAIL-1) as catalyst.

A good yielding procedure for the preparation of BIMs through a double nucleophilic addition of indoles to an *in situ* generated α-iminophosphonate was recently disclosed. [38] Using as starting substrates *N*-trityl substituted α-aminophosphonates, the authors found that the radical bromination by *N*-bromosuccinimide under inert atmosphere, led to α-iminophosphonates **1.92** by a subsequent spontaneous β-elimination of hydrogen bromide. The reaction of **1.92** with two equivalents of the appropriated indole, using phosphoric acid as catalyst, gave rise to the desired BIMs **1.93** in yields ranging from 53 to 72% (Scheme 1.44).



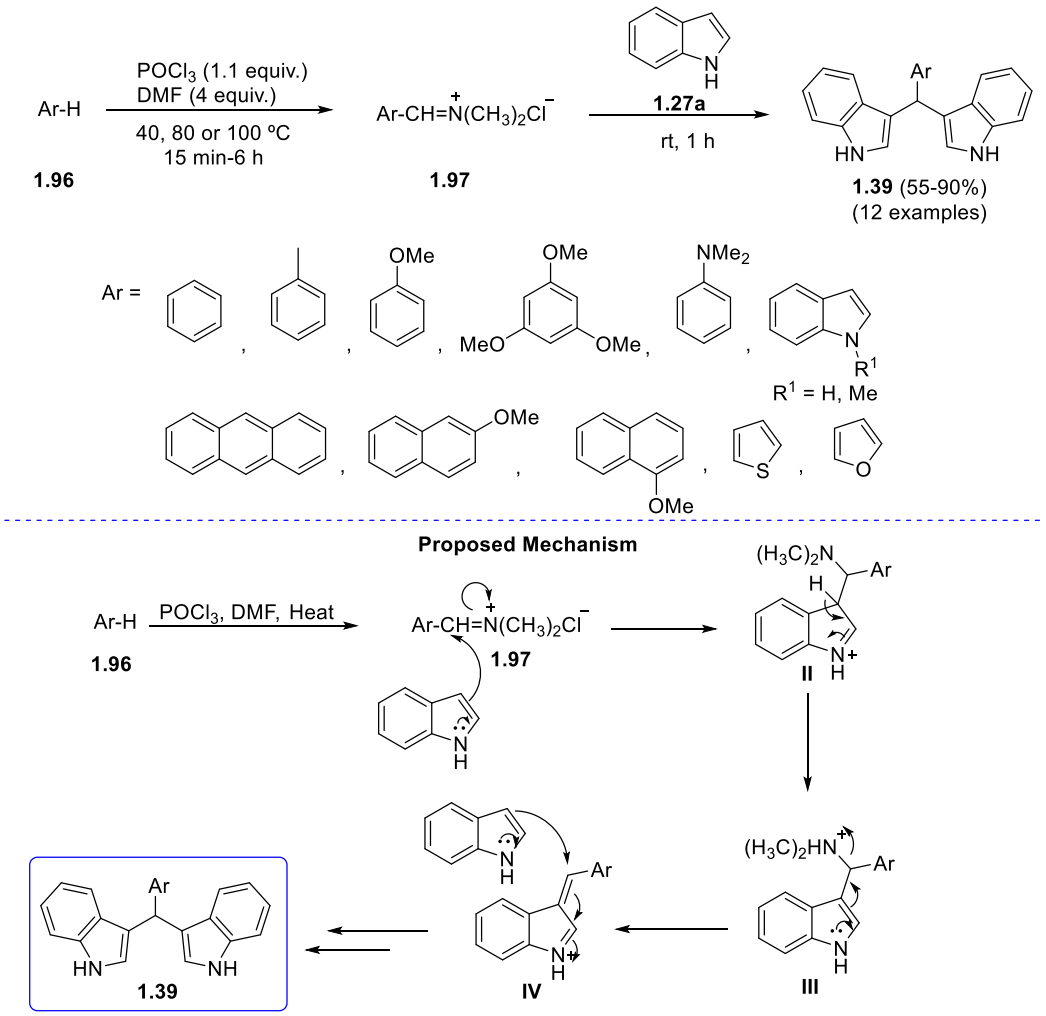

**Scheme 1.44** The double nucleophilic addition of indoles to an *in situ* generated α-iminophosphonate.

BIMs have also been prepared through *p*-toluenesulfonic acid (PTSA)-catalysed Markovnikov addition and C-N bond cleavage of indoles **1.27** with dehydroalanine esters **1.94** (Scheme 1.45).[197] Under the optimal conditions the target BIMs **1.95** were obtained in good to excellent yields (40-95%). The methodology provided the target compounds starting from indoles substituted at position 5, 6, or 7 with either electron-donating or -withdrawing substituents. However, reactions with indoles bearing electron donating-substituents required longer reaction times leading to lower yields than those of their electron-deficient counterparts. The same pattern was observed when the reaction was performed with *N*-methyl indole. Surprisingly, the authors also reported that methylindol-3-yl-acrylates were obtained when a bulky substituent was introduced at indole´s C-2 or C-4. This result was explained considering an elimination reaction after a hampered attack of the second indole molecule (omitted in the scheme). Therefore, using 1*H*-indole as template, the proposed mechanism suggests that after tautomerization of *N*-acetylamidoacrylate (step A), the indole aza-Friedel–Crafts reaction occurs (step B) and through the loss of the amide leaving group (step C) the cationic alkylideneindoleninium **IV** is generated. A subsequent attack of a second molecule of indole on these electrophilic iminiums **IV** leads to the desired BIMs **1.95** (Scheme 1.45).




**Scheme 1.45** PTSA-catalysed Markovnikov addition and C-N bond cleavage of indoles with dehydroalanine esters.

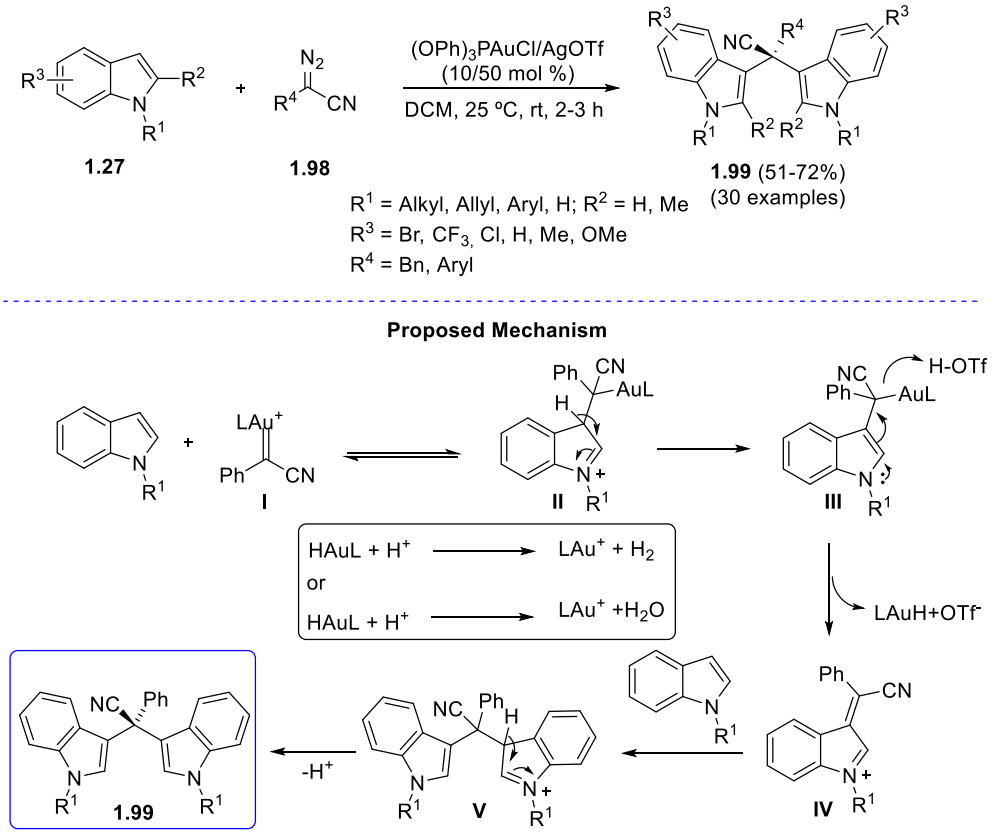
A metal-free one-pot procedure for the preparation of BIMs via the Vilsmeier-Haack reaction of electron-rich aromatics compounds and indoles has also been reported.[198] BIMs were obtained in moderate to very good yields (55-90%) after treatment of the electron-rich aromatics with $POCl_3$ and DMF, followed by addition of indole at room temperature (Scheme 1.46). The initial step of the eventual mechanism involves the Vilsmeier–Haack reaction in order to generate the aromatic *N*,*N*-dimethyliminium salt **1.97** which smoothly reacts with indole **1.27a** to form the corresponding azafulvene **II**, which further reacts with another molecule of indole **1.27a** to produce the corresponding BIM **1.39** (Scheme 1.46).




**Scheme 1.46** Metal-free one-pot procedure for the preparation of BIMs from the Vilsmeier-Haack reaction of electron-rich aromatics compounds and indoles.

A gold-catalysed oxidative coupling of indoles with a α-cyano gold carbene under oxidant-free conditions to form BIMs was described by Singh *et al.* (Scheme 1.47).[199] The results showed the compatibility of this gold catalysis with both *N*-aryl and *N*-alkyl-indoles in the reaction with diazocyanides **1.98**. The scope of this catalytic transformation was also expanded to diverse aryl- and alkyl-substituted diazocyanides. In general, the BIMs were obtained in reasonable yields (42-72%). This synthetic route was also applied to the synthesis of unsymmetrical BIMs from the reaction between *N*-aryl-indoles and phenyl diazocyanide **1.98a**. However, the correspondent adduct of *N*-aryl-hydroarylation was also obtained, as minor product. This suggested that *N*-aryl-indoles reacted faster with gold carbenes than *N*-alkyl-indoles. Thus, the nucleophilic addition of indole to the α-cyano gold carbene **I** led to the formation of intermediate **II**. Indolyl C3–H protons of **II** are

highly acidic and easily deprotonated by ion $TfO^-$ generating the gold-containing indole intermediate **III** which react with HOTf cleaving the Au–C bond to form the unsaturated iminiums **IV** and LAu–H. A subsequent attack of a second molecule of indole on these electrophilic iminiums **IV** leads to BIMs **1.99** (Scheme 1.47).[199]




**Scheme 1.47** Gold-catalysed oxidative coupling of indoles with a α-cyano gold carbene under oxidant-free conditions towards BIMs.

## 1.3 Final remarks and goals of the PhD project

The presentation of this literature review is introductory to this PhD thesis, whose main objective was the development of novel synthetic methodologies for the preparation of new bis(indolyl)methanes with potential biological activity.

Although several elegant, efficient and environmentally friendly methods producing good yields and selectivity have been reported, most of the above mentioned synthetic strategies still suffer from several drawbacks such as severe reaction conditions, complex catalyst and multi-step manipulations. On the other hand, the resulting BIMs generally lacked functionalities, especially at the methylene bridge, which could allow an easy manipulation or conversion into more elaborate compounds, eventually of greater interest and value.

Over the past decades, conjugated azo- and nitroso alkenes have been explored as electron-deficient heterodienes in inverse electron demand Diels-Alder reactions or as Michael-type acceptors in conjugate 1,4-addition reactions and emerged as powerful synthetic tools. The adducts and cycloadducts so formed have also been used as building blocks for the synthesis of a vast range of compounds with chemical and biological interest. Therefore, our strategy was to explore the chemistry of conjugated azo- and nitroso-alkenes to develop novel routes to functionalized bis(indolyl)methane oximes and hydrazones (Chapter 2).

Considering the high significance of natural bis(indolyl)methanes and their synthetic analogues, it is particularly desirable to look for more efficient and sustainable synthetic approaches to this class of heterocycles. Therefore, we set out to improve the efficiency and sustainability of the one-pot hetero-Diels–Alder strategy to BIMs using Natural Deep Eutetic Solvents (NADES) as reaction media (Chapter 3).

After the results obtained in the BIMs synthesis, we intended to extent this synthetic strategy to the synthesis bis- and tris(pyrazolyl)methanes (BPMs and TPMs) through the 1,4-conjugate addition of pyrazoles to α-halogenated nitroso- and azoalkenes (Chapter 4).

One strategy to find new compounds with biological activity was to explore the carboxylic acid/ tetrazole bioisosterism. Therefore, it was decided to investigate the reactivity of the tetrazolyl-2*H*-azirines towards arynes in order to obtain a new class of indole derivatives bearing a tetrazole moiety (Chapter 5).

## 1.4 References

1. Gribble, G. W., Hetorocyclic scaffolds II: Reaction and aplication of indoles. *Springer: Berlin, Edt. Gordon W. Gribble* **2010,** *Vol. 26*.

2. Kerru, N.; Gummidi, L.; Maddila, S.; Gangu, K. K.; Jonnalagadda, S. B., A Review on Recent Advances in Nitrogen-Containing Molecules and Their Biological Applications. *Molecules* **2020,** *25* (8), 1909-1951.

3. Vitaku, E.; Smith, D. T.; Njardarson, J. T., Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014,** *57* (24), 10257-10274.

4. Baeyer, A., Ueber die reduction aromatischer verbindungen mittelst zinkstaub. *Eur. J. Org. Chem.* **1866,** *140*, 295-296.

5. Kaushik, N.; Kaushik, N.; Attri, P.; Kumar, N.; Kim, C.; Verma, A.; Choi, E., Biomedical importance of indoles. *Molecules* **2013,** *18* (6), 6620-6662.

6. Sravanthi, T. V.; Manju, S. L., Indoles - A promising scaffold for drug development. *Eur. J. Pharm. Sci.* **2016,** *91*, 1-10.

7. Kaur, J.; Utreja, D.; Ekta; Jain, N.; Sharma, S., Recent developments in the synthesis and antimicrobial activity of indole and its derivatives. *Curr. Org. Synth.* **2019,** *16* (1), 17-37.

8. Wan, Y.; Li, Y.; Yan, C.; Yan, M.; Tang, Z., Indole: A privileged scaffold for the design of anti-cancer agents. *Eur. J. Med. Chem.* **2019,** *183*, 111691-11708.

9. Thanikachalam, P. V.; Maurya, R. K.; Garg, V.; Monga, V., An insight into the medicinal perspective of synthetic analogs of indole: A review. *Eur. J. Med. Chem.* **2019,** *180*, 562-612.

10. Kumari, A.; Singh, R. K., Medicinal chemistry of indole derivatives: Current to future therapeutic prospectives. *Bioorg. Chem.* **2019,** *89*, 103021-103055.

11. Han, Y.; Dong, W.; Guo, Q.; Li, X.; Huang, L., The importance of indole and azaindole scaffold in the development of antitumor agents. *Eur. J. Med. Chem.* **2020,** *203*, 112506-112528.

12. Li, J. Y.; Sun, X. F.; Li, J. J.; Yu, F.; Zhang, Y.; Huang, X. J.; Jiang, F. X., The antimalarial activity of indole alkaloids and hybrids. *Arch. Pharm.* **2020,** *353*, 1-11.

13. Zhu, Y.; Zhao, J.; Luo, L.; Gao, Y.; Bao, H.; Li, P.; Zhang, H., Research progress of indole compounds with potential antidiabetic activity. *Eur. J. Med. Chem.* **2021,** *223*, 113665-113698.

14. Chauhan, M.; Saxena, A.; Saha, B., An insight in anti-malarial potential of indole scaffold: A review. *Eur. J. Med. Chem.* **2021,** *218*, 113400.

15. Slominski, A.; Semak, I.; Pisarchik, A.; Sweatman, T.; Szczesniewski, A.; Wortsman, J., Conversion of L-tryptophan to serotonin and melatonin in human melanoma cells. *FEBS Lett.* **2002,** *511* (1-3), 102-106.

16. Kasahara, H., Current aspects of auxin biosynthesis in plants. *Biosci. Biotechnol. Biochem.* **2016,** *80* (1), 34-42.

17. McDonald, K. J.; Jardine, A. G., The use of fluvastatin in cardiovascular risk management. *Expert Opin. Pharmacother.* **2008,** *9* (8), 1407-1414.

18. Summ, O.; Evers, S., Mechanism of action of indomethacin in indomethacin-responsive headaches. *Curr. Pain Headache Rep.* **2013,** *17* (4), 327-333.

19. Klinger, J. R., Tadalafil for the treatment of pulmonary arterial hypertension. *Expert Rev. Respir. Med.* **2011,** *5* (3), 315-328.

20. Roila, F.; Del Favero, A., Ondansetron clinical pharmacokinetics. *Clin. Pharmacokinet.* **1995,** *29* (2), 95-109.

21. Wong, G. W. K.; Boyda, H. N.; Wright, J. M., Blood pressure lowering efficacy of partial agonist beta blocker monotherapy for primary hypertension. *CDSR* **2014**, 1-70.

22. Liu, Y.; Zhou, X.; Zhu, D.; Chen, J.; Qin, B.; Zhang, Y.; Wang, X.; Yang, D.; Meng, H.; Luo, Q.; Xie, P., Is pindolol augmentation effective in depressed patients resistant to selective serotonin reuptake inhibitors? A systematic review and meta-analysis. *Hum. Psychopharmacol.* **2015,** *30* (3), 132-142.

23. Sears, J. E.; Boger, D. L., Total synthesis of Vinblastine, related natural products, and key analogues and development of inspired methodology suitable for the systematic study of their structure–function properties. *Acc. Chem. Res.* **2015,** *48* (3), 653-662.

24. Nachshon-Kedmi, M.; Yannai, S.; Haj, A.; Fares, F. A., Indole-3-carbinol and 3,3′-diindolylmethane induce apoptosis in human prostate cancer cells. *FCT* **2003,** *41* (6), 745-752.

25. Queiroz, M. M. F.; Queiroz, E. F.; Zeraik, M. L.; Ebrahimi, S. N.; Marcourt, L.; Cuendet, M.; Castro-Gamboa, I.; Hamburger, M.; da Silva Bolzani, V.; Wolfender, J.-L.,

Chemical composition of the bark of tetrapterys mucronata and identification of acetylcholinesterase inhibitory constituents. *J. Nat. Prod.* **2014,** *77* (3), 650-656.

26. Majik, M.; Parameswaran, P.; Praveen, P., Bis(indolyl)methane alkaloids: isolation, bioactivity, and syntheses. *Synthesis* **2015,** *47* (13), 1827-1837.

27. Osawa, T.; Namiki, M., Structure elucidation of streptindole, a novel genotoxic metabolite isolated from intestinal bacteria. *Tetrahedron Lett.* **1983,** *24* (43), 4719-4722.

28. Kang, J.; Gao, Y.; Zhang, M.; Ding, X.; Wang, Z.; Ma, D.; Wang, Q., Streptindole and its derivatives as novel antiviral and anti-phytopathogenic fungus agents. *J. Agric. Food Chem.* **2020,** *68* (30), 7839-7849.

29. Arffmann, K.; Andrus, G. M., Treatment of fibromyalgia and related disorders. *WO 9917768* **1999**.

30. Bell, R.; Carmeli, S.; Sar, N., Vibrindole A, a metabolite of the marine bacterium, vibrio parahaemolyticus, isolated from the toxic mucus of the boxfish ostracion cubicus. *J. Nat. Prod.* **2004,** *57* (11), 1587-1590.

31. Talley, J. J.; Sprott, k.; Pearson, J. P.; Milne, G. T.; Yang, J. J.; Kim, C.; Barden, T.; Lundigran, R., Indole compounds *WO 2008019357* **2008**.

32. Khuzhaev, B. U.; Aripova, S. F.; Shakirov, R. S., Arundine — A new dimeric alkaloid from the roots ofArundo donax. *Chem. Nat. Compd.* **1994,** *30* (5), 635-636.

33. Wattenberg, L. W.; Loub, W. D., Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res.* **1978,** *38*, 1410-1413.

34. Safe, S.; Papineni, S.; Chintharlapalli, S., Cancer chemotherapy with indole-3-carbinol, bis(3′-indolyl)methane and synthetic analogs. *Cancer Lett.* **2008,** *269* (2), 326-338.

35. Kandekar, S.; Preet, R.; Kashyap, M.; M. U, R. P.; Mohapatra, P.; Das, D.; Satapathy, S. R.; Siddharth, S.; Jain, V.; Choudhuri, M.; Kundu, C. N.; Guchhait, S. K.; Bharatam, P. V., Structural elaboration of a natural product: Identification of 3,3′-diindolylmethane aminophosphonate and urea derivatives as potent anticancer agents. *Chem. Med. Chem.* **2013,** *8* (11), 1873-1884.

36. Praveen, C.; DheenKumar, P.; Muralidharan, D.; Perumal, P. T., Synthesis, antimicrobial and antioxidant evaluation of quinolines and bis(indolyl)methanes. *Bioorg. Med. Chem. Lett.* **2010,** *20* (24), 7292-7296.

37. Sarva, S.; Harinath, J. S.; Sthanikam, S. P.; Ethiraj, S.; Vaithiyalingam, M.; Cirandur, S. R., Synthesis, antibacterial and anti-inflammatory activity of bis(indolyl)methanes. *Chin. Chem. Lett.* **2016,** *27* (1), 16-20.

38. Maestro, A.; Martín-Encinas, E.; Alonso, C.; Martinez de Marigorta, E.; Rubiales, G.; Vicario, J.; Palacios, F., Synthesis of novel antiproliferative hybrid bis-(3-indolyl)methane phosphonate derivatives. *Eur. J. Med. Chem.* **2018,** *158*, 874-883.

39. Sathiyaraj, S.; Shanavas, A.; Kumar, K. A.; Sathiyaseelan, A.; Senthilselvan, J.; Kalaichelvan, P. T.; Nasar, A. S., The first example of bis(indolyl)methane based hyperbranched polyurethanes: Synthesis, solar cell application and anti-bacterial and anti-oxidant properties. *Eur. Polym. J.* **2017,** *95*, 216-231.

40. Sujatha, k.; Perumal, P. T.; Muralidharan, D.; Rajendran, M., Synthesis, analgesic and anti-inflammatory activities of bis(indolyl)methanes. *Indian J. Chem.* **2009,** *48B*, 267-272.

41. Giannini, G.; Marzi, M.; Marzo, M. D.; Battistuzzi, G.; Pezzi, R.; Brunetti, T.; Cabri, W.; Vesci, L.; Pisano, C., Exploring bis-(indolyl)methane moiety as an alternative and innovative CAP group in the design of histone deacetylase (HDAC) inhibitors. *Bioorg. Med. Chem. Lett.* **2009,** *19* (10), 2840-2843.

42. He, X.; Hu, S.; Liu, K.; Guo, Y.; Xu, J.; Shao, S., Oxidized bis(indolyl)methane: A simple and efficient chromogenic-sensing molecule based on the proton transfer signaling mode. *Org. Lett.* **2005,** *8* (2), 333-336.

43. Martínez, R.; Espinosa, A.; Tárraga, A.; Molina, P., Bis(indolyl)methane derivatives as highly selective colourimetric and ratiometric fluorescent molecular chemosensors for Cu2+ cations. *Tetrahedron* **2008,** *64* (9), 2184-2191.

44. Lee, G. W.; Kim, N.-K.; Jeong, K.-S., Synthesis of biindole−diazo conjugates as a colorimetric anion receptor. *Org. Lett.* **2010,** *12* (11), 2634-2637.

45. Kaur, P.; Kaur, S.; Singh, K., Bis(*N*-methylindolyl)methane-based chemical probes for $Hg^{2+}$ and $Cu^{2+}$ and molecular IMPLICATION gate operating in fluorescence mode. *Org. Biomol. Chem.* **2012,** *10* (8), 1497–1501.

46. Oliveira, E.; Baptista, R. M. F.; Costa, S. P. G.; Raposo, M. M. M.; Lodeiro, C., Synthesis and solvatochromism studies of novel bis(indolyl)methanes bearing functionalized arylthiophene groups as new colored materials. *Photochem. Photobiol. Sci.* **2014,** *13* (3), 492-498.

47. Shiri, M.; Zolfigol, M. A.; Kruger, H. G.; Tanbakouchian, Z., Bis- and Trisindolylmethanes (BIMs and TIMs)†. *Chem. Rev.* **2010,** *110* (4), 2250-2293.

48. Gong, H.; Xie, Z., Research progress of synthesis of bis(indolyl)methanes. *Chin. J. Org. Chem.* **2012,** *32* (07), 1195−1207.

49. Kaishap, P. P.; Dohutia, C., synthetic approached for bis(indolyl)methane. *Int. J. Pharm. Scienc. Res.* **2013,** *4*, 1312-1322.

50. Petrini, M.; Palmieri, A., Recent advances in the synthesis of unsymmetrical bisindolylmethane derivatives. *Synthesis* **2018,** *51* (04), 829-841.

51. Singh, A.; Kaur, G.; Banerjee, B., Recent developments on the synthesis of biologically significant bis/tris(indolyl)methanes under various reaction conditions: A review. *Curr. Org. Chem.* **2020,** *24* (6), 583-621.

52. Sarmah, D.; Bora, U., Methylene surrogates for the synthesis of 3,3'-diindolylmethanes. *ChemistrySelect* **2020,** *5* (28), 8577-8603.

53. Jia, Z.; Yang, J.; Wu, Z.; Zhang, T.; Liu, S.; Yuan, Y.; Zong, X.; Liu, X.; Zhang, Z., Recent advance on the synthesis of 3,3'-bisindolylmethane derivatives under transition-metal-free catalytic conditions. *Chin. J. Org. Chem.* **2021,** *41* (1), 52-64.

54. Khanna, L.; Mansi; Yadav, S.; Misra, N.; Khanna, P., "In water" synthesis of bis(indolyl)methanes: a review. *Synth. Commun.* **2021,** *51* (19), 2892-2923.

55. Fischer, H. E., Ueber einige reactionen der indole. *Chem. Ber.* **1886,** *19*, 713-718.

56. von Walther, R.; Clemen, J., Beitrage ZPF kenntniss des a-methylketols; . *j. Prakt.* **1900,** *61*, 249-289.

57. Kamal, A.; Ali Qureshi, A., Syntheses of some substituted di-indolylmethanes in aqueous medium at room temperature. *Tetrahedron* **1963,** *19* (4), 513-520.

58. Chen, D.; Yu, L.; Wang, P. G., Lewis acid-catalyzed reactions in protic media. Lanthanide-catalyzed reactions of indoles with aldehydes or ketones. *Tetrahedron Lett.* **1996,** *37* (26), 4467-4470.

59. Yadav, J. S.; Reddy, B. V. S.; Murthy, C. V. S. R.; Kumar, G. M.; Madan, C., Lithium perchlorate catalyzed reactions of indoles: an expeditious synthesis of bis(indolyl)methanes. *Synthesis* **2001,** *2001* (05), 783-787.

60. Bandgar, B. P.; Shaikh, K. A., Molecular iodine-catalyzed efficient and highly rapid synthesis of bis(indolyl)methanes under mild conditions. *Tetrahedron Lett.* **2003,** *44* (9), 1959-1961.

61. Azizian, J.; Teimouri, F.; Mohammadizadeh, M. R., Ammonium chloride catalyzed one-pot synthesis of diindolylmethanes under solvent-free conditions. *Catal. Commun.* **2007,** *8* (7), 1117-1121.

62. Yadav, J. S.; Gupta, M. K.; Jain, R.; Yadav, N. N.; Reddy, B. V. S., A practical synthesis of bis(indolyl)methanes employing boric acid. *Monatsh. Chem.* **2010,** *141* (9), 1001-1004.

63. Shelke, G. M.; Rao, V. K.; Tiwari, R. K.; Chhikara, B. S.; Parang, K.; Kumar, A., Bismuth triflate-catalyzed condensation of indoles with acetone. *RSC Adv.* **2013,** *3* (44), 22346-22352.

64. Xu, X.-F.; Xiong, Y.; Ling, X.-G.; Xie, X.-M.; Yuan, J.; Zhang, S.-T.; Song, Z.-R., A practical synthesis of bis(indolyl)methanes catalyzed by BF3·Et2O. *Chin. Chem. Lett.* **2014,** *25* (3), 406-410.

65. Beltrá, J.; Gimeno, M. C.; Herrera, R. P., A new approach for the synthesis of bisindoles through AgOTf as catalyst. *Beilstein J. Org. Chem.* **2014,** *10*, 2206-2214.

66. Swetha, A.; Babu, B. M.; Meshram, H. M., An efficient and rapid protocol for the synthesis of diversely functionalized bisindolylmethanes. *Tetrahedron Lett.* **2015,** *56* (14), 1775-1779.

67. Tornquist, B. L.; de Paula Bueno, G.; Manzano Willig, J. C.; de Oliveira, I. M.; Stefani, H. A.; Rafique, J.; Saba, S.; Almeida Iglesias, B.; Botteselle, G. V.; Manarin, F., Ytterbium (III) triflate/sodium dodecyl sulfate: a versatile recyclable and water-tolerant catalyst for the synthesis of bis(indolyl)methanes (BIMs). *ChemistrySelect* **2018,** *3* (23), 6358-6363.

68. Panov, A. A.; Lavrenov, S. N.; Mirchink, E. P.; Isakova, E. B.; Korolev, A. M.; Trenin, A. S., Synthesis and antibacterial activity of novel arylbis(indol-3-yl)methane derivatives. *J. Antibiot.* **2020,** *74* (3), 219-224.

69. Lee, S. O.; Choi, J.; Kook, S.; Lee, S. Y., Lewis acid-catalyzed double addition of indoles to ketones: synthesis of bis(indolyl)methanes with all-carbon quaternary centers. *Org. Biomol. Chem.* **2020,** *18* (44), 9060-9064.

70. Pasha, M. A.; Jayashanka, V. P., p-Toluenesulfonic acid (*p*-TSA) catalyzed efficient synthesis of bis(indolyl)methanes under grindstone method. *J. Pharmacol. Toxicol.* **2006,** *1* (6), 585-590.

71. Khaksar, S.; Ostad, S. M., Pentafluorophenylammonium triflate as an efficient, environmentally friendly and novel organocatalyst for synthesis of bis-indolyl methane derivatives. *J. Fluor. Chem.* **2011,** *132* (11), 937-939.

72. Ekbote, S. S.; Deshmukh, K. M.; Qureshi, Z. S.; Bhanage, B. M., Polyvinylsulfonic acid as a novel Brønsted acid catalyst for the synthesis of bis(indolyl)methanes. *Green Chem. Lett.* **2011,** *4* (2), 177-183.

73. Suresh Kumar, G. S.; Antony Muthu Prabhu, A.; Seethalaksmi, P. G.; Bhuvanesh, N.; Kumaresan, S., In situ Bronsted–Lowry acid catalyzed syntheses, characterization, single crystal XRD, electronic spectral-, DPPH radical scavenging-, and DNA protection studies of aryl-3,3′-bis(indolyl)methanes. *Spectrochim. Acta A Mol.* **2014,** *123*, 249-256.

74. Karthikeyan, K.; Sivaprasad, G., Synthesis of somebis(Indolyl)methanes Catalyzed by Ascorbic Acid under Mild Conditions. *Org. Prep. Proced. Int.* **2015,** *47* (6), 449-453.

75. El Sayed, M. T.; Ahmed, K. M.; Mahmoud, K.; Hilgeroth, A., Synthesis, cytostatic evaluation and structure activity relationships of novel bis-indolylmethanes and their corresponding tetrahydroindolocarbazoles. *Eur. J. Med. Chem.* **2015,** *90*, 845-859.

76. Banari, H.; Kiyani, H.; Pourali, A., Efficient synthesis of bis(indolyl)methanes, bispyrazoles and biscoumarins using 4-sulfophthalic acid. *Res. Chem. Intermed.* **2016,** *43* (3), 1635-1649.

77. Simha, P. R.; Mangali, M. S.; Kuppireddy Gari, D.; Venkatapuram, P.; Adivireddy, P., Benzenesulfonic acid: a versatile catalyst for the synthesis of bis(indolyl)methanes as antioxidants. *J. Heterocycl. Chem.* **2017,** *54* (5), 2717-2724.

78. Noland, W. E.; Brown, C. D.; DeKruif, R. D.; Lanzatella, N. P.; Gao, S. M.; Zabronsky, A. E.; Tritch, K. J., Condensation reactions of indole with acetophenones affording mixtures of 3,3-(1-phenylethane-1,1-diyl)bis(1H-indoles) and 1,2,3,4-tetrahydro-3-(1H-indol-3-yl)-1-methyl-1,3-diphenylcyclopent[b]indoles. *Synth. Commun.* **2018,** *48* (14), 1755-1765.

79. Pore, D. M.; Desai, U. V.; Thopate, T. S.; Wadgaonkar, P. P., A mild, expedient, solventless synthesis of bis(indolyl)alkanes using silica sulfuric acid as a reusable catalyst. *Arkivoc* **2006,** *2006* (12), 75-80.

80. Talukdar, D.; Thakur, A. J., A green synthesis of symmetrical bis(indol-3-yl)methanes using phosphate-impregnated titania catalyst under solvent free grinding conditions. *Green Chem. Lett.* **2013,** *6* (1), 55-61.

81. Siddiqui, Z. N.; Tarannum, S., Xanthan sulfuric acid: an efficient and biodegradable solid acid catalyst for the synthesis of bis(indolyl)methanes under solvent-free conditions. *C. R. Chim.* **2013,** *16* (9), 829-837.

82. Banothu, J.; Gali, R.; Velpula, R.; Bavantula, R.; Crooks, P. A., An Eco-friendly improved protocol for the synthesis of bis(3-indolyl)methanes using poly(4-vinylpyridinium)hydrogen sulfate as efficient, heterogeneous, and recyclable solid acid catalyst. *ISRN Org. Chem.* **2013,** *2013*, 1-5.

83. Siadatifard, S. H.; Abdoli-Senejani, M.; Bodaghifard, M. A.; Weaver, G., An efficient method for synthesis of bis(indolyl)methane and di-bis(indolyl)methane derivatives in environmentally benign conditions using TBAHS. *Cogent Med.* **2016,** *2* (1), 1-7.

84. Banari, H.; Kiyani, H.; Pourali, A. R., Bisindolization reaction employing phthalimide-*N*-sulfonic acid as an efficient catalyst. *Curr. Organocatalysis* **2020,** *7* (2), 124-133.

85. Azizi, N.; Gholibeghlo, E.; Manocheri, Z., Green procedure for the synthesis of bis(indolyl)methanes in water. *Sci. Iran* **2012,** *19* (3), 574-578.

86. Bandgar, B. P.; Patil, A. V.; Kamble, V. T., Fluoroboric acid adsorbed on silica gel catalyzed synthesis of bisindolyl alkanes under mild and solvent-free conditions. *Arkivoc* **2008,** *2007* (16), 252-259.

87. Kamble, V. T.; Kadam, K. R.; Joshi, N. S.; Muley, D. B., $HClO_4$–$SiO_2$ as a novel and recyclable catalyst for the synthesis of bis-indolylmethanes and bis-indolylglycoconjugates. *Catal. Commun.* **2007,** *8* (3), 498-502.

88. Khalafi-Nezhad, A.; Nourisefat, M.; Panahi, F., l-Proline-modified magnetic nanoparticles (LPMNP): a novel magnetically separable organocatalyst. *RSC Adv.* **2014,** *4* (43), 22497-22500.

89. Mohammadi Ziarani, G.; Hajiabbasi, P.; Badiei, A., Application of SBA-Pr-$NH_2$ as a nanoporous base silica catalyst in the development of 2,2-Bis(1*H*-indol-3-yl)acenaphthen-1(2*H*)-ones syntheses. *J. Iran. Chem. Soc.* **2015,** *12* (9), 1649-1654.

90. Ravi, K.; Krishnakumar, B.; Swaminathan, M., $BiCl_3$-loaded montmorillonite K10: A new solid acid catalyst for solvent-free synthesis of bis(indolyl)methanes. *Res. Chem. Intermed.* **2014,** *41* (8), 5353-5364.

91. Kandasamy, K.; Ganesabaskaran, S.; Pachamuthu, M. P.; Maheswari, R.; Ramanathan, A., Synthesis of pyrazolylbisindoles over mesoporous Lewis acidic ZrTUD-

1: Potential application in selective Cu2+ colorimetric detection. *Spectrochim. Acta A Mol.* **2015,** *150,* 34-39.

92. Vaid, R.; Gupta, M.; Chambyal, O. S.; Gupta, R., $SiO_2$-Diphenic acid: an efficient and recyclable heterogeneous catalyst for one-pot synthesis of bis-(indolyl)methane derivatives in liquid phase. *Chem. Sci. J.* **2015,** *127* (6), 987-997.

93. Soliman, H. A.; Mubarak, A. Y.; Elmorsy, S. S., An efficient synthesis of bis(indolyl) methanes and N,N′-alkylidene bisamides by Silzic under solvent free conditions. *Chin. Chem. Lett.* **2016,** *27* (3), 353-356.

94. Esmaielpour, M.; Akhlaghinia, B.; Jahanshahi, R., Green and efficient synthesis of aryl/alkylbis(indolyl)methanes using Expanded Perlite-PPA as a heterogeneous solid acid catalyst in aqueous media. *Chem. Sci. J.* **2017,** *129* (3), 313-328.

95. Wang, Y.; Sang, R.; Zheng, Y.; Guo, L.; Guan, M.; Wu, Y., Graphene oxide: an efficient recyclable solid acid for the synthesis of bis(indolyl)methanes from aldehydes and indoles in water. *Catal. Commun.* **2017,** *89,* 138-142.

96. Yaghoubi, A.; Dekamin, M. G.; Arefi, E.; Karimi, B., Propylsulfonic acid-anchored isocyanurate-based periodic mesoporous organosilica (PMO-ICS-Pr-SO3H): A new and highly efficient recoverable nanoporous catalyst for the one-pot synthesis of bis(indolyl)methane derivatives. *J. Colloid Interface Sci.* **2017,** *505*, 956-963.

97. Khatab, T. K.; Abdelghany, A. M.; Soliman, H. A., $V_2O_5/SiO_2$ as a heterogeneous catalyst in the synthesis of bis(indolyl)methanes under solvent free condition. *Silicon* **2017,** *10* (3), 703-708.

98. Saiadian, S.; Khorshidi, A., Comparison of the catalytic activity of copper nanostructures in Friedel-Crafts-type condensation reactions. *ChemistrySelect* **2018,** *3* (1), 142-146.

99. Gambaro, S.; La Manna, P.; De Rosa, M.; Soriente, A.; Talotta, C.; Gaeta, C.; Neri, P., The hexameric resorcinarene capsule as a brønsted acid catalyst for the synthesis of bis(heteroaryl)methanes in a nanoconfined space. *Front. Chem.* **2019,** *7*, 1-9.

100. Deb, B.; Debnath, S.; Chakraborty, A.; Majumdar, S., Bis-indolylation of aldehydes and ketones using silica-supported FeCl3: molecular docking studies of bisindoles by targeting SARS-CoV-2 main protease binding sites. *RSC Adv.* **2021,** *11* (49), 30827-30839.

101. Reyes-Mercado, E.; Rivas-Loaiza, J. A.; García-Merinos, J. P.; López, Y.; González-Campos, J. B., Chitosan-supported copper salt and copper metal

nanoparticles/copper (I) oxide microcrystals: Efficient and recyclable heterogeneous catalysts for the synthesis of bis(indolyl)methanes. *Chem. Eng. Process.* **2021,** *159*, 108201-108214.

102. Vijender Reddy, A.; Ravinder, K.; Niranjan Reddy, V. L.; Venkateshwer Goud, T.; Ravikanth, V.; Venkateswarlu, Y., Zeolite catalyzed synthesis of bis(Indolyl)methanes. *Synth. Commun.* **2003,** *33* (21), 3687-3694.

103. Karthik, M.; Palanichamy, M.; Murugesan, V., A mild, eco-friendly and efficient zeolite catalyzed synthesis of vibrindole A and bis(indolyl)methanes. In *Nanoporous Materials IV, Proceedings of the 4th International Symposium on Nanoporous Materials*, 2005; pp 873-878.

104. Magesh, C. J.; Nagarajan, R.; Karthik, M.; Perumal, P. T., Synthesis and characterization of bis(indolyl)methanes, tris(indolyl)methanes and new diindolylcarbazolylmethanes mediated by Zeokarb-225, a novel, recyclable, eco-benign heterogenous catalyst. *Appl. Catal. A* **2004,** *266* (1), 1-10.

105. Kidwai, M.; Chauhan, R.; Bhatnagar, D., Nafion-H® catalyzed efficient condensation of indoles with aromatic aldehydes in PEG-water solvent system: A green approach. *Arab. J. Chem.* **2016,** *9*, S2004-S2010.

106. Indurthi, H. K.; Virdi, R.; Koli, P.; Nageswara Rao, D.; Sharma, D. K., Seralite SRC-120 resin catalyzed synthesis of bis(indolyl)methanes using indoles and low/high boiling point carbonyl compounds under solvent free conditions. *Synth. Commun.* **2020,** *51* (1), 139-150.

107. Sobhani, S.; Safaei, E.; Hasaninejad, A.-R.; Rezazadeh, S., An eco-friendly procedure for the efficient synthesis of bis(indolyl)methanes in aqueous media. *J. Organomet. Chem.* **2009,** *694* (18), 3027-3031.

108. Sheikhshoaie, I.; Khabazzadeh, H.; Saeid-Nia, S., Iron(III)(salen)Cl as an efficient catalyst for synthesis of bis(indolyl)methanes. *Transit. Met. Chem.* **2009,** *34* (5), 463-466.

109. Yang, Y. L.; Wan, N. N.; Wang, W. P.; Xie, Z. F.; De Wang, J., Synthesis of bis(indolyl) methanes catalyzed by Schiff base–Cu(II) complex. *Chin. Chem. Lett.* **2011,** *22* (9), 1071-1074.

110. Jafarpour, M.; Rezaeifard, A.; Gorzin, G., Enhanced catalytic activity of Zr(IV) complex with simple tetradentate Schiff base ligand in the clean synthesis of indole derivatives. *Inorg. Chem. Commun.* **2011,** *14* (11), 1732-1736.

111. Mandal, S. M.; Pegu, R.; Porto, W. F.; Franco, O. L.; Pratihar, S., Novel boronic acid derivatives of bis(indolyl) methane as anti-MRSA agents. *Bioorg. Med. Chem. Lett.* **2017,** *27* (10), 2135-2138.

112. Sai Manoj Gorantla, N. V. T.; Guruprasad Reddy, P.; Abdul Shakoor, S. M.; Mandal, R.; Roy, S.; Mondal, K. C., Tetranuclear 3 d/4 f coordination complexes as homogeneous catalysts for bis(indolyl)methane synthesis. *ChemistrySelect* **2019,** *4* (26), 7722-7727.

113. Qiao, J.; Gao, S.; Wang, L.; Wei, J.; Li, N.; Xu, X., Air-stable μ-oxo-bridged binuclear titanium(IV) salophen perfluorooctanesulfonate as a highly efficient and recyclable catalyst for the synthesis of bis(indolyl) methane derivatives. *J. Organomet. Chem.* **2020**, 121039-121045.

114. Nguyen, H. T. D.; Nguyen, T. T.; Nguyen, P. T. K.; Tran, P. H., A highly active copper-based metal-organic framework catalyst for a friedel–crafts alkylation in the synthesis of bis(indolyl)methanes under ultrasound irradiation. *Arab. J. Chem.* **2020,** *13* (1), 1377-1385.

115. Boroujeni, K. P.; Shahrokh, M.; Kiani, K.; Farokhnia, A.; Kazemi, R.; Kheiri, F., Synthesis and catalytic application of bimetallic and trimetallic magnetic nanoalloys for the preparation of bis(indolyl)methanes. *Russ. J. Org. Chem.* **2020,** *56* (10), 1823-1831.

116. Azizi, N.; Torkian, L.; Saidi, M. R., Highly efficient synthesis of bis(indolyl)methanes in water. *J. Mol. Catal.* **2007,** *275* (1-2), 109-112.

117. Giri, B. Y.; Devi, B. L. A. P.; Vijaylakshmi, K.; Prasad, R. B. N.; Lingaiah, N.; Prasad, P. S. S., A mild and efficient synthesis of bis(indolyl)methane derivatives catalyzed by monoammonium salt of as-tungstophosphoric acid. *Indian J. Chem.* **2012,** *51B*, 1731-1737.

118. Tayebee, R.; Amini, M. M.; Abdollahi, N.; Aliakbari, A.; Rabiei, S.; Ramshini, H., Magnetic inorganic–organic hybrid nanomaterial for the catalytic preparation of bis(indolyl)arylmethanes under solvent-free conditions: Preparation and characterization of H5PW10V2O40/pyridino-Fe3O4 nanoparticles. *Appl. Catal. A* **2013,** *468*, 75-87.

119. Tayade, Y. A.; Patil, D. R.; Wagh, Y. B.; Jangle, A. D.; Dalal, D. S., An efficient synthesis of 3-indolyl-3-hydroxy oxindoles and 3,3-di(indolyl)indolin-2-ones catalyzed by sulfonated β-CD as a supramolecular catalyst in water. *Tetrahedron Lett.* **2015,** *56* (5), 666-673.

120. Xiang, Z.; Liu, Z.; Chen, X.; Wu, Q.; Lin, X., Biocatalysts for cascade reaction: Porcine pancreas lipase (PPL)-catalyzed synthesis of bis(indolyl)alkanes. *Amino Acids* **2013,** *45* (4), 937-945.

121. Xie, Z.-B.; Sun, D.-Z.; Jiang, G.-F.; Le, Z.-G., Facile synthesis of bis(indolyl)methanes catalyzed by α-chymotrypsin. *Molecules* **2014,** *19* (12), 19665-19677.

122. Sun, D.; Jiang, G.; Xie, Z.; Le, Z., α-Chymotrypsin-catalyzed synthesis of bis(indolyl)alkanes in water. *Chin. J. Chem.* **2015,** *33* (4), 409-412.

123. Naeimi, A.; Honarmand, M.; Salandari, N., First biomimetic electrospun polymer from Carthamus tinctorius plant for sustainable synthesis of bis (1H-indol-3-yl)methanes. *Polymer* **2018,** *159*, 181-190.

124. Patil, M.; Karhale, S.; Bhenki, C.; Kumbhar, A.; Helavi, V., Sulfonic acid@pericarp-pomegranate: A natural supported catalyst for synthesis of bis(indolyl)alkanes. *React. Kinet. Mech. Catal.* **2020,** *130* (2), 993-1007.

125. Fu, Y.; Lu, Z.; Fang, K.; He, X.; Xu, H.; Hu, Y., Enzymatic approach to cascade synthesis of bis(indolyl)methanes in pure water. *RSC Adv.* **2020,** *10* (18), 10848-10853.

126. Chia, P. W.; Lim, B. S.; Tan, K. C.; Yong, F. S. J.; Kan, S.-Y., Water extract of onion peel for the synthesis of bisindolylmethanes. *J. King Saud Univ. Sci.* **2019,** *31* (4), 642-647.

127. Pal, R., Tamarind fruit juice as a natural catalyst: An excellent catalyst for efficient and green synthesis of bis-, tris-, and tetraindolyl compounds in water. *Indian J. Chem.* **2014,** *53B*, 763-768.

128. Mi, X.; Luo, S.; He, J.; Cheng, J.-P., $Dy(OTf)_3$ in ionic liquid: an efficient catalytic system for reactions of indole with aldehydes/ketones or imines. *Tetrahedron Lett.* **2004,** *45* (23), 4567-4570.

129. Chakraborti, A. K.; Roy, S. R.; Kumar, D.; Chopra, P., Catalytic application of room temperature ionic liquids: [bmim][MeSO4] as a recyclable catalyst for synthesis of bis(indolyl)methanes. Ion-fishing by MALDI-TOF-TOF MS and MS/MS studies to probe the proposed mechanistic model of catalysis. *Green Chem.* **2008,** *10* (10), 1111-1118.

130. Gupta, G.; Chaudhari, G.; Tomar, P.; Gaikwad, Y.; Azad, R.; Pandya, G.; Waghulde, G.; Patil, K., Synthesis of bis(indolyl)methanes using molten N-butylpyridinium bromide. *Eur. J. Chem.* **2012,** *3* (4), 475-479.

131. Das, P. J.; Das, J., Synthesis of aryl/alkyl(2,2′-bis-3-methylindolyl)methanes and aryl(3,3′-bis indolyl)methanes promoted by secondary amine based ionic liquids and microwave irradiation. *Tetrahedron Lett.* **2012,** *53* (35), 4718-4720.

132. Shi, X.-L.; Lin, H.; Li, P.; Zhang, W., Friedel-Crafts alkylation of indoles exclusively in water catalyzed by ionic liquid supported on a polyacrylonitrile fiber: a simple "release and catch" catalyst. *Chem. Cat. Chem.* **2014,** *6* (10), 2947-2953.

133. Ying, A.; Li, Z.; Ni, Y.; Xu, S.; Hou, H.; Hu, H., Novel multiple-acidic ionic liquids: Green and efficient catalysts for the synthesis of bis-indolylmethanes under solvent-free conditions. *J. Ind. Eng. Chem.* **2015,** *24*, 127-131.

134. Tran, P. H.; Nguyen, X.-T. T.; Chau, D.-K. N., A Brønsted-acidic ionic liquid gel as an efficient and recyclable heterogeneous catalyst for the synthesis of bis(indolyl)methanes under solvent-free sonication. *Asian J. Org. Chem.* **2018,** *7* (1), 232-239.

135. Chatterjee, R.; Mahato, S.; Santra, S.; Zyryanov, G. V.; Hajra, A.; Majee, A., Imidazolium zwitterionic molten salt: an efficient organocatalyst under neat conditions at room temperature for the synthesis of dipyrromethanes as well as bis(indolyl)methanes. *ChemistrySelect* **2018,** *3* (21), 5843-5847.

136. Azizi, N.; Manocheri, Z., Eutectic salts promote green synthesis of bis(indolyl) methanes. *Res. Chem. Intermed.* **2012,** *38* (7), 1495-1500.

137. Seyedi, N.; Khabazzadeh, H.; Saeednia, S., ZnCl2/Urea as a deep eutectic solvent for the preparation of bis(indolyl)methanes under ultrasonic conditions. *Synth. React. Inorg. Me.* **2015,** *45* (10), 1501-1505.

138. Nasreen, A.; Varala, R.; Rao, K. S., A green protocol for the synthesis of bis(indolyl)methanes catalyzed by succinic acid under microwave irradiation. *Org. Commun.* **2017,** *10* (2), 104-113.

139. Zahran, M.; Abdin, Y.; Salama, H., Eco-friendly and efficient synthesis of bis(indolyl)methanes under microwave irradiation. *Arkivoc* **2008,** *2008* (11), 256-265.

140. Zhang, D.-W.; Zhang, Y.-M.; Zhang, Y.-L.; Zhao, T.-Q.; Liu, H.-W.; Gan, Y.-M.; Gu, Q., Efficient solvent-free synthesis of bis(indolyl)methanes on SiO2 solid support under microwave irradiation. *Chem. Pap.* **2015,** *69* (3), 470-478.

141. Nikpassand, M.; Fekri, L. Z.; Nabatzadeh, M., Efficient and green synthesis of novel derivatives of 3,3′-((aryl-1-phenyl-1H-pyrazol-4-yl)methylene)bis(1H-indole) under ultrasound irradiation. *Synth. Commun.* **2016,** *47* (1), 29-36.

142. Hote, B. S.; Siddiqui, T. A. J.; Pisal, P. M.; Mandawad, G. G., Green approach of solvent- and catalyst free synthesis of bis(indolyl)methanes under visible light irradiation. *Polycycl. Aromat. Compd.* **2020**, 1-9.

143. Nam, S. M.; Jang, Y. S.; Son, G. E.; Song, C. H.; In, I.; Park, C. P., Droplet-based continuous flow synthesis of biologically active Bis(indolyl)methanes and Tris(indolyl)methanes. *Tetrahedron Lett.* **2020,** *61* (32), 152178-152181.

144. Mohapatra, S. S.; Wilson, Z. E.; Roy, S.; Ley, S. V., Utilization of flow chemistry in catalysis: New avenues for the selective synthesis of Bis(indolyl)methanes. *Tetrahedron* **2017,** *73* (14), 1812-1819.

145. Mhaldar, S. N.; Mandrekar, K. S.; Gawde, M. K.; Shet, R. V.; Tilve, S. G., Solventless mechanosynthesis of bis(Indolyl)Methanes. *Synth. Commun.* **2019,** *49* (1), 94-101.

146. Naidu, K.; Khalivulla, S.; Rasheed, S.; Fakurazi, S.; Arulselvan, P.; Lasekan, O.; Abas, F., Synthesis of bisindolylmethanes and their cytotoxicity properties. *Int. J. Mol. Sci.* **2013,** *14* (1), 1843-1853.

147. Hirashita, T.; Ogawa, M.; Hattori, R.; Okochi, S.; Araki, S., Condensation of indoles and aldehydes in subcritical water without the addition of catalysts. *Bull. Chem. Soc. Jpn.* **2015,** *88* (12), 1760-1764.

148. Brahmachari, G.; Banerjee, B., Facile and one-pot access of 3,3-bis(indol-3-yl)indolin-2-ones and 2,2-bis(indol-3-yl)acenaphthylen-1(2*H*)-one derivatives via an eco-friendly pseudo-multicomponent reaction at room temperature using sulfamic acid as an organo-catalyst. *ACS Sustain. Chem. Eng.* **2014,** *2* (12), 2802-2812.

149. Yu, J.; Shen, T.; Lin, Y.; Zhou, Y.; Song, Q., Rapid and efficient synthesis of 3,3-di(1*H*-indol-3-yl)indolin-2-ones and 2,2-di(1*H*-indol-3-yl)-2*H*-acenaphthen-1-ones catalyzed by p-TSA. *Synth. Commun.* **2014,** *44* (14), 2029-2036.

150. Xiang, J.; Wang, J.; Wang, M.; Meng, X.; Wu, A., One-pot total synthesis of streptindole, arsindoline B and their congeners through tandem decarboxylative deaminative dual-coupling reaction of amino acids with indoles. *Org. Biomol. Chem.* **2015,** *13* (14), 4240-4247.

151. Jiang, C.; Li, J.; Lü, G.; Zheng, Y.; Yu, X.; Lü, S.; Hai, L.; Wu, Y., Potassium *tert*-butanolate promoted reaction of benzaldehydes and indoles: a new strategy for synthesis of bis(indolyl)arylmethanes. *Chem. Res. Chin. Univ.* **2017,** *33* (2), 200-205.

152. Deb, M. L.; Deka, B.; Saikia, P. J.; Baruah, P. K., Base-promoted three-component cascade approach to unsymmetrical bis(indolyl)methanes. *Tetrahedron Lett.* **2017,** *58* (20), 1999-2003.

153. Nguyen, N.-K.; Ha, M.-T.; Bui, H. Y.; Trinh, Q. T.; Tran, B. N.; Nguyen, V. T.; Hung, T. Q.; Dang, T. T.; Vu, X. H., Magnetically recyclable $CuFe_2O_4$ catalyst for efficient synthesis of bis(indolyl)methanes using indoles and alcohols under mild condition. *Catal. Commun.* **2021,** *149*.

154. Sekar, G.; Badigenchala, S.; Ganapathy, D.; Das, A.; Singh, R., Iron(II) chloride–1,1′-binaphthyl-2,2′-diamine ($FeCl_2$–BINAM) complex catalyzed domino synthesis of bisindolylmethanes from indoles and primary alcohols. *Synthesis* **2013,** *46* (01), 101-109.

155. Qiang, W.; Liu, X.; Loh, T.-P., Supported iridium catalyst for the green synthesis of 3,3′-bis(indolyl)methanes using methanol as the bridging methylene source. *ACS Sustain. Chem. Eng.* **2019,** *7* (9), 8429-8439.

156. Mondal, A.; Sharma, R.; Dutta, B.; Pal, D.; Srimani, D., Well-defined *NNS*-Mn complex catalyzed selective synthesis of C-3 alkylated indoles and bisindolylmethanes using alcohols. *J. Org. Chem.* **2022,** *87* (6), 3989-4000.

157. Zhang, S.; Fan, W.; Qu, H.; Xiao, C.; Wang, N.; Shu, L.; Hu, Q.; Liu, L., One-pot synthesis of bis(indolyl)methanes: ruthenium-catalyzed reaction of benzylic alcoholes and indoles. *Curr. Org. Chem.* **2012,** *16* (7), 942-948.

158. Putra, A. E.; Takigawa, K.; Tanaka, H.; Ito, Y.; Oe, Y.; Ohta, T., Transition-metal-catalyzed regioselective alkylation of indoles with alcohols. *Eur. J. Org. Chem.* **2013,** *2013* (28), 6344-6354.

159. Biswas, N.; Sharma, R.; Srimani, D., Ruthenium pincer complex catalyzed celective synthesis of C-3 alkylated indoles and bisindolylmethanes directly from indoles and alcohols. *Adv. Synth. Catal.* **2020,** *362* (14), 2902-2910.

160. Nikoofar, K.; Ghanbari, K., A domino electro-oxidative synthesis of 3,3′-bis(indolyl)methane nanoparticles. *Monatsh. Chem.* **2015,** *146* (12), 2021-2027.

161. Konwar, D.; Bora, U., Free radical triggered convenient synthesis of bis(indolyl)methane with potassium peroxodisulfate as catalyst. *ChemistrySelect* **2020,** *5* (25), 7460-7466.

162. Yang, T.; Lu, H.; Shu, Y.; Ou, Y.; Hong, L.; Au, C.-T.; Qiu, R., $CF_3SO_2Na$-Mediated, UV-light-induced Friedel–Crafts alkylation of indoles with ketones/aldehydes and bioactivities of products. *Org. Lett.* **2020,** *22* (3), 827-831.

163. Ling, Y.; An, D.; Zhou, Y.; Rao, W., $Ga(OTf)_3$-Catalyzed temperature-controlled regioselective Friedel–Crafts alkylation of trifluoromethylated 3-indolylmethanols with 2-substituted indoles: divergent synthesis of trifluoromethylated unsymmetrical 3,3′-and 3,6′-bis(indolyl)methanes. *Org. Lett.* **2019,** *21* (9), 3396-3401.

164. Li, Z.; Shi, Z.; He, C., Addition of heterocycles to electron deficient olefins and alkynes catalyzed by gold(III). *J. Organomet. Chem.* **2005,** *690* (23), 5049-5054.

165. Ferrer, C.; Amijs, C. H. M.; Echavarren, A. M., Intra- and intermolecular reactions of indoles with alkynes catalyzed by gold. *Chem. Eur. J.* **2007,** *13* (5), 1358-1373.

166. Bhuvaneswari, S.; Jeganmohan, M.; Cheng, C.-H., Platinum-catalyzed multistep reactions of indoles with alkynyl alcohols. *Chem. Eur. J.* **2007,** *13* (29), 8285-8293.

167. Gao, R.; Yi, C. S., Regioselective formation of α-vinylpyrroles from the ruthenium-catalyzed coupling reaction of pyrroles and terminal alkynes involving C−H bond activation. *J. Org. Chem.* **2010,** *75* (9), 3144-3146.

168. Xia, D.; Wang, Y.; Du, Z.; Zheng, Q.-Y.; Wang, C., Rhenium-catalyzed regiodivergent addition of indoles to terminal alkynes. *Org. Lett.* **2012,** *14* (2), 588-591.

169. Vicenzi, D.; Sgarbossa, P.; Biffis, A.; Tubaro, C.; Basato, M.; Michelin, R. A.; Lanza, A.; Nestola, F.; Bogialli, S.; Pastore, P.; Venzo, A., Platinum(II) Complexes with Novel Diisocyanide Ligands: Catalysts in Alkyne Hydroarylation. *Organometallics* **2013,** *32* (23), 7153-7162.

170. Srivastava, A.; Patel, S. S.; Chandna, N.; Jain, N., Copper-catalyzed anti-Markovnikov hydroindolation of terminal alkynes: regioselective synthesis of bis(indolyl)alkanes. *J. Org. Chem.* **2016,** *81* (23), 11664-11670.

171. McLean, E. B.; Cutolo, F. M.; Cassidy, O. J.; Burns, D. J.; Lee, A.-L., Selectivity control in gold-catalyzed hydroarylation of alkynes with indoles: application to unsymmetrical bis(indolyl)methanes. *Org. Lett.* **2020,** *22* (17), 6977-6981.

172. Zeng, M.; Xue, J.-W.; Jiang, H.; Li, K.; Chen, Y.; Chen, Z.; Yin, G., Decarboxylative addition of propiolic acids with indoles to synthesize bis(indolyl)methane derivatives with a Pd(II)/LA catalyst. *J. Org. Chem.* **2021,** *86* (12), 8333-8350.

173. Ling, F.; Xiao, L.; Fang, L.; Feng, C.; Xie, Z.; Lv, Y.; Zhong, W., $B(C_6F_5)_3$-catalyzed Markovnikov addition of indoles to aryl alkynes: an approach toward bis(indolyl)alkanes. *Org. Biomol. Chem.* **2018,** *16* (47), 9274-9278.

174. Maiti, G.; Kayal, U.; Karmakar, R.; Bhattacharya, R. N., An efficient one pot conversion of alkynes to bis(indolyl) and bis(pyrrolyl)alkanes in aqueous ethanol. *Indian J. Chem.* **2013,** *52B*, 122-128.

175. Lu, W.; Jia, C.; Kitamura, T.; Fujiwara, Y., Pd-Catalyzed selective addition of heteroaromatic C−H bonds to C−C triple bonds under mild conditions. *Org. Lett.* **2000,** *2* (19), 2927-2930.

176. Jari, T.; Amrollahi, M. A.; Kheilkordi, Z., ZrOCl2·SiO2-catalyzed synthesis of bis(indoles) via conjugate addition of indole with electron-deficient alkenes in water. *Heterocycl. Comm.* **2015,** *21* (2), 89–92.

177. Jia, K.-Y.; Yu, J.-B.; Jiang, Z.-J.; Su, W.-K., Mechanochemically activated oxidative coupling of indoles with acrylates through C–H activation: synthesis of 3-vinylindoles and β,β-diindolyl propionates and study of the mechanism. *J. Org. Chem.* **2016,** *81* (14), 6049-6055.

178. Zhang, S.; Chen, Z.; Qin, S.; Lou, C.; Senan, A. M.; Liao, R.-Z.; Yin, G., Non-redox metal ion promoted oxidative coupling of indoles with olefins by the palladium(ii) acetate catalyst through dioxygen activation: experimental results with DFT calculations. *Org. Biomol. Chem.* **2016,** *14* (17), 4146-4157.

179. Ma, S.; Yu, S., $Sc(OTf)_3$-Catalyzed indolylation of 1,2-allenic ketones: controlled highly selective synthesis of β-indolyl-α,β-unsaturetated (*E*)-enones and β,β-bisindolyl ketones. *Org. Lett.* **2005,** *7* (22), 5063-5065.

180. Paz Muñoz, M.; de la Torre, M. C.; Sierra, M. A., New platinum-catalysed dihydroalkoxylation of allenes. *Adv. Synth. Catal.* **2010,** *352* (13), 2189-2194.

181. Muñoz, M. P.; de la Torre, M. C.; Sierra, M. A., Platinum-catalysed bisindolylation of allenes: A complementary alternative to gold catalysis. *Chem. Eur. J.* **2012,** *18* (15), 4499-4504.

182. Gopalaiah, K.; Chandrudu, S.; Devi, A., Iron-catalyzed oxidative coupling of benzylamines and indoles: Novel approach for synthesis of bis(indolyl)methanes. *Synthesis* **2015,** *47* (12), 1766-1774.

183. Liao, M.; Zhang, X.; Yue, P., TEMPO/CuI synergetic catalyzed oxidative cross-coupling of indoles with benzylamines: Synthesis of bis(indolyl)phenylmethanes. *Synth. Commun.* **2018,** *48* (13), 1694-1700.

184. Saini, P.; Kumari, P.; Hazra, S.; Elias, A. J., Oxidative coupling of benzylamines with indoles in aqueous medium to realize bis-(indolyl)methanes using a water-soluble cobalt catalyst and air as the oxidant. *Chem. Asian J.* **2019,** *14* (23), 4154-4159.

185. Kadu, V. D.; Gund, M. S.; Godage, A. S., A sustainable iodine catalyzed C( sp3 )-H bond functionalization of benzylamines towards synthesis of bis(indolyl)methanes and tris(indolyl)methanes. *ChemistrySelect* **2021,** *6* (43), 11954-11960.

186. Kadu, V. D.; Chandrudu, S. N.; Hublikar, M. G.; Raut, D. G.; Bhosale, R. B., Metal-free oxidative coupling of arylmethylamines with indoles: a simple, environmentally benign approach for the synthesis of 3,3′-bis(indolyl)methanes. *RSC Adv.* **2020,** *10* (39), 23254-23262.

187. Chen, C. C.; Hong, B. C.; Li, W. S.; Chang, T. T.; Lee, G. H., Synthesis of biologically active bis(Indolyl)methane derivatives by bisindole alkylation of tetrahydroisoquinolines with visible-light induced ring-opening fragmentation. *Asian J. Org. Chem.* **2016,** *6* (4), 426-431.

188. Lei, L. S.; Wang, B. W.; Jin, D. P.; Gao, Z. P.; Huan-Liang; Wang, S. H.; Xu, X. T.; Zhang, K.; Zhang, X. Y., $Al(OTf)_3$ -Catalyzed tandem coupling reaction between *N*,*N*-disubstituted aminomalonitriles and substituted arenes: a synthesis of 1-cyano-bisindolylmethane analogues. *Adv. Synth. Catal.* **2020,** *362* (14), 2870-2875.

189. Guo, X.; Pan, S.; Liu, J.; Li, Z., One-pot synthesis of symmetric and unsymmetric 1,1-bis-indolylmethanes via tandem iron-catalyzed C−H bond oxidation and C−O bond cleavage. *J. Org. Chem.* **2009,** *74* (22), 8848-8851.

190. Ye, L.; Cai, S.-H.; Wang, D.-X.; Wang, Y.-Q.; Lai, L.-J.; Feng, C.; Loh, T.-P., Photoredox catalysis induced bisindolylation of ethers/alcohols via sequential C–H and C–O bond cleavage. *Org. Lett.* **2017,** *19* (22), 6164-6167.

191. Du, K.-S.; Huang, J.-M., Electrochemical synthesis of bisindolylmethanes from indoles and ethers. *Org. Lett.* **2018,** *20* (10), 2911-2915.

192. Zhuo, M.-H.; Jiang, Y.-J.; Fan, Y.-S.; Gao, Y.; Liu, S.; Zhang, S., Enantioselective synthesis of triarylmethanes by chiral imidodiphosphoric acids catalyzed friedel–crafts reactions. *Org. Lett.* **2014,** *16* (4), 1096-1099.

193. Zhuo, M.-H.; Liu, G.-F.; Song, S.-L.; An, D.; Gao, J.; Zheng, L.; Zhang, S., Chiral imidodiphosphoric acids-catalyzed Friedel-Crafts reactions of indoles/pyrroles with 3-hydroxy-indolyloxindoles: Enantioselective synthesis of 3,3-diaryloxindoles. *Adv. Synth. Catal.* **2016,** *358* (5), 808-815.

194. Sun, F.-L.; Zheng, X.-J.; Gu, Q.; He, Q.-L.; You, S.-L., Enantioselective synthesis of unsymmetrical triarylmethanes by chiral brønsted acids. *Eur. J. Org. Chem.* **2010,** *2010* (1), 47-50.

195. Kim, Y.; Lee, J.; Jung, J.; Kim, S.-G., Chiral Brønsted acid-catalyzed Friedel–Crafts reaction of 3-indolylsulfamidates with indoles: Synthesis of enantioenriched bisindolylmethane sulfamates. *Tetrahedron Lett.* **2019,** *60* (25), 1625-1630.

196. Chatterjee, R.; Santra, S.; Zyryanov, G. V.; Majee, A., Brønsted acidic ionic liquid–catalyzed tandem trimerization of indoles: An efficient approach towards the synthesis of indole 3,3′-trimers under solvent-free conditions. *J. Heterocycl. Chem.* **2020,** *57* (4), 1863-1874.

197. Lucarini, S.; Mari, M.; Piersanti, G.; Spadoni, G., Organocatalyzed coupling of indoles with dehydroalanine esters: synthesis of bis(indolyl)propanoates and indolacrylates. *RSC Adv.* **2013,** *3* (41), 19135-19144.

198. Veisi, H.; Ataee, M.; Darabi-Tabar, P.; Amiri, E.; Faraji, A. R., One-pot conversion of aromatic compounds to the corresponding bis(indolyl)methanes by the Vilsmeier–Haack reaction. *C. R. Chim.* **2014,** *17* (4), 305-309.

199. Singh, R. R.; Liu, R.-S., Gold-catalyzed oxidative couplings of two indoles with one aryldiazo cyanide under oxidant-free conditions. *Chem. Commun.* **2017,** *53* (33), 4593-4596.

# Chapter 2

## Hetero-Diels-Alder Approach to Bis(indolyl)methanes


## Abstract

In this chapter, a novel synthetic approach to bis(indolyl)methanes is presented. The one-pot synthetic strategy based on two consecutive hetero-Diels-Alder cycloaddition reactions of electrophilic conjugated nitrosoalkenes with indoles was extended to a range of new 1-hydroxyiminomethyl-bis(indolyl)methanes. Furthermore, a similar and broad range approach was applied to the synthesis of previously unknown 1-hydrazonomethyl-bis(indolyl)methanes. The biological evaluation of the new bis(indolyl)methanes as anti-cancer agents was investigated.

# Chapter 2. Hetero-Diels-Alder Approach to Bis(indolyl)methanes

# 2.1 Introduction

### 2.1.1 Diels-Alder reactions

The Diels−Alder (DA) reaction, a versatile method to achieve structural diversity, is recognized as one of the most powerful reactions in modern organic chemistry for the preparation of carbo- and heterocyclic compounds. Discovered by Otto Diels and Kurt Alder in 1928, DA reaction has been intensively explored and become an important pillar of organic synthetic methodologies, being frequently used as key step for the construction of complex biologically active molecules including natural products.[1-3]

The DA reaction is generally described as a concerted process, although the cycloaddition between asymmetrically substituted dienes or heterodienes and/or asymmetrically substituted dienophiles takes place through highly asymmetric transition state structures occurring via an asynchronous mechanism. In extreme cases where the substitution of the diene and/or dienophile allows the stabilization of eventual biradical or zwitterionic intermediates the reaction becomes stepwise, with the formation of the first σ-bond between the most electrophilic centre of one reagent with the nucleophilic centre of its counterpart as the initial step followed by the ring closure, a mechanistic pathway supported by DFT calculations.[4-6]

In general, DA reactions can be classified into two types of suprafacial [π4s + π2s] cycloadditions: (a) the *normal*- and (b) *inverse*-electron-demand DA reactions (Figure 2.2), according to the relative energies of the frontier molecular orbitals (FMOs) of the diene and the dienophile in the Huckel molecular orbital model. While in the first case electron-donating substituents on the diene and electron-withdrawing substituents on the dienophile favour the reactivity, inverse-electron-demand DA reactions are favoured with dienes bearing electron-withdrawing substituents and dienophiles with electron-donating substituents. Based on the Woodward−Hoffmann rules, both types of DA reactions are thermally allowed. The FMO theory predicts that the normal [π4s + π2s] cycloaddition could be controlled by a $HOMO_{diene}$−$LUMO_{dienophile}$ interaction between electron-rich dienes and electron-deficient dienophiles, while the *inverse*-electron-demand DA reaction is dominated by the $LUMO_{diene}$ -$HOMO_{dienophile}$ interaction (Figure 2.1).[1, 2]

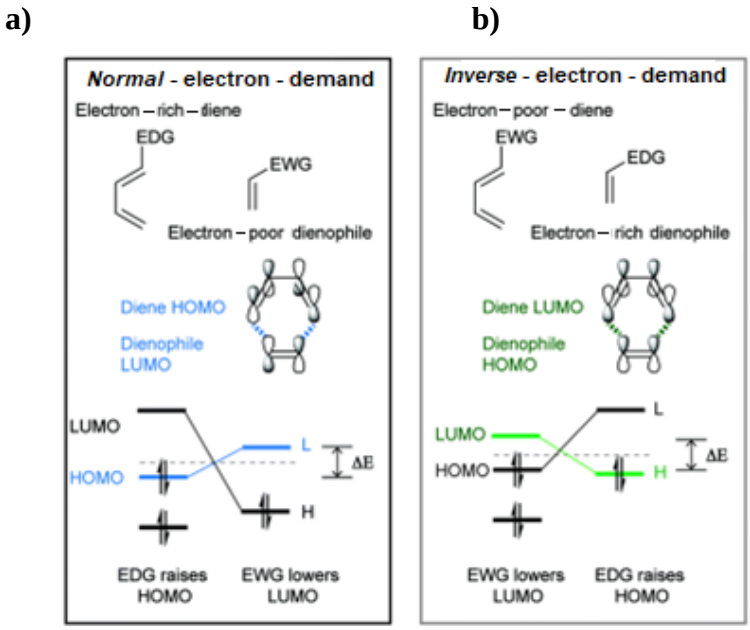
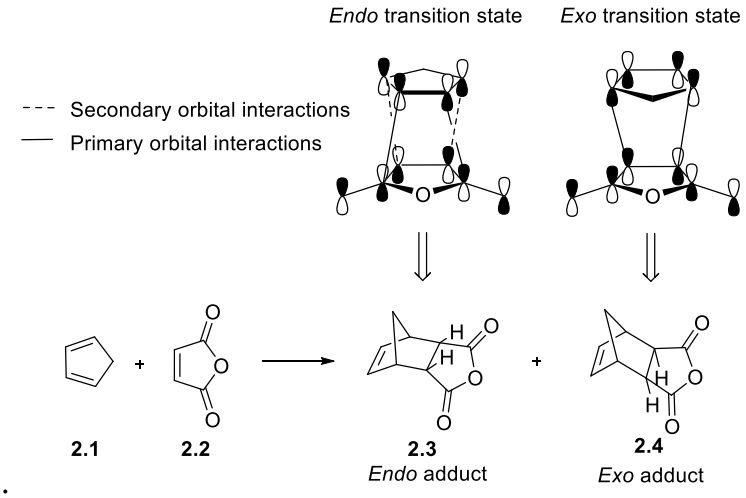



**Figure 2.1** Classification of the main types of Diels-Alder reactions.[7]

In general, both normal- and inverse-electron-demand DA reactions, are characterized by *endo* selectivity.[8, 9] A classic example is the Diels-Alder reaction between cyclopentadiene (**2.1**) and maleic anhydride (**2.2**), described by Arrieta and Cossio (Scheme 2.1).[8] The diene and dienophile approach each other in parallel planes and the most stable transition state is the one corresponding to the maximum orbital overlap. The *endo* product is less stable than the *exo* product yet it is the main product of the reaction, which can be explained by favourable secondary orbital interactions lowering the energy of the transition state. Thus, as in most Diels-Alder reactions, this cycloaddition is kinetically controlled.




**Scheme 2.1** DA reaction between cyclopentadiene (**2.1**) and maleic anhydride (**2.2**).

The DA reaction can be classified as homo-Diels-Alder when involving only all carbon dienes/dienophiles and hetero-Diels-Alder (HDA) when heteroatoms are present either on the 4 π-component (ex. nitroso- and azoalkenes) or 2 π-component.

Inverse-electron-demand HDA reactions of nitroso- and azoalkenes (heterodienes) is a synthetic strategy that allows the preparation of a wide range of adducts and cycloadducts with wide variety of substituents that, in addition to being highly important structures, are also very useful as intermediates in the synthesis of a great variety of cyclic and acyclic systems.

### 2.1.2 Nitroso- and azoalkenes

Over the past decades, conjugated nitroso- and azoalkenes emerged as powerful synthetic tools, particularly when carrying electron-withdrawing substituents, being used as intermediates, or building blocks for the synthesis of a plethora of new heterocycles systems with chemical and biological interest.[10-19]

The general structure of nitroso- and azoalkenes is represented in Figure 2.2.

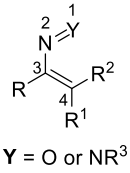
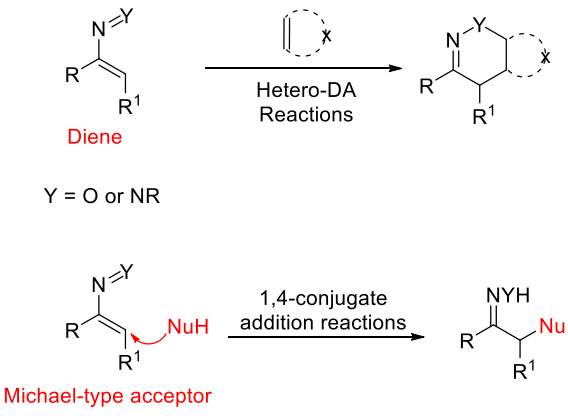


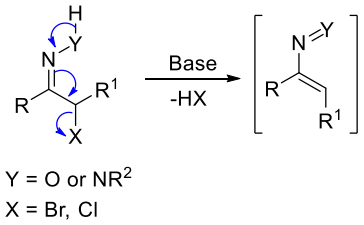

**Figure 2.2** Schematic representation of nitroso- or azoalkenes intermediates**.**

A common feature of nitroso- and azoalkenes is the highly electrophilic centre at C4, which allows to explore its reactivity either as electron-deficient heterodienes in HDA reactions, with an enormous variety of electron-rich alkenes and heterocycles, or as Michael-type acceptors in conjugate 1,4-addition reactions (Scheme 2.2).[10, 11, 13, 14, 17, 20-27]




**Scheme 2.2** Typical reactivity of azo- and nitrosoalkenes.

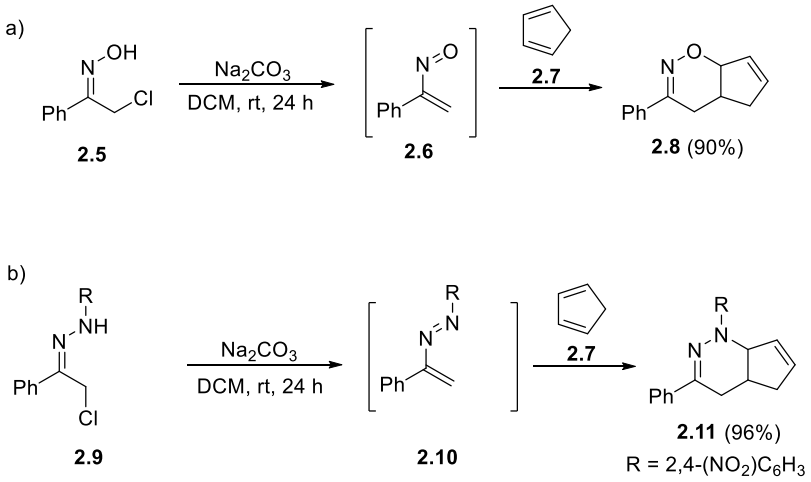
Generally, nitroso or azoalkenes are generated *in situ* due to their unstable characteristics. However, some substituted nitroso or azoalkene derivatives are very often stable enough to be isolated or even purified by column chromatography. The main method for their generation is the base-mediated dehydrohalogenation of α-halooximes or α-halohydrazones (Scheme 2.3).[11-13, 20, 21, 28]




**Scheme 2.3** Main method for the azo- and nitrosoalkenes generation.

The [4+2] cycloaddition reaction with alkenes is probably the most important reaction of nitroso- and azoalkenes. In many cases, nitroso and azoalkenes participate in this type of reactions as conjugated heterodienes originating 5,6-dihydro-4*H*-1,2-oxazine or 1,4,5,6-tetrahydropyridazine derivatives, respectively. Besides their structural interest, these products are also valuable intermediates in the synthesis of various functionalised cyclic and acyclic compounds.[10]

In the pioneering work of Gilchrist's group, in 1979, it has been shown that nitrosoalkene **2.6**, generated *in situ* from the corresponding α-halooxime **2.5**, reacts with cyclopentadiene (**2.7**) giving rise to 1,2-oxazine derivative **2.8** in excellent yield (Scheme 2.4 a). A similar reactivity was observed when the study was extended to azoalkene **2.10** that, after being generated *in situ* from the α-halohydrazone **2.9**, afforded pyridazine derivative **2.8** also in excellent yield (Scheme 2.4 b).[29]




**Scheme 2.4** Pioneering work of Gilchrist´s group.

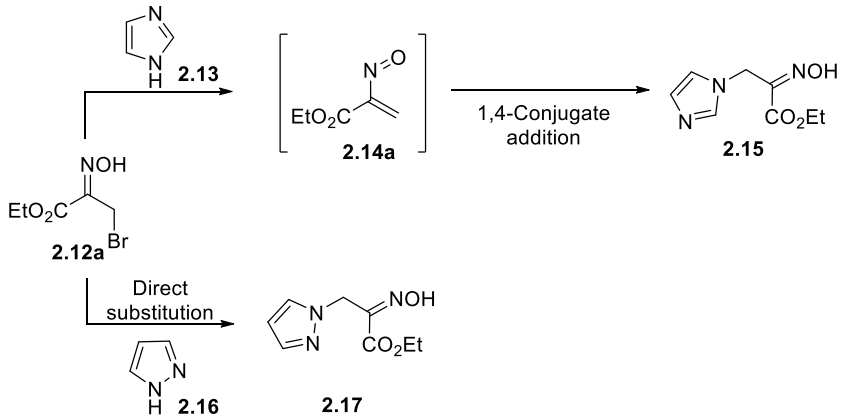
Conjugated nitroso and azoalkenes, unsubstituted or bearing either C- or P-bonded functional groups at C4 position, have been widely explored for the construction of a vast range of new heterocycles systems.[12, 17, 21, 23, 27, 30] In particular, the study of reactions between these reactive intermediates with heterocycles as dienophiles has been subject of interest.[17]

### 2.1.3 Hetero-Diels-Alder versus 1,4-conjugate addition reaction

DA reactions are characterized by asynchronous bond formation as explained above. An example of this feature is the well-established reactions between conjugated nitrosoalkenes, generated *in situ* by base-mediated dehydrobromination of α-bromooximes, with electron rich olefins which have been explored as a general route to 1,2-oxazine cycloadducts.[10-12, 17]

However, reaction of nucleophiles with α-halooximes may involve two different mechanism pathways namely the generation of nitrosoalkenes followed by 1,4-conjugate addition or the direct substitution of the halogen, as illustrated by Gilchrist *et al*. in the reaction between ethyl bromopyruvate oxime and imidazole. This study has shown that oxime **2.12a** reacts with imidazole **2.13** faster than with the corresponding *O*-alkylated oxime, for which dehydrobromination is blocked. This result indicates that the reaction with imidazole follows an elimination-addition mechanism, while the *O*-alkylated oxime undergoes direct substitution. Moreover, imidazole proved to be a strong enough base to eliminate HBr from the α-bromooxime **2.12a**, generating the corresponding heterodiene

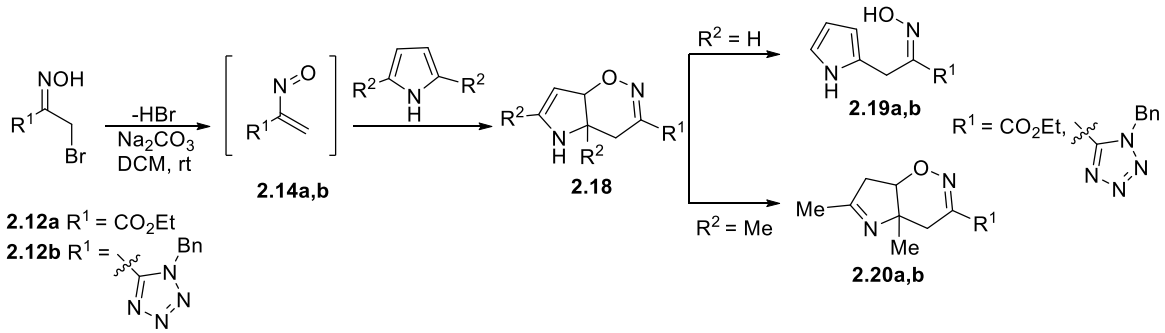
**2.14.** Furthermore, a direct halogen displacement was observed when basic azoles (e.g. pyrazole **2.16**) were used (Scheme 2.5).[31]




**Scheme 2.5** Reactions between ethyl bromopyruvate with azoles.

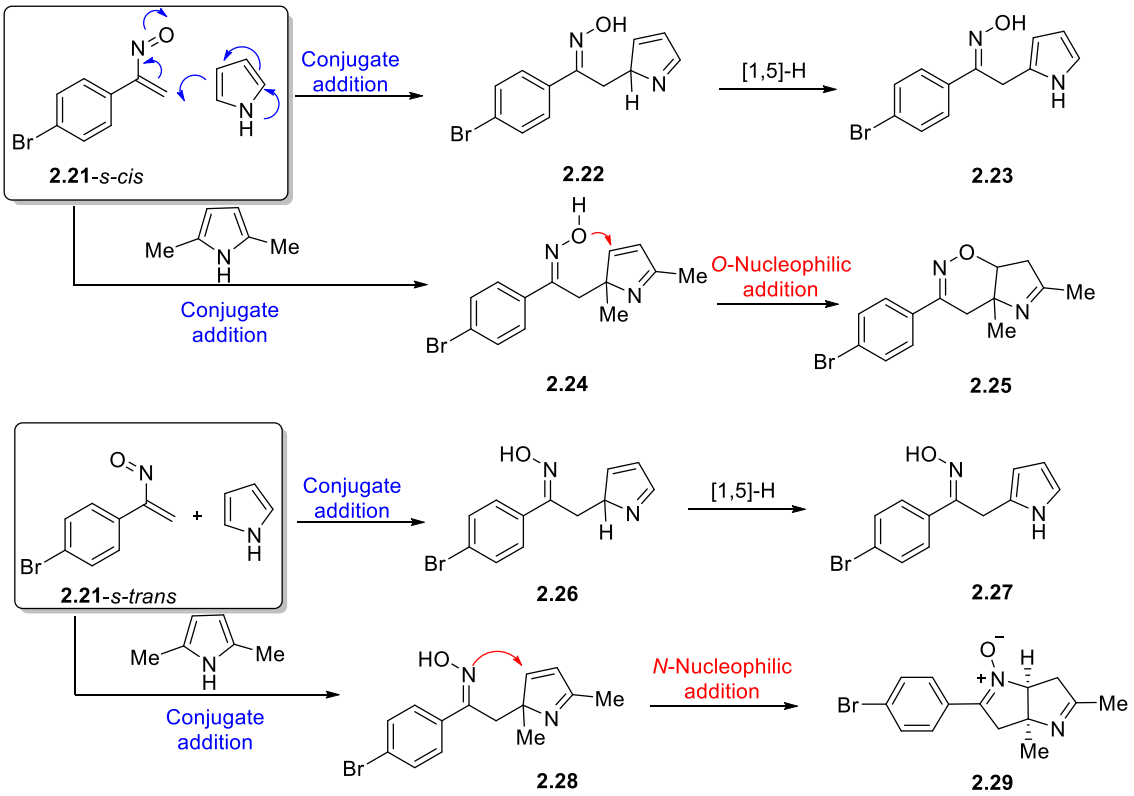
However, some of the most pertinent questions are: which mechanism pathway takes place? The reaction follows a HDA cycloaddition or a 1,4-conjugate addition pathway? Although most studies indicate that DA reactions are involved, this has always been subject of discussion.

Nitrosoalkenes **2.14a**,**b** are known to react with electron-rich heterocycles such as pyrrole giving open chain oximes **2.19.** The formation of these products can be rationalised considering a rearomatisation of the initially formed DA cycloadduct, the bicyclic 1,2-oxazines **2.18.** Oximes **2.19a**,**b** were isolated as single stereoisomers as expected after a ring-opening reaction of bicyclic 1,2-oxazines. The reaction between nitrosoalkene **2.14a**,**b** and 2,5-dimethylpyrrole led to 5,6-dihydro-4*H*-1,2-oxazines **2.20a**,**b** (Scheme 2.6).[32-34]




**Scheme 2.6** HDA reactions between nitroalkenes **2.14a**,**b** with pyrroles.

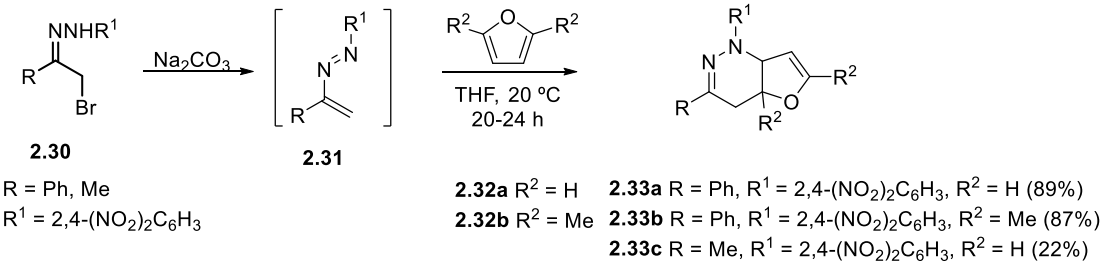
Recently, Pinho e Melo and co-workers[35] demonstrated that 1-(*p*-bromophenyl)nitrosoethylene **(2.21)** shows a different reactivity towards pyrrole since two isomeric oximes **2.23** and **2.27** were formed. Thus, this result cannot be explained by a single process involving HDA reaction but rather by a 1,4-conjugate addition reaction followed by a 1,5-sigmatropic hydrogen-shift as shown in Scheme 2.7. In the reaction of this nitrosoalkene with 2,5-dimethylpyrrole, after an initial conjugate addition, an intramolecular *O*- and *N*-nucleophilic addition occurs giving rise to the corresponding bicyclic oxazine **2.25** and five-membered cyclic nitrone **2.29**, respectively. Quantum chemical calculations allowed to rationalize these experimental results concluding that the DA reaction is favoured when starting from ethyl nitrosoacrylate, while 1-(*p*-bromophenyl)nitrosoethylene **(2.21)** an alternative mechanistic pathway, corroborated by quantum chemical calculations, at the DFT level, should be considered.[35]




**Scheme 2.7** Mechanism proposal for the reaction of 1-(*p*-bromophenyl)nitrosoethylene with pyrrole and 2,5-dimethylpyrrole.

The HDA cycloaddition reaction of azoalkenes and heterocycles as dienophiles was explored by Gilchrist *et al* as outlined in Scheme 2.8. The reaction between azoalkene **2.31**

and furan (**2.32a**) or 2,5-dimethyl furan (**2.32b**), led to the corresponding cycloadducts **2.33** in moderate to excellent yields (22%-89%).[29]




**2.30**
R = Ph, Me
$R^1$ = 2,4-$(NO_2)_2C_6H_3$

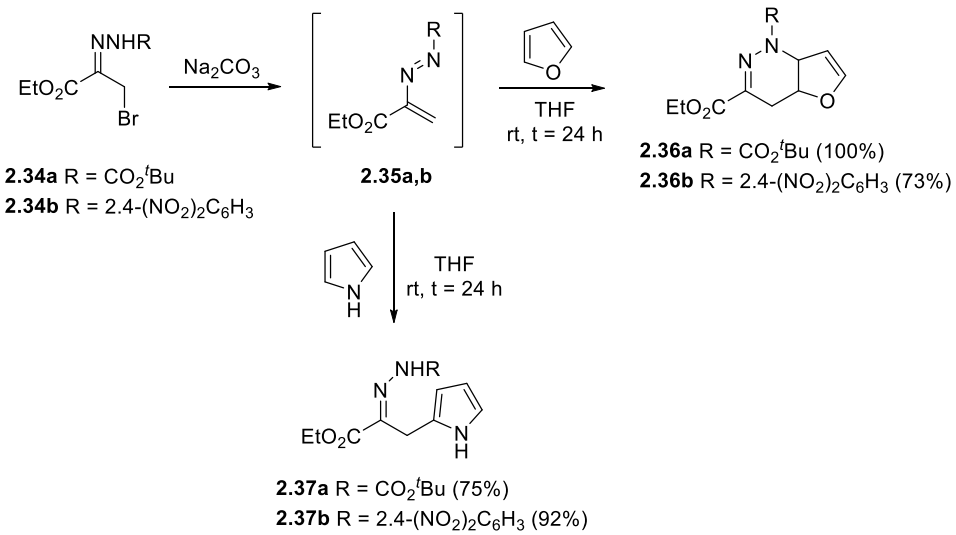
**2.31**

**2.32a** $R^2$ = H
**2.32b** $R^2$ = Me

**2.33a** R = Ph, $R^1$ = 2,4-$(NO_2)_2C_6H_3$, $R^2$ = H (89%)
**2.33b** R = Ph, $R^1$ = 2,4-$(NO_2)_2C_6H_3$, $R^2$ = Me (87%)
**2.33c** R = Me, $R^1$ = 2,4-$(NO_2)_2C_6H_3$, $R^2$ = H (22%)

**Scheme 2.8** HDA cycloaddition reaction of azoalkenes and furan or 2,5-dimethylfuran.

Clarke *et al* also described the synthesis of new tetrahydropyridazines **2.36** via hetero-Diels-Alder of azoalkenes **2.35** bearing an ethyl ester at the C-3.[36] The synthesis of these heterocycles **2.36** was carried out *in situ*, through dehydrohalogenation of α-halohydrazones in presence of a molecule of furan, in yields ranging from 73 to 100%. When heterodyne **2.35** reacted with pyrrole, open chain hydrazones **2.37** were obtained as a result of cycloadduct ring-opening reaction of the HDA cycloadducts with the concomitant pyrrole rearomatisation (Scheme 2.9).




**2.34a** R = $CO_2{}^t$Bu
**2.34b** R = 2.4-$(NO_2)_2C_6H_3$

**2.35a,b**

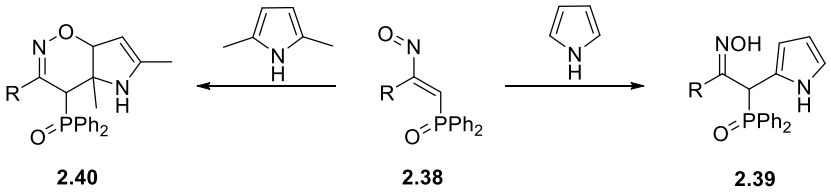
**2.36a** R = $CO_2{}^t$Bu (100%)
**2.36b** R = 2.4-$(NO_2)_2C_6H_3$ (73%)

**2.37a** R = $CO_2{}^t$Bu (75%)
**2.37b** R = 2.4-$(NO_2)_2C_6H_3$ (92%)

**Scheme 2.9** Synthesis of tetrahydropyridazines and open chain hydrazones via HDA reaction of azoalkenes with an ethyl ester at C-3.

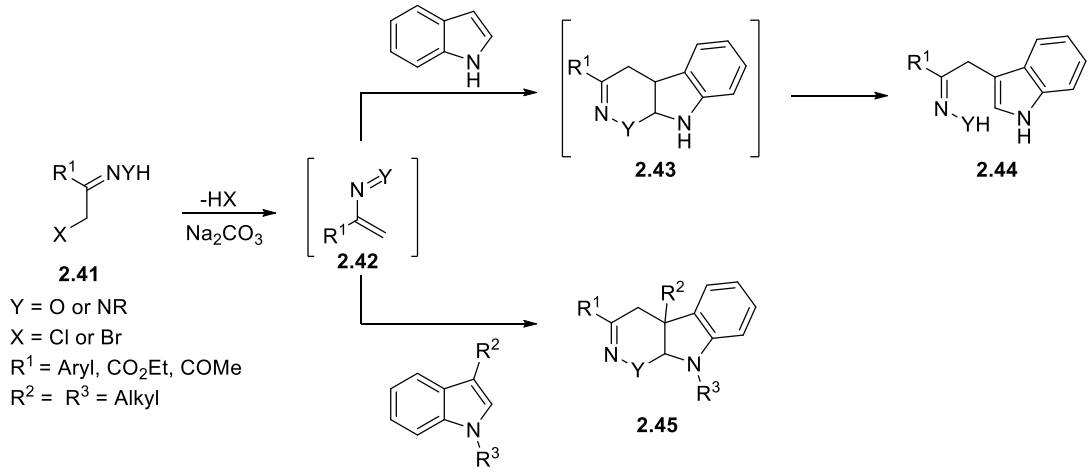
Palacios and co-workers[37] also reported a combined experimental and theoretical study regarding the reaction of electrophilic conjugate heterodienes such as phosphinyl

nitrosoalkenes **2.38** with electron-rich heterocycles namely pyrrole and 2,5-dimethylpyrrole. In this study, the quantum chemical calculations showed very good agreement with the experimental findings and the proposed mechanisms. In this case, the reaction mechanism seems to occur through a concerted asynchronous [4 + 2] cycloaddition process, which is kinetically favoured. Therefore, the nature of the nitrosoalkenes substituent at 3- and/or 4-positions has a strong influence on the reaction pathway and outcome of these transformations (Scheme 2.10).




**Scheme 2.10** HDA reaction of phosphinylated nitrosoalkenes with pyrrole and 2,5-dimethylpyrrole.

Further studies focused on reactions of 3-substituted nitroso- and azoalkenes **2.42** with indoles were also described. These heterodienes participate in HDA reaction with indole leading to (*E*)-oximes **2.44** as single products. These experimental results were also corroborated by quantum chemical calculations, at the DFT level of theory. In addition, reactions of these reactive intermediates with 3-alkylindoles gave rise to the expected hetero-Diels-Alder cycloadducts **2.45** (Scheme 2.11).[38-41]



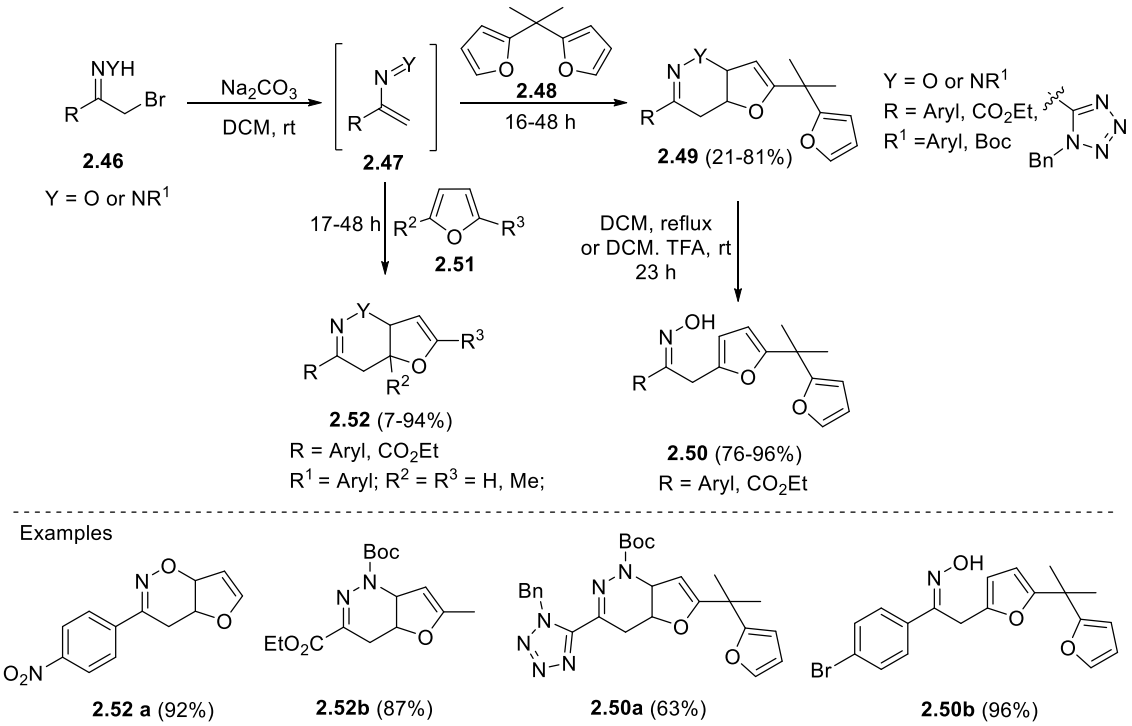

**Scheme 2.11** HDA reactions of 3-substituted nitroso- and azoalkenes with indoles.

### 2.1.4 Hetero-Diels-Alder (HDA) reactions of nitroso- and azoalkenes with heterocycles

In this section HDA reactions in which nitroso- and azoalkenes participate as 4π component and aromatic heterocycles as 2π component will be present and discuss. The most recent and relevant contributions on the subject will be presented.

#### 2.1.4.1 With furan

The reactivity between nitroso- or azoalkenes **2.47** and 2,2-bis(furan-2-yl)propane (**2.48**) leading to the corresponding 1,2-oxazine- or 4a,7a-dihydrofuro[3,2-*c*]-pyridazines **2.49** was described by Pinho e Melo *et al* .[17, 42, 43] The 1,2-oxazines were efficiently converted into the corresponding open-chain oximes **2.50** by treatment with TFA, through ring-opening rearrangement and concomitant rearomatisation of the furan ring. Reactions of nitroso and azoalkenes **2.47** with furan ($R^2 = R^3 = H$), 2-methylfuran ($R^2 = R^3 = Me$) and 2,5-dimethylfuran ($R^2 = R^3 = Me$) were also explored giving rise to the corresponding cycloadducts **2.52** in low to excellent yields (Scheme 2.12).

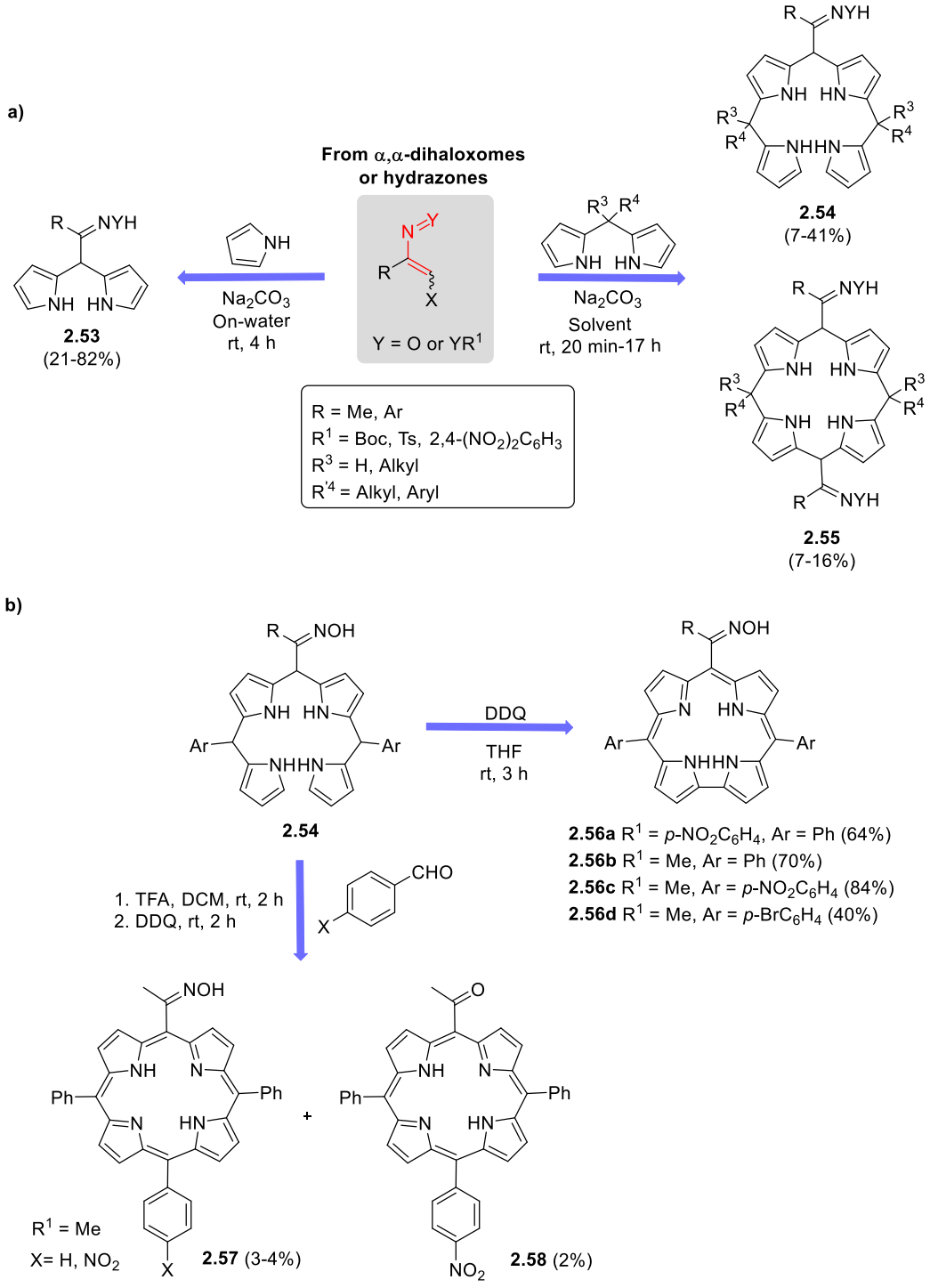



**Scheme 2.12** Reactivity of nitroso- and azoalkenes **2.47** towards 2,2-bis(furan-2-yl)propane, furan, 2-methylfuran and 2,5-dimethylfuran.

### 2.1.4.2 With pyrrole

An efficient methodology for the preparation of 5-substituted-dypirromethanes **2.53** based on two consecutive HDA reactions of azo- and nitrosoalkenes with pyrrole, was also stablished by Pinho e Melo research group (Scheme 2.13 a).[17, 44] The approach to these dipyrromethanes was carried out in dichloromethane (DCM), in absence of solvent or under on-water conditions. Surprisingly, the latter proved to be the most effective reaction media affording the target dipyrromethanes **2.53** in much shorter reaction times, higher efficiency, and with easier isolation and purification methods (Scheme 2.13 a).[44]

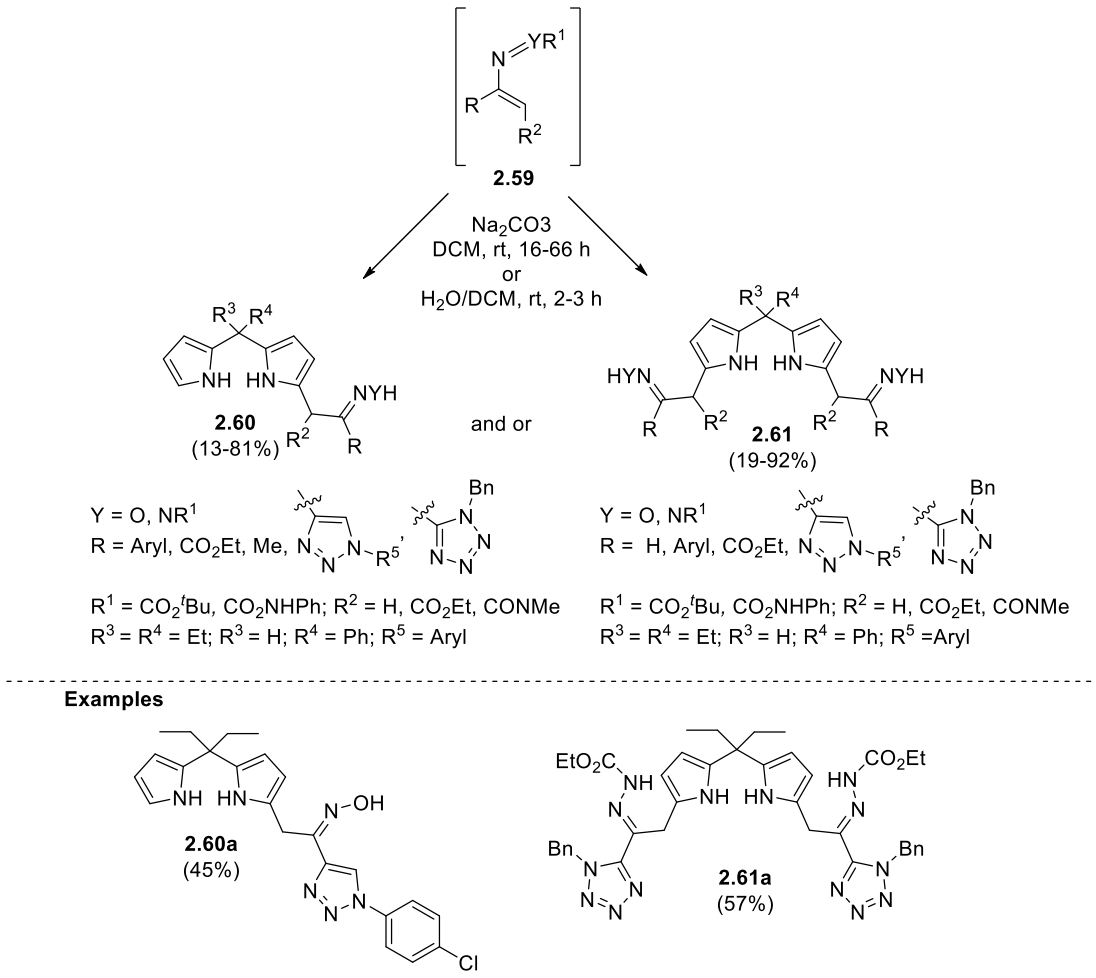
This broad and versatile methodology was successfully applied to the synthesis of bilanes **2.54** and calix[4]pyrroles **2.55**. While bilanes **2.54** were obtained from the reaction of monofunctionalised dipyrromethanes with another molecule of dipyrromethane, an initial bisfunctionalisation followed by a second DA reaction with dipyrromethane gave rise to calix[4]pyrroles **2.55** (Scheme 2.13 a). [19, 45] Furthermore, bilanes **2.54** ($R^1$ = Me) containing an oxime functionality, prepared by two consecutive hetero-Diels−Alder reactions (or conjugate additions) between nitrosoalkenes and dipyrromethane, led to corroles **2.56** or porphyrins **2.57** and **2.58** via oxidative macrocyclization, or treatment with aldehydes followed by oxidation, respectively (Scheme 2.13 b). [19]




**Scheme 2.13**a) HDA reactions of azo- and nitroso-alkenes with pyrroles. b) synthesis of corroles and porphyrins.

This synthetic strategy was also applied to the synthesis of mono- or bis-functionalised dipyrromethanes **2.60** and **2.61**, respectively, which depending on the starting oxime or hydrazone, were obtained in low to excellent yields.[17, 45, 46] These functionalised

dipyrromethanes were screened for their *in vitro* activity as anticancer agents against leukemia and lymphoma. In fact, **2.60a** showed high activity against Staphylococcus species including methicillin resistant strains. (Scheme 2.14).[15]

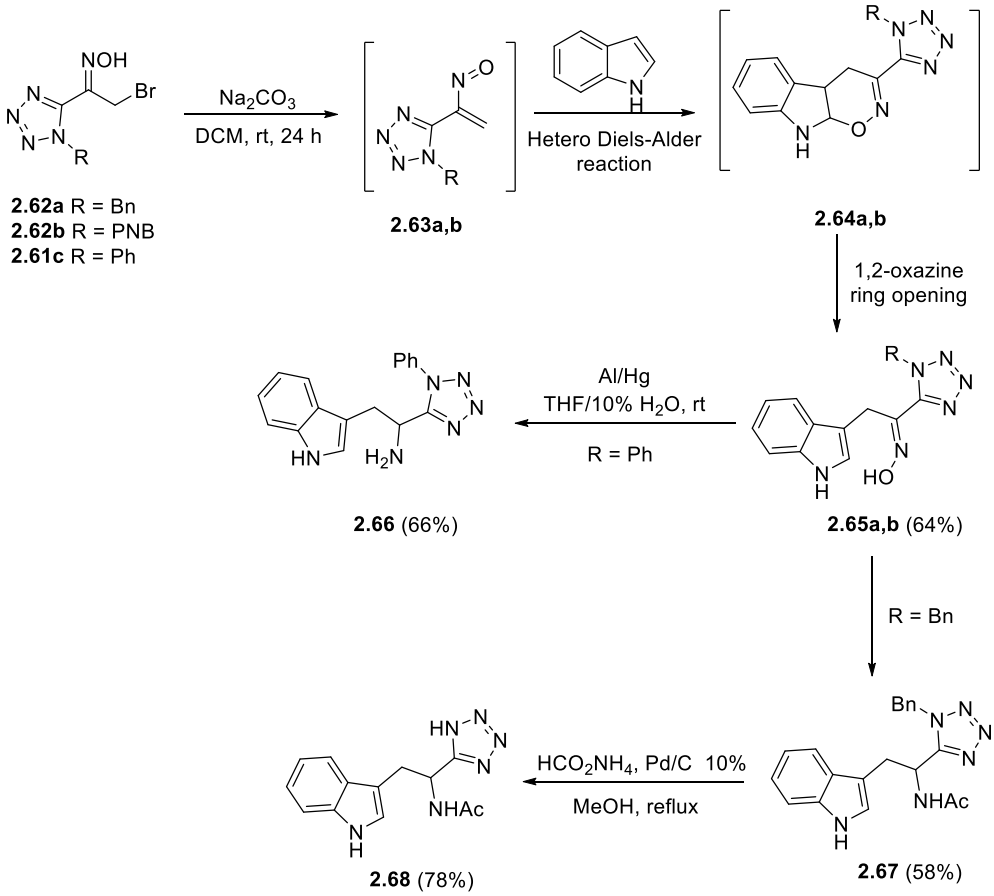



**Scheme 2.14** Functionalization of dipyrromethanes.

### 2.1.4.3 With indole

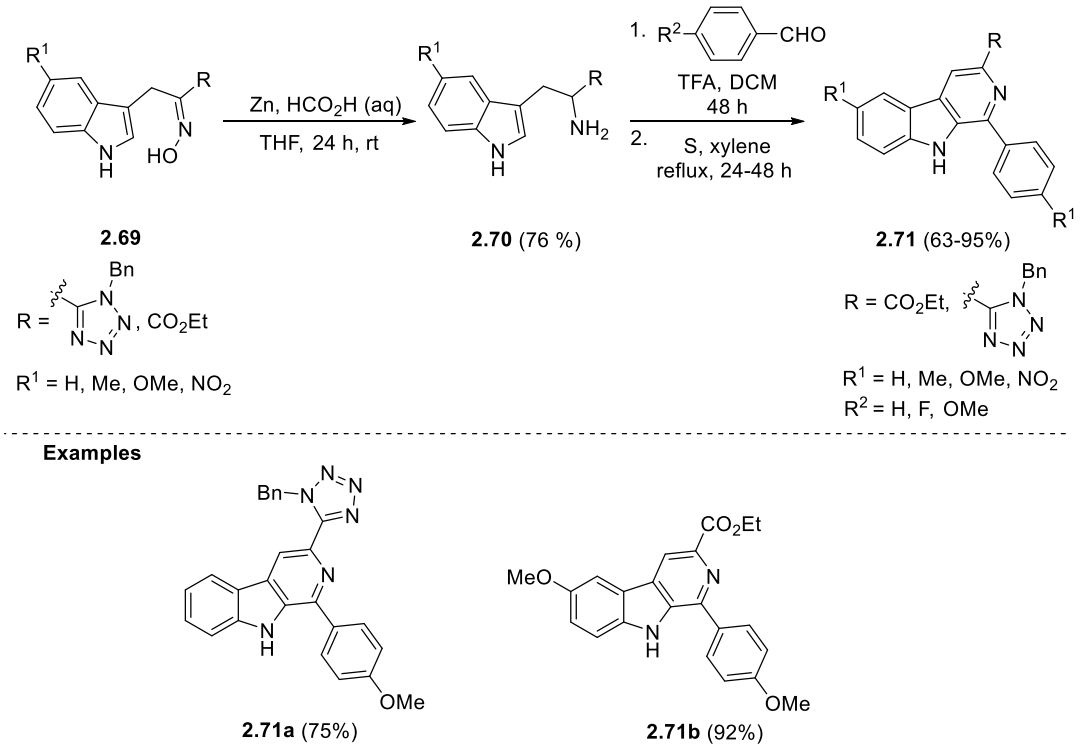
Reaction between nitroso-azoalkenes and indole was also explored by Pinho e Melo and co-workers.[18, 33, 34, 47] Examples of this kind of reactivity were the generation of novel 3-tetrazolyl-nitrosoalkenes and their reactivity towards indoles. The aim of this study was the synthesis of tryptophan analogues where the carboxylic group was replaced by the bioisosteric tetrazolyl group. The reaction of *in situ* generated 3-tetrazolyl-nitrosoalkenes **2.63** with indole gave the corresponding open-chain oximes **2.65**, resulting from the ring-opening and concomitant rearomatization of the indole unit of the initially formed hetero-Diels-Alder cycloadducts **2.64**. The reduction of the oxime moiety of functionalized

indoles **2.65** afforded the desired tryptophan analogues **2.66** and **2.67**. The deprotection of the tetrazolyl moiety of compound **2.67** was also carried out affording tetrazolyl-tryptamine **2.68** (Scheme 2.15). This type of building block is a particularly interesting synthetic tool to explore carboxylic acid/tetrazole bioisosterism in drug discovery.[18, 33, 34, 47]




**Scheme 2.15** Synthesis of Tryptophan analogues derived from nitrosovinyltetrazoles.

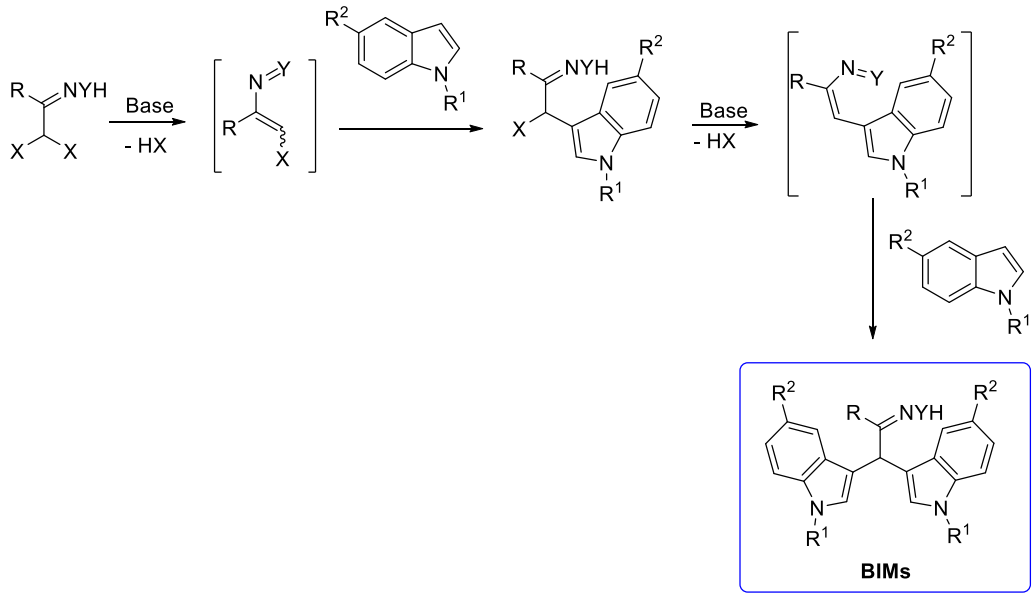
The synthesis of tryptophan analogues based on the chemistry of nitrosoalkenes and the use of these building blocks in the preparation of novel functionalized β-carbolines **2.71** was recently reported. The structural diversity that was achieved allowed the discovery of interesting activities against a range of cancer cell lines with the selectivity depending on the type of substitution pattern of the β-carboline core. For example, compound **2.71a** proved to be a very promising anticancer agent against breast adenocarcinoma (MCF-7), lung carcinoma (NCIeH460) and ovarian carcinoma (OVCAR-3) with GI50 between 1.32 and 1.62 μM (Scheme 2.16).[18]




**Scheme 2.16** Synthetic sequence to obtain 3-tetrazolyl-β-carbolines.

## 2.2 Rationale and goals

As part of our continuing interest and investigation on HDA reaction of electron-deficient conjugated nitroso and azoalkenes, we envisioned that a novel methodology to the synthesis of new bis(indolyl)methanes (BIMs) bearing oxime or hydrazone moieties could be developed based on the chemistry of these heterodienes. On the other hand, this study could lead to a library of new BIMs with eventual interesting biological activities. The aim of this PhD project was to establish a one-pot synthetic strategy to these BIMs based on two consecutive hetero-Diels-Alder cycloaddition reactions between nitroso- and azoalkenes, generated *in situ* from the corresponding α,α'-dihalooximes or hydrazones, and indoles as outlined in scheme 2.17.



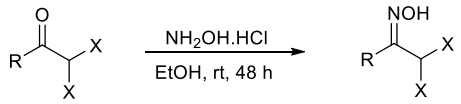

**Scheme 2.17** Synthetic approach to BIMs oximes or hydrazones.

## 2.3 Novel approach to bis(indolyl)methanes: synthesis of 1-hydroxyiminomethyl and 1-hydrazonomethyl-bis(indolyl)methanes derivatives with anti-cancer properties

### 2.3.1 Synthesis of α,α'-dihalooximes and α,α'-dihalohydrazones

This research work began with the synthesis of the precursors, namely the preparation of the α,α'-dihalooximes and α,α'-dihalohydrazones.

The α,α'-dihalooximes **2.73a-e** were prepared following known procedures.[44, 48] The experimental method consists in the condensation between the appropriate α,α'-haloketone **2.72** and hydroxylamine hydrochloride, as shown in Scheme 2.18.

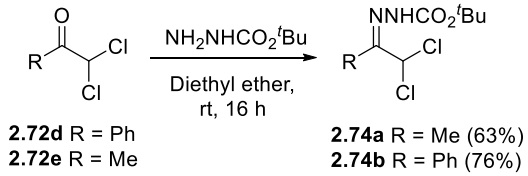



**2.72a** X = Br, R = *p*-$BrC_6H_4$
**2.72b** X = Br, R = *p*-$ClC_6H_4$
**2.72c** X = Br, R = *p*-$FC_6H_4$
**2.72d** X = Cl, R = Ph
**2.72e** X = Cl, R = Me

**2.73a** X = Br, R = *p*-$BrC_6H_4$ (72%)
**2.73b** X = Br, R = *p*-$ClC_6H_4$ (78%)
**2.73c** X = Br, R = *p*-$FC_6H_4$ (75%)
**2.73d** X = Cl, R = Ph (75%)
**2.73e** X = Cl, R = Me (55%)

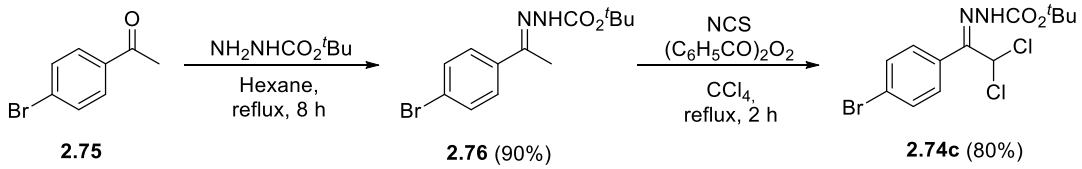
**Scheme 2.18** Synthesis of the α,α'-dihalooximes.

α,α'-Dihalohydrazones **2.74a** and **2.74b** were prepared by reacting 2,2-dichloro-1-phenylethan-1-one (**2.72d**) or 1,1-dichloropropan-2-one (**2.72e**), respectively, with *tert*-butyl hydrazinecarboxylate at room temperature, as outlined in Scheme 2.19.[49]



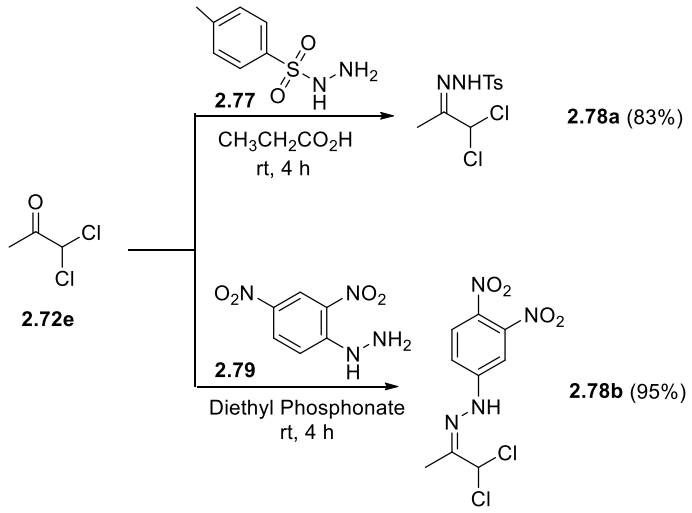

**Scheme 2.19** Synthesis of the α,α'-dihalohydrazones.

The α,α-dichlorohydrazone **2.74c** was prepared in two steps. Firstly, the hydrazone **2.76** was synthesised by the reaction of 4-bromoacetophenone (**2.75**) with *tert*-butyl hydrazinecarboxylate, in refluxing hexane.[50] The halogenation of **2.76** with *N*-chlorosuccinimide, in the presence of a catalytic amount of benzoyl peroxide, led to the target hydrazone **2.74c** (80%), as described by South *et al*.[51] (Scheme 2.20).




**Scheme 2.20** Synthesis of the α,α'-dihalohydrazone **2.74c**.

Additionally, hydrazones **2.78** were also prepared following established experimental procedures. Carrying out the reaction of 1,1-dichloropropan-2-one (**2.72e**) with *p*-toluenesulfonyl hydrazide **2.77** in propionic acid, at room temperature for 4 h, led to the desired tosylhydrazone **2.78a** in very good yield ( 83%).[52] The reaction of 1,1-dichloropropan-2-one (**2.72e**) with 2,4-dinitrofenylhydrazine (**2.79**) in diethyl phosphonate, afforded hydrazone **2.78b** in 95% yield (Scheme 2.21).[53]




**Scheme 2.21** Synthesis of the α,α'-dihalohydrazones **2.78a**,**b**.

### 2.3.2 Synthesis of 1-hydroxyiminomethyl bis(indolyl)methanes

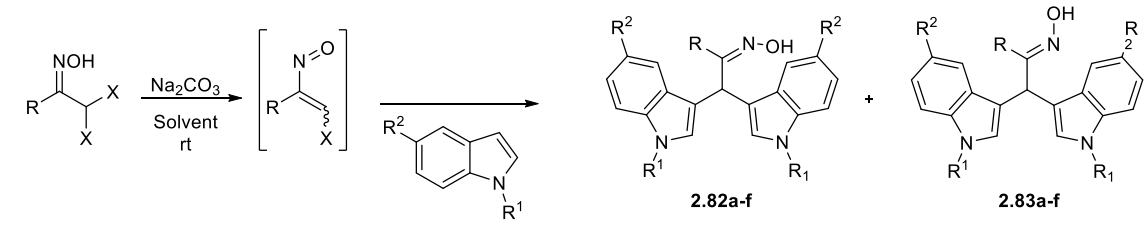
Initially, the reactivity between transient nitrosoalkenes, generated *in situ* from α,α'-dihalooximes **2.73a-f**, and indoles **2.81a**,**b** in presence of a base ($Na_2CO_3$), using dichloromethane (DCM) as solvent and carrying out the reaction at room temperature during 36 h was explored. New 1-hydroxyiminomethyl-bis(indolyl)methanes (BIMs) **2.82** and **2.83** were obtained as outlined in Table 2.1. In general, the reaction yields were considered excellent (28-80%) (Table 2.1, entries 1, 3, 5, 7, 9, 11), considering that the synthetic approach involves two consecutive DA cycloaddition reactions. The results also have shown that no other products were obtained, revealing the regioselectivity of these reactions. On the other hand, both alkyl and aryl oximes could be used in this methodology. Starting from aryl oximes **2.73a-d** the BIMs **2.82a-e** (R and NOH in *trans* geometry) were obtained as single or major products (Table 2.1, entries 1-10), whereas the isomer **2.83f** (R and NOH in *cis* geometry) was obtained as major product when alkyl oximes were used (Table 2.1, entries 11 and 12).

Looking for more sustainable reaction media, it was decided to explore these reactions using water as solvent.

The use of water as reaction medium has attracted deep interest of researchers in the last decades leading to unexpected results. The expected low solubility of the reagents has been shown not to be an obstacle, and it has also been found that both reactivity and selectivity of the reactions are generally improved when compared with reactions carried out in organic solvents.[54-56] In particular, carrying out DA reactions in aqueous medium

has been shown to be advantageous, not only environmentally but also because induces major improvements in yields, reaction times and selectivity.[16, 44, 57] Thus, the reactivity of nitrosoalkenes **2.80a-e** towards indoles **2.81a**,**b** was also studied using water as solvent although the use of a co-solvent was required as previously observed for other nitrosoalkenes.[19, 35] In fact, the DA reactions carried out using the water/DCM solvent system (Table 2.1, entries 2, 4, 6, 8, 10, 12), result in significantly shorter reaction times (the reaction time was reduced from 36 h to 3 h) and in general gave better yields (42-73% overall yield) compared to those carried out in DCM (29-80% overall yield) (Figure 2.3). These results also clearly show that the efficiency of the reaction, using the $H_2O$/DCM system, between the nitrosoalkenes bearing halogenated aryl substituents at C3 position and indoles increased in the order F > Cl > Br > H, which is the order of electron withdrawing ability and consequently the order of expected effectiveness for an *inverse*-electron-demand DA reaction (Table 2.1, entries 2, 4, 6, 8). However, this correlation could not be observed when reactions were carried out in DCM. In fact, the isolated yields do not reflect the expected reactivity and efficiency which may be explained by considering the differences in the isolation and purification procedures.

**Table 2.1** - One-pot synthesis of new 1-hydroxyiminomethyl-bis(indolyl)methanes 2.**82a-f** and **2.83a-f**.




**2.73a** R = *p*-$BrC_6H_4$, X = Br
**2.73b** R = *p*-$ClC_6H_4$, X = Br
**2.73c** R = *p*-$FC_6H_4$, X = Br
**2.73d** R = Ph, X = Cl
**2.73e** R = Me, X = Cl

**2.80a-e**

**2.81a** $R^1 = R^2 = H$
**2.81b** $R^1$ = Me, $R^2 = H$

**2.82a/2.83a** R = *p*-$BrC_6H_4$, $R^1 = R^2 = H$
**2.82b/2.83b** R = *p*-$ClC_6H_4$, $R^1 = R^2 = H$
**2.82c/2.83c** R = *p*-$FC_6H_4$, $R^1 = R^2 = H$
**2.82d/2.83d** R = Ph, $R^1 = R^2 = H$
**2.82e/2.83e** R = *p*-$BrC_6H_4$, $R^1$ = Me, $R^2 = H$
**2.82f/2.83f** R = Me, $R^1 = R^2 = H$

| Entry | R | X | $R^1$ | $R^2$ | Time (h) | Solvent | yield | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | **2.82** | **2.83** |
| 1 | *p*-$BrC_6H_4$ | Br | H | H | 36 | DCM | **2.82a** 34% | - |
| 2 | *p*-$BrC_6H_4$ | Br | H | H | 3 | $H_2O$/DCM | **2.82a** 55% | - |
| 3 | *p*-$ClC_6H_4$ | Br | H | H | 36 | DCM | **2.82b** 80% | - |
| 4 | *p*-$ClC_6H_4$ | Br | H | H | 3 | $H_2O$/DCM | **2.82b** 64% | **2.83b** 9% |
| 5 | *p*-$FC_6H_4$ | Br | H | H | 36 | DCM | **2.82c** 33% | - |
| 6 | *p*-$FC_6H_4$ | Br | H | H | 3 | $H_2O$/DCM | **2.82c** 71% | - |
| 7 | Ph | Cl | H | H | 36 | DCM | **2.82d** 44% | - |
| 8 | Ph | Cl | H | H | 3 | $H_2O$/DCM | **2.82d** 42% | **2.83d** 12% |
| 9 | *p*-$BrC_6H_4$ | Br | Me | H | 36 | DCM | **2.82e** 63% | - |
| 10 | *p*-$BrC_6H_4$ | Br | Me | H | 3 | $H_2O$/DCM | **2.82e** 70% | - |
| 11 | Me | Cl | H | H | 36 | DCM | **2.82f** 9% | **2.83f** 19% |
| 12 | Me | Cl | H | H | 3 | $H_2O$/DCM | - | **2.83f** 57% |

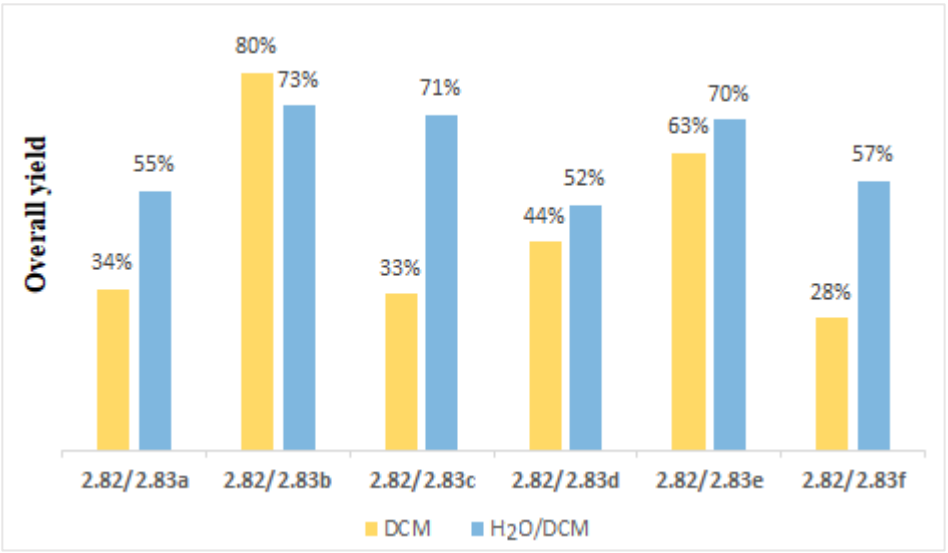
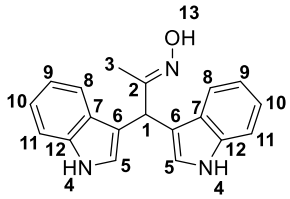



**Figure 2.3** Synthesis of new 1-hydroxyiminomethyl-bis(indolyl)methanes **2.82**/**2.83** (DCM versus $H_2O$/DCM).

The unequivocal determination of the structure of these BIMs was achieved by nuclear magnetic resonance (NMR) spectroscopy. The NMR data of compound **2.83f** is discussed in more detail as an illustrative example. The assignment of the signals was done using two-dimensional techniques such as COSY, NOESY, HMQC and HMBC. Table 2.2 shows the assignments of the chemical shifts observed in the $^{1}H$ and $^{13}C$ NMR spectra.

**Table 2.2** $^{1}H$ and $^{13}C$ NMR signal assignments of BIM **2.83f**.

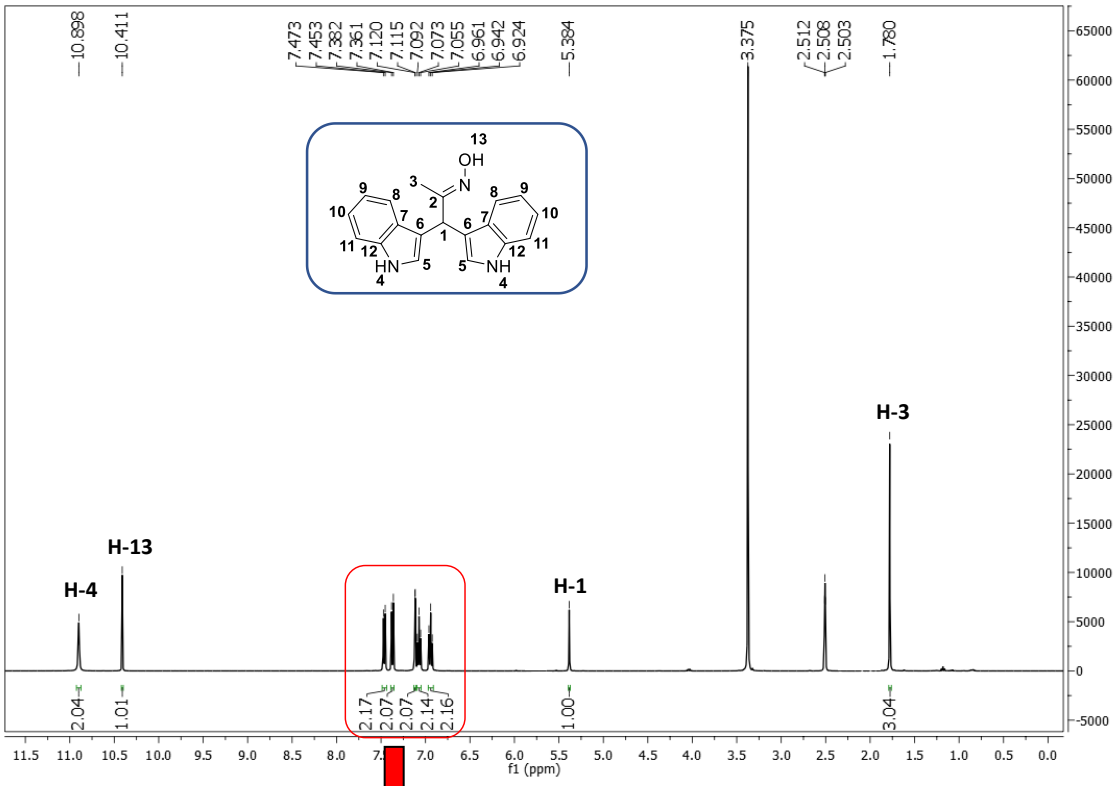



| Position | δ(ppm) $^{1}H$ NMR | δ(ppm) $^{13}C$ NMR |
|---|---|---|
| 3 | 1.78 (s, 3H) | 12.2 ($CH_3$) |
| 1 | 5.38 (s, 1H) | -* |
| 10 | 6.94 (pseudo t, *J* = 7.4 Hz, *J* = 7.2 Hz, 2H) | 118.2 (CH, Ar) |
| 9 | 7.07 (pseudo t, *J* = 7.4 Hz, *J* = 7.2 Hz, 2H) | 120.9 (CH, Ar) |
| 5 | 7.12 (d, *J* = 2.0 Hz, 2H) | 123.4 (CH, Ar) |
| 8 | 7.37 (d, *J* = 8.4 Hz, 2H) | 111.4 (CH, Ar) |
| 11 | 7.46 (d, J = 8.0 Hz, 2H) | 118.9 (CH, Ar) |
| 13 | 10.41 (s, 1H, OH) | - |
| 4 | 10.90 (br s, 2H, NH) | - |
| 6 | - | 114.2 (C, Ar) |
| 7 | - | 126.8 (C, Ar) |
| 12 | - | 136.2 (C, Ar) |
| 2 | - | 156.9 (C) |

*Under DMSO-$d_6$ signal.

In the $^{1}H$ NMR spectrum (Figure 2.4a) of BIM **2.83f** the singlet signals corresponding to the [(H-4 (NH)] protons at 10.90 ppm, the oxime moiety proton (H-13) at 10.41 ppm, the *meso* proton (H-1) at 5.38 ppm and the methyl group protons (H-3) at 1.78 ppm, are easily identified. The $^{13}C$ NMR spectrum (Figure 2.5) shows a quaternary carbon (C-2) at 156.9 ppm corresponding to the carbon of the C=N bond of the oxime moiety and a signal at 12.2 ppm corresponding to the C-3 (methyl group).

a)




b)

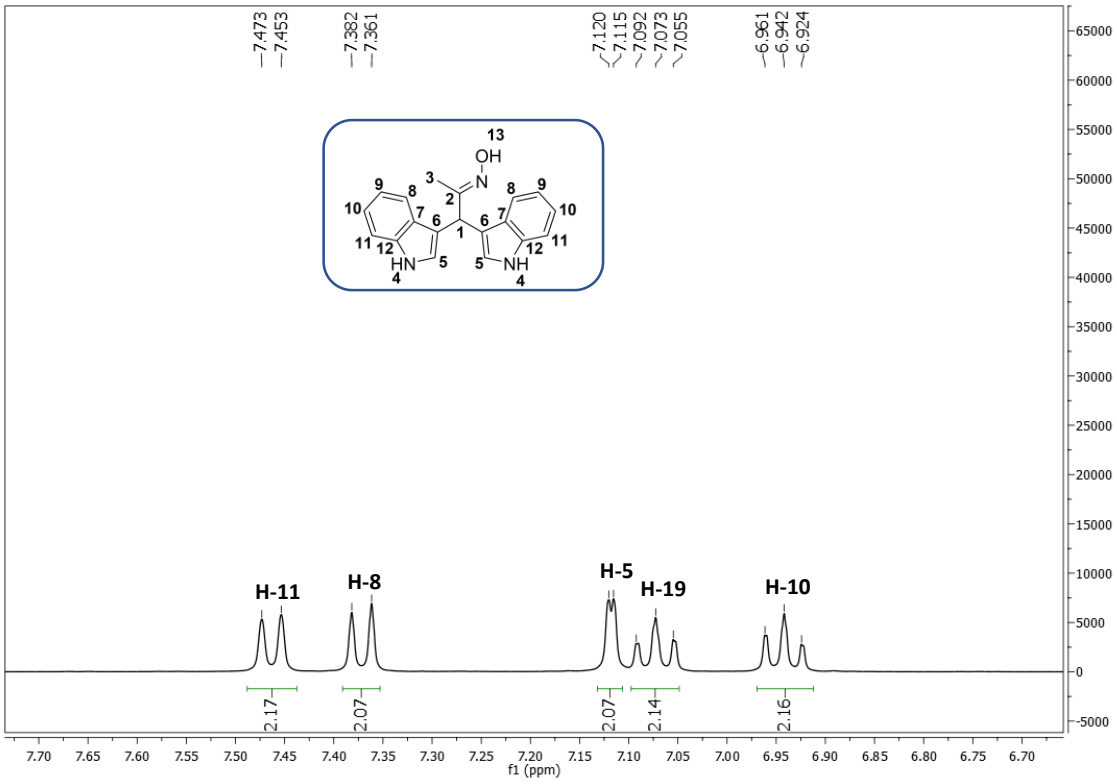



**Figure 2.4** a) $^{1}$H NMR spectra of BIM **2.83f**. b) $^{1}$H NMR expansion of BIM **2.83f**.

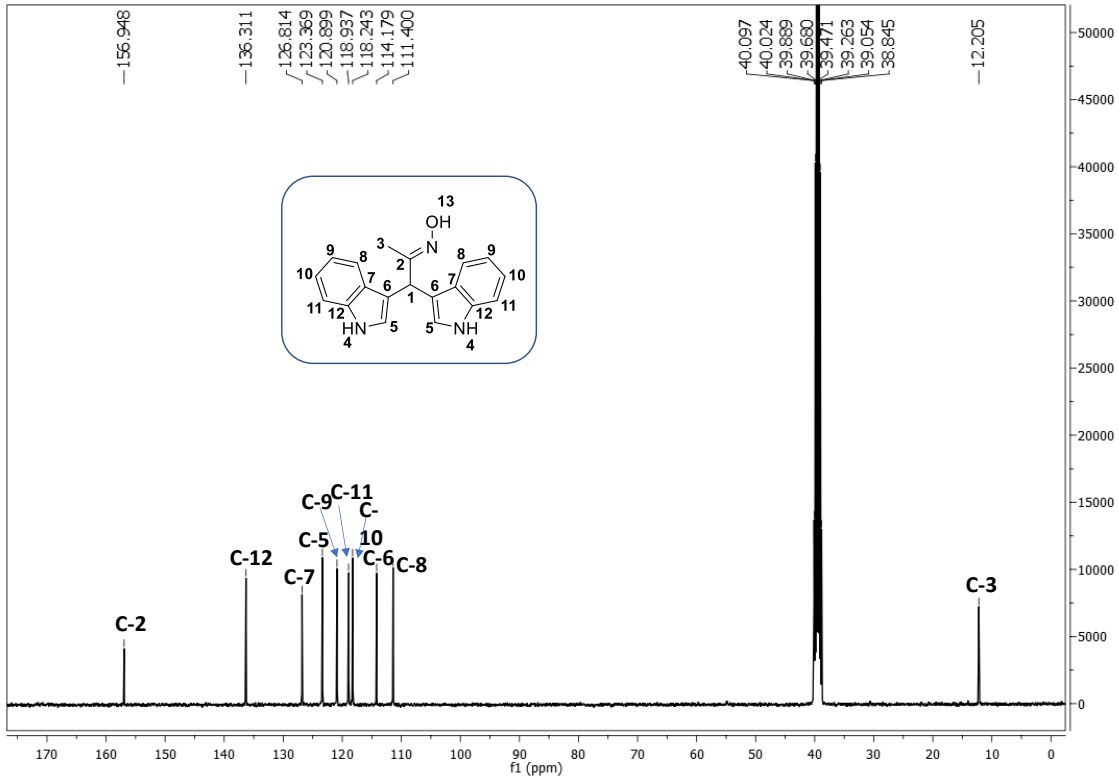



**Figure 2.5** $^{13}$C NMR spectrum of BIM **2.83f**.

The main interactions observed in the bi-dimensional NMRs represented in Figure 2.6, allowed the assignment of protons corresponding to the indole core.

Through the analysis of the COSY spectrum (Figure 2.7) it was possible to observe the correlation between the H-4 (NH) (singlet at 10.90 ppm) proton with H-5 (doublet at 7.12 ppm) which in turn showed correlation with the *meso* proton H-1 (singlet at 5.38 ppm). On the other hand, correlation between the H-11 proton (doublet at 7.46 ppm) and the H-10 proton (*pseudo* triplet at 6.94 ppm) was also identify. The latter also showed correlation with H-9 (*pseudo* triplet at 7.07 ppm) which in turn showed correlation with H-8 (doublet at 7.37 ppm). In the NOESY spectrum, connectivity between H-4 proton (broad singlet at 10.90 ppm) and H-11 (doublet at 7.46 ppm) and between the latter and H-10 proton (*pseudo* triplet at 6.94 ppm) were observed. Furthermore, it was also possible to observe connectivities between H-11 and H-1 (singlet at 5.38 ppm), H-8 (doublet at 7.37 ppm) and H-9 (*pseudo* triplet at 7.07 ppm) and between H-5 (doublet 7.12 ppm) and H-1 (singlet at 5.38 ppm).

By analysis of the NOESY spectra of **2.83f**, connectivity was observed between the hydroxyl proton H-13 ( singlet at 10.41 ppm) and the methyl protons H-3 (singlet at 1.78

ppm), which allowed the stereochemistry assignment of the BIM oxime **2.83f** (this topic will be further discussed).

The carbons of the indole nuclei were identified by analysis of the HMQC (Figure 2.9) and HMBC (Figure 2.10) spectra. The C-8, C-9, C-10, and C-11 carbons were easily identified by analysis of HMQC spectra, whereas the assignment of the quaternary carbons was made by analysing the HMBC spectra. Therefore, the main connectivities observed in the latter spectrum were between the C-2 (156.9 ppm) with both H-1 and H-13 protons, C-6 (114.2 ppm) carbon and the proton H-1 as well as with the H-4 proton, C-7 (126.8 ppm) with H-1 and H-8 and finally, C-12 carbon with H-4, H-9 and H-11 protons (Figure 2.10).

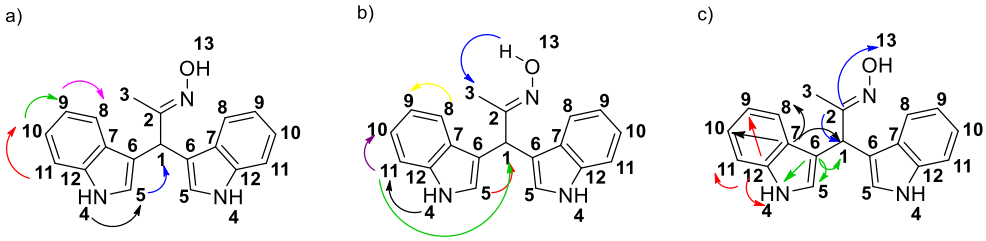
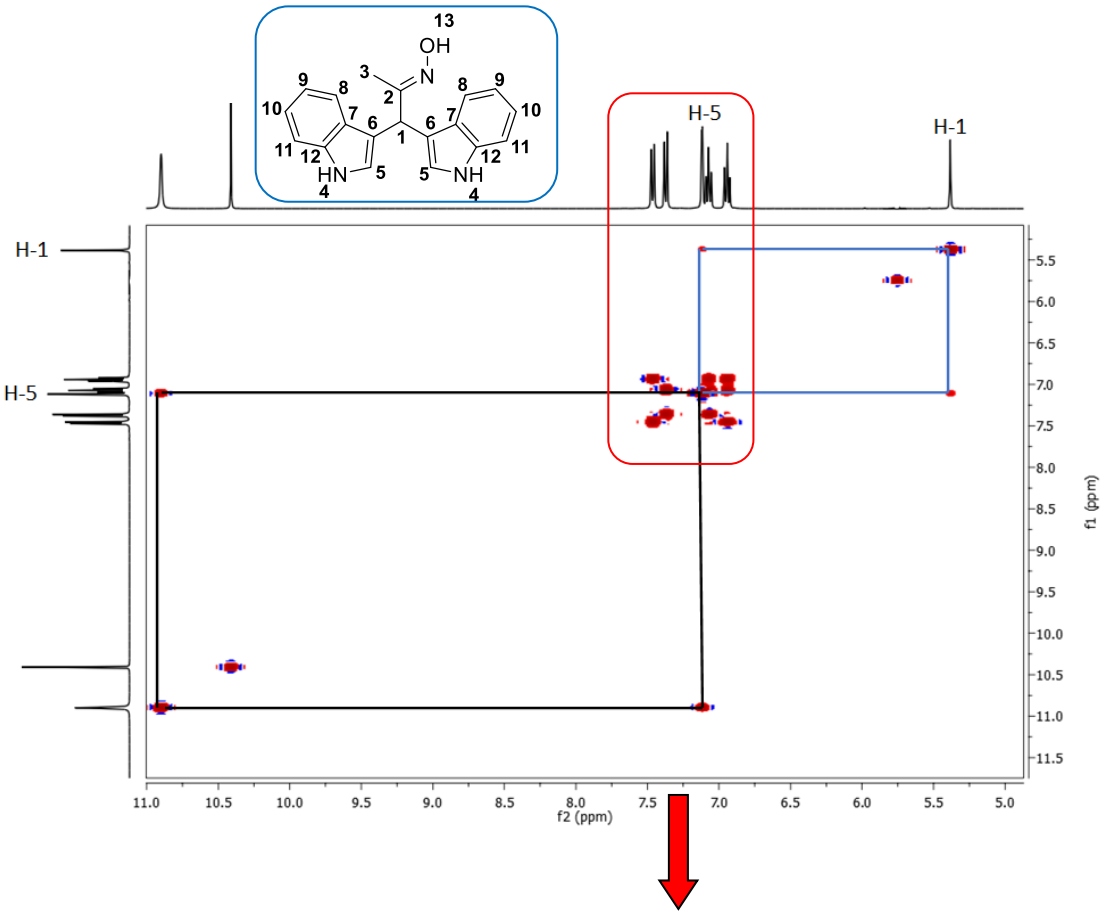



**Figure 2.6** Main observed connectivities in the COSY (a), NOESY (b) and HMBC (c) of compound **2.83f**.



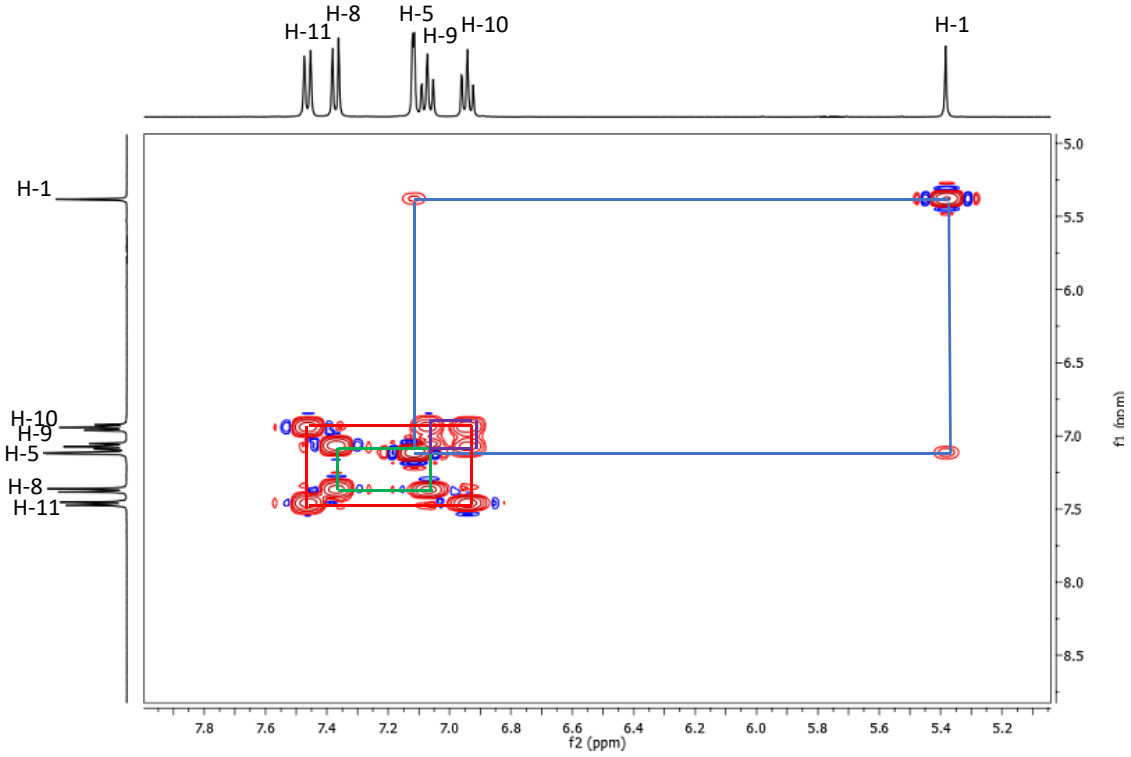
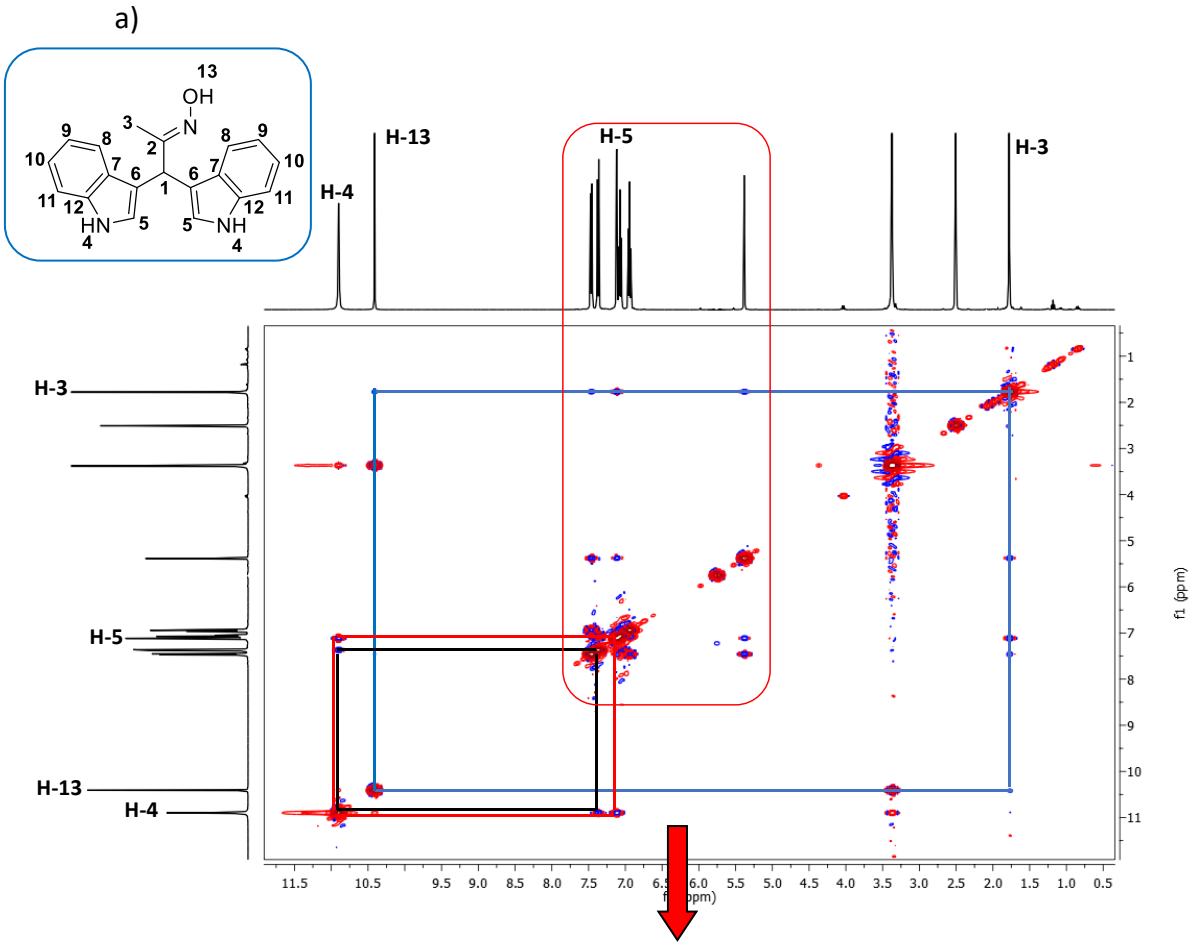



**Figure 2.7** Expansions of the COSY spectrum of BIM **2.83f**.



b)

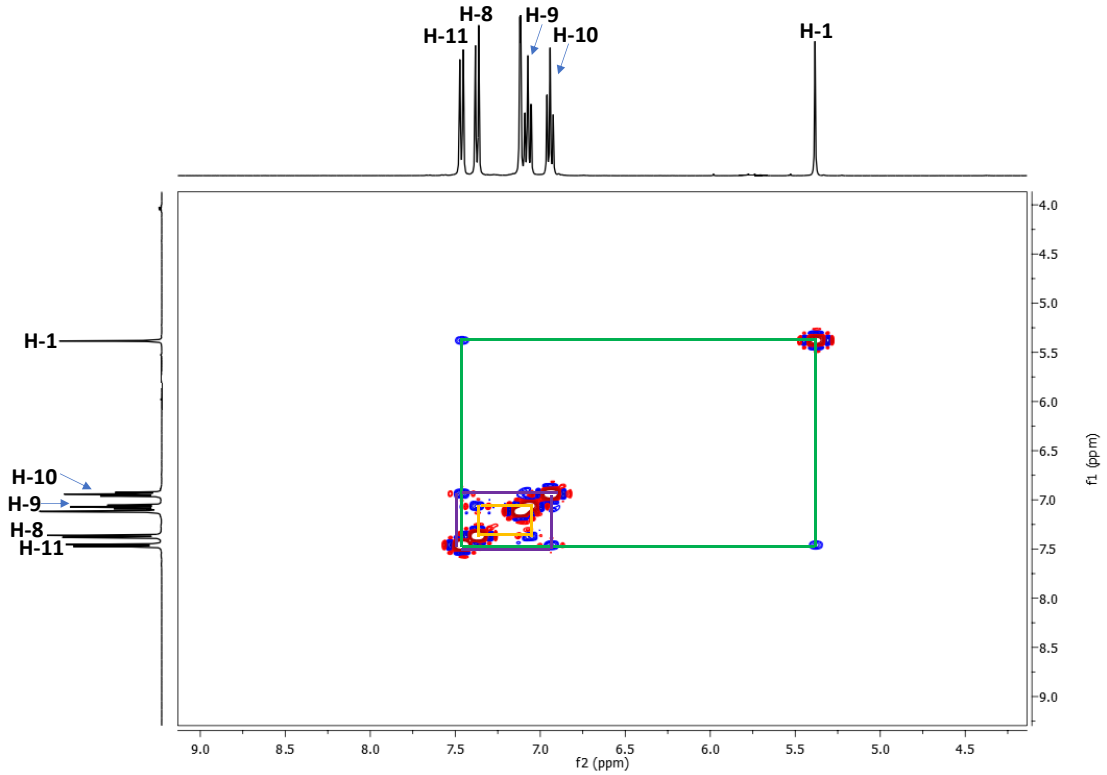



**Figure 2.8** a) NOESY spectrum of BIM **2.83f** b) expansion of the NOESY spectrum of BIM **2.83f**.

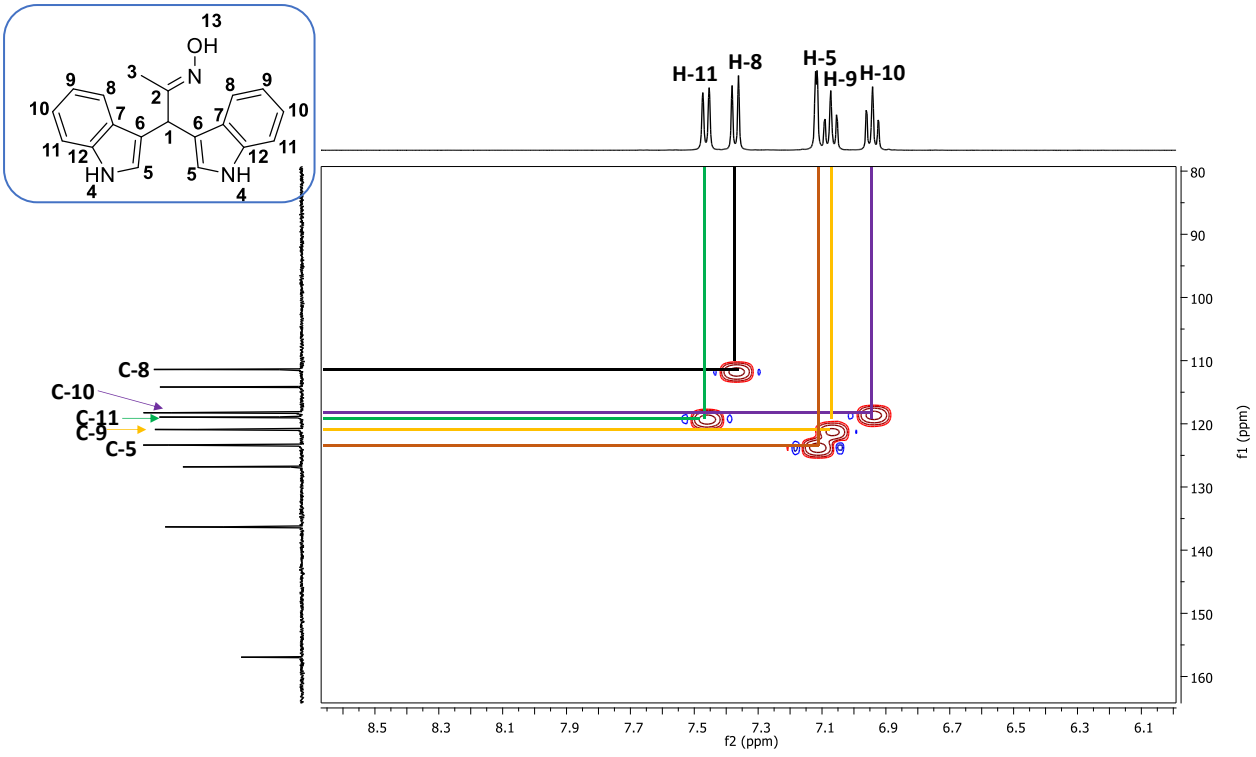



**Figure 2.9** Expansion of the HMQC spectrum of BIM **2.83f**.

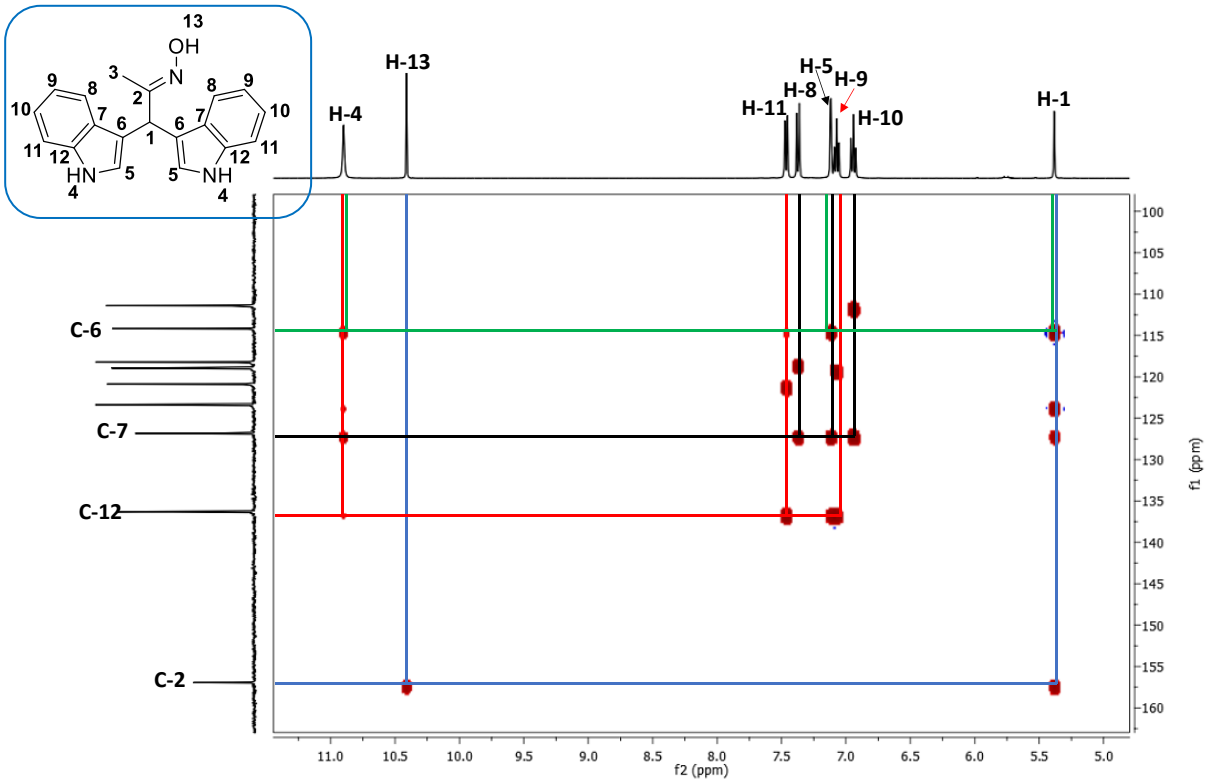



**Figure 2.10** Expansion of the HMBC spectrum of BIM **2.83f**.

In order to confirm the stereochemistry assignment of BIMs **2.82** and **2.83**, the $^{1}$H NMR (Figure 2.11) and NOESY spectra of compounds **2.82f** and **2.83f** as well as the ones corresponding to compounds **2.82d** (Figure 2.12) and **2.83d** (Figures 2.13 and 2.14) were analysed. In fact, in the NOESY spectrum of **2.82f** no connectivity between the hydroxyl proton H-13 and the methyl group protons H-3 was observed, contrary to what was observed for oxime **2.83f** (see Figure 2.8). Similarly, in the NOESY spectra of aryl BIM oximes, no connectivity was observed between the hydroxyl proton and the phenyl protons in **2.82d**, whereas that connectivity was observed in BIM **2.83d** (Figure 2.14). Moreover, the two isomeric-oximes **2.82** and **2.83** are also characterized by $^{1}$H NMR spectra with different features. The chemical shift of the *meso* proton H-1 appears at higher value for isomers **2.82** (**2.82d**: $\delta$ = 6.82 ppm; **2.82f**: $\delta$ = 6.39 ppm) (Figures 2.12 and 2.11) than for the corresponding isomers **2.83 (2.83d**: 5.77 ppm; **2.83f**: 5.38 ppm) (Figures 2.13 and 2.4).

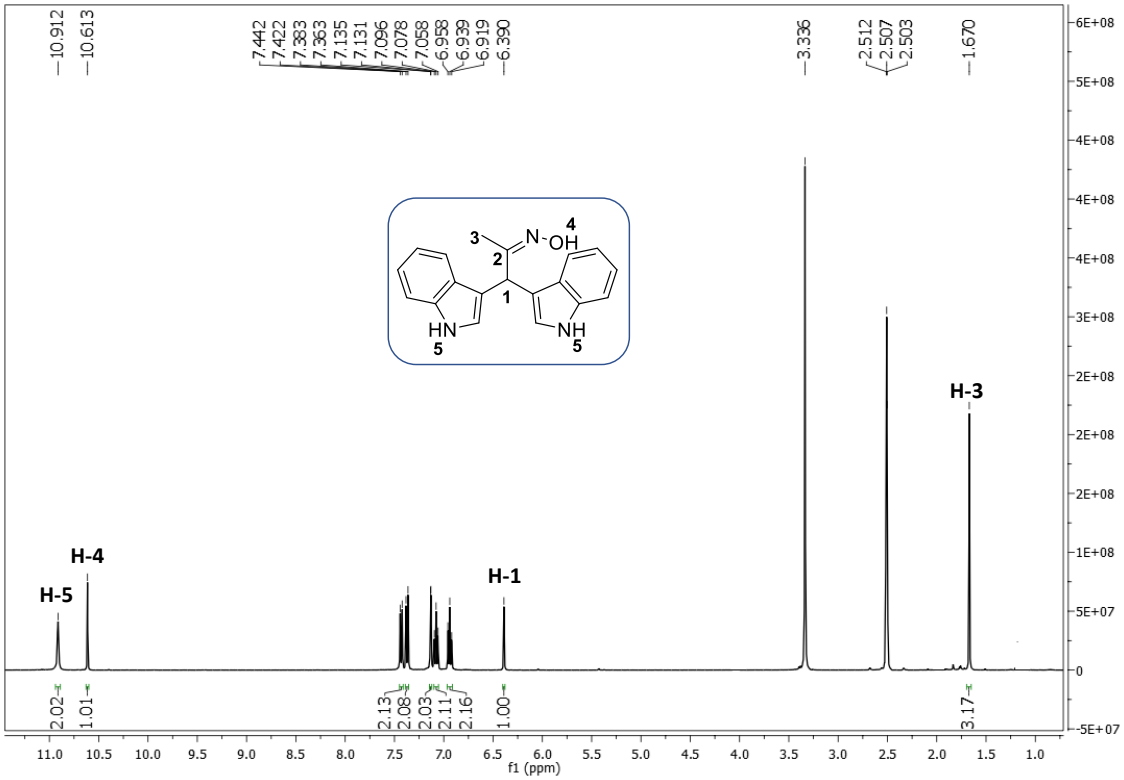



**Figure 2.11** $^{1}$H NMR spectrum of compound **2.82f**.

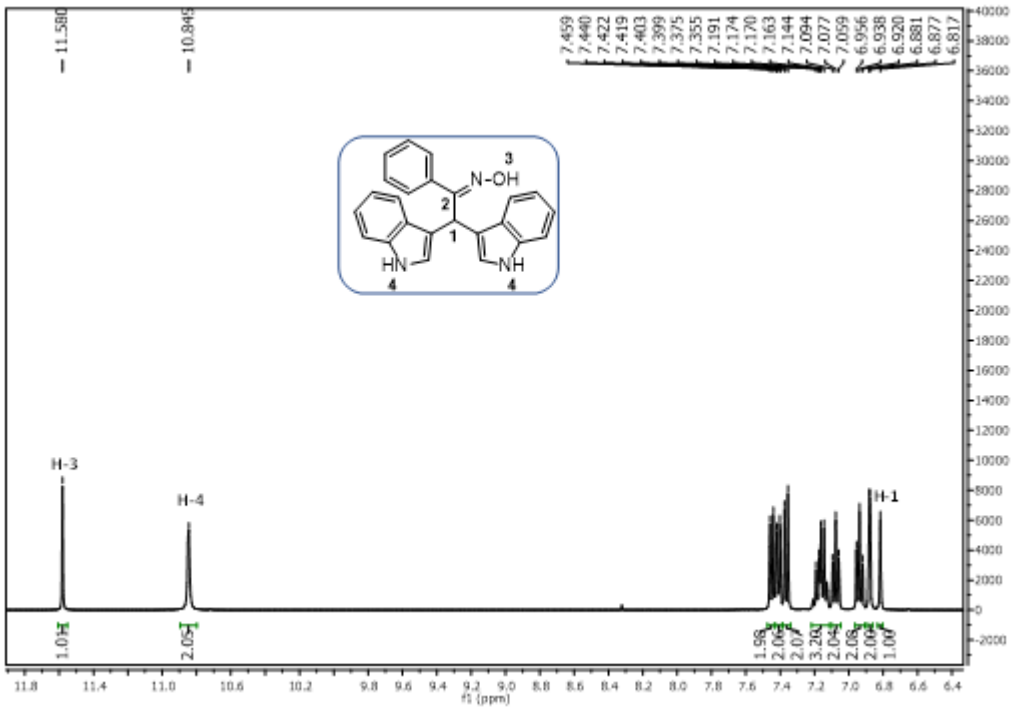



**Figure 2.12** Expansion of the $^{1}$H NMR spectrum of compound **2.82d**.

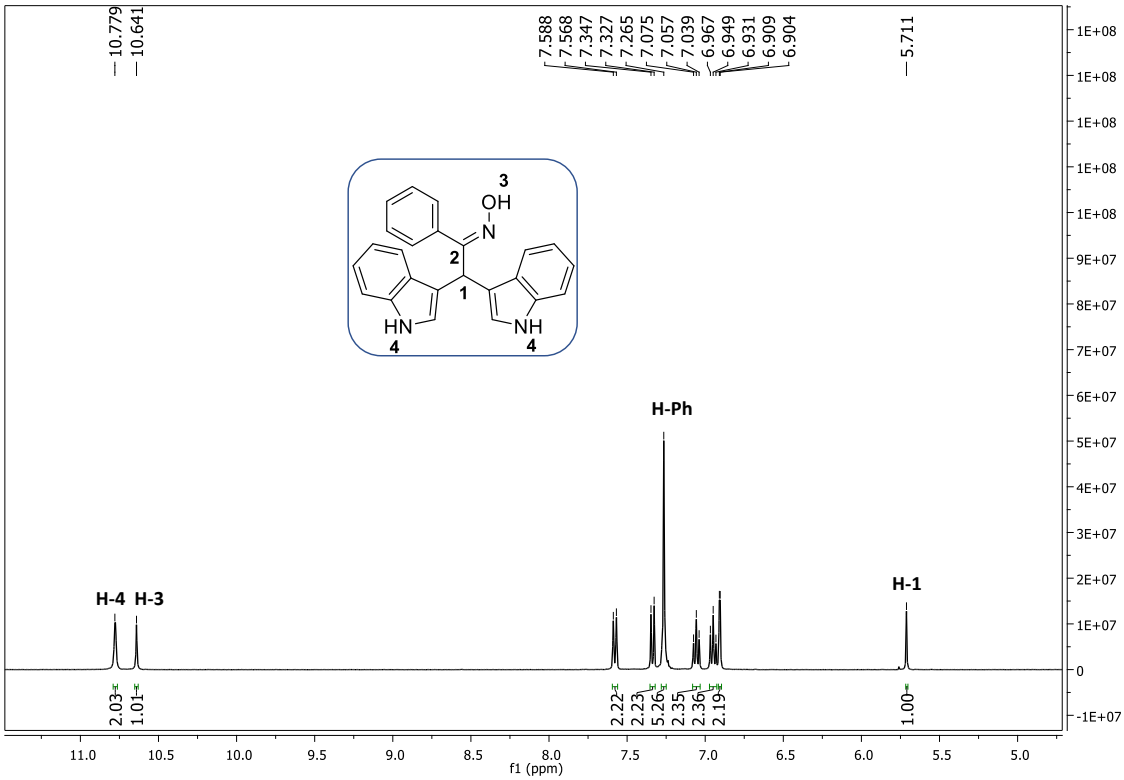



**Figure 2.13** Expansion of the $^{1}$H NMR spectrum of compound **2.83d**.

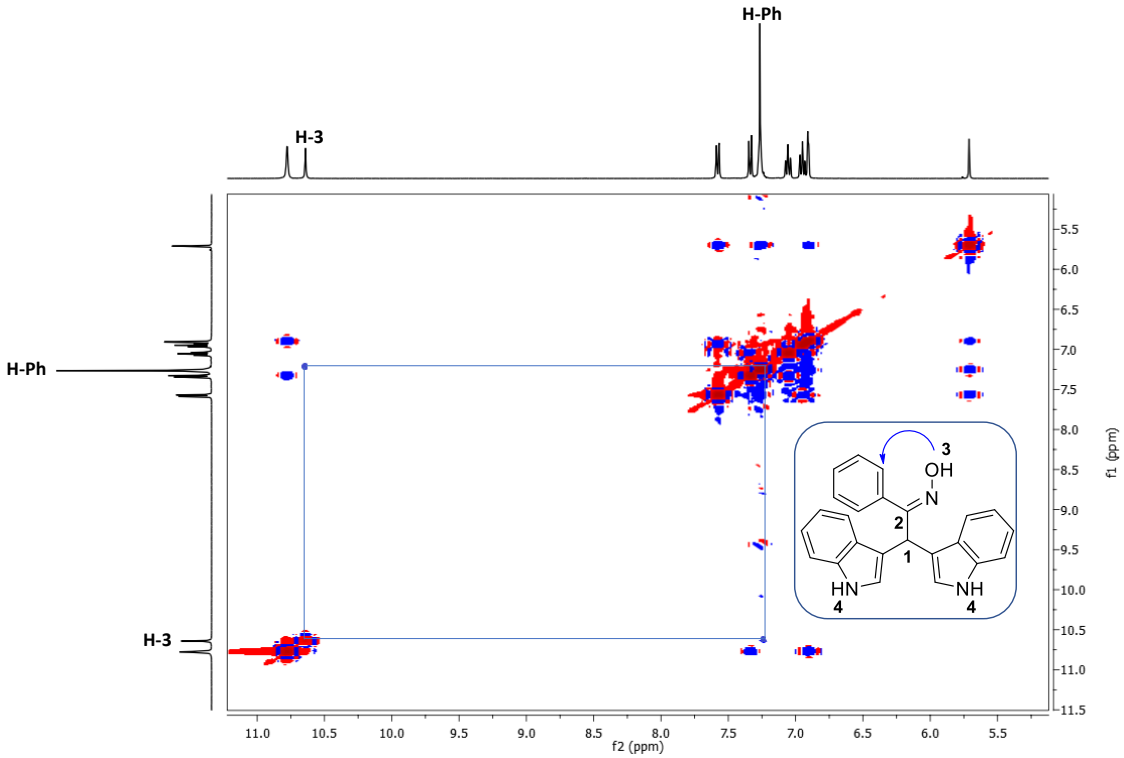
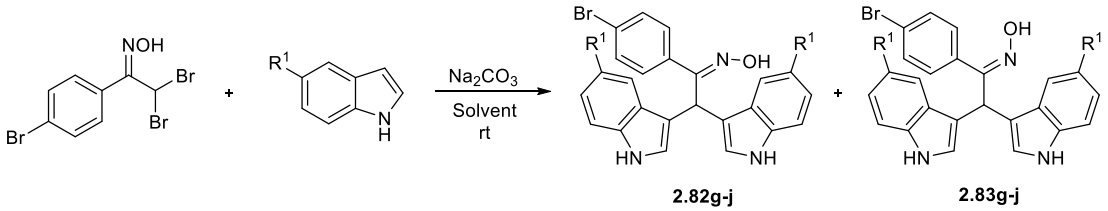



**Figure 2.14** Expansion of the NOESY spectrum of compound **2.83d**.

After the cytotoxicity evaluation of some of these BIMs (see Section 2.3.5), BIM **2.82a** was identified as promising scaffold for anticancer activity. Taking in account these results, reactivity studies for the synthesis of new BIM derivatives **2.82g-j** and **2.83g-j** were carried out in order to establish a structure-activity relationship. These involved the reaction between nitrosoalkenes, generated *in situ* from 2,2-dibromo-1-(4-bromophenyl)ethanone oxime **2.73a**, and 5-substituted indoles **2.81**. Reactions were carried out in DCM and in water using DCM as co-solvent (9:1), at room temperature, as outline in Table 2.3. As already observed in the previous reactions, the latter solvent system led to better or comparable yields (Table 2.3, entries 2, 4, 6 and 8) and shorter reaction times (36 h *versus* 3 h) than when DCM was used as solvent (Table 2.3, entries 1, 3, 5 and 7) (Figure 2.15). Furthermore, the synthesis of BIM **2.82g**, using 5-bromoindole, could only be achieved in $H_2O$/DCM (Table 2.3, entries 1 and 2). The reactions were regioselective and stereoselective, since (*E*)-oximes were obtained as single or major product. Only in the case of the reaction with 5-bromoindole two isomeric oximes **2.82g** (41%) and **2.83g** (10%) were isolated (Table 2.3 entry 2). The efficiency of the reaction increases with the presence of stronger electron donating substituents on the indole, as expected for the inverse electron-demand DA reaction. In fact, starting from oxime **2.73a** and 5-metoxy- (**2.81d**) or 5-hydroxyindole (**2.81f**), the corresponding BIMs were obtained in 78% yield (Table 2.3, entries 4 and 8) while the reaction with 5-bromo- (**2.81a**) or 5-methylindole (**2.81e**) gave the target BIMs in 51% (overall) and 55% yield, respectively (Table 2.3, entries 2 and 6).

**Table 2.3** – One-pot synthesis of new 1-hydroxyiminomethyl-bis(indolyl)methanes **2.82g-j** and **2.83g**.




**2.73a**

**2.81c** $R^1$ = Br
**2.81d** $R^1$ = OMe
**2.81e** $R^1$ = Me
**2.81f** $R^1$ = OH

**2.82g-j**

**2.83g-j**

**2.82g/2.83g** $R^1$ = Br
**2.82h/2.83h** $R^1$ = OMe
**2.82i/2.83i** $R^1$ = Me
**2.82j/2.83j** $R^1$ = OH

| Entry | $R^1$ | Time (h) | Solvent | Yield (%) | |
|---|---|---|---|---|---|
| | | | | **2.82** | **2.83** |
| 1 | Br | 36 | DCM | - [a] | - |
| 2 | Br | 3 | $H_2O$/DCM | **2.82g** 41% | **2.83g** 10% |
| 3 | OMe | 36 | DCM | **2.82h** 71% | - |
| 4 | OMe | 3 | $H_2O$/DCM | **2.82h** 78% | - |
| 5 | Me | 36 | DCM | **2.82i** 20% | - |
| 6 | Me | 3 | $H_2O$/DCM | **2.82i** 55% | - |
| 7 | OH | 36 | DCM | **2.82j** 23% | - |
| 8 | OH | 3 | $H_2O$/DCM | **2.82j** 78% | |

[a] No evidence of the target compound was observed.

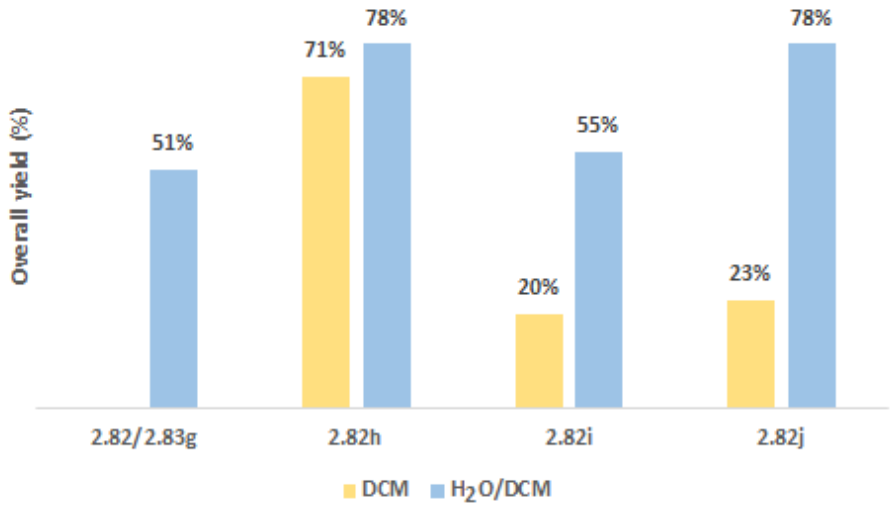
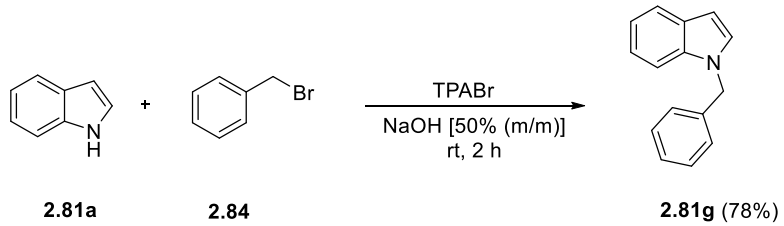



**Figure 2.15** Synthesis of new 1-hydroxyiminomethyl-bis(indolyl)methanes **2.82g-j/2.83g** (DCM versus $H_2O$/DCM).

### 2.3.3 Synthesis of 1-hydrazonomethyl-bis(indolyl)methanes

The study also aimed at the synthesis of unknown 1-hydrazonomethyl-bis(indolyl)methanes **2.85** based on a similar methodology (Table 2.4). Gilchrist *et al.*,

reported that the reaction of indole with azoalkenes, generated in *situ* from ethyl pyruvate derived α-halohydrazones, usually leads to *C*- and *N*-alkylated products.[58] Thus, our first approach was to carried out the hetero-Diels-Alder cycloaddition reactions using *N*-protected indoles as dienophiles, namely the commercially available *N*-methyl indole and *N*-benzylindole prepared as outline in scheme 2.22. The reaction between indole **2.81a**, and benzyl bromide **2.84**, in alkaline aqueous solution after stirring at room temperature using tetrapropylammonium bromide (TPABr) as catalyst, during 2 h, led to the *N*-benzylindole **2.81g** in 78% yield (Scheme 2.22).[59]




**2.81a** **2.84** **2.81g** (78%)

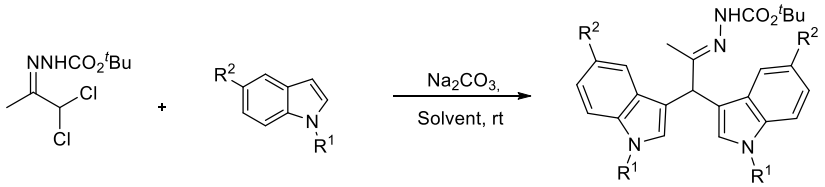
**Scheme 2.22** Synthesis of *N*-benzylindole.

Reactions between the azoalkene, generated *in situ* from dichlorohydrazone **2.74a** with *N*-methyl- **2.81b** or *N*-benzylindole **2.81g**, using DCM or the $H_2O$/DCM solvent system, led to the corresponding *N*-substituted BIMs hydrazones **2.85a**,**b** in yields ranging from 27 to 31% yield (Table 2.4 entries 1, 2, 4 and 5). The reaction of *N*-methylindole and hydrazone **2.74a** was carried out in water affording BIM hydrazone **2.85a** in 49% yield (Table 2.6, entry 3). The "on-water" conditions could only be applied in this case because *N*-methylindole is a liquid at room temperature. The use of DCM as co-solvent was required to improve the efficacy of reactions when all the reagents were solids, as previously observed.

The first anti-tumoral activity studies showed that the presence of *N*-unsubstituted indole moieties in BIM oximes is fundamental to ensure high cytotoxicity against cancer cell lines (Section 6.3). Therefore, reactions of azoalkene, generated *in situ*, from dichlorohydrazone **2.74a** and *N*-unsubstituted indoles were carried out. This would be a better alternative to the use of *N*-protected indoles in the DA cycloaddition reaction, followed by deprotection reaction to obtain the target *N*-unprotected BIM hydrazones. Remarkably, the dichlorohydrazone **2.74a** reacted with *N*-unprotected indoles leading to the desired BIM hydrazones as single products (Table 2.4, entries 6-11). Reactions performed in $H_2O$/DCM with indole and 5-hydroxyindole were more effective (55%) and

needed shorter reaction time (3 h) than those performed in DCM (22% and 21% in 24 h) (Table 2.4, entries 6-9). Under the optimizing reaction conditions, the reaction between **2.74a** and 5-methylindole (**2.81e**) and 5-bromoindole (**2.81c**) led to the corresponding BIMs in 43% and 63% yield, respectively (Table 2.4, entries 10 and 11). Particularly interesting was to observe the formation of a secondary product **2.86** in 17 % yield, apparently resulting from the hydrolysis of BIM **2.85** (Figure 2.16). The results showed that the substitution pattern of the starting indoles had no impact on the efficiency of the reaction. Furthermore, complete regioselectivity was observed in these transformations since the (*E*)-hydrazones **2.85** were obtained as single product.

**Table 2.4** - One-pot synthesis of new 1-hydrazonomethyl-bis(indolyl)methanes **2.85a-f**.



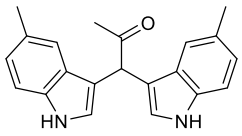

**2.74a**

**2.81a** $R^1 = R^2 = H$
**2.81b** $R^1$ = Me, $R^2$ = H
**2.81c** $R^1$ = H, $R^2$= Br
**2.81e** $R^1$ = H, $R^2$ = Me
**2.81f** $R^1$ = H, $R^2$ = OH
**2.81g** $R^1$ = Bn; $R^2$ = H

**2.85a** $R^1$ = Me, $R^2$ = H
**2.85b** $R^1$ = Bn, $R^2$ = H
**2.85c** $R^1 = R^2 = H$
**2.85d** $R^1$ = H, $R^2$ = OH
**2.85e** $R^1$ = H, $R^2$ = Me
**2.85f** $R^1$ = H, $R^2$ = Br

| Entry | $R^1$ | $R^2$ | Time (h) | Solvent | Yield (%) |
|---|---|---|---|---|---|
| | | | | | **2.85** |
| 1 | Me | H | 24 | DCM | **2.85a** 29% |
| 2 | Me | H | 3 | $H_2O$/DCM | **2.85a** 27% |
| 3 | Me | H | 2 | $H_2O$ | **2.85a** 49% |
| 4 | Bn | H | 24 | DCM | **2.85b** 31% |
| 5 | Bn | H | 3 | $H_2O$/DCM | **2.85b** 29% |
| 6 | H | H | 24 | DCM | **2.85c** 22% |
| 7 | H | H | 3 | $H_2O$/DCM | **2.85c** 55% |
| 8 | H | OH | 24 | DCM | **2.85d** 21% |
| 9 | H | OH | 3 | $H_2O$/DCM | **2.85d** 55% |
| 10 | H | Me | 3 | $H_2O$/DCM | **2.85e** 43%* |
| 11 | H | Br | 3 | $H_2O$/DCM | **2.85f** 63% |

*Compound **2.86** resulting from the hydrolysis of BIM hydrazone **2.85e**, was also obtained in 17% yield.




**2.86** (17%)

**Figure 2.16** Chemical structure of carbonyl BIM derivative.

The stereochemistry assignment of alkyl hydrazone **2.85f** was confirmed by the analysis of the proton NMR (Figure 2.17) and NOESY (Figure 2.18) spectra.

In the $^{1}$H NMR spectrum (Figure 2.17) of BIM **2.85f**, the singlet signals corresponding to the proton of the hydrazone moiety (H-13) at 9.40 ppm, the protons of the indole core (H-4) at 11.15 ppm, the *meso* proton (H-1) at 5.32 ppm, the methyl group protons (H-3) at 1.83 ppm and the signal corresponding to the *tert*-butyl protons (H-15) at 1.47 ppm are easily identified. In addition, analysing the NOESY spectrum was possible to observe the connectivity between $HNCO_2{}^{t}Bu$ (H-13) proton and the methyl group protons (H-3). The same stereochemistry was assigned by analogy to compounds **2.85a-e**.

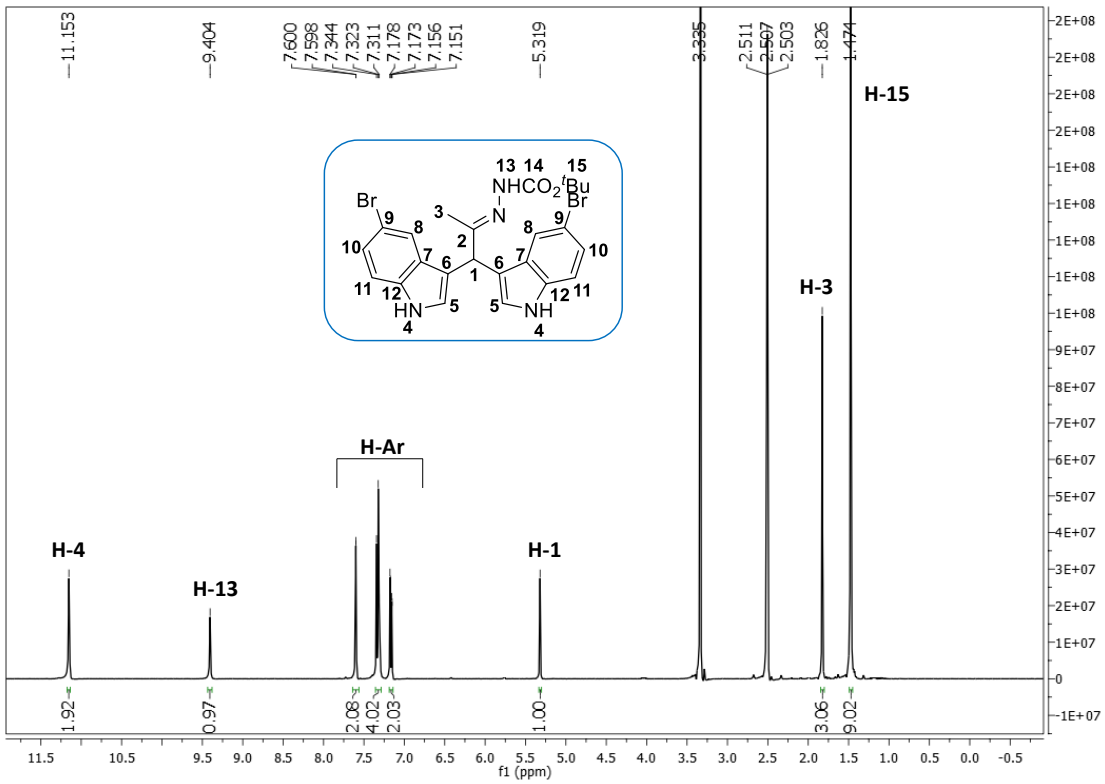



**Figure 2.17** $^{1}$H NMR of the BIM hydrazone **2.85f**.

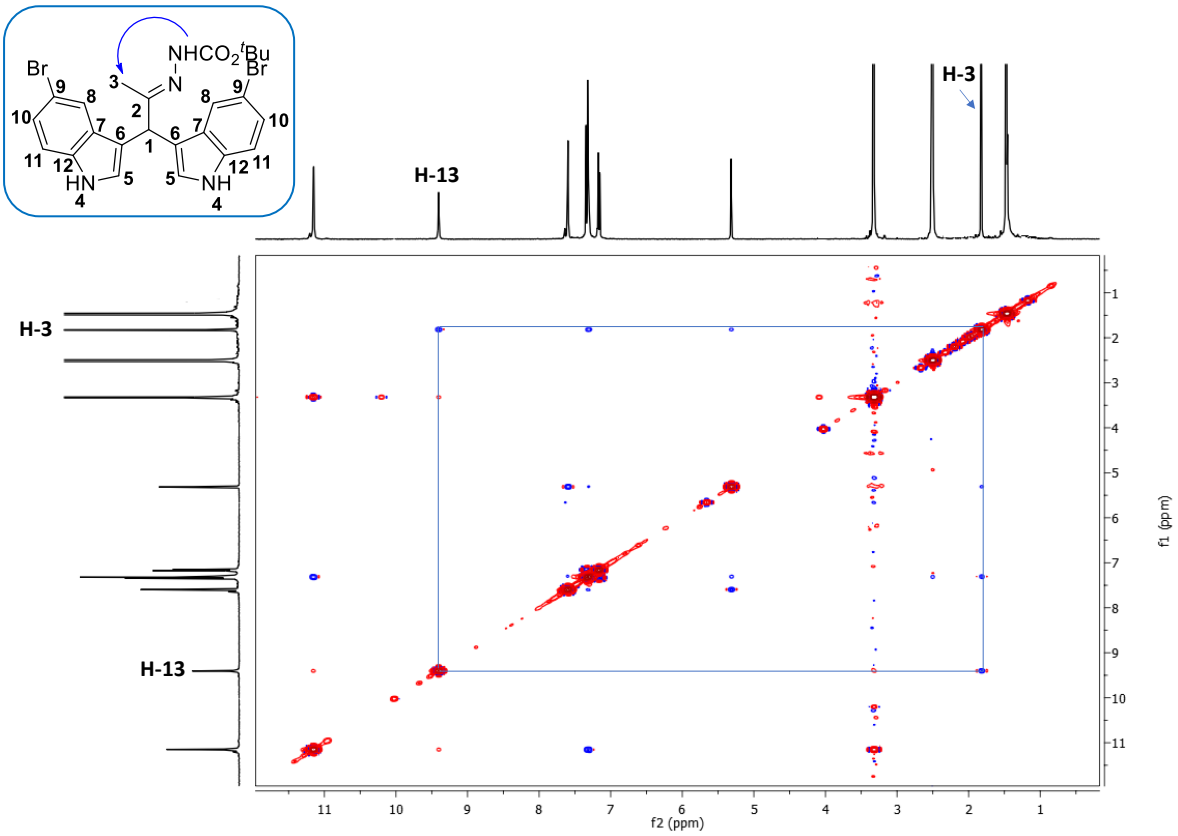
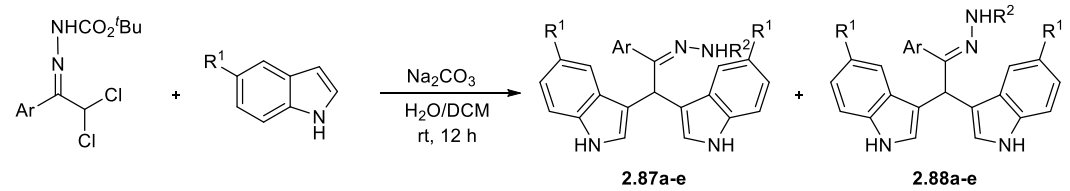



**Figure 2.18** NOESY spectrum of BIM hydrazone **2.85f**.

The work was then extended to the reaction of azoalkenes, generated *in situ* from α,α-dihalohydrazones **2.74b** and **2.74c**, towards indoles (Table 2.5). Under the previous optimised reaction conditions ($H_2O$/DCM), the aryl BIM hydrazones were also obtained albeit in lower yields and longer reaction time (12 h). Hydrazone **2.74b**, in the presence of base, reacted with 1*H*-indole (**2.81a**), 5-bromoindole (**2.81c**) and 5-methylindole (**2.81e**) giving selectively the target BIMs **2.87a-c** in 29%, 7 % and 11% yield, respectively (Table 2.5, entries 1-3). Starting from α,α'-dichlorohydrazone **2.74c**, BIMs **2.87d** (17%) and **2.87e** (13%) were obtained from the reaction with indoles **2.81a** and **2.81c**, respectively (Table 2.5, entries 4 and 5). Moreover, from the reaction of hydrazone **2.74c** with indole (**2.81a**), the (*Z*)-isomer hydrazone **2.88d** was also obtained in 12% yield (Table 2.5, entry 4).

**Table 2.5** - One-pot synthesis of new 1-hydrazonomethyl-bis(indolyl)methanes **2.87a-e** and **2.88d**.




**2.74b** Ar = Ph
**2.74c** Ar = *p*-$BrC_6H_4$

**2.81a** $R^1$ = H
**2.81c** $R^1$ = Br
**2.81e** $R^1$ = Me

**2.87a/2.88a** Ar = Ph, $R^1$ = H, $R^2$ = $CO_2{}^t$Bu
**2.87b/2.88b** Ar = Ph, $R^1$ = Me, $R^2$ = $CO_2{}^t$Bu
**2.87c/2.88c** Ar = Ph, $R^1$ = Br, $R^2$ = $CO_2{}^t$Bu
**2.87d/2.88d** Ar = *p*-$BrC_6H_4$, $R^1$ = H, $R^2$ = $CO_2{}^t$Bu
**2.87e/2.88e** Ar = *p*-$BrC_6H_4$, $R^1$ = Me, $R^2$ = $CO_2{}^t$Bu

| **Entry** | **Ar** | **$R^1$** | **Yield (%)** | |
|---|---|---|---|---|
| | | | **2.87** | **2.88** |
| 1 | Ph | H | **2.87a** 29% | - |
| 2 | Ph | Me | **2.87b** 7% | - |
| 3 | Ph | Br | **2.87c** 11% | - |
| 4 | *p*-$BrC_6H_4$ | H | **2.87d** 17% | **2.88d** 12% |
| 5 | *p*-$BrC_6H_4$ | Me | **2.87e** 13% | - |

The structure elucidation of BIM hydrazone **2.87b** was based in the $^1$H NMR spectrum (Figure 2.19) in which it was possible to identify the signal corresponding to the *tert*-butyl group protons H-4 (singlet at 1.39 ppm), the methyl group protons H-6 (singlet at 2.33 ppm), the *meso* proton H-1 (singlet at 5.59 ppm), the hydrazone proton H-3 (singlet at 8.21 ppm) and H-5 protons (singlet at 10.69 ppm). The stereochemistry assignment of BIM **2.87b** was confirmed by the analysis of the NOESY spectrum, in which no connectivity was observed between the $NHCO_2{}^t$Bu proton and the phenyl group protons (H-Ph). The same stereochemistry was assigned by analogy to BIM hydrazones **2.87a** and **2.87d-e**. The observed stereoselectivity is in agreement with that obtained for the 1-hydroxyliminomethyl-bis(indolyl)methanes synthesis, where isomers with the substituent group (R) in *trans* position relative to the oxime functionality were obtained as single or major products.

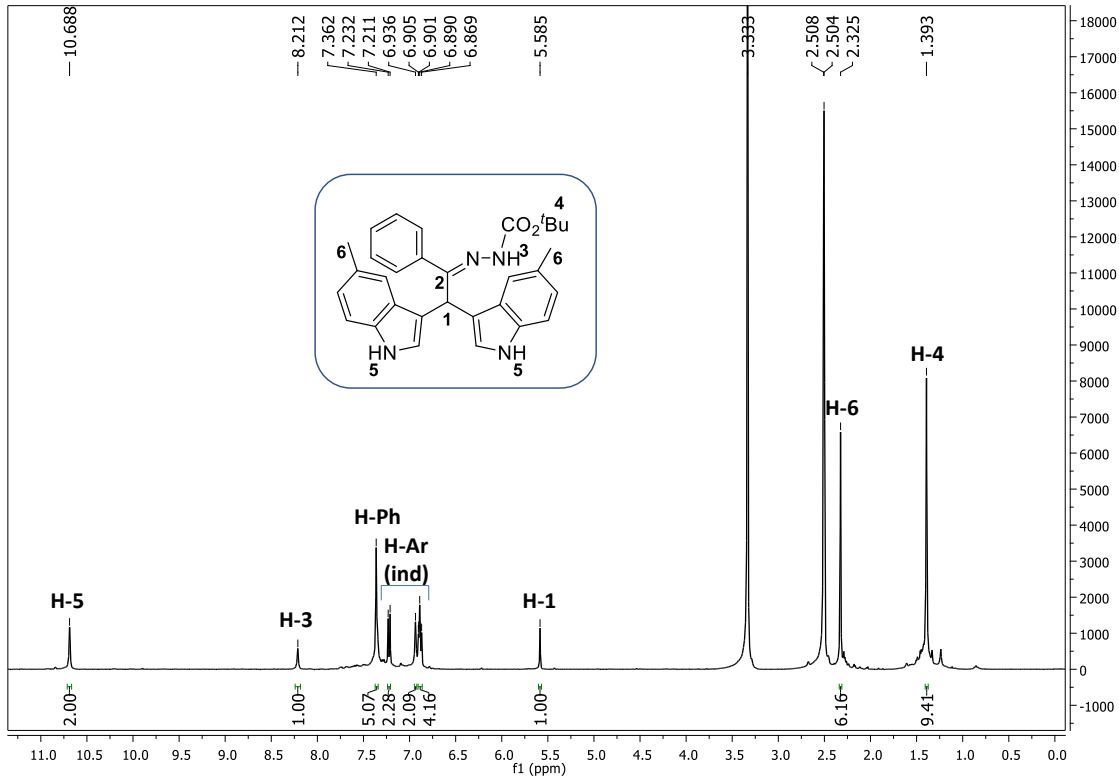
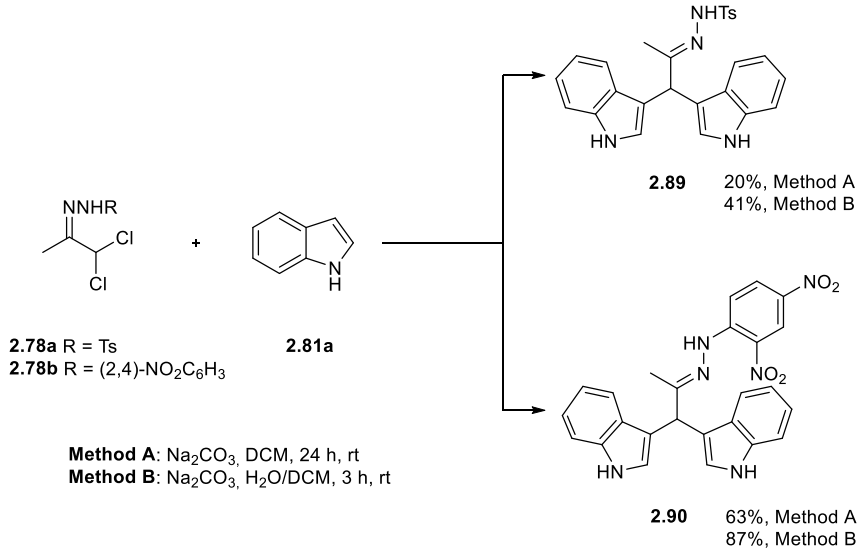



**Figure 2.19** $^{1}$H NMR spectrum of BIM hydrazone **2.87b**.

The results showed that reactions were more efficient when starting from alkyl hydrazone **2.74a** than from aryl hydrazones **2.73b** and **2.73c**. Therefore, it was decided to explore the reactivity of alkyl hydrazones **2.78a** and **2.78b** derived from dichloroacetone. The base-induced dehydrohalogenation was carried out both in DCM and in the $H_2O$/DCM solvent system as outlined in Scheme 2.23. Therefore, azoalkenes bearing tosyl and 2,4-dinitrophenyl substituents, generated *in situ* from the corresponding hydrazones, reacted with indole leading to the target BIMs **2.89** and **2.90**, respectively. As expected, the reactions carried out in $H_2O$/DCM (method B) were more efficient than the ones performed in DCM (Method A). In fact, using the method B, the BIMs **2.89** and **2.90** were obtained in 41% and 87%, respectively (Scheme 2.23).




**Scheme 2.23** One-pot synthesis of hydrazones **2.89** and **2.90**.

These results showed that the substitution pattern of the starting indoles has a minimal influence on the efficiency of these transformations, whereas it was shown that the hydrazone substituent plays a major role and a proper balance between electrophilicity and stability of the heterodienes should be achieved. The efficiency of these transformations, in terms of isolated products followed the order tosyl < *tert*-butyloxycarbonyl < 2,4-dinitrophenyl substituent.

### 2.3.4 Mechanistic proposal

The one pot synthesis of bis(indolyl)methanes can be explained considering that the starting α,α'-dihalooximes or α,α'-dihalohydrazone, in presence of base, are converted *in situ* into the corresponding transient and reactive 4-halonitrosoalkenes or 4-haloazoalkenes, which react as heterodienes *via* HDA reaction with a first molecule of the appropriate indole, giving the corresponding cycloadduct. The initial formed cycloadducts undergo ring-opening to the corresponding 3-alkylated indole under the driving force of indole rearomatisation. The latter undergo a subsequent dehydrohalogenation to give the corresponding heterodiene followed by a second HDA reaction with another molecule of indole affording the target bis(indolyl)methanes.

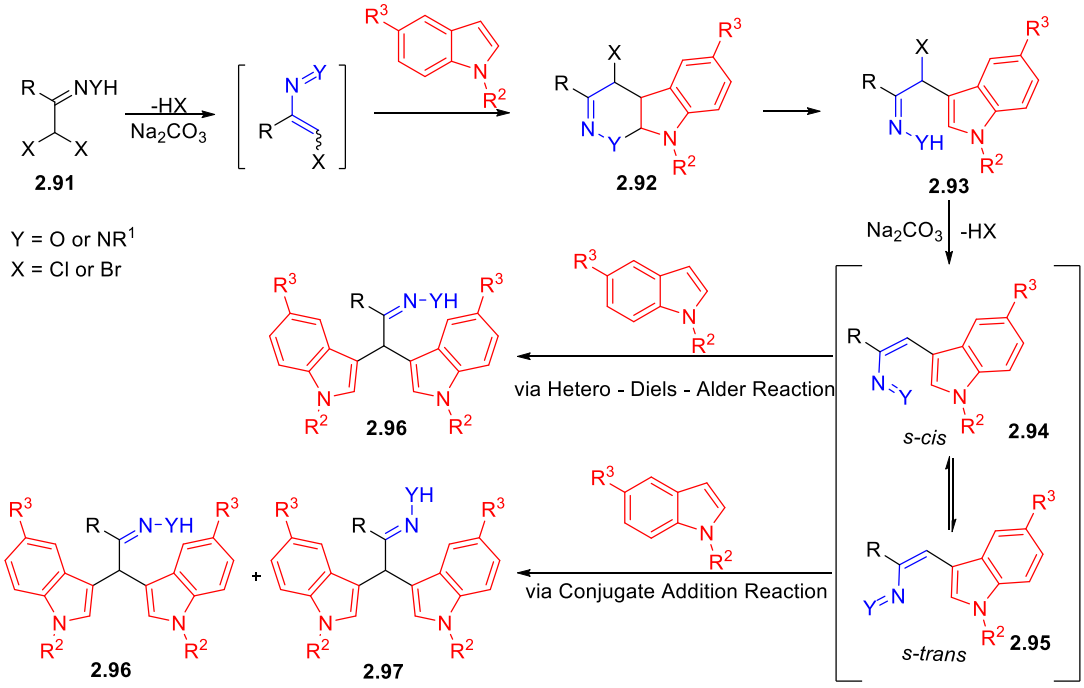
However, the isolation of two isomeric oximes or hydrazones in some of these transformations cannot be explained by a process involving only HDA cycloaddition reactions.

The generation of 4-haloazoalkenes from α,α'-dihalohydrazones has been proposed as an intermediate in various reactions.[19, 44, 45, 52, 60-62] Furthermore, the unprecedent catalytic

asymmetric inverse-electron-demand Diels Alder reaction between indole and a 4-chloroazoalkene, generated *in situ* from the appropriate α,α'-dichlorohydrazones, affording the corresponding chiral cycloadduct was reported by Wang *et al*..[40] On the other hand, similar studies in which HDA cycloadducts were isolated from the reaction of α,α'-dihalooximes with indoles via the generation of 4-halonitrosoalkenes, including the asymmetric synthesis of 4-halo-5,6-dihydro-4*H*-oxazines were also reported.[39, 63] The mechanistic proposal of the first alkylation step leading to 3-alkylated indoles via HDA reactions is therefore corroborated by these reaction outcomes.

However, the question is whether the reaction with the second indole molecule occurs via competitive cycloaddition or conjugate addition reaction. It is well established that HDA reaction should lead to BIMs **2.96** as single products, whereas conjugate addition allows the synthesis of the two stereoisomeric BIMs **2.96** /or **2.97** (Scheme 2.24).

The reaction of aryl α,α'-dihalooximes or α,α'-dihalohydrazones with indoles gave the isomer **2.96** as major products or single products. This indicate that HDA reaction must be the main mechanistic pathway, although in some cases the conjugate addition can also occur in a competitive fashion. On the other hand, starting from alkyl α,α'-dihalooximes or α,α'-dihalohydrazones the reaction with indoles afforded isomers **2.97** as single or major products. Thus, the formation of these bis(indolyl)methanes can only be explained considering that, in the second alkylation step, indoles participate in conjugate addition reactions with 3-alkylheterodienes, at the *s*-trans conformation, followed by 1,5-sigmatropic hydrogen shift (Scheme 2.24).




**Scheme 2.24** Proposed mechanism for the one-pot synthesis of BIMs.

### 2.3.5 Anti-cancer activity of BIMs

Initially, the cytotoxicity of compounds **2.82a**, **2.82e** and **2.82d** was evaluated in different tumoral cell lines, namely HepG2 (hepatocellular carcinoma), MDA-MB-468 (human breast carcinoma), RAW 264.7 (murine leukaemic monocyte macrophages), THP1 (human acute monocytic leukaemia), U937 (human leukaemic monocytic lymphoma) and EL4 (murine T-Lymphoma). The compounds selectivity towards tumoral cells was assessed determining their cytotoxicity with respect to two non-tumoral derived cell lines S17 (murine bone marrow) and N9 cells (murine microglial).

Results of the half maximal inhibitory concentrations ($IC_{50}$) are shown in Table 2.6 together with the toxicity of etoposide, a known anti-tumoral drug. Compound **2.82e** was considerably less cytotoxic against the tumoral cells than the other two compounds with $IC_{50}$ values ranging from 35.7 (HepG2) to 124 μM (THP1) and no selectivity was observed.

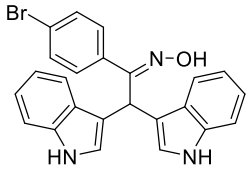
However, compounds **2.82a** and **2.82d** were considerably cytotoxic to all cells tested, with $IC_{50}$ values varying from 1.62 (THP1) to 23.9 μM (RAW) and from 10.7 (MDA) to 34.1 μM (U937), respectively.

Surprisingly, compound **2.82a** showed particularly cytotoxicity against leukaemia and lymphoma cell lines, showing $IC_{50}$ values in range of 1.6 μM. In addition, the highest selectivity indexes 9.86-14.2 were also observed for compound **2.82a** (Table 2.6 and Figure 2.23). This study allowed to establish the new 1-hydroxyliminomethyl-

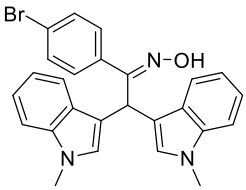
bis(indolyl)methane **2.82a** as the most promising scaffold for the design of new anticancer compounds against leukaemia and lymphoma.

Some conclusions regarding structure-activity relationships were also redrawn based on the biological evaluation of these BIMs. In fact, there is a dramatic difference in the anticancer activity when comparing *N*-unsubstituted BIM **2.82a** and *N*-methyl substituted BIM derivative **2.82e**, since **2.82a** present high $IC_{50}$ values. On the other hand, the significantly lower $IC_{50}$ values achieved for **2.82a** in comparison with the ones obtained for **2.82d** demonstrate that the presence of the bromo substituent is crucial to ensure high cytotoxic activity (Table 2.6).

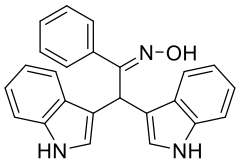
**Table 2.6** - Cytotoxicity of BIMs **2.82a**, **2.82e** and **2.82d** and etoposide (positive control) against several tumoral and non-tumoral cell lines. Results are expressed as $IC_{50}$ values (mean ± SEM) in μM and the selectivity index was calculated for two non-tumour cell lines (S17 and N9).




**2.82a**




**2.82e**




**2.82d**

| | **Etoposide** | | | **2.82a** | | | **2.82e** | | | **2.82d** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | SI (S17) | SI (N9) | $IC_{50}$ (μM) | SI (S17) | SI (N9) | $IC_{50}$ (μM) | SI (S17) | SI (N9) | $IC_{50}$ (μM) | SI (S17) | SI (N9) |
| **HepG2** | 1.40 ± 0.10 | 8.97 | 1.71 | 16.6 ± 0.07 | 0.98 | 1.38 | 35.7 ± 0.40 | 1.44 | 0.56 | 28.0 ± 0.49 | 0.94 | 1.05 |
| **MDA** | 6.69 ± 0.44 | 3.68 | 0.88 | 22.1 ± 0.18 | 0.73 | 1.04 | 36.7 ± 0.57 | 0.81 | 0.31 | 10.7 ± 0.19 | 2.45 | 2.72 |
| **RAW** | 0.82 ± 0.03 | 9.55 | 1.88 | 23.9 ± 0.30 | 0.68 | 0.96 | 42.2 ± 0.41 | 1.22 | 0.47 | 12.2 ± 0.05 | 2.16 | 2.40 |
| **THP1** | 1.82 ± 0.06 | 8.55 | 1.81 | 1.62 ± 0.08 | 10.04 | 14.2 | 124 ± 11.5 | 0.41 | 0.16 | 18.1 ± 0.26 | 1.45 | 1.61 |
| **EL4** | 4.90 ± 0.47 | 5.47 | 0.80 | 1.65 ± 0.02 | 9.86 | 13.94 | 106 ± 10.5 | 0.48 | 0.19 | 11.9 ± 0.07 | 2.21 | 2.45 |
| **U937** | 1.10 ± 0.04 | 9.27 | 1.85 | 1.64 ± 0.08 | 9.92 | 14.02 | 97.0 ± 4.14 | 0.53 | 0.20 | 34.1 ± 0.69 | 0.77 | 0.86 |
| **S17** | 10.4 ± 0.15 | | | 16.3 ± 0.14 | | | 51.4 ± 1.07 | | | 26.3 ± 0.23 | | |
| **N9** | 1.95 ± 0.12 | | | 23.0 ± 0.34 | | | 19.8 ± 0.74 | | | 29.2 ± 0.29 | | |

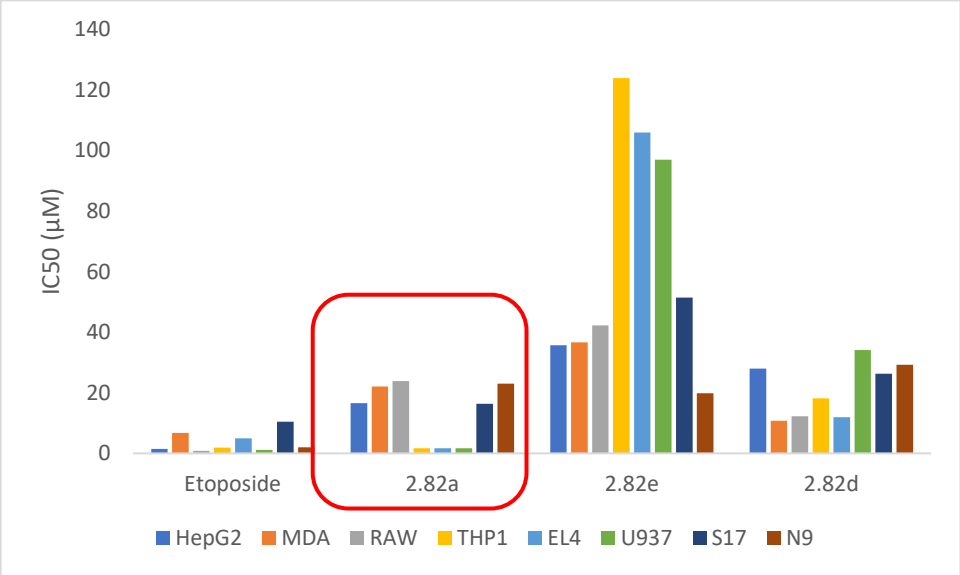
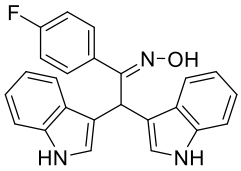


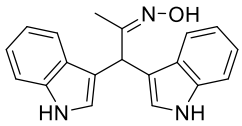

**Figure 2.20** Comparison between compounds **2.82a**, **2.82e** and **2.82d** and etoposide (positive control) and cell lines. Graphical representation of table 2.6.

The observed high cytotoxicity of BIM **2.82a** against THP1, EL4 and U937 cell lines led us to extend this study to BIMs **2.82c**, **2.82** and **2.83f** (Table 2.7). The compound **2.82c**, bearing a 4-fluorphenyl substituent and C-3 position, showed moderate cytotoxicity against the mentioned cell lines and was not selective. These results emphasize the fact that the presence of the 4-bromophenyl is vital to ensure low $IC_{50}$ values. On the other hand, alkyl oximes **2.82f** and **2.83f**. were even less active against THP1, EL4 and U937 cell lines and showed no significant difference between isomeric compounds.

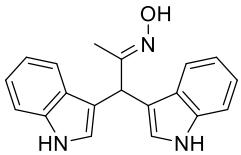
**Table 2.7** Cytotoxicity of compounds **2.82c**, **2.82f** and **2.83f** and etoposide (positive control) against three tumoral cell lines (THP1, EL4 and U937). Results are expressed as $IC_{50}$ values (mean ± SEM) in μM and the selectivity index was calculated for non-tumour cell line S17.




**2.82c**




**2.82f**




**2.83f**

| | **Etoposide** | | **2.82c** | | **2.82f** | | **2.83f** | |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | SI (S17) | $IC_{50}$ (μM) | SI (S17) | $IC_{50}$ (μM) | SI (S17) | $IC_{50}$ (μM) | SI (S17) |
| **THP1** | 1.82 ± 0.06 | 8.55 | 23.9 ± 0.9 | 0.89 | 30.7 ± 0.1 | 0.62 | 57.6 ± 0.5 | 0.60 |
| **EL4** | 4.90 ± 0.47 | 5.47 | 16.9 ± 1.6 | 1.47 | 23.9 ± 0.9 | 1.09 | 39.9 ± 3.7 | 0.92 |
| **U937** | 1.10 ± 0.04 | 9.27 | 28.0 ± 1.6 | 1.08 | 42.5 ± 0.5 | 0.85 | 59.3 ± 0.5 | 0.62 |
| **S17** | 10.4 ± 0.15 | | 24.9 ± 0.1 | | 26.1 ± 0.4 | | 35.8 ± 0.4 | |

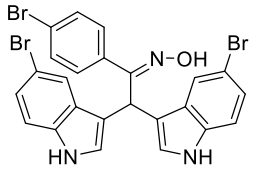
The cytotoxicity of BIMs **2.82g–2.82j**, **2.85a–2.85d**, **2.85f**, **2.89** and **2.90** were also evaluated in the three cell lines for which BIM **2.82a** (scaffold) showed significant anti-tumoral activity. Results of $IC_{50}$ for these BIMs are shown in table 2.8 together with the toxicity of etoposide. As with BIMs **2.82** and **2.83** , the selectivity of these compounds against tumour cells was evaluated by determining their cytotoxicity with respect to the non-tumour cell line S17. Compounds **2.82g-j** showed an interesting anti-tumoral activity particularly against lymphoma cell lines (EL4), with values of $IC_{50}$ ranging from 3.23 μM to 15.6 μM. Among the studied BIMs, the most promising was BIM **2.82c** with $IC_{50}$ values of 3.23 μM (EL4), 6.34 μM (THP-1) and 9.79 μM (U-937). However, scaffold **2.82a** is still a more efficient anti-cancer agent than BIM derivatives **2.82g-2.82j**. On the other hand, BIM oxime **2.82a** showed high selectivity whereas low selectivity was observed for 1-hydroxyiminomethyl-bis(indolyl)methanes **2.82g-2.82j**.

The cytotoxicity of 1-*tert*-butoxycarbonylhydrazonomethyl-bis(indolyl)methanes **2.85a–2.85d** and **2.85f** was also evaluated in the same cell lines and although the results indicated moderate anti-cancer activity, compound **2.85b** derived from *N*-benzylindole, presented significant cytotoxic against EL4 with a $IC_{50}$ of 5.56 μM (Table 2.8). However, this compound was less cytotoxic against THP-1 cell ($IC_{50}$ = 38.8 μM) and no cytotoxic against U937 cell line was observed. BIM **2.85a**, derived from *N*-methylindole was also active for EL4, with $IC_{50}$ value of 9.99 μM. Particularly surprising was the result obtained

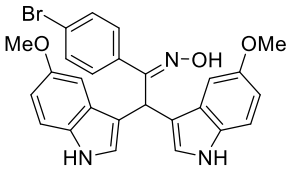
with compound **2.85c**, derived from *N*-unsubstituted indole, showing lower activity than **2.85a** and **2.85b**, which contrast with the previously results obtained for BIM oximes where *N*-unsubstituted BIMs were the most active. Regarding BIMs **2.85d** and **2.85f**, derived from 5-bromo and 5-hydroxyindole, respectively, moderate anti-cancer activity was observed ($IC_{50}$ = 15.4 μM and 22.5 μM, respectively). Nevertheless, BIM hydrazone **2.85d** showed the highest selectivity (SI > 6.49).

Finally, to explore the impact of the hydrazone moiety substituent, the cytotoxicity of bis(indolyl)methanes **2.89** and **2.90** was also evaluated. Compound **2.89**, bearing a tosyl substituent did not showed significant differences in anti-cancer activity against the three cell lines, whereas BIM **2.90**, bearing 2,4-dinitrophenyl group as substituent, showed a considerable cytotoxicity against EL4 with $IC_{50}$ value of 5.45 μM .

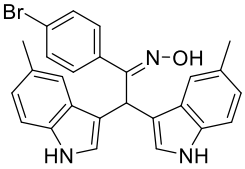
**Table 2.8** - Cytotoxicity of compounds **2.82g–2.82j**, **2.85a–2.85d**, **2.85f**, **2.89** and **2.90** and etoposide (positive control) against three tumoral and one non-tumoral cell lines. Results are expressed as $IC_{50}$ (μM). Values and the selectivity index were calculated using a non-tumour cell line (S17).



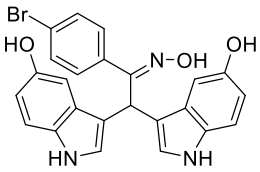

**2.82g**



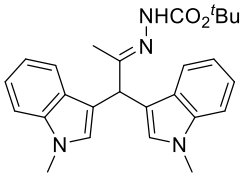

**2.82h**



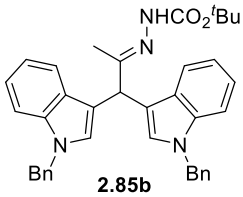

**2.82i**



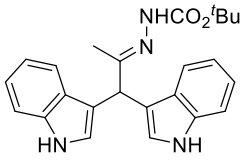

**2.82j**



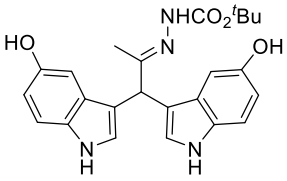

**2.85a**



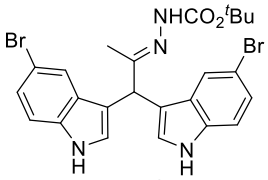

**2.85b**



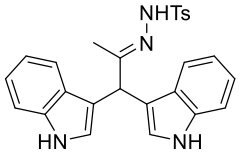

**2.85c**



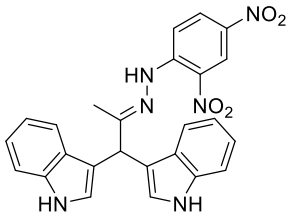

**2.85d**




**2.85f**




**2.89**



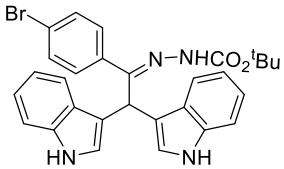

**2.90**

| Compound/Cell lines | EL4 | THP-1 | U937 | S17 | Selectivity | | |
|---|---|---|---|---|---|---|---|
| | | | | | EL4 | THP-1 | U937 |
| **Etoposide** | 4.90 | 1.82 | 1.10 | 10.4 | 5.47 | 8.55 | 9.27 |
| **2.82g** | 7.65 | 12.2 | 18.6 | N.D. | N.D. | N.D. | N.D. |
| **2.82h** | 9.29 | 22.6 | 18.1 | 17.7 | 1.91 | < 1 | < 1 |
| **2.82i** | 3.23 | 6.34 | 9.79 | 11.5 | 3.56 | 1.81 | 1.17 |
| **2.82j** | 15.6 | 15.5 | 12.8 | 21.9 | 1.40 | 1.41 | 1.71 |
| **2.85a** | 9.99 | 25.3 | 14.2 | N.D. | N.D. | N.D. | N.D. |
| **2.85b** | 5.56 | 38.8 | N.A.[a] | 7.65 | 1.38 | < 1 | < 1 |
| **2.85c** | 15.3 | 30.0 | 25.0 | 50.0 | 3.27 | 1.67 | 2.00 |
| **2.85d** | 15.4 | 28.6 | 156.9 | N.A.[b] | 6.49 | 3.50 | < 1 |
| **2.85f** | 22.5 | 36.2 | 28.3 | 41.3 | 1.84 | 1.14 | 1.46 |
| **2.89** | 18.9 | 23.5 | 22.9 | 25.0 | 1.32 | 1.06 | 1.09 |
| **2.90** | 5.45 | 14.0 | 13.3 | 7.92 | 1.45 | < 1 | < 1 |

N.D. – not determined; N.A. – not active.
[a] Cell viability: 66.6% at 100 μM.
[b] Cell viability: 48.4% at 100 μM.

Additionally, the cytotoxicity of BIM **2.87d** was evaluated in three colorectal cancer stem cells (CSCs) lines HT-29, SW-620, HCT116. Salinomycin (anti-CSC agent) and 5-FU (traditional chemotherapeutic) were used as a positive control and negative control, respectively. In CSC-enriched spheroids derived from HT-29, HCT116 and SW-620 cell lines, BIM **2.87d** displayed $IC_{50}$ values (0.24 μM, 0.45 μM, 0.56 μM) similar to the positive control salinomycin ( 0.25 μM , 0.74 μM , 0.09 μM) (Table 2.9).

**Table 2.9 -** Cytotoxicity of compound **2.87d**, salinomycim (anti-CSC agent and positive control) and 5-FU (tradicional chemotherapeutic and negative control) against CSC-enriched spheroids derived from three colorectal cancer cell lines HT-29, SW-620, HCT116. $IC_{50}$ (μM) values and confidence intervals at 95% ($CI_{95}$) are presented.




**2.87d**

| | 3D (CSC-enriched conditions) | | | | | |
|---|---|---|---|---|---|---|
| | **HT-29** | | **SW-620** | | **HCT116** | |
| | **$IC_{50}$ (μM)** | $CI_{95}$ | **$IC_{50}$ (μM)** | $CI_{95}$ | **$IC_{50}$ (μM)** | $CI_{95}$ |
| **Salinomycin** | **0.25** | 0.001-0.46 | **0.74** | 0.61-0.88 | **0.09** | 0.04-0.13 |
| **5-FU** | **1.74** | 0.24-2.77 | **0.61** | 0.46-0.83 | **1.35** | 0.85-2.10 |
| **2.87d** | **0.24** | 0.15-0.35 | **0.45** | 0.38-0.51 | **0.56** | 0.40-0.75 |

To evaluate the potential compound toxicity, the $IC_{50}$ determination in primary mouse hepatocytes was carried out. The results showed that the $IC_{50}$ of compound **2.87d** (3.02 μM ) is higher than salinomycin (1.74 μM) (Table 2.10).

**Table 2.10** – $IC_{50}$ determination in primary mouse hepatocytes of BIM **2.87d**, salinomycim (anti-CSC agent and positive control) and 5-FU (traditional chemotherapeutic and negative control).

| | Primary mouse hapatocytes | |
|---|---|---|
| | **$IC_{50}$ (μM)** | 95CI |
| **Salinomycin** | **1.74** | 0.93-3.24 |
| **5-FU** | **80.89** | - |
| **2.87d** | **3.02** | - |

Finally, tumorspheres numbers were counted and spheres area was determined. Although BIM **2.87d** reduced the tumorspheres area in all cell lines (Figure 2.21a), the number of HCT116-derived spheres significantly increased (Figure 2.21b).

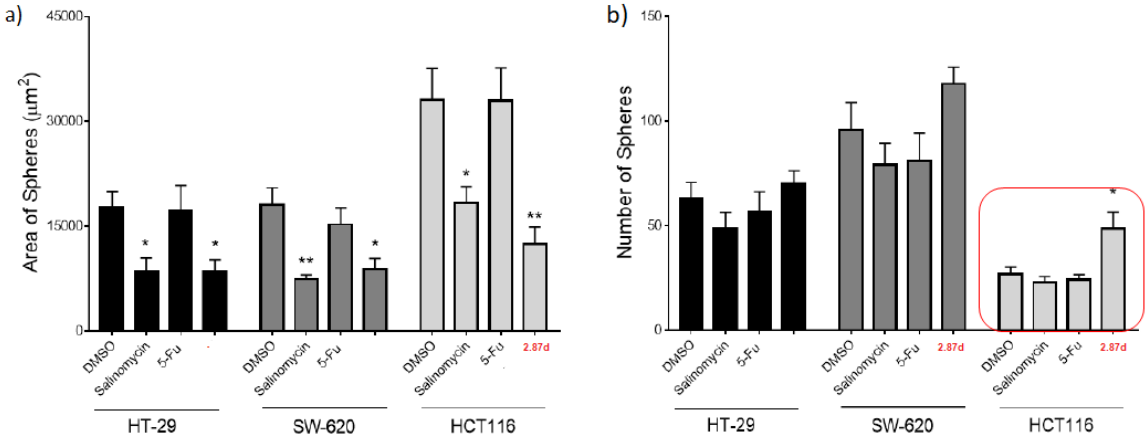
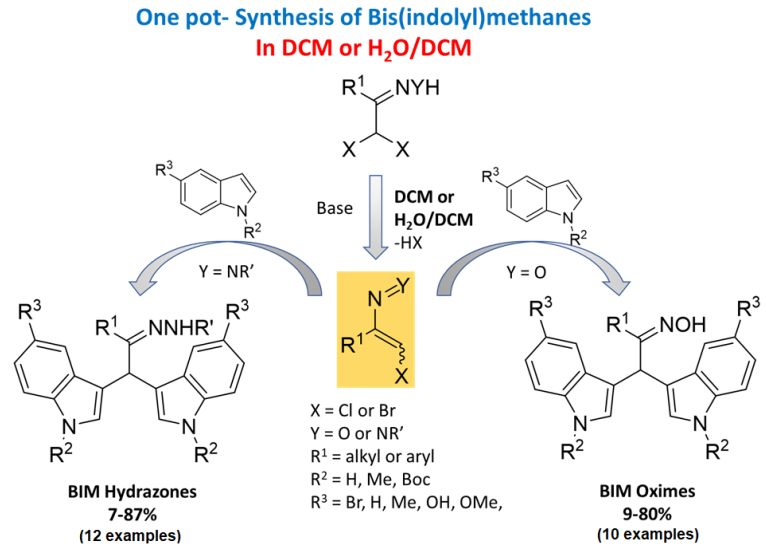


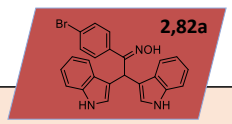

**Figure 2.21** a) Determined spheres area; b) Number of tumorspheres for BIM **2.87d**.

## 2.4 Conclusions

In summary, two consecutive HAD reactions or a HAD followed by a 1,4-conjugate addition reaction of electrophilic nitroso- an azoalkenes with electron rich indoles led to a new class of bis(indolyl)methanes. The methodology proved to be versatile and characterised by a very broad scope, leading to a library of new BIM oximes and hydrazones. On the other hand, the substitution pattern of the starting α,α'-dihalooximes and α,α'-dihalohydrazones and/or indoles allowed an easy modulation of the BIM structure. Reactions were carried out in dichloromethane (DCM) or in water using DCM as co-solvent. The latter solvent system proved to be the best reaction medium for the synthesis of these new BIMs, affording the desired products in higher yield, shorter reaction time and easier isolation procedures. Also, to our delight, under this methodology, novel BIM oximes and hydrazones were efficiently obtained using *N*-unsubstituted indoles.

Furthermore, the novel BIMs showed relevant "in vitro" anticancer activity, particularly against lymphoma cell lines, and a promising scaffold **2.82a** has been identified.[64, 65]




| | | | | Etoposide | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (µM) | SI (S17) | Si (N9) | $IC_{50}$ (µM) | SI (S17) | Si (N9) |
| THP1 | 1,62 ± 0,08 | 10,04 | 14,20 | 1,82 ± 0,06 | 8,55 | 1,81 |
| EL4 | 1,65 ± 0,02 | 9,86 | 13,94 | 4,90 ± 0,47 | 5,47 | 0,80 |
| U937 | 1,64 ± 0,08 | 9,92 | 14,02 | 1,10 ± 0,04 | 9,27 | 1,85 |

## 2.5 References

1. Gilchrist, T. L., "Heterocyclic Chemistry", 3Ed., Longmans, . **1997**.

2. Nicolaou, K. C.; Snyder, S. A.; Montagnon, T.; Vassilikogiannakis, G., The Diels-Alder reaction in total synthesis. *Angew. Chem., Int. Ed. Engl.* **2002**, *41* (10), 1668-1698.

3. Png, Z. M.; Zeng, H.; Ye, Q.; Xu, J., Inverse-electron-demand Diels-Alder reactions: Principles and applications. *Chem.: Asian J.* **2017**, *12* (17), 2142-2159.

4. Domingo, L. R.; Pérez, P.; Sáez, J. A., Understanding the regioselectivity in hetero Diels–Alder reactions. An ELF analysis of the reaction between nitrosoethylene and 1-vinylpyrrolidine. *Tetrahedron* **2013**, *69* (1), 107-114.

5. Domingo, L. R.; Pérez, P.; Sáez, J. A., Understanding the local reactivity in polar organic reactions through electrophilic and nucleophilic Parr functions. *RSC Adv.* **2013**, *3* (5), 1486-1494.

6. Jasiński, R., On the question of stepwise [4+2] cycloaddition reactions and their stereochemical aspects. *Symmetry* **2021**, *13* (10), 1911-1928.

7. Oliveira, B. L.; Guo, Z.; Bernardes, G. J. L., Inverse electron demand Diels–Alder reactions in chemical biology. *Chem. Soc. Rev.* **2017**, *46* (16), 4895-4950.

8. Arrieta, A.; Cossío, F. P.; Lecea, B., Direct evaluation of secondary orbital interactions in the Diels−Alder reaction between cyclopentadiene and maleic anhydride. *J. Org. Chem.* **2001**, *66* (18), 6178-6180.

9. Vidal, C.; Merz, L.; García-Álvarez, J., Deep eutectic solvents: Biorenewable reaction media for Au(i)-catalysed cycloisomerisations and one-pot tandem cycloisomerisation/Diels–Alder reactions. *Green Chem.* **2015**, *17* (7), 3870-3878.

10. Gilchrist, T. L., Nitroso-alkenes and nitroso-alkynes. *Chem. Soc. Rev.* **1983**, *12* (1), 53-73.

11. Lyapkalo, I. y. M.; Ioffe, S. L., Conjugated nitrosoalkenes. *Russ. Chem. Rev.* **1998**, *67* (6), 467-484.

12. Molander, G. A.; Trost, B. M.; Abramite, J. A.; Arrayás, R. G.; Avilov, D.; Block, E.; Carretero, J. C.; Collier, S. J.; Dittmer, D.; Drabowicz, J.; Gaumont, A. C.; Gulea, M.; Jubault, P.; Keglevich, G.; Kiełbasiński, P.; Leclerc, E.; McLaws, M. D.; Mikołajczyk, M.; Ono, N.; Quirion, J. C.; Reissig, H. U.; Sammakia, T.; Sammons, M. F.; Szelke, H.; Zimmer, R., Product subclass 2: 1-Nitrosoalkenes. In *Houben–Weyl Methods of Molecular Transformations*, 2007.

13. Attanasi, O. A.; De Crescentini, L.; Favi, G.; Filippone, P.; Mantellini, F.; Perrulli, F. R.; Santeusanio, S., Cultivating the passion to build heterocycles from 1,2-diaza-1,3-dienes: The force of imagination. *Eur. J. Org. Chem.* **2009**, *2009* (19), 3109-3127.

14. Lemos, A., Cycloaddition reactions of conjugated azoalkenes. In targets in heterocyclic systems: Chemistry and properties. *Attanasi, O. A., Spinelli, D., Eds.; Soc Chimica Italiana: Rome* **2010**, *14*, 1-18.

15. Jorda, R.; Lopes, S. M. M.; Řezníčková, E.; Kryštof, V.; Pinho e Melo, T. M. V. D., Biological evaluation of dipyrromethanes in cancer cell lines: antiproliferative and pro-apoptotic properties. *ChemMedChem* **2017**, *12* (9), 701-711.

16. Lopes, S. M. M.; Novais, J. S.; Costa, D. C. S.; Castro, H. C.; Figueiredo, A. M. S.; Ferreira, V. F.; Pinho e Melo, T. M. V. D.; da Silva, F. d. C., Hetero-Diels-Alder reactions of novel 3-triazolyl-nitrosoalkenes as an approach to functionalized 1,2,3-triazoles with antibacterial profile. *Eur. J. Med. Chem.* **2018**, *143*, 1010-1020.

17. Lopes, S. M. M.; Cardoso, A. L.; Lemos, A.; Pinho e Melo, T. M. V. D., Recent advances in the chemistry of conjugated nitrosoalkenes and azoalkenes. *Chem. Rev.* **2018**, *118* (23), 11324-11352.

18. Panice, M. R.; Lopes, S. M. M.; Figueiredo, M. C.; Goes Ruiz, A. L. T.; Foglio, M. A.; Nazari Formagio, A. S.; Sarragiotto, M. H.; Pinho e Melo, T. M. V. D., New 3-tetrazolyl-β-carbolines and β-carboline-3-carboxylates with anti-cancer activity. *Eur. J. Med. Chem.* **2019**, *179*, 123-132.

19. Lopes, S. M. M.; Pinho e Melo, T. M. V. D., Meso-substituted corroles from nitrosoalkenes and dipyrromethanes. *J. Org. Chem.* **2020**, *85* (5), 3328-3335.

20. Attanasi, O.; Filippone, P., Working twenty years on conjugated azo-alkenes (and environs) to find new entries in organic synthesis. *Synlett* **1997**, *1997* (10), 1128-1140.

21. Rai, K. M. L., Heterocycles via oxime cycloadditions. In *Synthesis of Heterocycles via Cycloadditions II*, 2008, 1-69.

22. M. de los Santos, J.; Vicario, J.; Alonso, C.; Palacios, F., *b*-Hydroxyimino phosphorus derivatives. An efficient tool in organic synthesis. *Curr. Org. Chem.* **2011**, *15* (10), 1644-1660.

23. Sukhorukov, A. Y.; Ioffe, S. L., Chemistry of six-membered cyclic oxime ethers. Application in the synthesis of bioactive compounds. *Chem. Rev.* **2011**, *111* (8), 5004-5041.

24. Belskaya, N. P.; Eliseeva, A. I.; Bakulev, V. A., Hydrazones as substrates for cycloaddition reactions. *Russ. Chem. Rev.* **2015**, *84* (12), 1226-1257.

25. Boyko, Y. D.; Dorokhov, V. S.; Sukhorukov, A. Y.; Ioffe, S. L., Conjugated nitrosoalkenes as Michael acceptors in carbon–carbon bond forming reactions: a review and perspective. *Beilstein J. Org. Chem.* **2017**, *13*, 2214-2234.

26. Naumovich, Y. A.; Ioffe, S. L.; Sukhorukov, A. Y., Michael addition of P-nucleophiles to conjugated nitrosoalkenes. *J. Org. Chem.* **2019**, *84* (11), 7244-7254.

27. Lemos, A., Addition and cycloaddition reactions of phosphinyl- and phosphonyl-2*H*-azirines, nitrosoalkenes and azoalkenes. *Molecules* **2009**, *14* (10), 4098-4119.

28. Waring, A.; Attanasi, O. A.; Crescentini, L. D.; Filippone, P.; Mantellini, F.; Santeusanio, S., 1,2-Diaza-1,3-butadienes: just a nice class of compounds, or powerful tools in organic chemistry? Reviewing an experience. *Arkivoc* **2003**, *2002* (11), 274-292.

29. Faragher, R.; Gilchrist, T. L., Cycloaddition reactions of nitrosoalkenes and azoalkenes with cyclopentadiene and other dienes. *J. Chem. Soc., Perkin trans.* **1979**.

30. G. Tsoungas, P., Synthesis of 1,2-oxazines and their *N*-oxides. *Heterocycles* **2002**, *57* (6).

31. Gilchrist, T. L.; Stretch, W.; Chrystal, E. J. T., Reaction of azoles with ethyl bromopyruvate oxime: alkylation by substitution and by elimination–addition. *J. Chem. Soc., Perkin Trans. 1* **1987**, (0), 2235-2239.

32. Gilchrist, T. L.; Roberts, T. G., Addition and cycloaddition reactions of the electrophilic vinyl nitroso compounds 3-nitrosobut-3-en-2-one, 2-nitrosopropenal, and ethyl 2-nitrosopropenoate. *J. Chem. Soc., Perkin trans.* **1983**.

33. Lopes, S. M. M.; Palacios, F.; Lemos, A.; Pinho e Melo, T. M. V. D., Diels–Alder reactions of 3-(1H-tetrazol-5-yl)-nitrosoalkenes: synthesis of functionalized 5-(substituted)-1H-tetrazoles. *Tetrahedron* **2011**, *67* (46), 8902-8909.

34. Lopes, S. M. M.; Lemos, A.; Melo, T. M. V. D. P. e., A hetero-Diels–Alder approach to functionalized 1H-tetrazoles: synthesis of tetrazolyl-1,2-oxazines, -oximes and 5-(1-aminoalkyl)-1H-tetrazoles. *Tetrahedron Lett.* **2010**, *51* (51), 6756-6759.

35. Nunes, S. C. C.; Lopes, S. M. M.; Gomes, C. S. B.; Lemos, A.; Pais, A. A. C. C.; Pinho e Melo, T. M. V. D., Reactions of nitrosoalkenes with dipyrromethanes and pyrroles: Insight into the mechanistic pathway. *J. Org. Chem.* **2014**, *79* (21), 10456-10465.

36. Clarke, S. J.; Gilchrist, T. L.; Lemos, A.; Roberts, T. G., Reactions of azoalkenes derived from hydrazones of ethyl bromopyruvate with electron rich alkenes and heterocycles. *Tetrahedron* **1991**, *47* (29), 5615-5624.

37. de los Santos, J. M.; Rubiales, G.; Sbai, Z. E.; Ochoa de Retana, A. M.; Palacios, F., Reaction of phosphinylated nitrosoalkenes with electron-rich heterocycles. Electrophilic aromatic substitution vs. cycloaddition. *Org. Biomol. Chem.* **2017**, *15* (3), 662-671.

38. Lopes, S. M. M.; Nunes, S. C. C.; Caratão, C. C.; Pais, A. A. C. C.; Pinho e Melo, T. M. V. D., Reactivity of 1-arylnitrosoethylenes towards indole derivatives. *Monatsh. Chem.* **2016**, *147* (9), 1565-1573.

39. Zhang, Y.; Stephens, D.; Hernandez, G.; Mendoza, R.; Larionov, O. V., Catalytic diastereo- and enantioselective annulations between transient nitrosoalkenes and indoles. *Chem. Eur. J.* **2012**, *18* (52), 16612-16615.

40. Tong, M.-C.; Chen, X.; Li, J.; Huang, R.; Tao, H.; Wang, C.-J., Catalytic asymmetric synthesis of [2,3]-fused indoline heterocycles throughiInverse-electron-demand aza-Diels-Alder reaction of indoles with azoalkenes. *Angew. Chem., Int. Ed. Engl.* **2014**, *53* (18), 4680-4684.

41. Wei, L.; Wang, C.-J., The catalytic asymmetric synthesis of tetrahydropyridazines via inverse electron-demand aza-Diels–Alder reaction of enol ethers with azoalkenes. *Chem. Comm.* **2015**, *51* (84), 15374-15377.

42. Lopes, S. M. M.; Henriques, M. S. C.; Paixão, J. A.; Pinho e Melo, T. M. V. D., Exploring the chemistry of furans: Synthesis of functionalized bis(furan-2-yl)methanes and 1,6-dihydropyridazines. *Eur. J. Org. Chem.* **2015**, *2015* (28), 6146-6151.

43. Alves, A. J. S.; Lopes, S. M. M.; Henriques, M. S. C.; Paixão, J. A.; Pinho e Melo, T. M. V. D., Hetero-Diels-Alder and ring-opening reactions of furans applied to the synthesis of functionalized heterocycles. *Eur. J. Org. Chem.* **2017**, *2017* (27), 4011-4025.

44. Pereira, N. A. M.; Lopes, S. M. M.; Lemos, A.; Melo, T. M. V. D. P. e., On-water synthesis of dipyrromethanes via bis-hetero-diels-alder reaction of azo- and nitrosoalkenes with pyrrole. *Synlett* **2014**, *25* (3), 423-427.

45. Lopes, S. M. M.; Lemos, A.; Pinho e Melo, T. M. V. D., Reactivity of dipyrromethanes towards azoalkenes: Synthesis of functionalized dipyrromethanes, calix[4]pyrroles, and bilanes. *Eur. J. Org. Chem.* **2014**, *2014* (31), 7039-7048.

46. Pereira, N. A. M.; Lemos, A.; Serra, A. C.; Pinho e Melo, T. M. V. D., Functionalization of dipyrromethanes via hetero-Diels–Alder reaction with azo- and nitrosoalkenes. *Tetrahedron Lett.* **2013**, *54* (12), 1553-1557.

47. Lopes, S. M. M.; Brigas, A. F.; Palacios, F.; Lemos, A.; Pinho e Melo, T. M. V. D., [4+2] Cycloadditions of 3-tetrazolyl-1,2-diaza-1,3-butadienes: synthesis of 3-tetrazolyl-1,4,5,6-tetrahydropyridazines. *Eur. J. Org. Chem.* **2012**, *2012* (11), 2152-2160.

48. Yoon, S. C.; Cho, J.; Kim, K., Reactions of 1-aryl-2,2-dihalogenoethanone oximes with tetrasulfur tetranitride (S4N4): a general method for the synthesis of 3-aryl-4-halogeno-1,2,5-thiadiazoles. *J Chem Soc Perk T 1* **1998**, (1), 109-116.

49. Lemos, A.; Lourenço, J. P., Cycloaddition reactions of nitrosoalkenes, azoalkenes and nitrile oxides mediated by hydrotalcite. *Arkivoc* **2010**, 170-182.

50. Matunas, R.; Lai, A. J.; Lee, C., Iridium-catalyzed selective *N*-allylation of hydrazines. *Tetrahedron* **2005**, *61* (26), 6298-6308.

51. South, M. S.; Jakuboski, T. L.; Westmeyer, M. D.; Dukesherer, D. R., Synthesis and reactions of haloazodienes. A new and general synthesis of substituted pyridazines. *J. Org. Chem.* **1996**, *61* (25), 8921-8934.

52. Sakai, K.; Hida, N.; Kondo, K., Reactions of alpha-polyhalo ketone tosylhydrazones with sulfide Ion and primary amines - cyclization to 1,2,3-thiadiazoles and 1,2,3-triazoles. *B. Chem. Soc. Jpn.* **1986**, *59* (1), 179-183.

53. Maynard, J. A., Use of diethyl phosphonate as a solvent and catalyst for preparation of arylhydrazones. *Aust. J. Chem.* **1962**, *15* (4), 867-&.

54. Chanda, A.; Fokin, V. V., Organic synthesis "On Water". *Chem. Rev.* **2009**, *109* (2), 725-748.

55. Zuo, Y.-J.; Qu, J., How Does Aqueous Solubility of Organic Reactant Affect a Water-Promoted Reaction? *J. Org. Chem.* **2014**, *79* (15), 6832-6839.

56. Narayan, S.; Muldoon, J.; Finn, M. G.; Fokin, V. V.; Kolb, H. C.; Sharpless, K. B., "On Water": Unique Reactivity of Organic Compounds in Aqueous Suspension. *Angew. Chem., Int. Ed. Engl.* **2005**, *44* (21), 3275-3279.

57. Attanasi, O. A.; De Crescentini, L.; Filippone, P.; Fringuelli, F.; Mantellini, F.; Matteucci, M.; Piermatti, O.; Pizzo, F., `Inverse-Electron-Demand'Diels-Alder Reactions of (E)-3-Diazenylbut-2-enes in Water. *Helv. Chim. Acta* **2001**, *84* (2), 513-525.

58. J., C. S.; Gilchrist, T. L.; Lemos, A.; Roberts, T. G., Reactions of Azoalkenes derived from Hydrazones of Ethyl Bromopyruvate with Electron Rich Alkenes and Heterocycles. *Tetrahedron* **1991**, *47*, 5615-5624.

59. Bocchi, V.; Casnati, G.; Dossena, A.; Villani, F., Synthesis of *N*-alkylindoles using tetraalkylammonium salt catalysis. *Synthesis-Stuttgart* **1976**, (6), 414-416.

60. Mantenuto, S.; Lucarini, S.; De Santi, M.; Piersanti, G.; Brandi, G.; Favi, G.; Mantellini, F., One-Pot synthesis of biheterocycles based on indole and azole scaffolds using tryptamines and 1,2-diaza-1,3-dienes as building blocks. *Eur. J. Org. Chem.* **2016**, *2016* (19), 3193-3199.

61. Hanselmann, R.; Job, G. E.; Johnson, G.; Lou, R.; Martynow, J. G.; Reeve, M. M., Synthesis of an antibacterial compound containing a 1,4-substituted 1*H*-1,2,3-triazole: a scaleable alternative to the "click" reaction. *Org. Process Res. Dev.* **2009**, *14* (1), 152-158.

62. Hatcher, J. M.; Coltart, D. M., Copper(I)-catalyzed addition of Grignard reagents to in situ-derived *N*-sulfonyl azoalkenes: An umpolung alkylation procedure applicable to the formation of up to three contiguous quaternary centers. *J. Am. Chem. Soc.* **2010**, *132* (13), 4546-4547.

63. Yoon, S. C.; Kim, K.; Park, Y. J., Hetero-Diels−Alder reactions of α-aryl-β-monohalo-α-nitrosoethylenes: Diastereoselective synthesis of 6-substituted 3-aryl-4-halo-5,6-dihydro-4*H*-1,2-oxazines. *J. Org. Chem.* **2001**, *66* (22), 7334-7341.

64. Grosso, C.; Cardoso, A. L.; Lemos, A.; Varela, J.; Rodrigues, M. J.; Custódio, L.; Barreira, L.; Pinho e Melo, T. M. V. D., Novel approach to bis(indolyl)methanes: De novo synthesis of 1-hydroxyiminomethyl derivatives with anti-cancer properties. *Eur. J. Med. Chem.* **2015**, *93*, 9-15.

65. Grosso, C.; Cardoso, A. L.; Rodrigues, M. J.; Marques, C.; Barreira, L.; Lemos, A.; Pinho e Melo, T. M. D. V., Hetero-Diels-Alder approach to bis(indolyl)methanes. *Bioorg. Med. Chem.* **2017**, *25* (3), 1122-1131.

# Chapter 3

## Natural Deep Eutectic Solvents in the Hetero-Diels-Alder Approach to Bis(indolyl)methanes


## Abstract

In this chapter, the use of natural deep eutectic solvents (NADES) as reaction media to carry out hetero-Diels-Alder reactions will be presented. This allowed to improve the efficiency and sustainability of the synthetic approach to bis(indolyl)methanes (BIMs) based on bis-hetero-Diels-Alder/conjugate addition reactions of nitroso- and azoalkenes with indoles. The ternary mixture of $H_2O$ with choline chloride/glycerol allowed the tuning of the physical properties of this NADES leading to a better solvent system, affording the target hydroxyiminomethyl- and hydrazonomethyl-BIMs, in much shorter reaction times, higher efficiency and easier isolation procedures. Furthermore, the direct access to carbonyl-BIMs was possible when 3-methyl-1-*tert*-butoxycarbonyl azoalkenes were used.

# Chapter 3. Natural Deep Eutectic Solvents in the Hetero-Diels-Alder Approach to Bis(indolyl)methanes

## 3.1 Deep eutectic solvents: solvents of the XXI century.

In the last decades, the pressure on the chemical industry to reduce the formation of waste, sewage, and toxic gases has been increasing due to the need to develop more sustainable processes. In this context, the scientific community has made enormous efforts to find more sustainable and less toxic reaction media than conventional organic solvents. Examples of these alternative reaction media are water, supercritical carbon dioxide, solvents derived from renewable resources, ionic liquids (ILs), and deep eutectic solvents (DESs).[1]

### 3.1.1 Definition and classification of deep eutectic solvents

DESs are low melting point temperature solids formed by mixing two or more phase-immiscible solid compounds, usually with higher melting points than the final eutectic mixture, which can become liquid, in certain ratio and at an exact temperature, the eutectic point. DESs are called natural deep eutectic solvents (NADES) when composed by natural products or therapeutic deep eutectic solvents (THEDES), when one of the components is an active pharmaceutical ingredient.[2-5] These remarkable solvents are usually formed from hydrogen bond acceptors (HBA) (e.g., choline chloride) or metal halides (e.g. $ZnCl_2$) and a hydrogen bond donor (HBD) (e.g. urea or carboxylic acids).[2-9] A schematic representation of a deep eutectic solvent is shown in Figure 3.1.

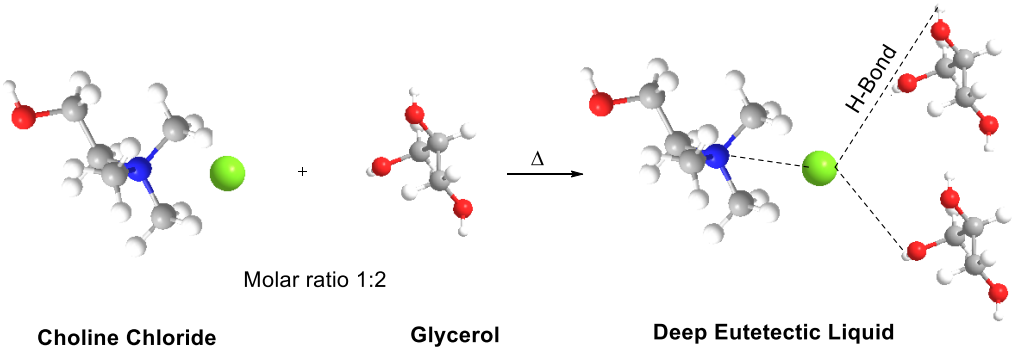



**Figure 3.1** Schematic representation of the formation of a natural deep eutectic solvent, illustrated with choline chloride and glycerol.

DES are generally described by the general formula $Cat^+X^-zY$, where $Cat^+$ is any ammonium, phosphonium or sulfonium cation, $X^-$ is a Lewis base, a halide anion, Y is either a Lewis or Bronsted base and, z is the number of Y molecules.[3, 5] Complex anionic species are formed between $X^-$ and Y, although hydrogen bonds are the main interactions between these components, occasionally electrostatic forces and van der Walls interactions can also be established.[8]

Experimental and computational studies of choline chloride-based deep eutectic solvents were carried out by Perkins *et al*.[10] Four hydrogen bond donors (HBD) were studied in order to determine the changes in the hydrogen bonding interactions when the HBD is different in the DES. In this study, urea, maleic acid, ethylene glycol and glycerol were chosen as the HBDs of interest. The results revealed that for all systems except for the DES choline chloride:urea, the largest fraction of hydrogen bond interactions occur between the HBD and the anion, since this DES still exhibits a great amount of urea−urea interactions, because the oxygen atom in urea also acts as a relatively strong hydrogen bond acceptor. Additionally, the two polyols have similar trends for the different hydrogen bond types (i.e., both systems have the largest contribution from HBD−anion interactions). The main difference being a larger contribution from the HBD−HBD hydrogen bonds in choline chloride: glycerol than in choline chloride: ethylene glycol, due to the additional OH functional group present in glycerol. On the other hand, choline chloride:maleic acid exhibited an extensive amount of HBD−anion and cation−anion interactions.

Taking into account the enormous number of different combinations of HBA and HBD to form the deep eutectic solvents, four types of DES have been often considered (Table 3.1). [5, 11]

**Table 3.1** Types of deep eutectic solvents

| DES Type | Composition |
|---|---|
| I | Quaternary salt + Metal halide ($MCl_x$, M = Zn, Sn,Fe, Al, Ga, In) |
| II | Quaternary salt + Hydrated metal halide ($MCl_x.yH_2O$, M = Cr, Co, Cu, Ni, Fe) |
| III | Quaternary salt + HBD ($R\text{-}CONH_2$, R-COOH, R-OH) |
| IV | Metal halide + HBD |

Examples of the most used components of DES (HBA and HBD) are shown in figure.3.2.

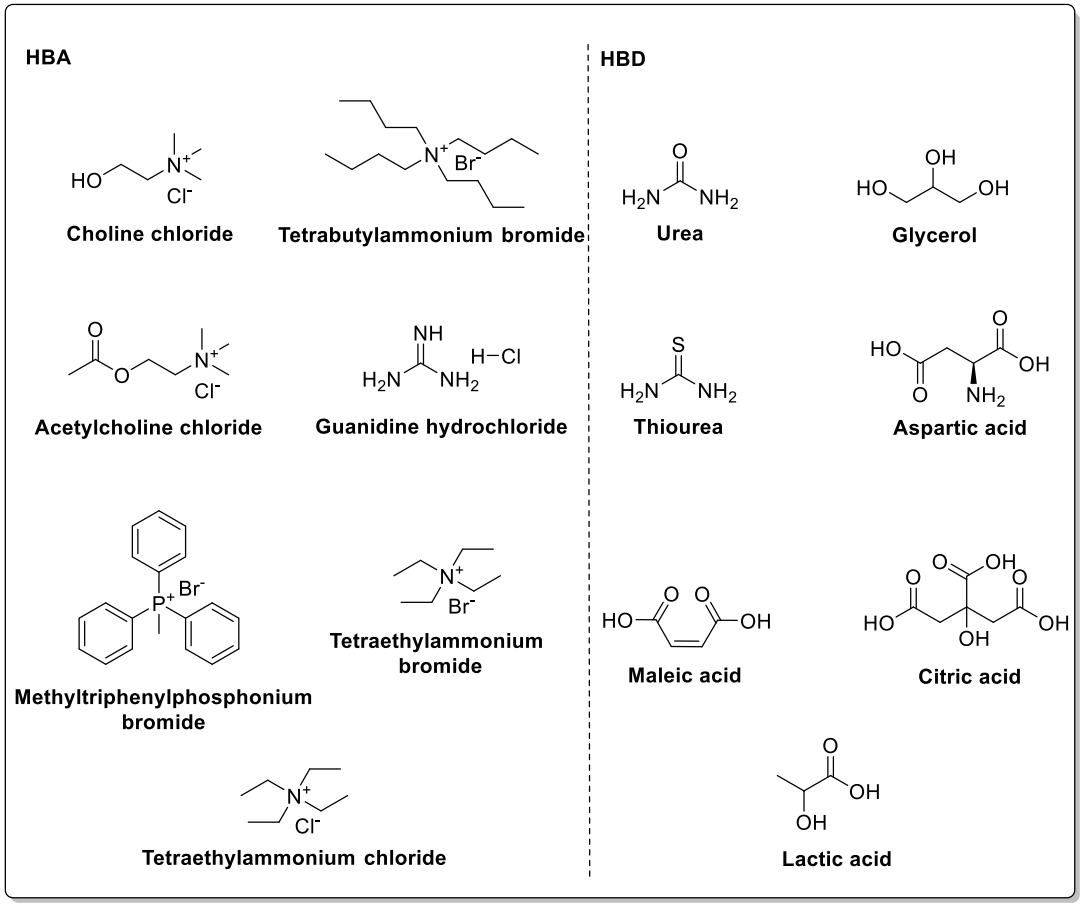



**Figure 3.2** Examples of common HBA and HBD for the synthesis of DES.

DES have been extensively accepted as a new class of ionic liquids (ILs) sharing many characteristics and properties with them. However, DES are considered as the solvents of the 21$^{st}$ century due to their versatility, nontoxicity, biodegradability and lower costs of the raw materials. Additionally, the possibility of tuning their physical properties namely freezing point, polarity, viscosity, density, solubility, and acidity/basicity, amongst other properties, by the correct selection and ratio of the HBA and the HBD components makes them very attractive solvents.[2-9, 12] Some of the differences and the similarities between DES and ILS are highlighted in Figure 3.3.

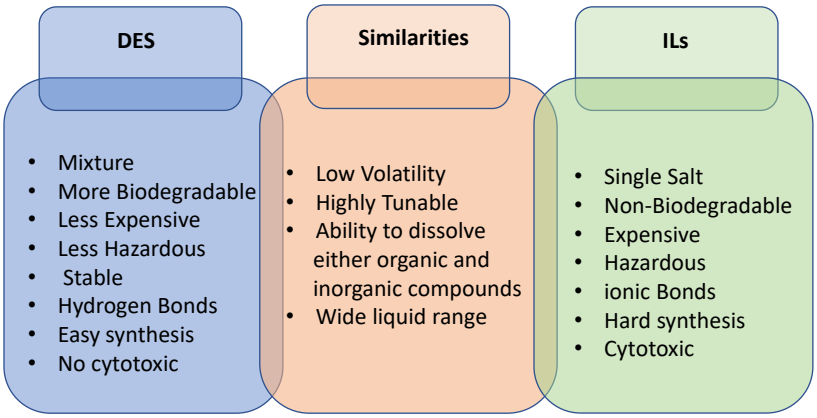



**Figure 3.3** Similarities and differences of DES and ILs.

## 3.2 Natural deep eutectic solvents

In 2011, as a result of an attempt to explain the solubility of intracellular compounds that are insoluble in water and lipid phases, Choi and co-workers reported the discovery of a new class of DES, which were described as natural deep eutectic solvents (NADES).[13] These are mixtures containing combinations of metabolites that occur in large amounts in cells, with a crucial role in biological processes such as cryoprotection, drought resistance, germination, and dehydration. Mixtures of ChCl with a range of natural products such as ChCl/organic acids or alcohols or sugars, organic acids/sugars, amino acids/organic acids or sugars were assessed, resulting in the discovery of more than 100 NADES.[13] Dai *et al* in 2013 provided additional information about NADES, by showing that they are excellent solvents for a wide range of metabolites of low to medium polarity that were non-soluble or poorly soluble in water.[14] In the years that followed, a huge amount of natural biodegradable, bio-renewable and non-toxic products, such as sugars, amino acids, choline chloride, urea, among others, were tested in the production of NADES.[2, 3, 5, 12, 15]

### 3.2.1 Preparation and main properties of NADES

NADES are usually very easy to prepare, and their components are all commercially available. The standard preparation methods for the synthesis of NADES involves vacuum evaporation, freeze-drying or heating and stirring.[14, 16-19] The vacuum evaporation method involves two steps. In the first step, the NADES components are dissolved in water, followed by heating (50 ºC), vortexing motion (25 ºC) and/or ultrasound (25 ºC). In the second step, the water is removed by rotatory evaporation or centrifugal vacuum. In the freeze-drying method, each component is initially dissolved in approximately 5 w/w% of

water. Then, the solutions are mixed, frozen, and subsequently freeze-dried to form a clear and homogeneous liquid. Finally, the most common preparation method involves the heating and stirring of the components under an inert atmosphere until a clear and homogenous liquid is formed. NADES components are commonly identified as a molar ratio (mol/mol), which reflects the contribution of the HBD and HBA. Examples of NADES obtained through these methods are presented in table 3.2.

**Table 3.2** Selected examples of NADES, preparation methods, and references.

| NADES | Method | Ref[a] |
|---|---|---|
| Choline chloride/urea (CC/U) | Direct mixing and stirring at 80 ºC | Abbott *et al* [20] |
| Choline chloride/glycerol (CC/Gly) | Direct mixing and stirring at 300 rpm and 70 ºC | Shahbaz *et al* [21] |
| Choline chloride/carboxylic acids | Direct mixing and stirring at 100 ºC | Abbott *et al* [18] |
| Choline chloride/urea (CC/U) | Freezing–drying method | Abbott *et al* [19] |
| Choline chloride/sucrose | Vacuum evaporating method | Dai *et al* [14] |

The choice of the components to produce these solvents as well as its molar ratio has been crucial to obtain NADES with enhanced physical properties that allowed expanding their application in the most diverse areas. Among others, physicochemical properties such as freezing point ($T_f$), viscosity, density, ionic conductivity, and surface tension, have been broadly analysed.[2, 4, 5, 8, 18, 20-32] Table 3.3 presents some selected properties of choline chloride-based NADES described in the literature.

**Freezing point**: DES or NADES producing consists of mixing two solids able to generate a new liquid phase by self-association via hydrogen bonds. The new phase is normally characterized by a lower freezing point than that of the individual components. For example, when choline chloride (CC) and glycerol (Gly) are mixed in a 1:2 molar ratio, the freezing point ($T_f$) of the eutectic is -1 ºC, which is significantly lower than the ones of CC and Gly ($T_f$ of CC and Gly are 302 and 17.8 ºC, respectively). The enormous decline of the freezing point is related to the interaction between the halide anion and the HBD

component. In general, NADES with a freezing point lower than 50 ºC have higher applicability since they can be used as low-cost and safe solvents in several fields.[4, 32]

**Density**: This propriety is one of the most important properties when choosing a solvent**.** Although the densities of NADES range from 0.785-1.63 g/cm$^3$ at room temperature, the vast majority have higher density than water (e.g. CC/U = 1.21 g/cm$^3$). These values can be in general ascribed to the different molecular organization or packing of NADES and DES. The molar ratio also has influence on density. For instance, the addition of CC to glycerol results in a decrease in the density (Table 3.3, entries 1-3).[8, 33]

**Viscosity**: Viscosity is also a parameter to be considered when choosing NADES. In general, these solvents have high viscosities due to their extensive network of hydrogen bonds, van der Walls, and electrostatic interactions. Thus, the viscosity is highly dependent on the nature of the HBD. The NADES formed by CC/EG show the lowest viscosity (Table 3.3, entry 5). On the contrary, the use of sugars such as glucose (table 3.3, entry 8) or carboxylic acids (e.g. Maleic acid) (Table 3.3, entry 7) significantly increase the NADES viscosity. The molar ratio also affects the viscosity in the same way as density (Table 3.3, entries 1-3). Another factor that has a strong influence on this parameter is temperature. As confirmed by Savi *et al*, in a study regarding the influence of the temperature on the formation, stability, and properties of functional NADES, the temperature increment results in a decrease in their viscosity.[22]

**Ionic Conductivity**: Considering the values presented in Table 3.3, it is clear that conductivity shows an inverse correlation with viscosity. An increase in viscosity results in a decrease in conductivity. Similarly, the molar ratio also has an enormous influence on this property (Table 3.3, entries 1-3) Therefore, in line with what has already been said, conductivities of NADES increase significantly with the temperature due to a decrease in the DES viscosity.

**Surface tension**: The strength of intermolecular interaction that controls the formation of DESs influences this property. It is expectable that the surface tension also increases with the increment of the molar composition of CC, as observed with viscosity. The addition of CC causes a cleavage of the hydrogen bonds between the glycerol molecules which results in a less ordered system and therefore less surface tension. Therefore, as well as the other cited properties, surface tensions of CC-based NADESs showed a linear correlation with temperature as demonstrated by Abbott *et al*. [34]

**Table 3.3** Selected physicochemical properties of some NADES, reported in literature

| Entry | NADES | $T_f$ (ºC) | Density ($g/cm^3$) | Viscosity (cP) | Conductivity (mS/cm) | Surface tension ($mN/m^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | CC/Gly (1:2) | -1 [26] | 1.18 (20 ºC) [26] | 376 (20 ºC) [26] | 1.05 (20 ºC) [26] | 55.8 (20 ºC) [26] |
| 2 | CC/Gly (1:3) | 1.7 [26] | 1.20 (20 ºC) [26] | 450 (20 ºC) [26] | 0.85 (20 ºC) [26] | 50.8 (20 ºC) [26] |
| 3 | CC/Gly (1:4) | 2 [26] | 1.21 (20 ºC) [26, 27] | 503 (20 ºC) [26] | 0.58 (20 ºC) [26] | 57.4 (20 ºC) [26] |
| 4 | CC/U (1:2) | 12 [28] | 1.25 (25 ºC) [28] | 750 (25 ºC) [28] | 0.199 (40 ºC) [28] | 66 (*) [29] |
| 5 | CC/EG (1:2) | -66 [21] | 1.12 ( 20 ºC) [21, 26] | 36 (20 ºC) [26] | 7.61 (20 ºC) [26] | 49 (20 ºC) [26] |
| 6 | CC/CA (1:1) | 69 [18] | 1.33 (30 ºC) [18] | 289 (75 ºC) [18] | 0.018 (30 ºC) [18] | -* |
| 7 | CC/MA (1:1) | 10 [31] | 1.25 (25 ºC) [31] | 1124 (25 ºC) [8] | 0.36 (25 ºC) [8] | 64.4 (50 ºC) [30] |
| 8 | CC/Glu (1:2) | 14 [35] | 1.21 (85 ºC) [35] | 8045 (25 ºC)[35] | -* | 71.7 (25 ºC) [35] |

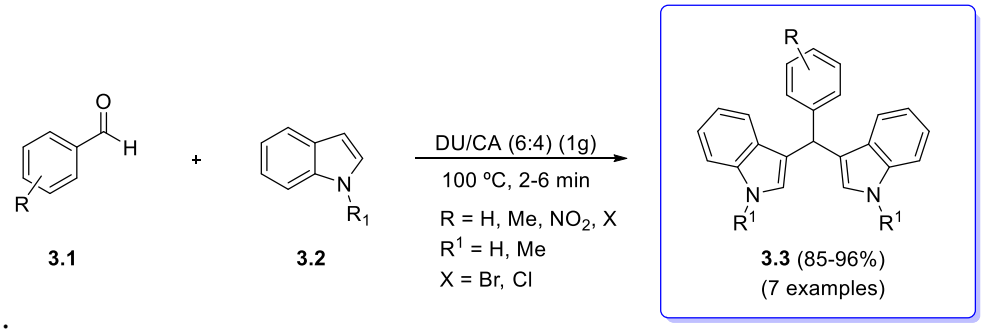
CC = Choline Chloride; Gly = Glycerol; U = Urea; EG = Ethylene glycol; CA = Citric acid; MA = Malonic acid; Glu = Glucose; * Data not available.

Due to their unique proprieties, NADES have attracted growing interests as solvents and/or organocatalysts in the fields of food processing, electrodeposition, biocatalysis, and organic synthesis.[5, 7, 23, 24, 32, 36-42] In addition, several studies demonstrated that some NADES can improve the solubility of poorly water-soluble drugs, increase drug delivery rates as well as skin and membrane permeation, which has raised the interest of researchers and professionals of biomedical and pharmaceutical areas.[15, 43-47]

### 3.3.2 NADES in the synthesis of bis(indolyl)methanes

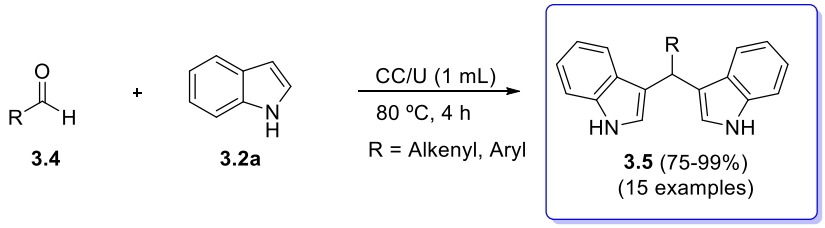
As already mentioned, bis(indolyl)methanes (BIMs) are considered a very important family of heterocyclic compounds present in various natural products having relevant biological activities (see Chapter 1). Thus, even though an enormous panoply of synthetic methods for the preparation of BIMs derivatives has been reported, the most explored so far has been the catalysed reaction of indoles with carbonyl compounds or their synthetic equivalents. Synthetic routes to BIMs involving NADES, acting as green solvents as well as organocatalysts, have also been reported.[42, 48-50]

Bafti *et al* reported an eco-friendly and efficient method for the electrophilic substitution reaction of indoles **3.2** with aldehydes **3.1** using dimethylurea/citric acid (DU/CA) (6:4) as NADES (Scheme 3.1).[51] This environmentally friendly procedure gave rise to BIMs in excellent yields with a short reaction time (2-6 min). The scope of the method includes aromatic aldehydes with electron-withdrawing or electron-donating groups and heterocyclic aldehydes.




**Scheme 3.1** Synthesis of BIMs using dimethylurea/citric acid as NADES.

Choline chloride/urea (CC/U) was also used as solvent/organocatalyst for the condensation of indole and alkenyl or aryl aldehydes to obtain the target BIMs (Scheme 3.2).[49] Under CC/U reaction medium, arylaldehydes **3.4** reacted with indole (**3.2a**) at 70 ºC for 4 h, giving rise to BIMs **3.5** in excellent yields (75-99%). However, when the same reaction conditions were applied to alkenyl aldehydes or ketones the method failed to afford the target BIMs.[49]




**Scheme 3.2** Synthesis of BIMs using choline chloride/urea as NADES.

Regarding the mode of action of the NADES, the authors proposed that the CC/U binary system can act as an organocatalytic solvent via hydrogen-bonding activation of the starting aldehyde (Figure 3.4).[49] Furthermore, when choline chloride/glycerol (CC/Gly) system was used, the reaction failed. This result was surprising since Li *et al* reported that glycerol, in the absence of any catalyst, was an effective medium to promote the electrophilic reactivity of aldehydes towards indoles affording the corresponding BIM derivatives.[52] In addition, the authors also reported very interesting results regarding the recyclability and reusability of NADES, tested in the reaction between *p*-methoxybenzaldehyde and indole. In fact, after recycling and reusing NADES four times, no considerable decrease in the reaction yield was observed.[49]

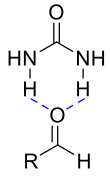
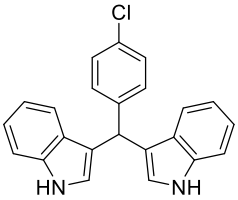
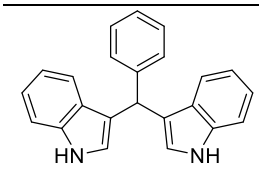


**Figure 3.4** Hydrogen-bonding activation of the starting aldehyde.

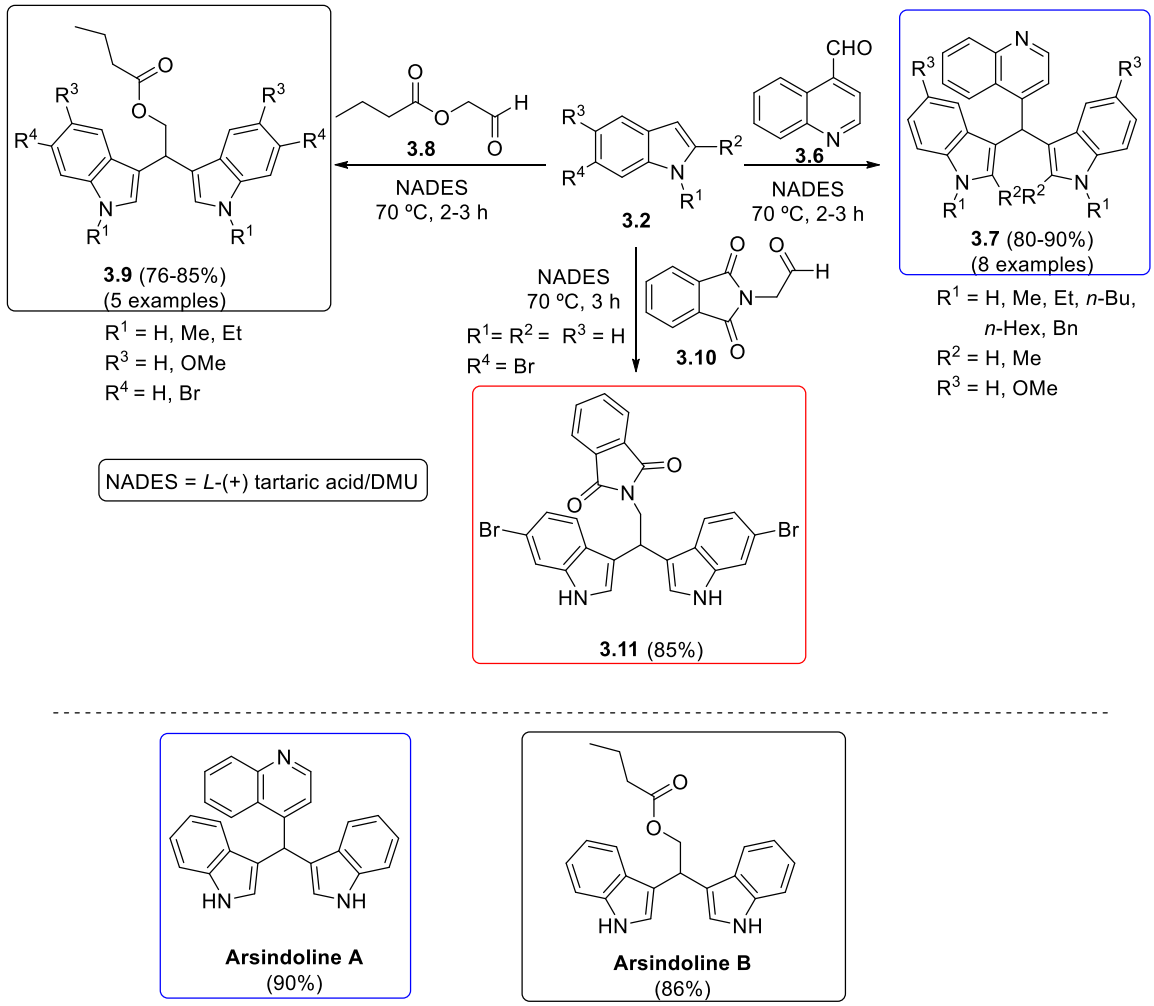
In fact, in most of the reported cases, the use of these solvents leads to a better efficiency of the reactions, namely increase yields and shorter reaction times, than using conventional solvents (Table 3.4).

**Table 3.4** Comparison between the use of NADES and other reported methods for the synthesis of BIMs.

| Product | Conditions | Catalyst | Solvent | Time | Yield (%)[ref a] |
|---|---|---|---|---|---|
|  | rt | --- | MeOH | 18 h | 75 [53] |
| | 75 ºC | $FePO_4$ | Glycerin | 8 h | 85 [54] |
| | rt | --- | $H_2O$ | 5 h | 55 [53] |
| | rt | $Fe(HSO_4)_3$ | $CH_2Cl_2$ | 30 min | 80 [55] |
| | **80 ºC** | --- | **Choline chloride/urea** | **4 h** | **93 [49]** |
| | **100 ºC** | **---** | **Dimethylurea/citric acid** | **2 min** | **95 [51]** |
| | rt/US | --- | $ZnCl_2$/Urea | 10 min | 90 [50] |
|  | rt | --- | MeOH | 12 h | 70 [53] |
| | MW | [bnmim][$HSO_4$ ]* | Solvent-free | 5 min | 93 [56] |
| | rt | $ZrOCl_2$ | $CH_3CN$ | 35 min | 93 [57] |
| | **100 ºC** | **---** | **Dimethylurea/citric acid** | **2 min** | **90 [51]** |
| | **rt** | **---** | **$ZnCl_2$/Urea** | **10 min** | **92 [50]** |
| | rt | --- | Choline chloride/$SnCl_2$ | 60 min | 97 [48] |

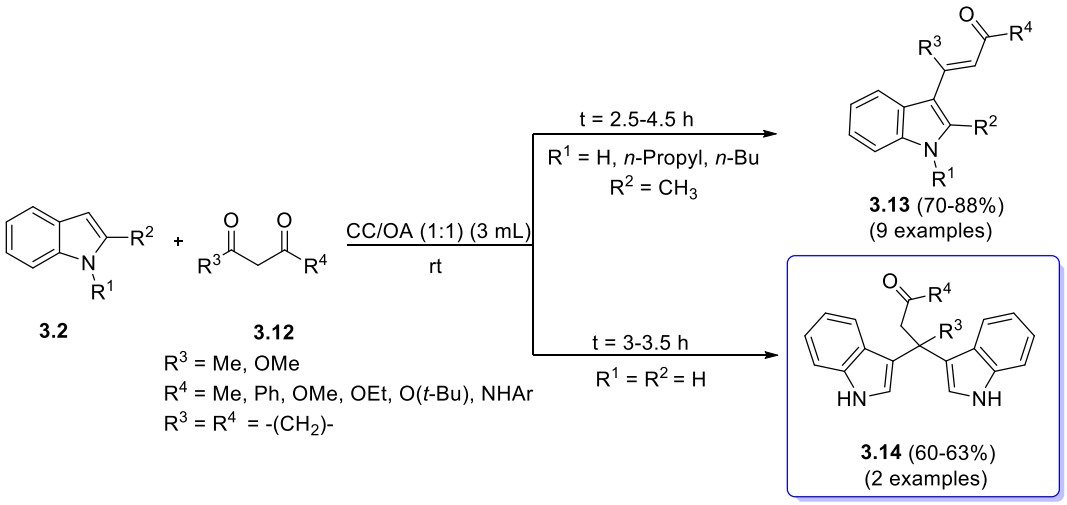
*[bnmim] [$HSO_4$] = 1-benzyl-3-methyl-imadazolium hydrogen sulphate

A synthesis of BIMS alkaloids arsindoline A, arsindoline B (natural bioactive products) and their analogues using *L*-(+)-tartaric acid/dimethyl urea as NADES was reported by Jella and Nagarajan (Scheme 3.3).[58] Under the optimised conditions, the reactions between indoles **3.2** and quinoline 4-aldehyde (**3.6**) afforded arsindoline A and their analogues **3.7** in excellent yields (80-90%). Similarly, arsindoline B derivatives **3.9** were also obtained in very good yields (76-85%) when indoles reacted with 2-oxoethyl butyrate (**3.8**). Finally, the strategy was efficiently applied in the synthesis of 1-(2,2-bis(6-bromo-1*H*-indol-3-yl)ethyl)indoline-2,3-dione (**3.11**) (85%).



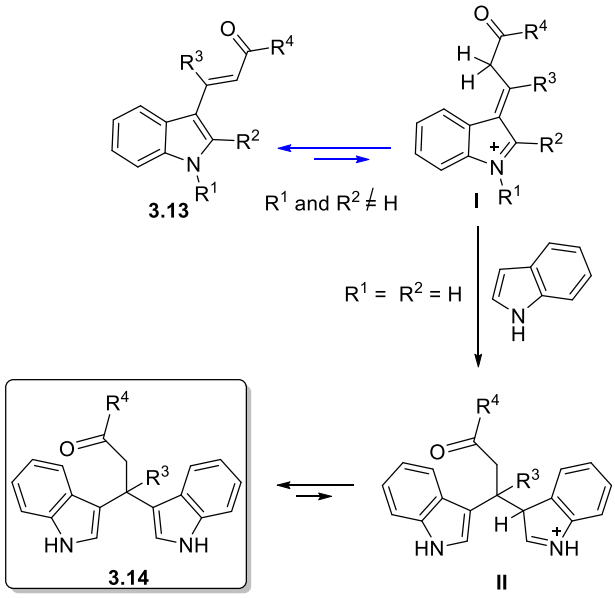

**Scheme 3.3** Synthesis of BIMS using *L*-(+)-tartaric acid/dimethyl urea as NADES.

The reaction between indoles **3.2** and 1,3-dicarbonyl **3.12** compounds using the NADES choline chloride/oxalic acid (CC/OA) as solvent was reported by Sanap and Shankarling .[59] The authors found that C3 alkenylation/alkylation of indoles required a substituent at indole C2 position giving products **3.13**, whereas C2-unsubstituted indoles gave indole derivatives **3.14** in good yields (Scheme 3.4).



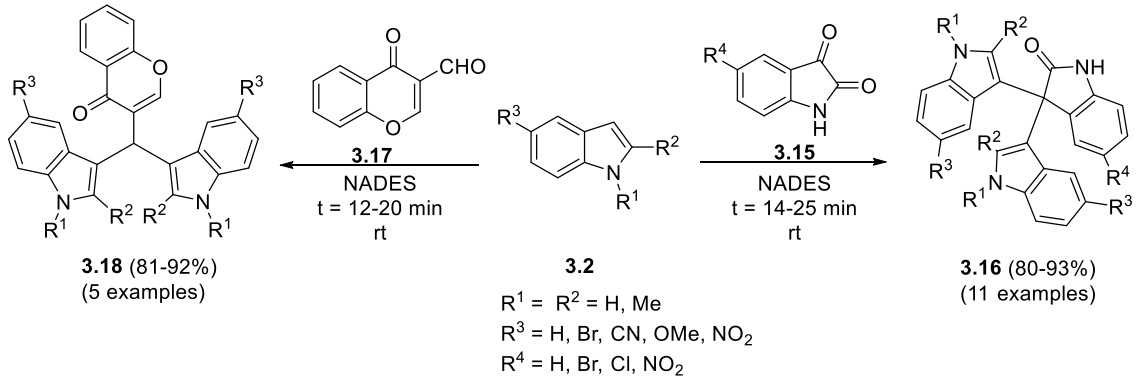

**Scheme 3.4** Synthesis of BIMs and C-3 alkenylated indoles using choline chloride/oxalic acid as NADES.

According to the authors, the electrophilicity of carbonyl carbon is increased by hydrogen-bonding activation, allowing the attack of indole. Subsequently, the hydroxyl group forms a hydrogen bond with DES eliminating water to form the intermediate **I**. In the reaction with 1,2-substituted indoles, the mechanism proceeds to form the C3-alkenylated derivatives **3.13** whereas, starting from unsubstituted indole the intermediate **I** is prone to be attacked by another indole molecule giving the target BIM **3.14**. At the end of the process, NADES was recovered and reused for several runs with no significant loss of the catalytic activity (Scheme 3.5).[59]




**Scheme 3.5** Proposed mechanism for the synthesis of BIMs and C-3 alkenylated indoles

A fast and efficient synthetic approach towards 3,3-diaryloxindole and chromone based bis(indolyl)alkanes **3.16** and **3.18**, respectively, using oxalic acid/proline as NADES was reported by Chandam *et al* in 2015 (Scheme 3.6).[60] The reaction between indoles **3.2** and isatins **3.15**, in oxalic acid dihydratate/proline (1:1) as NADES afforded **3.16** in yields ranging from 80 to 93%. This methodology was further extended to the synthesis of chromone-base BIMs **3.18** which were also obtained in excellent yields (81-92%). Recycling and reusability studies were also carried out and indicated that the NADES remains with high catalytic activity even after four successive runs.




**Scheme 3.6** Synthesis of BIMs 3,3-diaryloxindole and chromone-based BIMS using oxalic acid dihydrate/proline as NADES.

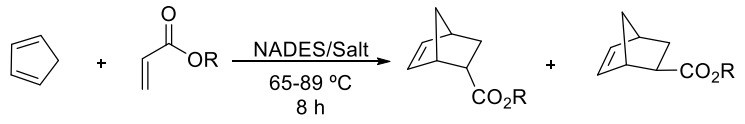
### 3.2.3 NADES in cycloaddition reactions

Diels–Alder reactions are some of the most effective carbon-carbon bond-forming reactions in organic synthesis being used in the synthesis of several natural products and biologically active molecules. The effects of conventional organic solvents on the rate and selectivity of Diels–Alder reactions are well established. However, due to environmental cautions, the search for alternate benign solvents and techniques to carry out this type of reaction has been growing in the last decades.

Although NADES have been widely used as solvents in numerous chemical transformations,[2-6, 15, 23, 32, 34, 36, 38-42, 61-64] there are few studies reporting their use in Diels-Alder reactions.[65-70]

One of these studies was reported by Imperato and co-workers who tested a new class of natural eutectic mixture of carbohydrates with urea or methylated urea (DMU) in the Diels-Alder reaction of cyclopentadiene (**3.19**) with methyl **3.20a** and *n*-butyl acrylate

**3.20b**. The reaction medium allowed a very efficient cycloaddition, leading to a good *endo-exo* selectivity.[66] The highest selectivity was obtained with the mixture sorbitol/DMU/$NH_4Cl$ (5.0:1). The use of Lewis acid as catalyst in Diels-Alder reactions in organic solvents is well established [71] and can also improve the rate and selectivity in alternative reaction media such as supercritical carbon dioxide [72] or water.[73] Indeed, carry out the cycloaddition in sorbitol/DMU/$NH_4Cl$ in presence of 10 mol% of $Sc(OTf)_3$ led to an improvement of the *endo-exo* selectivity to 6:1 and 10:1 when the reaction was performed using the acrylates **3.20a** and **3.20b**, respectively (Scheme 3.7).




**3.19** **3.20a** R = Me, **3.20b** R = *n*-Bu; **3.21a** R = Me, **3.21b** R = *n*-Bu; **3.22a** R = Me, **3.22b** R = *n*-Bu

| NADES | Temp (ºC) | Dienophile | Yiled (%) | *Endo*/*exo* ratio |
|---|---|---|---|---|
| **Sorbitol/DMU/$NH_4Cl$** | 67 | **3.20a** | Quant. | 5.0:1 (6.0:1)[a] |
| | | **3.20b** | 83 | 3:7:1 (10:1)[a] |

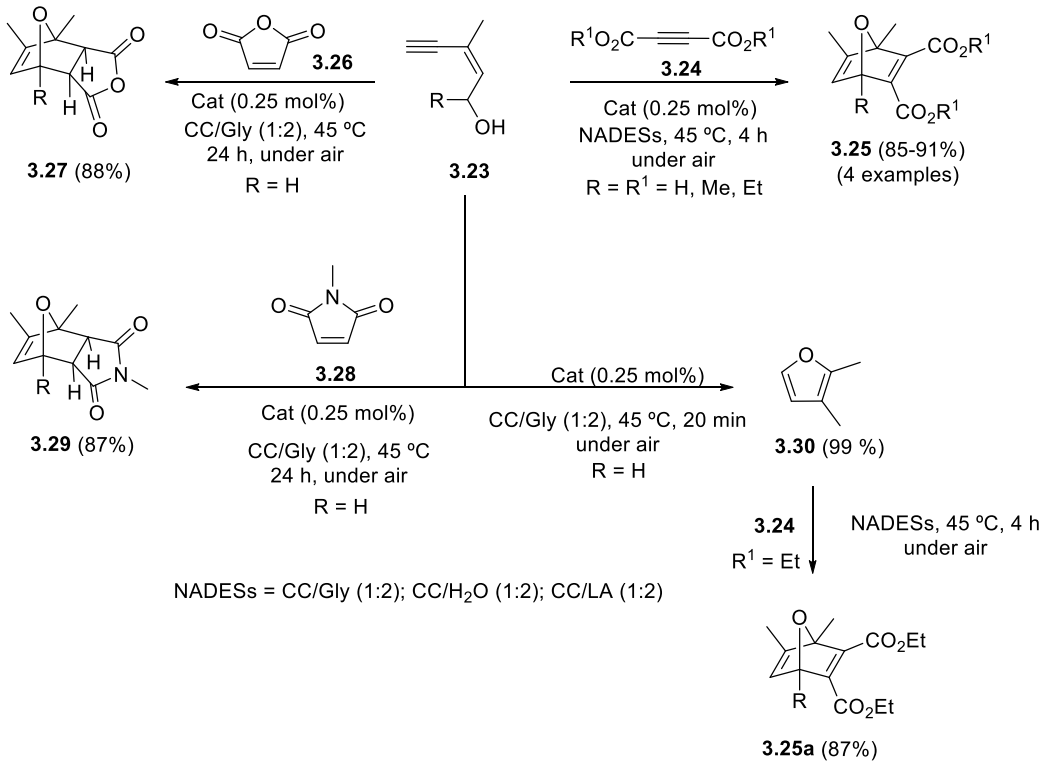
[a] Selectivity ratio with addition of 10 mol% of $Sc(OTf)_3$

**Scheme 3.7** Diels-Alder reactions between cyclopentadiene and acrylates in carbohydrates-urea-salt based NADES.

Nagare and Kumar also reported a similar study in which a eutectic mixture of carbohydrates with urea or methylated urea was used as the reaction media to perform the Diels-Alder reaction of cyclopentadiene with methyl acrylate. The authors proved that these reaction media can be very effective in increasing reaction rates.[68]

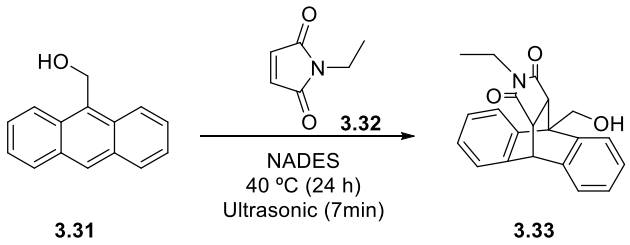
A one-pot tandem cycloisomerisation/Diels–Alder reaction in NADES for the selective synthesis of 7-oxanorbornadienes **3.25** and 7-oxanorbornenes **3.27** and **3.29** was described by Vidal *et al* (Scheme 3.8).[70] In this work, the treatment of the (*Z*)-enynols **3.23** with a slight excess of activated alkynes **3.24** gave rise to 7-oxanorbornadienes **3.25** in excellent yields (Scheme 3.8). Different reactive olefins (like maleic anhydride **3.26** or *N*-methylmaleimide **3.28**) were also active in the tandem cycloisomerisation/Diels–Alder reaction under the optimised conditions and led to the corresponding *exo*-regioisomers of the desired 7-oxanorbornenes **3.27** and **3.29** in good yields. In order to gain some insight into the role of the catalyst in the Diels–Alder cycloadditiom step, a new strategy involving the rection between **3.30** [isolated from the cycloisomerisation reaction of (Z)-3-methyl-2-

penten-4-yn-1-ol (**3.23**)] and the activated alkyne diethyl acetylenedicarboxylate (**3.24)** ($R^1$ = Et), in the absence of the catalyst was carried out affording the 7-oxanorbornadiene **3.25a** in similar yields and reaction times. This result leading the authors to conclude that the catalyst is not active in the cycloaddition step (Scheme 3.8).[70]




**Scheme 3.8** A One-pot tandem cycloisomerization/Diels–Alder reaction in the NADES for the selective synthesis of 7-oxanorbornadienes and 7-oxanorbornenes.

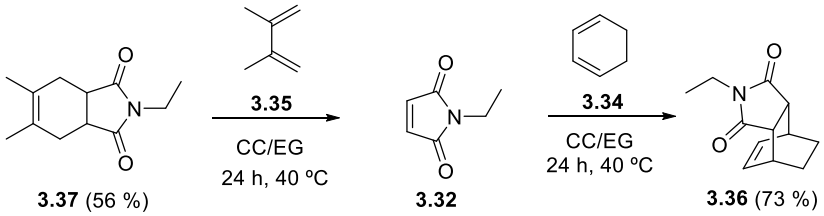
More recently, the Diels-Alder reaction using *N*-ethylmaleimide (**3.32**) as dienophile and 9-anthracenemethanol (**3.31**) as diene in NADES under conventional heating and ultrasonic activation was reported by Marullo *et al*.[67] In this study, NADES proved to be a suitable solvent for the purpose reaction, affording the target product **3.33** in yields higher than 60% when compared with the conventional organic solvents. However, the most impressive result was the significant decrease of the reaction times (conventional heating vs ultrasonic) when ultrasonic activation was tested (t = 70 min) (Scheme 3.9).




NADES: CC/EG, CC/Gly, CC/U, CC/Frutose (2:1), CC/PhAA, TBAC/EG, TBPCl/EG, AcCC/EG, Menthol/AA, $Ph_3PCl$/Gly

**Scheme 3.9** Diels-Alder reaction between 9-anthracenemethanol and *N*-ethylmaleimide at 40 ºC and ultrasonic activation in NADES.

This methodology was also extended using, 1,3-cyclohexadiene (**3.34**) and 2,3-dimethyl-1,3-butadiene (**3.35**) as dienes in CC/EG as NADES at 40 ºC. The results were analogous to the ones mentioned above and the products **3.36** and **3.37** were obtained in good yields (Scheme 3.10).[67]

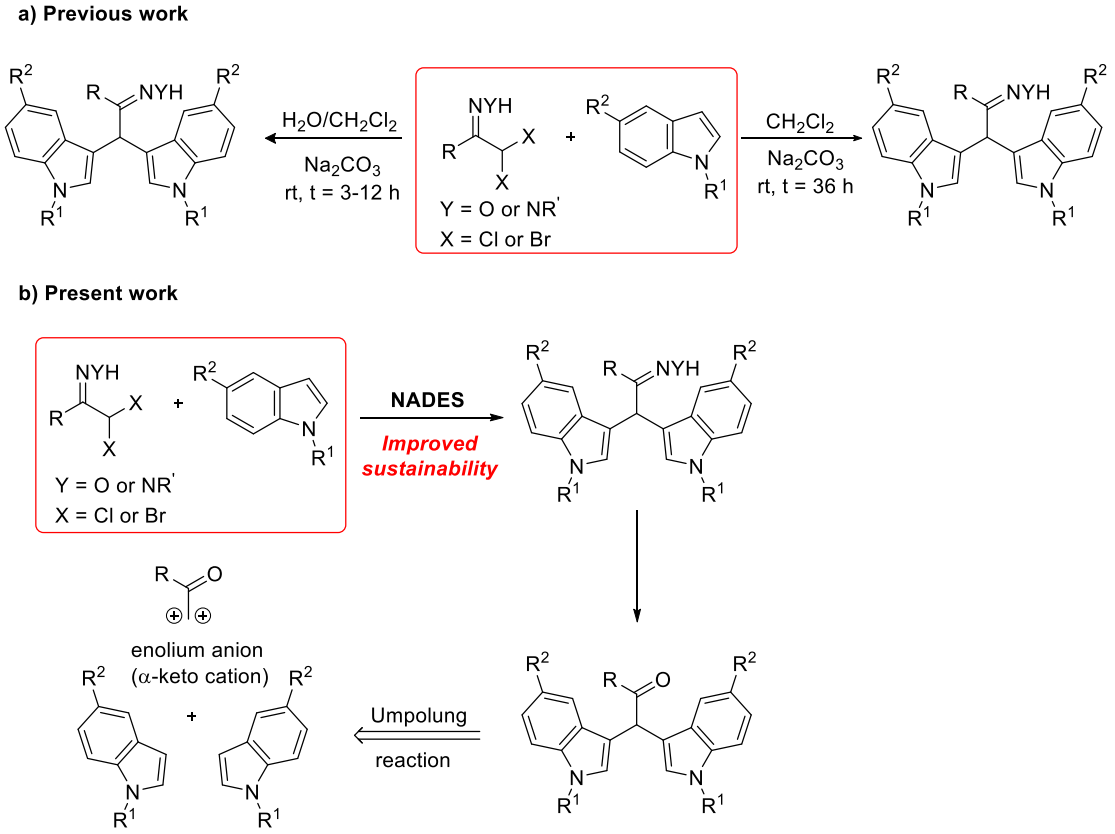



**Scheme 3.10** Diels-Alder reaction between *N*-ethylmaleimide, 1,3-cyclohexadiene and 2,3-dimethyl-1,3-butadiene as dienes in CC/EG under conventional heating.

## 3.3 Rationale and goals

The unprecedented approach to BIMs via bis-hetero-Diels–Alder reaction of azo- and nitrosoalkenes with indoles was described in Chapter 2 (Scheme 3.11a). Reactions between indoles and nitroso- or azoalkenes in dichloromethane (DCM) or water using DCM as co-solvent, were carried out. To our delight, the solvent system water/DCM led to significant decrease in reaction times. The described methodology proved to be versatile with a broad scope, leading to a library of new BIMs oximes and hydrazones. Additionally, the novel BIMs showed relevant "*in vitro*" anticancer activity and a promising scaffold was identified (see Chapter 2). Considering the high importance of bis(indolyl)methanes and their derivatives, it was particularly desirable to search for more efficient and sustainable synthetic strategies for this class of heterocycles. In continuation of our interest and investigation on the chemistry of BIMs, we decided to improve the efficiency and

sustainability of the one-pot hetero-Diels–Alder strategy by exploring NADESs as reaction media. Moreover, it was also our goal the conversion of the hydrazone and oxime moiety into carbonyl BIMs, originating new derivatives, opening the possibility to other synthetic transformations and reactivities (Scheme 3.11b).




**Scheme 3.11** Synthetic approach to BIMS using NADES.

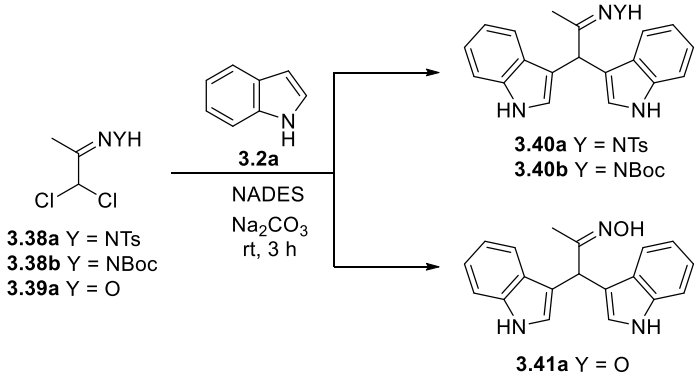
## 3.4 Synthesis of BIM derivatives using NADESs as solvents

The starting α,α'-dihalooximes or hydrazones were the same as those used in the study described in Chapter 2. The NADES used in this study were kindly provided by Professor Amadeus Brigas from the Department of Pharmacy and Chemistry, University of Algarve.

First, we decided to determine the best experimental conditions to carry out the reactions between alkyl α,α-dihalohydrazones **3.38a**, **3.38b** and oxime **3.39a** towards indole **3.2a** using pure NADES as solvents (Table 3.5). The NADES chosen for the screening included the mixture of choline chloride (CC) as HBA with glycerol (Gly), urea (U), or aspartic acid as the HBD as well as the mixture tetra-*n*-butylammonium bromide

(TBAB)/Gly. The reactions were carried out at room temperature for 3 h. Unfortunately, the reactions of alkyl *α,α*-dihalohydrazones **3.38a**, **3.38b** or oxime **3.39a** with indole **3.2a** using these NADES as solvents showed to be less efficient than under the previously optimised conditions presented in Chapter 2 (Table 3.5). Nevertheless, we observed that the highest yields were obtained with NADES based on CC:Gly (2:1) (Table 3.5, entries 1, 4, and 5). Surprisingly, it turned out that the isolation of the reaction products was extremely easy, as they were obtained by simple filtration, after dilution of the reaction with water, followed by crystallization.

**Table 3.5** Initial screening of NADES for the synthesis of bis(indolyl)methanes.




| Entry | Y | NADES | Product, Yield % | Previous work $H_2O$/DCM (3 h) |
|---|---|---|---|---|
| 1 | NTs | CC/Gly (2:1) | **3.40a** 24% | **3.40a** 41% |
| 2 | NTs | CC/U (1:1) | **3.40a** 16% | |
| 3 | NTs | CC/Aspartic acid (1:1) | -- | |
| 4 | NBoc | CC/Gly (2:1) | **3.40b** 22% | **3.40b** 55% |
| 5 | O | CC/Gly (2:1) | **3.41a** 28% | **3.41a** 57% |
| 6 | O | CC/U (1:1) | **3.41a** 14% | |
| 7 | O | TBAB/Gly (1:10) | **3.41a** 12% | |

Choline chloride:glycerol is probably the most studied and interesting eutectic system in the framework of green and sustainable chemistry. Choline chloride occurs naturally, is a white and water-soluble salt used mainly in animal feed particularly as an additive in chicken food to accelerate their growth. Glycerol is a colourless, odourless, and viscous liquid that is sweet-tasting and non-toxic and is a by-product of a range of hydrolysis and trans-esterification processes of oils and fats.[3, 32, 34] One of the major disadvantages of its use as a solvent itself is its high viscosity and high boiling point. However, the combination of choline chloride with glycerol significantly minimises these drawbacks. Some studies have shown that by adding CC to glycerol, a clear reduction of the viscosity is observed,

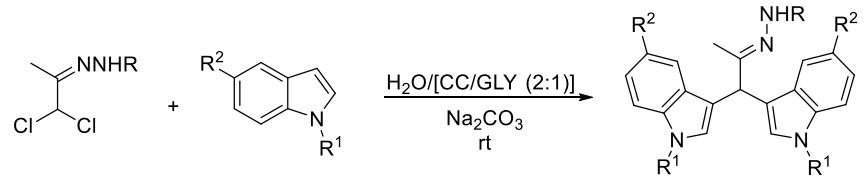
which can be explained by the broke of the 3D intermolecular H-bond interactions in glycerol by the addition of CC, resulting in a less ordered system. However, the CC:Gly mixture still presents a high degree of viscosity.

Recent studies have shown that the addition of water at low mole fractions to form a ternary mixture, allowed the possibility of tuning proprieties of NADES reducing their viscosity and increasing their conductivity. However, at higher mole fractions the presence of water weakens the hydrogen-bonding interactions of the two components of NADES leading to a severe change of their properties. Water acts as an additional H-bond donor and acceptor, which participates in the intermolecular network and gradually weakens the intermolecular interactions between all ionic and neutral species until all NADES components are entirely solvated at high dilutions.[74-78]

In this context, we decided to investigate the use of the ternary mixture $H_2O$/NADES to verify if this solvent system had a positive effect on the reaction under study. Thus, reactions between alkyl hydrazones **3.38a**, **3.38b** and indoles **3.2a-d** were carried out using two different solvent systems $H_2O$/[CC/Gly (2:1)] (3:1) and $H_2O$/[CC/Gly (2:1)] (1:3). We were delighted to observe that under both conditions the target BIMs **3.40** were obtained in good to excellent yields (50-80%) (Table 3.6). Further, we also verify a slight improvement in yields when the solvent system $H_2O$/[CC/Gly (2:1)] (3:1) was used when comparing with the ones obtained with the solvent system $H_2O$/DCM (Table 3.6, entries 1,3,5 and Figure 3.5). However, the most impressive result was the significant decrease of the reaction times being more significant when the solvent system $H_2O$/[CC/Gly (2:1)] (1:3) was used (10 min) (Table 3.6 entries 2, 4, 6, 8, 10). It was also observed that under both reaction conditions the *E* isomer was obtained as single product.

Regarding the isolation and purification process, it was found that the BIMs synthetized using the solvent system $H_2O$/[CC/Gly (2:1)] (3:1) had to be isolated by extraction with dichloromethane, whereas BIMs prepared with the ternary mixture $H_2O$/ [CC/Gly (2:1)] (1:3) precipitated in the reaction media and were isolated by a simpler filtration and further purified by crystallization.

**Table 3.6** Synthesis of bis(indolyl)methanes derived from alkylhydrazones using the ternary mixture $H_2O$/[CC/Gly (2:1)].




**3.38a** R = Ts
**3.38b** R = Boc

**3.2a** $R^1 = R^2 = H$
**3.2b** $R^1$ = Me, $R^2$ = H
**3.2c** $R^1$ = H, $R^2$ = Me
**3.2d** $R^1$ = H, $R^2$ = OH

**3.40a** R = Ts, $R^1$ = H, $R^2$ = H
**3.40b** R = Boc, $R^1$ = H, $R^2$ = H
**3.40c** R = Boc, $R^1$ = Me, $R^2$ = H
**3.40d** R = Boc, $R^1$ = H, $R^2$ = Me
**3.40e** R = Boc, $R^1$ = H, $R^2$ = OH

| Entry | R | $R^1$ | $R^2$ | $H_2O$/NADES | Reaction time | Product, Yield % | Previous work $H_2O$/DCM (3 h) |
|---|---|---|---|---|---|---|---|
| 1 | Ts | H | H | 3:1 | 1 h | **3.40a** 80% | **3.40a** 41% |
| 2 | Ts | H | H | 1:3 | 10 min | **3.40a** 70% | |
| 3 | Boc | H | H | 3:1 | 1 h | **3.40b** 54% | **3.40b** 55% |
| 4 | Boc | H | H | 1:3 | 10 min | **3.40b** 50% | |
| 5 | Boc | Me | H | 3:1 | 1 h | **3.40c** 56% | **3.40c** 49% |
| 6 | Boc | Me | H | 1:3 | 10 min | **3.40c** 50% | |
| 7 | Boc | H | Me | 3:1 | 1 h | **3.40d** 53% | **3.40d** 43% |
| 8 | Boc | H | Me | 1:3 | 10 min | **3.40d** 55% | |
| 9 | Boc | H | OH | 3:1 | 1 h | **3.40e** 71% | **3.40e** 55% |
| 10 | Boc | H | OH | 1:3 | 10 min | **3.40e** 64% | |

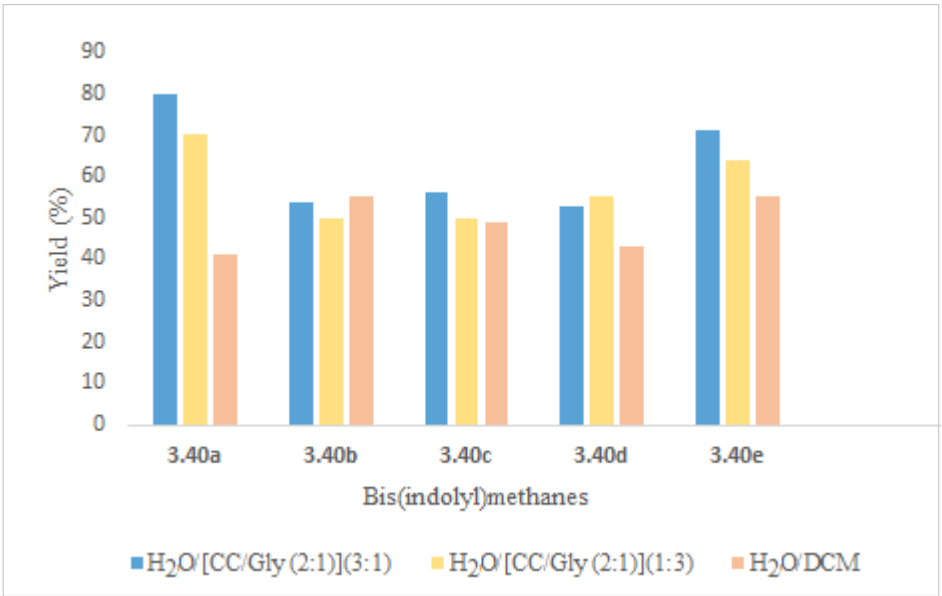


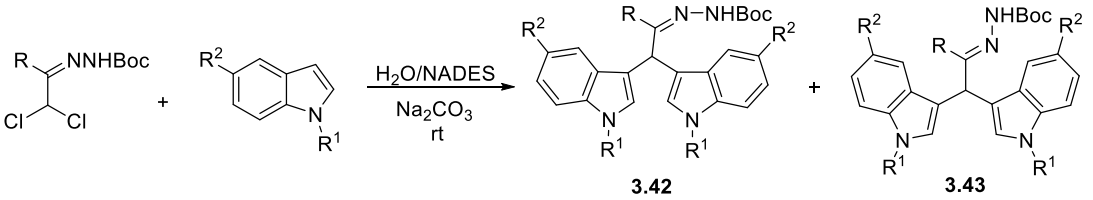

**Figure 3.5** Synthesis of BIMS **3.40a-e** (NADES versus $H_2O$/DCM).

As shown in Chapter 2, the drawbacks of the previous strategy were the moderate yields obtained in the synthesis of hydrazonoaryl-BIMs via hetero Diels–Alder reaction of indoles with conjugated azoalkenes. Thus, we decided to extend the use of NADES to these reactions hoping to observe an improvement in its efficiency. The results of this study are summarised in Table 3.7. The reactivity of arylhydrazones **3.38c** and **3.38d** towards 1*H*-indole (**3.2a**) and *N*-methyl-1*H*-indole (**3.2c**) was initially explored. We were pleased to observe that the use of both solvent systems, $H_2O$/[CC/Gly (2:1)] (3:1) and $H_2O$/[CC/Gly (2:1)] (1:3), led to a significant improvement of the reaction efficiency, and the target BIMs **3.42a–e** and **3.43a-d** were isolated in moderate to good overall yields (25-50%) (Table 3.7). However, it was found that this improvement was more significant when the ternary mixture $H_2O$/[CC/Gly (2:1)], in a 3:1 $H_2O$/NADES ratio was used (Table 3.7, entries 1, 3, 5). Once again, reactions using the solvent system $H_2O$/NADES (1:3) were faster, giving the desired products after 30 min of reaction albeit in lower yields (Table 3.7, entries 2, 4, 6). Finally, under both reaction conditions, the *E* isomer was obtained as single product or major product (Table 3.7, entries 1-6).

This strategy was also successfully extended to the synthesis of new BIM hydrazones derived from 1-methylindole (**3.2b**) (Table 3.7, entries 7-10). Starting from arylhydrazone **3.38c**, the reaction towards 1-methyl-1*H*-indole (**3.2b**) gave rise to the corresponding BIM **3.42d** as major product (44-60%) together with the isomeric derivative **3.43c** (18-21%) (Table 3.7, entries 7, 8). Arylhydrazone **3.38d** reacted with **3.2b** to give a mixture of isomeric BIMS hydrazones **3.42e**/**3.43d** in good overall yield (48-80%) (Table 3.7, entries 9,10). In this case, under both solvent systems, the reactions were completed after 1 h.

Unlike what was observed for methylhydrazones derived BIMS **3.40**, the purification method purification for these arylhydrazones derived BIMs was more complex, involving the extraction of the reaction mixture with DCM followed by flash chromatography.

**Table 3.7** Synthesis of bis(indolyl)methanes derived from arylhydrazones using the ternary mixture $H_2O$/[CC/Gly (2:1)].




**3.38c** R = $C_6H_5$
**3.38d** R = *p*-$BrC_6H_4$

**3.2a** $R^1$ = $R^2$ = H
**3.2b** $R^1$ = Me, $R^2$ = H
**3.2c** $R^1$ = H, $R^2$ = Me

**3.42a** R = $C_6H_4$, $R^1$ = H, $R^2$ = H
**3.42b/3.43a** R = *p*-$BrC_6H_4$, $R^1$ = H, $R^2$ = H
**3.42c/3.43b** R = *p*-$BrC_6H_4$, $R^1$= H, $R^2$ = Me
**3.42d/3.43c** R = $C_6H_4$, $R^1$ = Me, $R^2$ = H
**3.42e/3.43d** R = *p*-$BrC_6H_4$, $R^1$= Me, $R^2$ = H

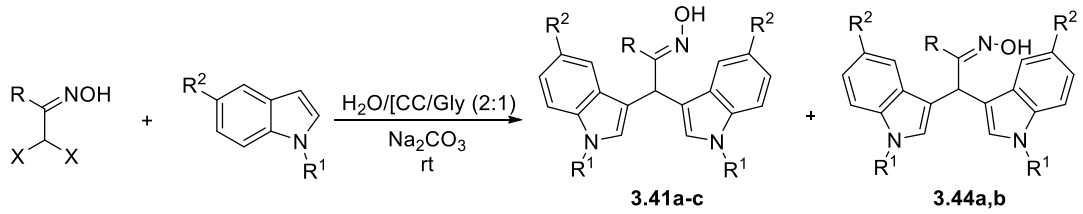
| Entry | R | $R^1$ | $R^2$ | $H_2O$/NADES | Reaction time | Product, Yield % | | Previous work $H_2O$/DCM (12 h) | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $C_6H_5$ | H | H | 3:1 | 1 h | **3.42a** 44% | - | **3.42a** 29% | - |
| 2 | $C_6H_5$ | H | H | 1:3 | 30 min | **3.42a** 25% | - | | |
| 3 | *p*-$BrC_6H_4$ | H | H | 3:1 | 1 h | **3.42b** 48% | - | **3.42b** 17% | **3.43a** 12% |
| 4 | *p*-$BrC_6H_4$ | H | H | 1:3 | 30 min | **3.42b** 43% | - | | |
| 5 | *p*-$BrC_6H_4$ | H | Me | 3:1 | 1 h | **3.42c** 42% | **3.43b** 8% | **3.42c** 13% | - |
| 6 | *p*-$BrC_6H_4$ | H | Me | 1:3 | 30 min | **3.42c** 23% | **3.43b** 10% | | |
| 7 | $C_6H_5$ | Me | H | 3:1 | 1 h | **3.42d** 60% | **3.43c** 18% | - | |
| 8 | $C_6H_5$ | Me | H | 1:3 | 30 min | **3.42d** 44% | **3.43c** 21% | | |
| 9 | *p*-$BrC_6H_4$ | Me | H | 3:1 | 1 h | **3.42e/3.43d** 80%[a] | | - | |
| 10 | *p*-$BrC_6H_4$ | Me | H | 1:3 | 1 h | **3.42e/3.43d** 48%[a] | | | |

[a]Mixture of isomeric hydrazones

Following these encouraging results, was decided to extend the use of NADES to the synthesis of hydroxyiminomethylbis(indolyl)methanes (Table 3.8). The application of the same optimised reaction conditions afforded the target BIMS in good overall yield. (45-83%), generally in similar or higher yields and shorter reaction times than the ones obtained using the solvent system $H_2O$/DCM (Table 3.8). This result was even more significant when the reaction was carried out using the solvent system $H_2O$/[CC/Gly (2:1)] (1:3). In fact, using this system, only 10 minutes were required for the synthesis of the target product **3.41a**, when previously 3 h were needed (Table 3.8, entry 1,2). Despite this, the efficiency of the reaction was always slightly higher when the system $H_2O$/[CC/Gly (2:1)] (3:1) was used, but a longer reaction time (1 h) was required.

Another interesting result was related to the stereochemistry of the products. Regardless of the used ternary mixture, the *E*-isomers **3.41** were obtained as single products when starting from alkyl oximes **3.39a** (Table 3.8, entries 1, 2, 9, 10) whereas aryl oximes **3.39b** and **3.39c** reacted with indoles to give the **3.44** Z-isomers as single or major products (Table 3.8, entries 3-8). The study was also extended to the synthesis of a new BIM oxime **3.41c** which was obtained from the reaction of the alkyl oxime **3.39a** with *N*-methyl indole **3.2c** (Table 3.8, entries 9, 10).

**Table 3.8** Synthesis of BIM oximes using the solvent system $H_2O$/[CC/Glycerol (2:1)].




**3.39a** R = Me, X = Cl
**3.39b** R = *p*-$FC_6H_4$, X = Br
**3.39c** R = *p*-$BrC_6H_4$, X = Br

**3.2a** $R^1 = R^2 = H$
**3.2b** $R^1 = H$, $R^2 = OH$
**3.2c** $R^1 = Me$, $R^2 = H$

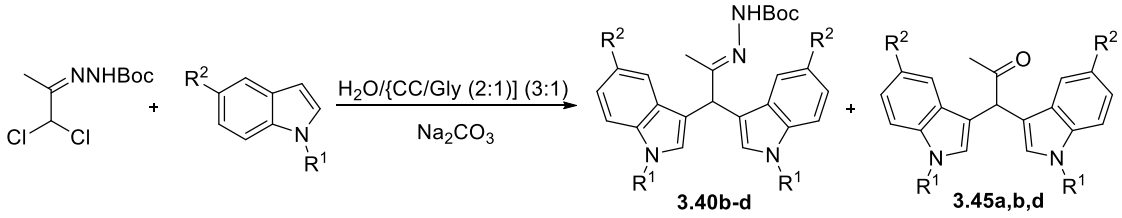
**3.41a** R = Me, $R^1 = H$, $R^2 = H$
**3.41b/3.44a** R = *p*-$FC_6H_4$, $R^1 = H$, $R^2 = H$
**3.44b** R = *p*-$BrC_6H_4$, $R^1 = H$, $R^2 = H$
**3.44c** R = *p*-$BrC_6H_4$, $R^1 = H$, $R^2 = OH$
**3.41c** R = Me, H, $R^1 = Me$, $R^2 = H$

| Entry | R | $R^1$ | $R^2$ | $H_2O$/NADES | Reaction time | Product, Yield % | | Previous Work $H_2O$/DCM (3 h) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **3.41** | **3.44** | **3.41** | **3.44** |
| **1** | Me | H | H | 3:1 | 1 h | **3.41a** 45% | - | **3.41a** 57% | - |
| **2** | Me | H | H | 1:3 | 10 min | **3.41a** 49% | - | | |
| **3** | *p*-$FC_6H_4$ | H | H | 3:1 | 1 h | **3.41b** 15% | **3.44a** 60% | - | **3.44a** 71% |
| **4** | *p*-$FC_6H_4$ | H | H | 1:3 | 10 min | **3.41b** 22% | **3.44a** 60% | | |
| **5** | *p*-$BrC_6H_4$ | H | H | 3:1 | 1 h | - | **3.44b** 55% | - | **3.44b** 55% |
| **6** | *p*-$BrC_6H_4$ | H | H | 1:3 | 30 min | - | **3.44b** 52% | | |
| **7** | *p*-$BrC_6H_4$ | H | OH | 3:1 | 1 h | - | **3.44c** 83% | - | **3.44c** 78% |
| **8** | *p*-$BrC_6H_4$ | H | OH | 1:3 | 30 min | - | **3.44c** 71% | | |
| **9** | Me | Me | H | 3:1 | 1 h | **3.41c** 56% | - | | |
| **10** | Me | Me | H | 1:3 | 30 min | **3.41c** 52% | - | | |

Since in our previous work (see Chapter 2), in a unique occasion, the synthesis of the hydrazone BIM was formed together with the corresponding acetyl BIM derivative, isolated as minor product, we decided to investigate the possibility of obtaining carbonyl-BIMs in one-pot reaction, using the ternary system $H_2O$/NADES.

Using the reaction between hydrazone **3.38b** and indole **3.2a** as model reaction, we carried out the reaction at room temperature for 6 h using the solvent system $H_2O$/[CC/Gly (2:1)] (3:1). Surprisingly, not only the BIM **3.40b** was obtained in 21% yield but also the carbonyl BIM derivative **3.45a** was isolated in 40% yield, as the major product (Table 3.9, entry 1). After this interesting result, an increase of either the reaction time or temperature was investigated. However, no improvement in the yield of the hydrolysis component was observed but rather an enhancement of the formation of the BIM derived hydrazone **3.40a** (Table 3.9, entries 2-4). Under the optimised conditions, the reactions of hydrazone **3.38b** with indoles **3.2b** and **3.2c** were carried out and the carbonyl BIMS **3.45b** and **3.45c** were obtained in 22 and 19 % yield, respectively, along with BIMs **3.40c** and **3.40d** isolated as major product (Table 3.9, entries 5, 6). In view of these results, we also decided to try to extend this methodology to the preparation of carbonyl BIMs derivatives from α,α-dihalo-oximes **3.39a**, but our attempts to obtain the desired carbonyl BIM derivatives were unsuccessful. Even so, the fact that this methodology allows the synthesis of two types of functionalised BIMs in a one-pot procedure must be highlighted.

**Table 3.9** Optimisation of the reaction conditions for the synthesis of hydrazone- and carbonyl-BIMs




**3.38b**

**3.2a** $R^1 = R^2 = H$
**3.2b** $R^1$ = Me, $R^2$ = H
**3.2c** $R^1$ = H, $R^2$ = Me

**3.40b/3.45a** $R^1$ = H, $R^2$ = H
**3.40c/3.45b** $R^1$ = Me, $R^2$ = H
**3.40d/3.45c** $R^1$ = H, $R^2$ = Me

| Entry | $R^1$ | $R^2$ | Reaction time (h) | Temperature (° C) | Product, Yield % | | Previous Work $H_2O$/DCM (3 h) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **3.40** | **3.45** | **3.40** | **3.45** |
| **1** | H | H | 6 h | rt | **3.40b** 21% | **3.45a** 40% | **3.40b 55%** | |
| **2** | H | H | 12 h | rt | **3.40b** 22% | **3.45a** 37% | | |
| **3** | H | H | 6 h | 50 ºC | **3.40b** 49% | **3.45a** 16% | | |
| **4** | H | H | 6 h | 70 ºC | **3.40b** 39% | **3.45a** 12% | | |
| **5** | Me | H | 6 h | rt | **3.40c** 42% | **3.45b** 22% | **3.40c** 49% | - |
| **6** | H | Me | 6 h | rt | **3.40d** 36% | **3.45c** 19% | **3.40d** 43% | **3.45c** 17% |

The structural elucidation of carbonyl-BIMs **3.45** was achieved through comparison with the NMR spectra of their hydrazone-derived BIM precursors **3.40**. Analysing the $^{1}$H NMR spectrum of **3.40a** (Figure 3.6), the signals corresponding to the protons of *tert*-butyl group (singlet, H-4) at 1.47 ppm and to the hydrazone moiety proton (singlet, H-13) at 9.35 ppm were easily identified. Furthermore, in the $^{13}$C NMR spectrum, it was possible to assign the chemical shifts corresponding to carbon C-2 at 155.2 ppm (C=N), C-14 at 153.3 ppm (C=O ester group), C-15 at 78.9 ppm (carbon quaternary from t*ert*-butyl group), and C-16 at 28.1 ppm ($CH_3$ from *tert*-butyl group, Figure 3.7). On the other hand, in the $^{1}$H NMR spectrum of **3.45a** (Figure 3.8) the signals corresponding to the protons of the methyl group (singlet, H-3) can be observed at 2.20 ppm, the *meso* proton (singlet, H-1) at 5.58 ppm, and the indole NH proton (broad singlet, H-4) at 10.97 ppm. However, the signals of the *tert*-butyl group at 1.47 ppm and from the hydrazone moiety at 9.35 ppm were not observed. In addition, in the $^{13}$C NMR of **3.45a** (Figure 3.9) it is possible to identify the signal corresponding to the carbonyl carbon group at 206.4 ppm. However, the signals corresponding to the BOC moiety were not detected, which led us to the conclusion that compound **3.45a** was obtained (Table 3.9).

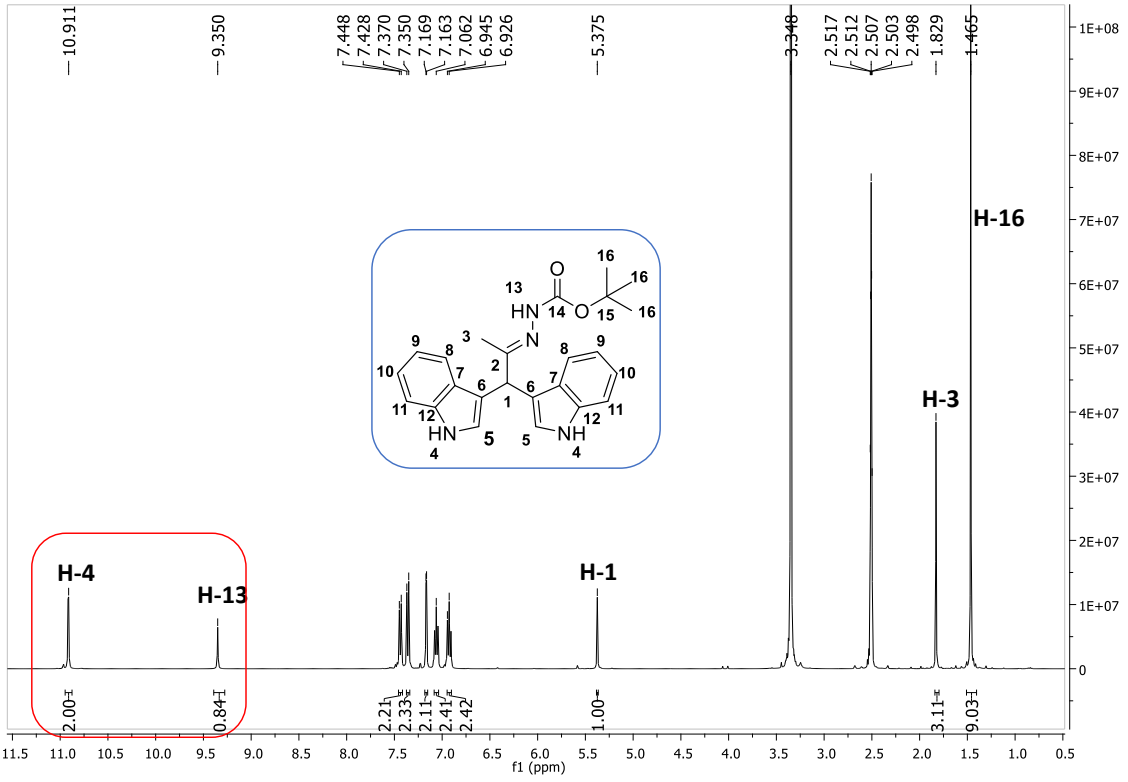



**Figure 3.6** $^{1}$H NMR spectrum of BIM **3.40a**

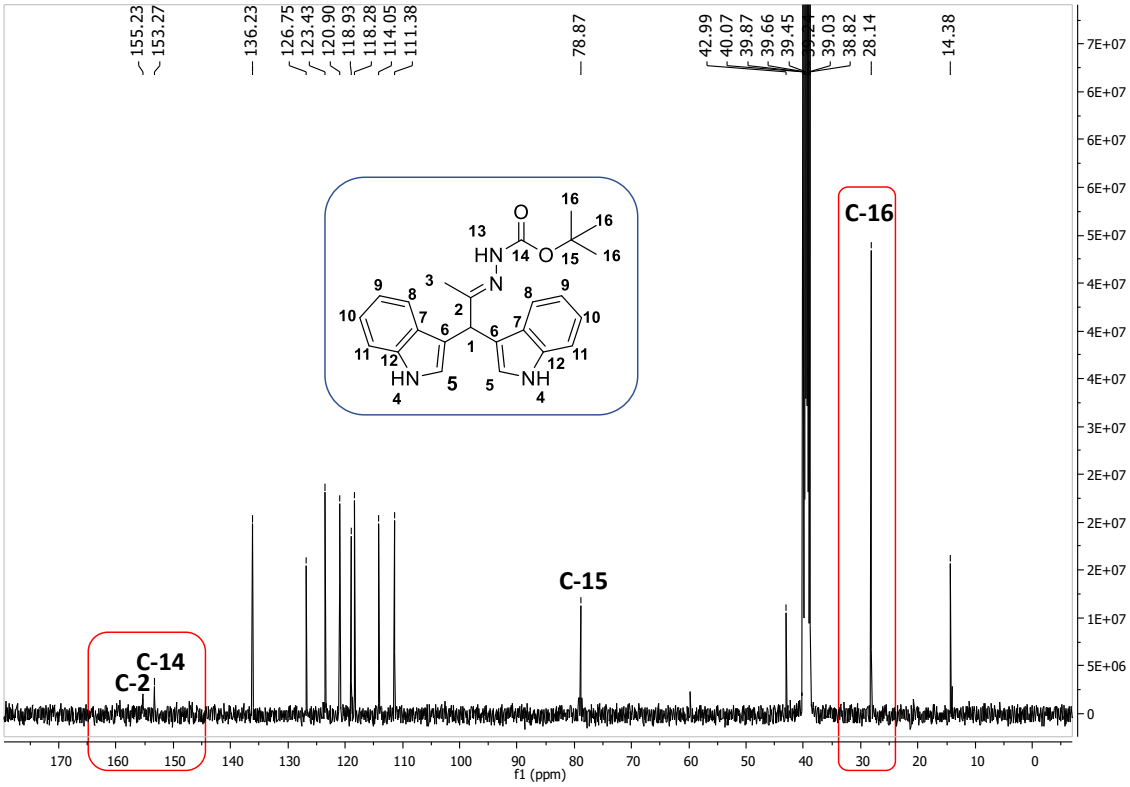



**Figure 3.7** $^{13}$C NMR spectrum of BIM **3.40a**.

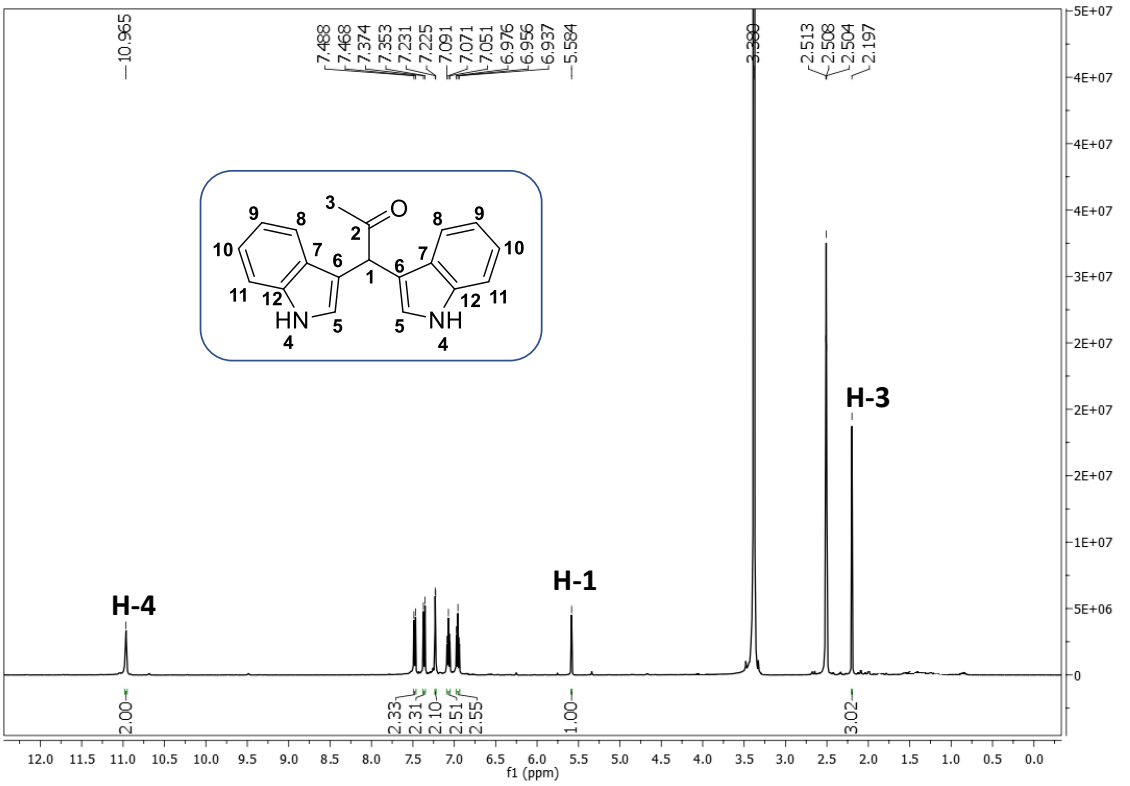



**Figure 3.8** $^{1}$H NMR spectrum of BIM **3.45a**.

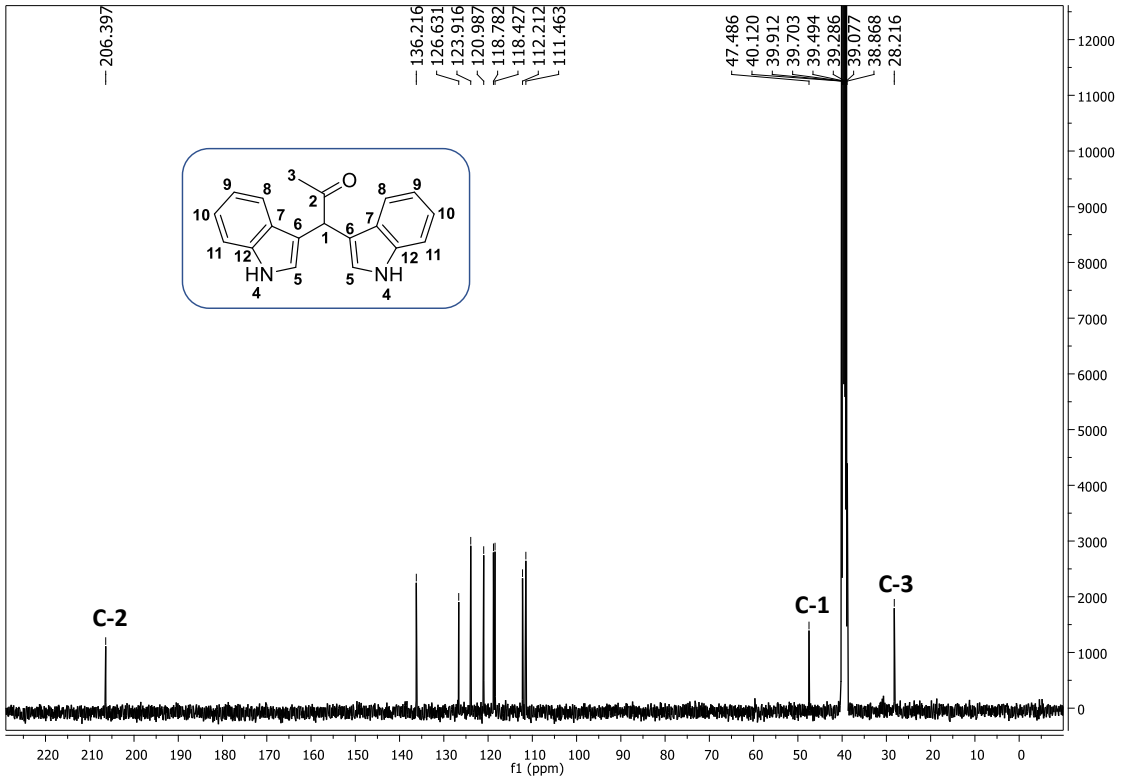
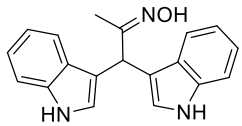
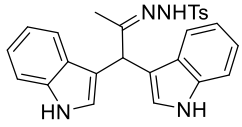
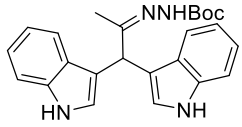


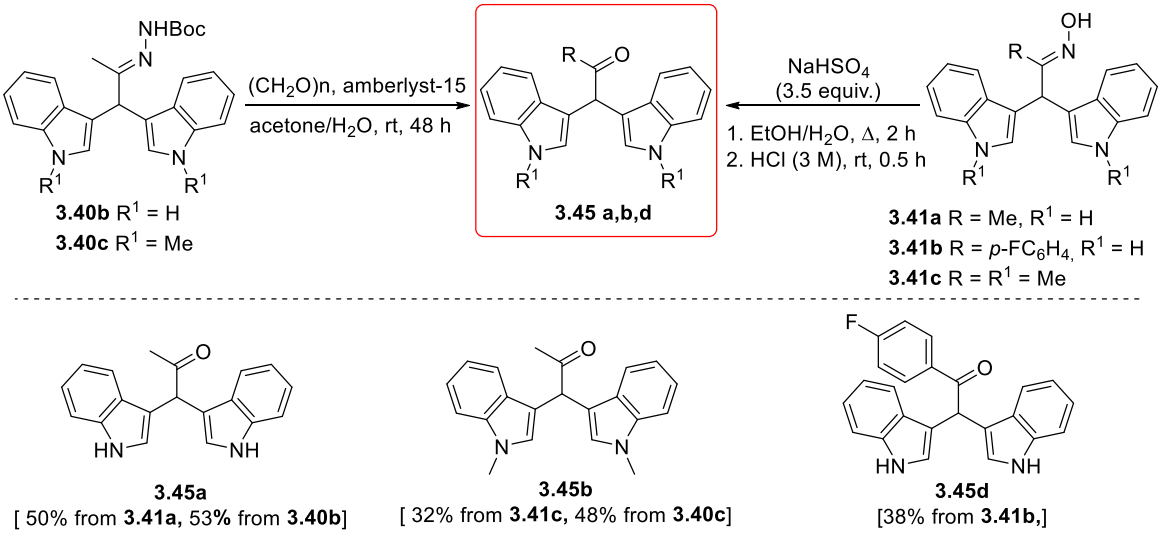

**Figure 3.9** $^{13}$C NMR spectrum of BIM **3.45a**.

Encouraged by the possibility of obtaining the carbonyl-BIMs, we decided to explore the ease/difficulty of the hydrolysis reaction of oxime and hydrazone BIMs by testing some methods reported in the literature.[79-95] Treating the BIM oxime **3.41a** with levelunic acid in HCl 1N[79], sodium hypochlorite (5%) in chloroform[82], $Mo(CO)_6$ in the presence of water[83], *N*-bromosuccinimide in acetone/$H_2O$[85], pyridinium chlorochromate (PCC) under microwave radiation[86], 70% *tert*-butyl hydroperoxide (TBHP) in carbon tetrachloride[84], silica chloride/wet $SiO_3$ in hexane[87], γ-picolinium chlorochromate (γ-PCC) in DCM[88], iodine in 30% of $H_2O_2$ in aqueous acetonitrile[89], aqueous 36% $H_2O_2$ and an 48% HBr solution[90], $Si_2Cl_6$ in anhydrous THF in the presence of $SiO_2$[91] or $K_3[Fe(CN)_6].3H_2O$ supported on silica gel in ethanol and water[92] did not lead to the desired carbonyl-BIM. Starting from BIM hydrazone **3.40a**, methods such as boron trifluoride etherate in acetone/$H_2O$[81] or 70% *tert*-butyl hydroperoxide (TBHP) in carbon tetrachloride[84] were tested but our goal was also not achieved. Finally, the reaction of BIM hydrazone **3.40b** in silica chloride/wet $SiO_3$[87] was also performed and like in the previous experiments, the corresponding carbonyl-BIM was not obtained (Table 3.10).

**Table 3.10** Tested methods for the hydrolysis reaction of oximes and hydrazones to carbonyls BIMs

| Starting BIMs | Method used [ref] |
|---|---|
|  **3.41a** | ○ (9/1) (v/v) Levelunic acid : HCl 1N[79]<br>○ Sodium hypochlorite (5%) in chloroform[82]<br>○ $Mo(CO)_6$ in presence of water[83]<br>○ *N*-bromosuccinimide in acetone/$H_2O$[85]<br>○ Pyridinium chlorochromate (PCC) under microwave radiation[86]<br>○ 70% *tert*-Butyl hydroperoxide (TBHP) in carbon tetrachloride[84]<br>○ Silica Chloride/Wet $SiO_2$ in hexane[87]<br>○ γ-picolinium chlorochromate (γ-PCC) in DCM[88]<br>○ Iodine in 30% of $H_2O_2$ in aqueous acetonitrile[89]<br>○ Aqueous 36% $H_2O_2$ and an aqueous 48% HBr solution[90]<br>○ $Si_2Cl_6$ in anhydrous THF in the presence of $SiO_2$[91]<br>○ $K_3[Fe(CN)_6].3H_2O$ supported on silica gel in ethanol and water[92] |
|  **3.40a** | ○ Boron trifluoride etherate in acetone/$H_2O$[81]<br>○ 70% *tert*-Butyl hydroperoxide (TBHP) in carbon tetrachloride[84] |
| \  **3.40b** | ○ Silica Chloride/Wet $SiO_2$ in hexane[87] |

For our delight, when BIM oximes **3.41a**, **3.41b**, and **3.41c** were treated with sodium bisulfite in EtOH/$H_2O$ at reflux during 2 h followed by treatment with HCl 3M at room temperature during 30 minutes [80] the hydrolysis was achieved, leading to the carbonyl-BIMs **3.45a**, **3.45b**, and **3.45d** in moderate to good yields (32-53%). Starting from hydrazones derived BIMs **3.40b** and **3.40c** using paraformaldehyde in presence of amberlyst-15 in acetone/$H_2O$ at room temperature during 48 h, [93] the target carbonyl-BIMs **3.45a** and **3.45b** were also obtained in good yields (48 and 53 %) (Scheme 3.12).



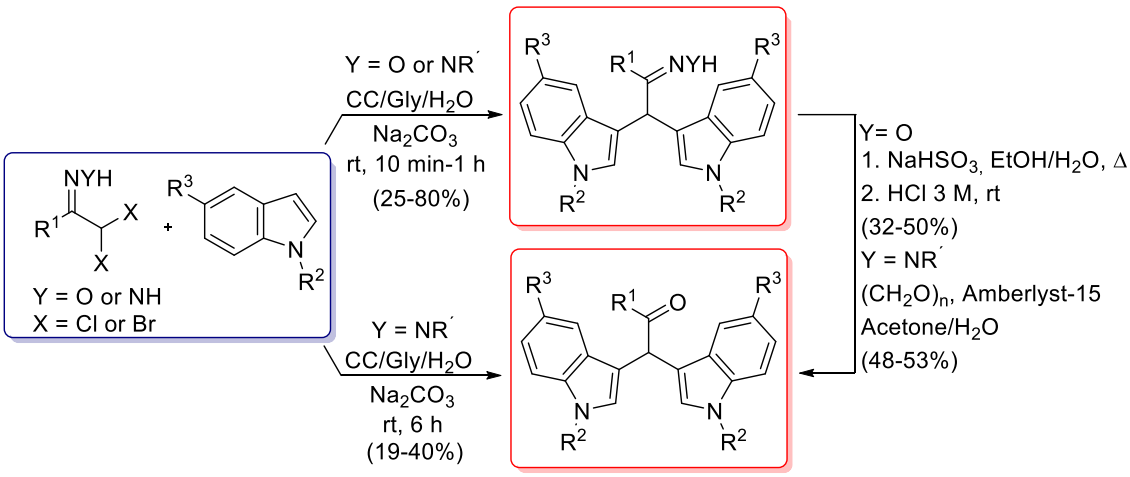

**Scheme 3.12** Hydrolysis reaction of BIM oximes and hydrazones.

The regeneration of carbonyl from hydroxyimino- and hydrazonomethyl-BIMs is a very challenging task, as shown by the presented results. Therefore, we believe that the $H_2O$/ NADES system represents an asset with added value.

## 3.5 Conclusions

The chapter describes an unprecedented use of natural deep eutectic solvents as an efficient medium for the synthesis of bis(indolyl)methanes, bearing hydroxyiminomethyl and hydrazonomethyl functionalities, via bis-hetero-Diels-Alder reaction between indoles and nitroso- azo-alkenes. The ternary mixture $H_2O$/ choline chloride:glycerol proved to be the best solvent system affording the desired BIM oximes and hydrazones, in much shorter reaction times, higher yields, allowing easier isolation procedures. We were also pleased to develop a novel strategy for the preparation of carbonyl-BIMs via one pot procedure using natural deep eutectic solvents.

Moreover, the hydrolysis of hydroxyiminomethyl and hydrazonomethyl-BIMs was also achieved by treatment with sodium bisulfite or paraformaldehyde in presence of amberlyst-15 in acetone/$H_2O$, respectively [96].



## 3.6 References

1. Andrade, C. K. Z.; Dar, A. R., Applying green processes and techniques to simplify reaction work-ups. *Tetrahedron* **2016**, *72* (47), 7375-7391.

2. Liu, Y.; Friesen, J. B.; McAlpine, J. B.; Lankin, D. C.; Chen, S.-N.; Pauli, G. F., Natural deep eutectic solvents: Properties, applications, and perspectives. *J. Nat. Prod.* **2018**, *81* (3), 679-690.

3. Tomé, L. I. N.; Baião, V.; da Silva, W.; Brett, C. M. A., Deep eutectic solvents for the production and application of new materials. *Appl. Mater. Today* **2018**, *10*, 30-50.

4. Hansen, B. B.; Spittle, S.; Chen, B.; Poe, D.; Zhang, Y.; Klein, J. M.; Horton, A.; Adhikari, L.; Zelovich, T.; Doherty, B. W.; Gurkan, B.; Maginn, E. J.; Ragauskas, A.; Dadmun, M.; Zawodzinski, T. A.; Baker, G. A.; Tuckerman, M. E.; Savinell, R. F.; Sangoro, J. R., Deep eutectic solvents: A review of fundamentals and applications. *Chem. Rev.* **2020**, *121* (3), 1232-1285.

5. Smith, E. L.; Abbott, A. P.; Ryder, K. S., Deep eutectic solvents (DESs) and their applications. *Chem. Rev.* **2014**, *114* (21), 11060-11082.

6. Fetisov, E. O.; Harwood, D. B.; Kuo, I. F. W.; Warrag, S. E. E.; Kroon, M. C.; Peters, C. J.; Siepmann, J. I., First-principles molecular dynamics study of a deep eutectic solvent: choline chloride/urea and its mixture with water. *J. Phys. Chem. B* **2018**, *122* (3), 1245-1254.

7. Soltanmohammadi, F.; Jouyban, A.; Shayanfar, A., New aspects of deep eutectic solvents: Extraction, pharmaceutical applications, as catalyst and gas capture. *Chem. Pap.* **2020**, *75* (2), 439-453.

8. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jérôme, F., Deep eutectic solvents: Syntheses, properties and applications. *Chem. Soc. Rev.* **2012**, *41* (21).

9. Chen, Y.; Mu, T., Revisiting greenness of ionic liquids and deep eutectic solvents. *GreenChE* **2021**, *2* (2), 174-186.

10. Perkins, S. L.; Painter, P.; Colina, C. M., Experimental and computational studies of choline chloride-based deep eutectic solvents. *J. Chem. Eng. Data* **2014**, *59* (11), 3652-3662.

11. Abbott, A. P.; Barron, J. C.; Ryder, K. S.; Wilson, D., Eutectic-based ionic liquids with metal-containing anions and cations. *Chem. Eur. J.* **2007**, *13* (22), 6495-6501.

12. Vanda, H.; Dai, Y.; Wilson, E. G.; Verpoorte, R.; Choi, Y. H., Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents. *C. R. Chim.* **2018**, *21* (6), 628-638.

13. Choi, Y. H.; van Spronsen, J.; Dai, Y.; Verberne, M.; Hollmann, F.; Arends, I. W. C. E.; Witkamp, G.-J.; Verpoorte, R., Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiol.* **2011**, *156* (4), 1701-1705.

14. Dai, Y.; van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H., Natural deep eutectic solvents as new potential media for green technology. *Anal. Chim. Acta* **2013**, *766*, 61-68.

15. Paiva, A.; Craveiro, R.; Aroso, I.; Martins, M.; Reis, R. L.; Duarte, A. R. C., Natural deep eutectic solvents – solvents for the 21st century. *ACS Sustain. Chem. Eng.* **2014**, *2* (5), 1063-1071.

16. Guedes, J. R.; Custodia, C. M.; Silva, R. J.; de Almeida, L. P.; Pedroso de Lima, M. C.; Cardoso, A. L., Early miR-155 upregulation contributes to neuroinflammation in Alzheimer's disease triple transgenic mouse model. *Hum. Mol. Genet.* **2014**, *23* (23), 6286-301.

17. Wikene, K. O.; Rukke, H. V.; Bruzell, E.; Tønnesen, H. H., Physicochemical characterisation and antimicrobial phototoxicity of an anionic porphyrin in natural deep eutectic solvents. *Eur. J. Pharm. Biopharm.* **2016**, *105*, 75-84.

18. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K., Deep eutectic solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids. *J. Am. Chem. Soc.* **2004**, *126* (29), 9142-9147.

19. Gutiérrez, M. C.; Ferrer, M. L.; Mateo, C. R.; del Monte, F., Freeze-drying of aqueous solutions of deep eutectic solvents: A suitable approach to deep eutectic suspensions of self-assembled structures. *Langmuir* **2009**, *25* (10), 5509-5515.

20. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixtures. *Chem. Comm.* **2003**, (1), 70-71.

21. Shahbaz, K.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M., Prediction of deep eutectic solvents densities at different temperatures. *Thermochim. Acta* **2011**, *515* (1-2), 67-72.

22. Savi, L. K.; Carpiné, D.; Waszczynskyj, N.; Ribani, R. H.; Haminiuk, C. W. I., Influence of temperature, water content and type of organic acid on the formation, stability

and properties of functional natural deep eutectic solvents. *Fluid Ph. Equilibria* **2019**, *488*, 40-47.

23. Khandelwal, S.; Tailor, Y. K.; Kumar, M., Deep eutectic solvents (DESs) as eco-friendly and sustainable solvent/catalyst systems in organic transformations. *J. Mol. Liq.* **2016**, *215*, 345-386.

24. Kalhor, P.; Ghandi, K., Deep eutectic solvents for pretreatment, extraction, and catalysis of biomass and food waste. *Molecules* **2019**, *24* (22).

25. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V., Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications* **2003**, (1), 70-71.

26. Abbott, A. P.; Harris, R. C.; Ryder, K. S., Application of hole theory to define ionic liquids by their transport properties. *J. Phys. Chem. B* **2007**, *111* (18), 4910-4913.

27. Chemat, F.; You, H. J.; Muthukumar, K.; Murugesan, T., Effect of l-arginine on the physical properties of choline chloride and glycerol based deep eutectic solvents. *J. Mol. Liq.* **2015**, *212*, 605-611.

28. D'Agostino, C.; Harris, R. C.; Abbott, A. P.; Gladden, L. F.; Mantle, M. D., Molecular motion and ion diffusion in choline chloride based deep eutectic solvents studied by $^{1}$H pulsed field gradient NMR spectroscopy. *Phys. Chem. Chem. Phys.* **2011**, *13* (48).

29. Arnold, T.; Jackson, A. J.; Sanchez-Fernandez, A.; Magnone, D.; Terry, A. E.; Edler, K. J., Surfactant behavior of sodium dodecylsulfate in deep eutectic solvent choline chloride/urea. *Langmuir* **2015**, *31* (47), 12894-12902.

30. Sanchez-Fernandez, A.; Hammond, O. S.; Jackson, A. J.; Arnold, T.; Doutch, J.; Edler, K. J., Surfactant–solvent interactioneEffects on the micellization of cationic surfactants in a carboxylic acid-based deep eutectic solvent. *Langmuir* **2017**, *33* (50), 14304-14314.

31. Shekaari, H.; Zafarani-Moattar, M. T.; Mokhtarpour, M., Experimental determination and correlation of acetaminophen solubility in aqueous solutions of choline chloride based deep eutectic solvents at various temperatures. *Fluid Ph. Equilibria* **2018**, *462*, 100-110.

32. Alonso, D. A.; Baeza, A.; Chinchilla, R.; Guillena, G.; Pastor, I. M.; Ramón, D. J., Deep eutectic solvents: The organic reaction medium of the century. *Eur. J. Org. Chem.* **2016**, *2016* (4), 612-632.

33. Tang, B.; Row, K. H., Recent developments in deep eutectic solvents in chemical sciences. *Monatsh. Chem.* **2013**, *144* (10), 1427-1454.

34. Abbott, A. P.; Harris, R. C.; Ryder, K. S.; D'Agostino, C.; Gladden, L. F.; Mantle, M. D., Glycerol eutectics as sustainable solvent systems. *Green Chem.* **2011**, *13* (1), 82-90.

35. Hayyan, A.; Mjalli, F. S.; Al-Nashef, I. M.; Al-Wahaibi, Y. M.; Al-Wahaibi, T.; Hashim, M. A., Glucose-based deep eutectic solvents: Physical properties. *J. Mol. Liq.* **2013**, *178*, 137-141.

36. Xu, P.; Zheng, G.-W.; Zong, M.-H.; Li, N.; Lou, W.-Y., Recent progress on deep eutectic solvents in biocatalysis. *Bioresour. Bioprocess.* **2017**, *4* (1), 1-18.

37. Ünlü, A. E.; Arıkaya, A.; Takaç, S., Use of deep eutectic solvents as catalyst: A mini-review. *Green Process. Synth.* **2019**, *8* (1), 355-372.

38. Molnar, M.; Lončarić, M.; Jakovljević, M.; Komar, M.; Lončar, M., Some applications of deep eutectic solvents in alkylation of heterocyclic compounds: A review of the past 10 years. *Heterocycl. Commun* **2021**, *27* (1), 45-56.

39. Ghosh, S. K.; Nagarajan, R., Deep eutectic solvent mediated synthesis of quinazolinones and dihydroquinazolinones: Synthesis of natural products and drugs. *RSC Adv.* **2016**, *6* (33), 27378-27387.

40. Kotha, S.; Chakkapalli, C., Application of Fischer indolization under green conditions using deep eutectic solvents. *Chem. Rec.* **2017**, *17* (10), 1039-1058.

41. Martínez, R.; Berbegal, L.; Guillena, G.; Ramón, D. J., Bio-renewable enantioselective aldol reaction in natural deep eutectic solvents. *Green Chem.* **2016**, *18* (6), 1724-1730.

42. Yadav, U. N.; Shankarling, G. S., Room temperature ionic liquid choline chloride–oxalic acid: A versatile catalyst for acid-catalyzed transformation in organic reactions. *J. Mol. Liq.* **2014**, *191*, 137-141.

43. Cherukuvada, S.; Nangia, A., Eutectics as improved pharmaceutical materials: Design, properties and characterization. *Chem. Commun.* **2014**, *50* (8), 906-923.

44. Li, Z.; Lee, P. I., Investigation on drug solubility enhancement using deep eutectic solvents and their derivatives. *Int. J. Pharm.* **2016**, *505* (1-2), 283-288.

45. Aroso, I. M.; Silva, J. C.; Mano, F.; Ferreira, A. S. D.; Dionísio, M.; Sá-Nogueira, I.; Barreiros, S.; Reis, R. L.; Paiva, A.; Duarte, A. R. C., Dissolution enhancement of

active pharmaceutical ingredients by therapeutic deep eutectic systems. *Eur. J. Pharm. Biopharm.* **2016**, *98*, 57-66.

46. Duarte, A. R. C.; Ferreira, A. S. D.; Barreiros, S.; Cabrita, E.; Reis, R. L.; Paiva, A., A comparison between pure active pharmaceutical ingredients and therapeutic deep eutectic solvents: Solubility and permeability studies. *Eur. J. Pharm. Biopharm.* **2017**, *114*, 296-304.

47. Emami, S.; Shayanfar, A., Deep eutectic solvents for pharmaceutical formulation and drug delivery applications. *Pharm. Dev. Technol.* **2020**, *25* (7), 779-796.

48. Azizi, N.; Manocheri, Z., Eutectic salts promote green synthesis of bis(indolyl) methanes. *Res. Chem. Intermed.* **2012**, *38* (7), 1495-1500.

49. Handy, S.; Westbrook, N. M., A mild synthesis of bis(indolyl)methanes using a deep eutectic solvent. *Tetrahedron Lett.* **2014**, *55* (35), 4969-4971.

50. Seyedi, N.; Khabazzadeh, H.; Saeednia, S., $ZnCl_2$/urea as a deep eutectic solvent for the preparation of bis(indolyl)methanes under ultrasonic conditions. *Synth. React. Inorg. Met. Org. Chem.* **2015**, *45* (10), 1501-1505.

51. Bafti, B.; Khabazzadeh, H., Dimethylurea/citric acid as a highly efficient deep eutectic solvent for the multi-component reactions. *J. Chem. Sci.* **2014**, *126* (3), 881-887.

52. He, F.; Li, P.; Gu, Y.; Li, G., Glycerol as a promoting medium for electrophilic activation of aldehydes: Catalyst-free synthesis of di(indolyl)methanes, xanthene-1,8(2H)-diones and 1-oxo-hexahydroxanthenes. *Green Chem.* **2009**, *11* (11), 1767–1773

53. Deb, M. L.; Bhuyan, P. J., An efficient and clean synthesis of bis(indolyl)methanes in a protic solvent at room temperature. *Tetrahedron Lett.* **2006**, *47* (9), 1441-1443.

54. Behbahani, K.; Sasani, M., Facile synthesis of bis(indolyl)methanes using iron(III) phosphate. *J. Serb. Chem. Soc.* **2012**, *77* (9), 1157-1163.

55. Rahimizadeh, M.; Eshghi, H.; Bakhtiarpoor, Z.; Pordel, M., Ferric hydrogensulfate as a recyclable catalyst for the synthesis of some new bis(indolyl)methane derivatives. *J. Chem. Res.* **2009**, (5), 269-270.

56. Sadaphal, S.; Shelke, K.; Sonar, S.; Shingare, M., Ionic liquid promoted synthesis of bis(indolyl)methanes. *Open Chem. J.* **2008**, *6* (4), 622-626.

57. Nagawade, R. R.; Shinde, D., Zirconium (IV) chloride - catalysed reaction of indoles: An expeditious synthesis of bis(indolyl)methanes. *Bull. Korean Chem. Soc.* **2005**, *26* (12), 1962-1964.

58. Jella, R. R.; Nagarajan, R., Synthesis of indole alkaloids arsindoline A, arsindoline B and their analogues in low melting mixture. *Tetrahedron* **2013**, *69* (48), 10249-10253.

59. Sanap, A. K.; Shankarling, G. S., Choline chloride based eutectic solvents: Direct C-3 alkenylation/alkylation of indoles with 1,3-dicarbonyl compounds. *RSC Adv.* **2014**, *4* (66), 34938-34943.

60. Chandam, D.; Mulik, A.; Patil, P.; Jagdale, S.; Patil, D.; Sankpal, S.; Deshmukh, M., Oxalic acid dihydrate: Proline (LTTM) as a new generation solvent for synthesis of 3,3-diaryloxindole and chromone based bis(indolyl)alkanes: Green, chromatography free protocol. *J. Mol. Liq.* **2015**, *207*, 14-20.

61. Liu, P.; Hao, J.-W.; Mo, L.-P.; Zhang, Z.-H., Recent advances in the application of deep eutectic solvents as sustainable media as well as catalysts in organic reactions. *RSC Adv.* **2015**, *5* (60), 48675-48704.

62. Punzi, A.; Coppi, D. I.; Matera, S.; Capozzi, M. A. M.; Operamolla, A.; Ragni, R.; Babudri, F.; Farinola, G. M., Pd-catalyzed thiophene–aryl coupling reaction via C–H bond activation in deep eutectic solvents. *Org. Lett.* **2017**, *19* (18), 4754-4757.

63. Faraone, A.; Wagle, D. V.; Baker, G. A.; Novak, E. C.; Ohl, M.; Reuter, D.; Lunkenheimer, P.; Loidl, A.; Mamontov, E., Glycerol hydrogen-bonding network dominates structure and collective dynamics in a deep eutectic solvent. *J. Phys. Chem. B* **2018**, *122* (3), 1261-1267.

64. Ramón, D. J.; Guillena, G., *Organic synthesis in DESs*. Università di Bari "Aldo Moro", Consorzio C.I.N.M.P.I.S., Dipartimento di Farmacia–Scienze del Farmaco: **2019**.

65. Mohamadpour, F., Green and convenient one-pot access to polyfunctionalized piperidine scaffolds via glutamic acid catalyzed knoevenagel-intramolecular [4+2] aza-Diels-Alder imin-based multi-component reaction under ambient temperature. *Polycycl. Aromat. Compd.* **2018**, *40* (3), 681-692.

66. Imperato, G.; Eibler, E.; Niedermaier, J.; König, B., Low-melting sugar–urea–salt mixtures as solvents for Diels–Alder reactions. *Chem. Commun.* **2005**, (9), 1170-1172.

67. Marullo, S.; Meli, A.; D'Anna, F., A joint action of deep eutectic solvents and ultrasound to promote Diels–Alder reaction in a sustainable way. *ACS Sustain. Chem. Eng.* **2020**, *8* (12), 4889-4899.

68. S., N. A.; A., K., Eutectic mixture-directed kinetics of Diels-Alder reaction. *Indian J. Chem.* **2011**, *50A*, 788-792.

69. Torres, P.; Balcells, M.; Canela-Garayoa, R., Effect of novel deep eutectic solvents on the endo/exo ratio of Diels–Alder reactions at room temperature. *ACS Omega* **2021**, *6* (30), 19392-19399.

70. Vidal, C.; Merz, L.; García-Álvarez, J., Deep eutectic solvents: Biorenewable reaction media for Au(i)-catalysed cycloisomerisations and one-pot tandem cycloisomerisation/Diels–Alder reactions. *Green Chem.* **2015**, *17* (7), 3870-3878.

71. Houk, K. N.; Strozier, R. W., Lewis acid catalysis of Diels-Alder reactions. *J. Am. Chem. Soc.* **2002**, *95* (12), 4094-4096.

72. Oakes, R. S.; Heppenstall, T. J.; Shezad, N.; Clifford, A. A.; Rayner, C. M., Use of scandium tris(trifluoromethanesulfonate) as a Lewis acid catalyst in supercritical carbon dioxide: Efficient Diels–Alder reactions and pressure dependent enhancement of endo:exo stereoselectivity. *Chem. Comm.* **1999**, (16), 1459-1460.

73. Manabe, K.; Mori, Y.; Kobayashi, S., Effects of Lewis acid-surfactant-combined catalysts on aldol and Diels-Alder reactions in water. *Tetrahedron* **1999**, *55* (37), 11203-11208.

74. Dai, Y.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H., Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chem.* **2015**, *187*, 14-19.

75. Zhekenov, T.; Toksanbayev, N.; Kazakbayeva, Z.; Shah, D.; Mjalli, F. S., Formation of type III deep eutectic solvents and effect of water on their intermolecular interactions. *Fluid Ph. Equilibria* **2017**, *441*, 43-48.

76. Gabriele, F.; Chiarini, M.; Germani, R.; Tiecco, M.; Spreti, N., Effect of water addition on choline chloride/glycol deep eutectic solvents: Characterization of their structural and physicochemical properties. *J. Mol. Liq.* **2019**, *291*, 111301-111308.

77. Di Pietro, M. E.; Tortora, M.; Bottari, C.; Colombo Dugoni, G.; Pivato, R. V.; Rossi, B.; Paolantoni, M.; Mele, A., In competition for water: Hydrated choline chloride:urea vs choline acetate:urea deep eutectic solvents. *ACS Sustain. Chem. Eng.* **2021**, *9* (36), 12262-12273.

78. Ferreira, A. S. D.; Craveiro, R.; Duarte, A. R.; Barreiros, S.; Cabrita, E. J.; Paiva, A., Effect of water on the structure and dynamics of choline chloride/glycerol eutectic systems. *J. Mol. Liq.* **2021**, *342*, 117463-117476.

79. DePuy, C. H.; Ponder, B. W., Levulinic acid as a reagent for the hydrolysis of oximes and 2,4-dinitrophenylhydrazones. *J. Am. Chem. Soc.* **2002**, *81* (17), 4629-4631.

80. Pines, S. H.; Chemerda, J. M.; Kozlowsk.Ma, Cleavage of oximes with bisulfite. A general procedure. *J. Org. Chem.* **1966**, *31* (10), 3446-3447.

81. Sacks, C. E.; Fuchs, P. L., Carbonyl regeneration from p-toluenesulfonylhydrazones (tosylhydrazones). *Synthesis* **1976**, *1976* (07), 456-457.

82. Khurana, J. M.; Ray, A.; Sahoo, P. K., Oxidative deoximation with sodium hypochlorite. *B. Chem. Soc. Jpn.* **1994**, *67* (4), 1091-1093.

83. Geneste, F.; Racelma, N.; Moradpour, A., $Mo(Co)_6$ i nduced cleavage of oximes. *Synth. Commun.* **2006**, *27* (6), 957-960.

84. Barhate, N. B.; Gajare, A. S.; Wakharkar, R. D.; Sudalai, A., Facile regeneration of carbonyl compounds from oximes and tosylhydrazones with TBHP. *Tetrahedron Lett.* **1997**, *38* (4), 653-656.

85. Bandgar, B. P.; Kale, R. R.; Kunde, L. B., ChemInformabstract: Selective regeneration of carbonyl compounds from oximes with *N*-bromosuccinimide under neutral and mild conditions. *ChemInform* **2010**, *30* (3), 1057-1060.

86. Chakraborty, V.; Bordoloi, M., Deoximation by pyridinium chlorochromate under microwave irradiation. *J. Chem. Res.* **1999**, (2), 120-121.

87. Shirini, F.; Zolfigol, M. A.; Khaleghi, M.; Mohammadpour-Baltork, I., Silica chloride/wet $SiO_2$ as a novel heterogeneous system for deprotection of oximes, hydrazones, and semicarbazones. *Synth. Commun.* **2003**, *33* (11), 1839-1844.

88. Salehi, P.; Khodaei, M. M.; Goodarzi, M., A mild and selective deoximation method using γ-picolinium chlorochromate (γ-Pcc). *Synth. Commun.* **2002**, *32* (8), 1259-1263.

89. Ganguly, N. C.; Nayek, S.; Barik, S. K., Convenient, mild catalytic deprotection of oximes to carbonyl compounds with hydrogen peroxide and iodine catalyst in aqueous acetonitrile. *Synth. Commun.* **2009**, *39* (22), 4053-4061.

90. Terent'ev, A.; Krylov, I.; Vil', V.; Pastukhova, Z.; Fastov, S.; Nikishin, G., Synthesis of dibromo ketones by the reaction of the environmentally benign $H_2O_2$-HBr system with oximes. *Open Chem. J.* **2012**, *10* (2), 360-367.

91. Du, L.; Gao, J.; Yang, S.; Wang, D.; Han, X.; Xu, Y.; Ding, Y., An efficient approach to deoximation using hexachlorodisilane under mild conditions. *Russ. J. Gen. Chem.* **2014**, *84* (11), 2200-2204.

92. Manesh, A. A.; Shaghasemi, B. S., $K_3[Fe(CN)_6].3H_2O$ supported on silica gel: An efficient and selective reagent for the cleavage of oximes to their corresponding carbonyl compounds in aqueous medium. *J. Chem. Sci.* **2015**, *127* (3), 493-497.

93. Miles, D. H.; Guasch, J.; Toste, F. D., A nucleophilic strategy for enantioselective intermolecular alpha-amination: Access to enantioenriched alpha-arylamino ketones. *J. Am. Chem. Soc.* **2015**, *137* (24), 7632-7635.

94. Joy, B.; Balaganesh, M.; Joseph Selvaraj, S., Electrocatalytic oxidation of benzaldehyde oxime using potassium perchlorate in biphasic medium. *Mater. Today: Proc.* **2017**, *4* (13), 12426-12436.

95. Sahu, S.; Sahu, S.; Patel, S.; Dash, S.; K., M. B., Deoximation of keto- and aldoximes to carbonyl compounds. *indian J. Chem. B* **2008**, *47B*, 259-271.

96. Grosso, C.; Brigas, A.; de los Santos, J. M.; Palacios, F.; Lemos, A.; Pinho e Melo, T. M. V. D., Natural deep eutectic solvents in the hetero-Diels–Alder approach to bis(indolyl)methanes. *Monatsh. Chem.* **2019**, *150* (7), 1275-1288.

# Chapter 4

## Conjugate Addition of Pyrazoles to Halogenated Nitroso- and Azoalkenes: A New Entry to Novel Bis(pyrazol-1-yl)methanes

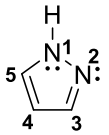

## Abstract

In this chapter, a new and versatile synthetic approach towards bis(pyrazolyl)methanes, based on 1,4-addition of pyrazoles to conjugated α-halogenated nitroso- and azoalkenes, is described. Tris(pyrazolyl)methanes could also be obtained by this methodology.

# Chapter 4. Conjugate Addition of Pyrazoles to Halogenated Nitroso and Azoalkenes: A New Entry to Novel Bis(pyrazol-1-yl)methanes

## 4.1 Pyrazoles

Pyrazoles are an important class of heterocyclic compounds having a 5-membered ring structure with three carbon and two nitrogen atoms in adjacent positions. They are electron-rich heterocyclic systems widely employed as building blocks in organic synthesis due to their versatile and rich chemistry. The pyrazole scaffold **4.1** (Figure 4.1) has three centres displaying nucleophilic features (N1, N2 and C4) and two electrophilic centres (C3 and C5) which allows it to participate in a large range of organic reactions.




**4.1**

**Figure 4.1** Chemical structure of 1*H*-pyrazole.

The occurrence in nature of the pyrazole core is rare due to the difficulty of living organisms to make a *N*-*N* bond.[1] Although scarce in nature, 1*H*-pyrazole is considered a privileged scaffold in medicinal chemistry. In fact , pyrazole derivatives exhibit a wild range of biological activities such as anti-tubercular, anti-viral, anti-Alzheimer, anti-diabetic, anti-tumour, anti-depressant, anti-microbial and anti-inflammatory.[2-20]

Pyrazole scaffold is also present in several synthetic marketed drugs such as rimonabant **4.2**, an inverse agonist for the cannabinoid receptor CB1 and an anorectic anti-obesity drug;[21] fomepizole **4.3**, a competitive inhibitor of the enzyme alcohol dehydrogenase used to treat ethylene glycol and methanol poisoning;[22] sulfaphenazole **4.4**, a sulfonamide antibacterial;[2] celecoxib (Celebrex®) **4.5**, a COX-2 inhibitor and nonsteroidal anti-inflammatory drug (NSAID),[3] and sildenafil (Viagra®) **4.6** a drug used to treat erectile dysfunction and pulmonary arterial hypertension[23] (Figure 4.2).

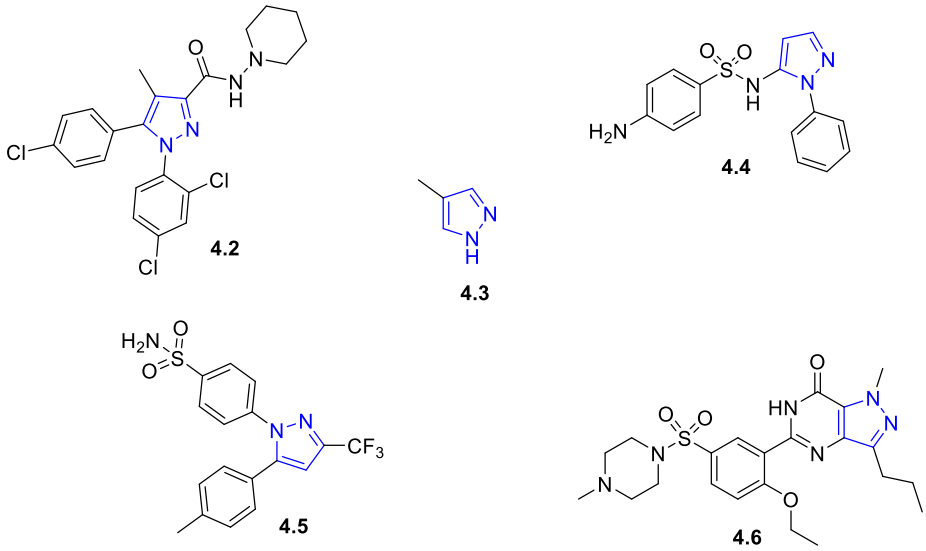



**Figure 4.2** Examples of marketed drugs containing the pyrazole scaffold.

Moreover, pyrazoles are also applied as fungicides,[24] pesticides,[4] insecticides[25] as well as chelating and extracting reagents for different metal ions.[26] More recently, pyrazoles have also been explored for the development of Positron Emission Tomography (PET) tracers.[27]

## 4.2 Synthesis of functionalised bis(pyrazolyl)methanes

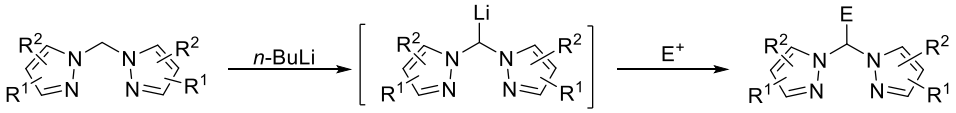
An important class of pyrazole derivatives are the bis(pyrazolyl)methanes (BPMs) which are one of the most fascinating family of stable and flexible bidentate ligands, isoelectronic and isosteric with the well-known bis(pyrazolyl)borates, firstly reported by Trofimenko.[28] Since then, their metal coordination chemistry with the main group and transition metals have been widely explored.[29-32]

The interest in BPM derivatives bearing an organic functional substituent at the central methylene bridge, has increased dramatically in the last decades. Their metal complexes have found broad applications in inorganic,[33-35] organometallic[36-39] and supramolecular chemistry,[40-43] as well as in metalloenzyme models [44-47] and metal-based antitumor compounds.[48-50] In this section will deal with synthetic approaches to BPMs.

The synthesis of BPMs was first reported by Trofimenko,[28] prepared by heating at 150 ºC a solution 1*H*- pyrazole in dichloromethane.

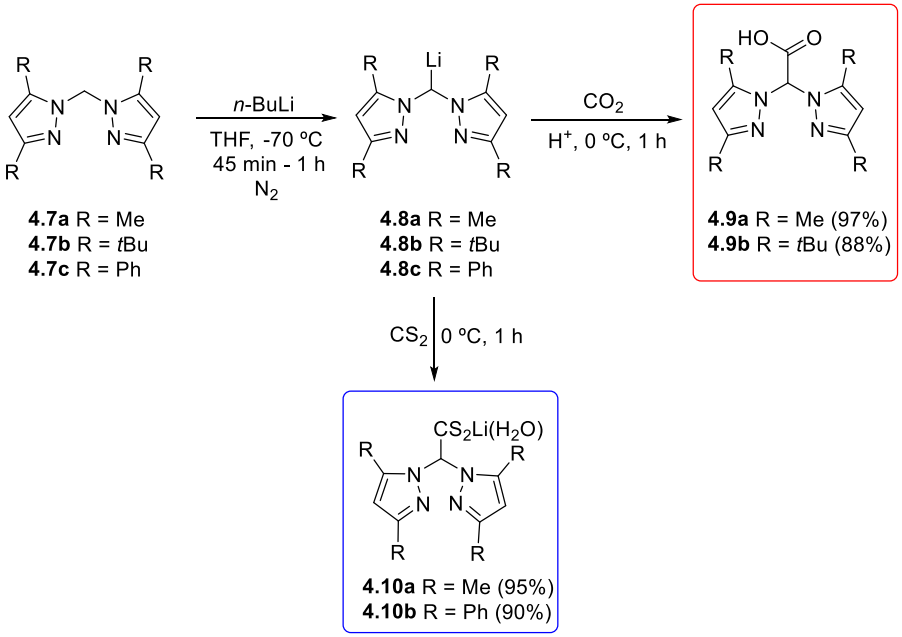
The main method for the functionalisation of the central methylene bridge of BPMs is outlined in Scheme 4.1. The formation of the anionic methylene bridge species from

deprotonation of BPM with *n*-butyllithium, followed by the reaction with the appropriate electrophile produces the desired BPM derivatives.[29-32]




**Scheme 4.1** Main method for the functionalisation of the central methylene bridge of BPMs.

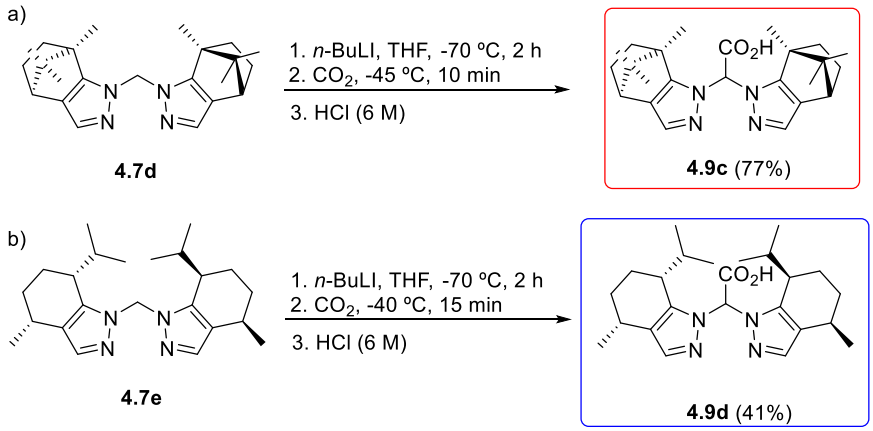
Carbon dioxide or carbon disulfide are examples of electrophiles employed to prepare these functionalised BPMs.[30, 51, 52] Deprotonation at the methylene group of the methylenebispyrazole systems **4.7** with *n*-BuLi in THF at -70 ºC, followed by treatment with carbon dioxide [30, 52] or carbon disulfide,[51] at 0 ºC, yielded the carboxylic acid-BPMs **4.9a** and **4.9b** or dithioacetate lithium salt BPMs **4.10a** and **4.10b**, respectively, in high yields (Scheme 4.2).




**Scheme 4.2** Synthesis of functionalised BPMs using carbon dioxide or carbon disulfide as electrophiles.

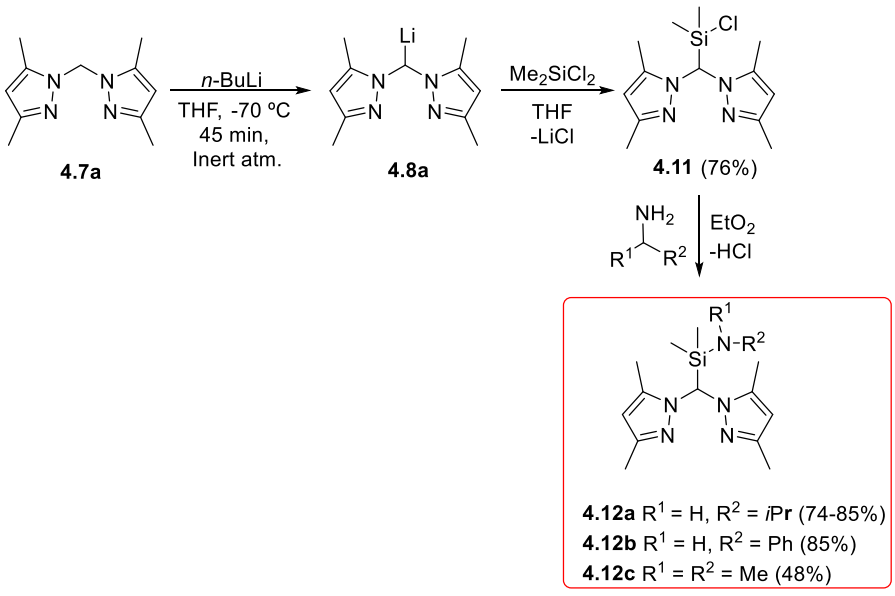
The synthesis of enantiopure chiral tripod ligands, obtained from $C_2$-symetric bis(camphorpyrazol-1-yl)methane **4.7d** or bis(menthonepyrazol-1-yl)methane **4.7e** by introduction of a carboxylate group at the methylene bridge was described (Scheme

(Scheme 4.3).[53, 54] Following this strategy, a prochiral centre is formed rather than an additional stereocentre (Scheme 4.3).



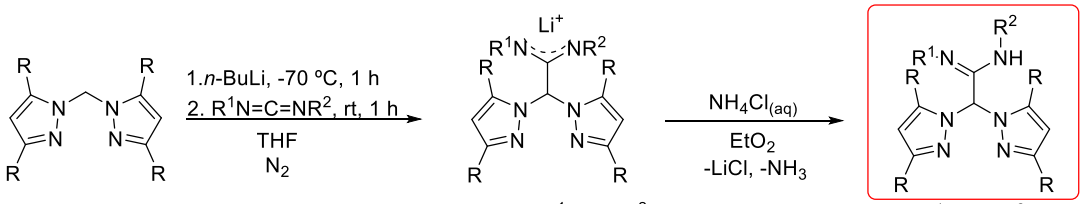

**Scheme 4.3** Synthesis of enantiopure chiral BPMs obtained from $C_2$-symmetric bis(camphorpyrazol-1-yl)methane or bis(menthonepyrazol-1-yl)methane.

Functionalised silicon-amine BPMs were also reported.[55, 56] As shown in Scheme 4.4, BPM **4.8a** reacts with $Me_2SiCl_2$ to form BPM **4.11** in good yield which then reacts smoothly with excess of primary alkyl or aryl amines to afford BPMs **4.12** in good to excellent yields (45-85%).




**Scheme 4.4** Synthesis of functionalised silicon-amine BPMs.

Several amidinate- and aminate-based BPMs (Scheme 4.5) were prepared as lithium salts or neutral BPMs[57]. Bis(3,5-dimethylpyrazol-1-yl)methane **4.7a** or bis(3,5-di-*tert*-butylpyrazol-1-yl)methane **4.7b** in THF in presence of *n*-BuLi reacted with the asymmetric *N*,*N*'-1-*tert*-butyl-3-ethylcarbodiimine ($R^1$ = Et, $R^2$ = *t*Bu) or symmetric carbodiimides *N*,*N*'-diisopropylcarbodiimine ($R^1 = R^2 =$ *i*Pr) and *N*,*N*'-diciclohexylcarbodiimine ($R^1 = R^2$ = Cy) leading to lithium acetamidinate BPMs **4.13a-c** in high yields (**4.13d** and **4.13e** were not isolated). These compounds, upon treatment with saturated aqueous ammonium chloride solution, in diethyl ether, were converted into the neutral BPMs **4.14** in excellent yields (Scheme 4.5).



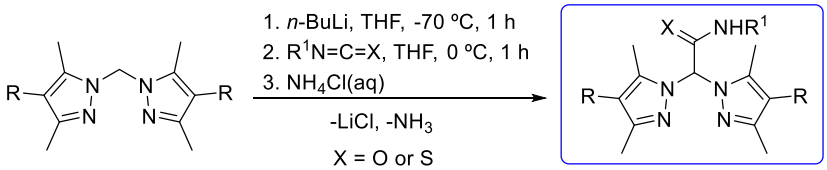

**4.7a** R = Me
**4.7b** R = *t*Bu

**4.13a** R = Me, $R^1$ = Et, $R^2$ = *t*Bu (85%)
**4.13b** R = Me, $R^1 = R^2$ = *i*Pr (86%)
**4.13c** R = Me, $R^1 = R^2$ = Cy (82%)
**4.13d** R = *t*Bu, $R^1$ = Et, $R^2$ = *t*Bu
**4.13e** R = *t*Bu, $R^1 = R^2$ = *i*Pr

**4.14a** R = Me, $R^1$ = Et, $R^2$ = *t*Bu (90%)
**4.14b** R = Me, $R^1 = R^2$ =*i*Pr (88%)
**4.14c** R = *t*Bu, $R^1$ = Et, $R^2$ =*t*Bu (96%)
**4.14d** R = *t*Bu, $R^1 = R^2$ = *i*Pr (98%)

**Scheme 4.5** Synthesis of amidinate- and aminate-based BPMs.

Following a two-step-one-pot methodology, it was possible to synthesise the acetamine- or thioacetamide-functionalised BPMs **4.15** (Scheme 4.6).[58, 59] Therefore, the one-pot reaction of BPMs **4.7** with *n*-BuLi, followed by the addition of diverse isocyanates ( $R^1$ = phenyl, *t*Bu, sBu, or (*S*)-(-)-α-methylbenzylisocyanates) or isothiocyanates ($R^1$ = phenyl or *tert*-butyl-isothiocyanates) and treatment with saturated aqueous ammonium chloride solution, led to the target BPMs **4.15** in good to excellent yields (60-90%).

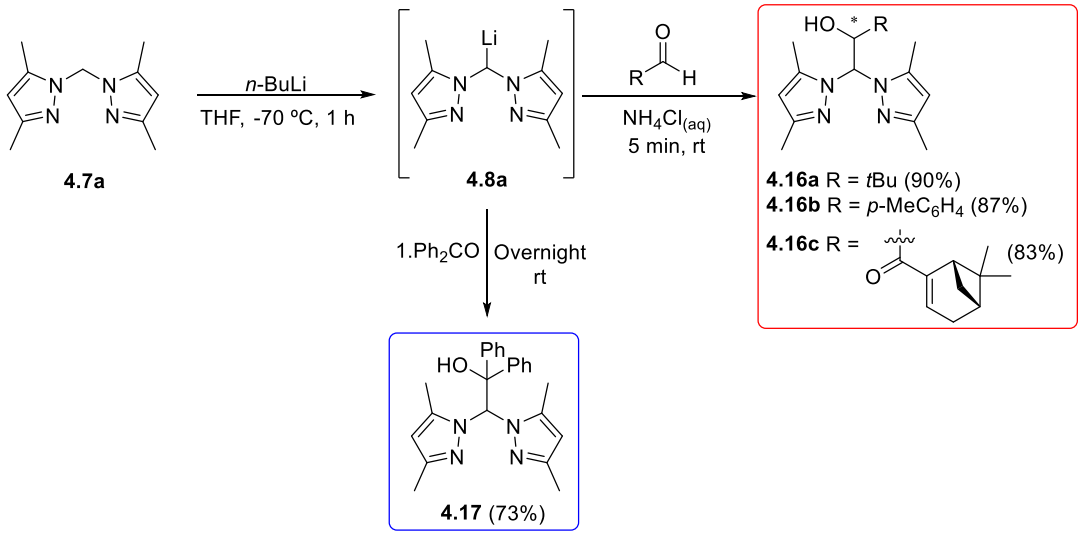



**4.7a** R = H
**4.7f** R = Me

**4.15a** R = H, $R^1$ = Ph, X = S (65%)
**4.15b** R = Me $R^1$ = Ph, X = S (60%)
**4.15c** R = H, $R^1$ = Ph, X = O (82%)
**4.15d** R = H, $R^1$ = Ph, X = S (84%)
**4.15e** R = H, $R^1$ = *t*Bu, X = O (81%)
**4.15f** R = H, $R^1$ = *t*Bu, X = S (81%)
**4.15g** R = H, $R^1$ = *s*Bu, X = O (85%)
**4.15h** R = H , $R^1$ = (*S*)-MePhCH, X = O (90%)

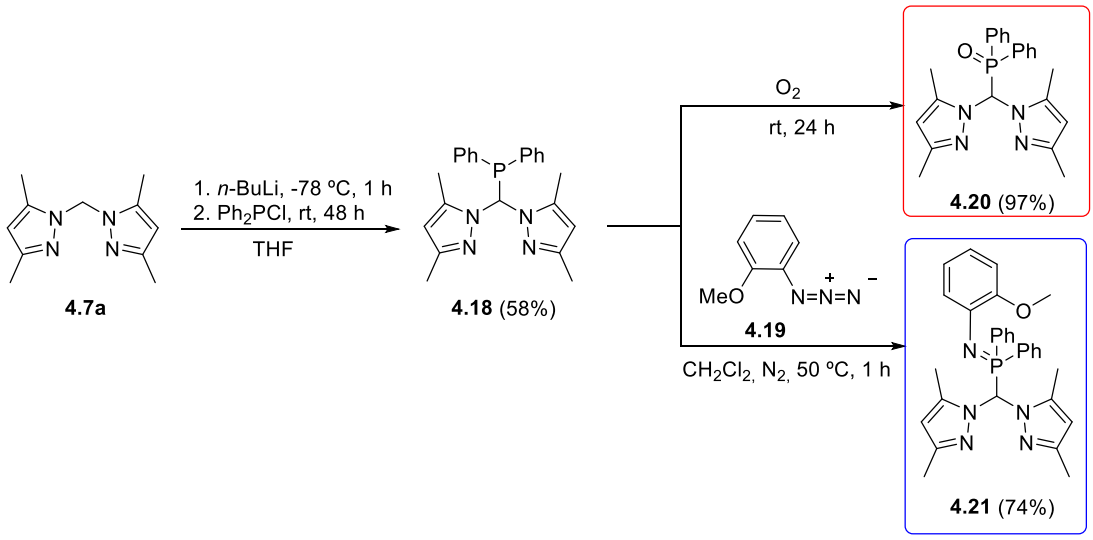
**Scheme 4.6** Synthesis of amidinate- and aminate-based BPMs.

Moreover, chiral BPMs bearing a secondary alcohol were synthesised following an identical synthetic strategy, as outlined in Scheme 4.7. Thus, in a simple high-yielding procedure involving the 1,2-addition of organometallics to diverse aldehydes, the chiral BPMs **4.16** were obtained (Scheme 4.7).[60, 61] On the other hand, the addition of benzophenone gave 2,2-[bis(3,5-dimethylpyrazol-1-yl)]-1,1-diphenylethanol (**4.17**) in 73% yield (Scheme 4.7).[62]



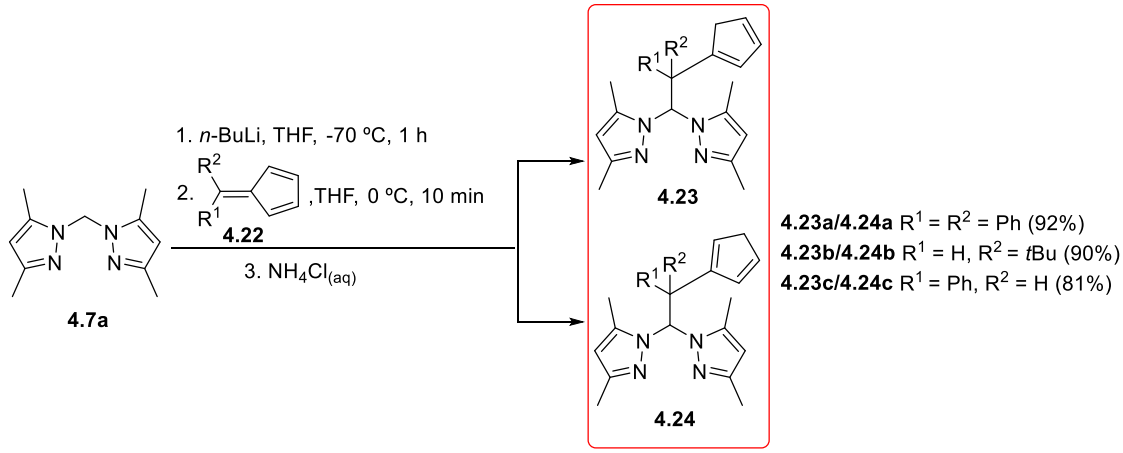

**Scheme 4.7** Synthesis of chiral BPMs bearing an alcohol moiety.

The preparation of phosphine, oxosphophine and iminophosphine based BPMs was also attained following an identical strategy (Scheme 4.8) [63] In the first step, BPMs **4.7a** was lithiated with *n*-BuLi producing *in situ* the lithium salt which then reacted with diphenylphospine chloride ($Ph_2PCl$) to afford the target BPM **4.18** in 56% yield. Subsequent oxidation with atmospheric oxygen or treatment with 2-azidoanisole **4.19** led to the transformation of **4.18** into the corresponding oxo-derivative **4.20** (97%) or the iminophosphine **4.21** derivative (74%), respectively (Scheme 4.8).[63]




**Scheme 4.8** Synthesis of phosphine, oxosphophine and iminophosphine based BPMs.

A new access to fulvene-based BPMs was described by Otero *et al.*[64 65] Reacting BPM **4.7a** with *n*-BuLi, followed by addition of three different fulvene derivatives **4.22** and treatment with saturated aqueous ammonium chloride solution led to a mixture of regioisomers **4.23** and **4.24** in a 3:1 ratio, in an overall yield ranging from 81 to 90% (Scheme 4.9).



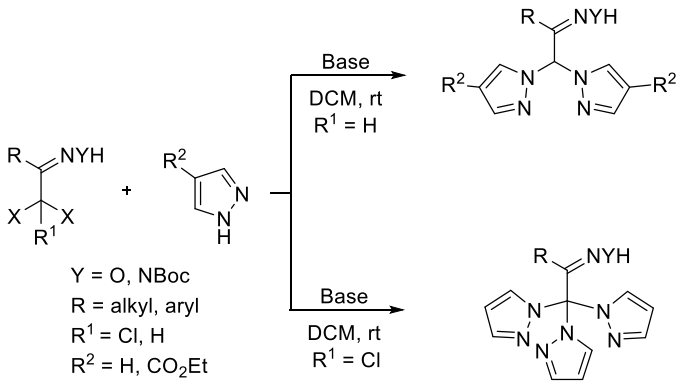

**Scheme 4.9** Synthesis of fulvene-based BPMs.

## 4.3 Rationale and goals

The aforementioned methodologies present several drawbacks, such as vulnerability of the pyrazole substituents to the strong base, the requirement of working in very low temperatures and/or inert atmospheres, the limited range of electrophiles that can be used, as well as problems with scale-up. Thus, the search for new methodologies for the synthesis of functionalised BPMs is highly desirable.

As already mentioned in Chapter 2, electrophilic conjugated nitroso- and azoalkenes have been used for the preparation of a plethora of new heterocyclic systems, participate in conjugate 1,4-addition reactions as Michael-type acceptors. Despite reports on chemistry of nitroso- and azoalkenes with azoles,[66] reactions of pyrazoles with α-halo or α,α'-dihalonitroso- or azoalkenes have not been disclosed. Thus, we set out to investigate a new and versatile approach to previously unknown bis- and tris-(pyrazolyl)methanes bearing a hydroxyamino or hydrazono functionality at the methylene bridge, based on the 1,4-conjugate addition of pyrazoles to these reactive intermediates.

The envisaged synthetic strategy for BPMs is shown in Scheme 4.10 and is based on the aforementioned methodology in Chapter 2 for the synthesis of bis(indolyl)methanes and dipyrromethanes, which explored sequential *in situ* generation of heterodienes followed by reaction with electron-rich heterocycles.[67]




**Scheme 4.10** Synthetic strategy towards bis- and tris(pyrazolyl)methanes.

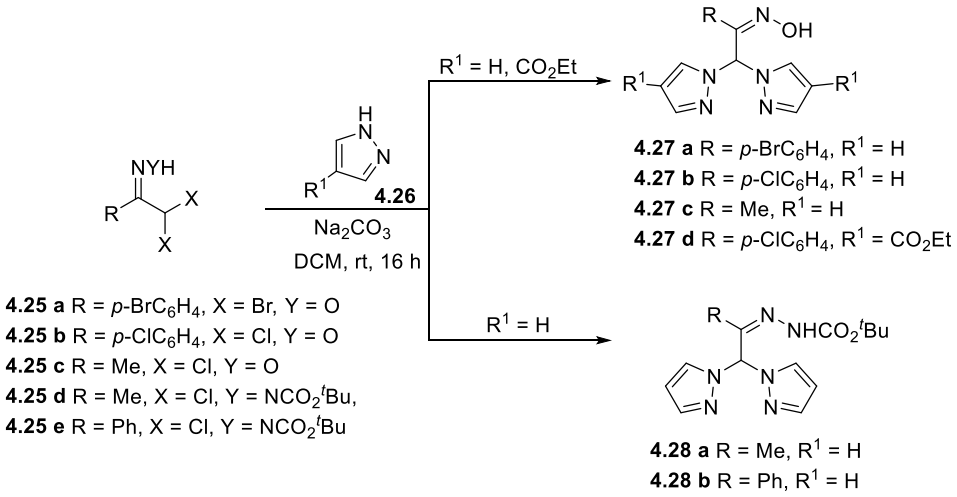
## 4.4 Synthesis of bis(pyrazolyl)methane oximes or hydrazones

Initially, the reactivity of pyrazoles **4.26** towards α,α'-dihalooximes and hydrazones **4.25**, in DCM at room temperature during 16 h, was explored (Table 4.1). The results show that starting from aryl α,α'-dichlorooximes **4.25a** and **4.25b** the target BPMs **4.27a** (67%) and **4.27b** (53%), could be obtain in good yields ( Table 4.1, entries 1 and 2). Starting from

oxime **4.25c** bearing a methyl group at C-3, the BPM **4.27c** was obtained in 43% yield (Table 4.1, entry 3). Comparing the reactions in which the α,α'-dichlorooxime **4.25c** participates (Table 4.1 entries 2 and 4), the results showed that the reaction with the less electron-rich 4-ethoxycarbonyl pyrazole produced BPM **4.27d** in lower yield (47%), isolated as mixture of isomeric oximes (Table 4.1, entry 4). These results clearly confirm that the efficiency of the reaction between pyrazoles and the conjugated heterodiene depends on the nucleophilicity of the first as well as on the electrophilicity of the later.

Enthusiastic about these results, it was decided to extend the study to reactions between pyrazole with electrophilic conjugated azoalkenes having a *tert*-butoxycarbonyl withdrawing group at N1 (Table 4.1, entries 5 and 6). The reaction of pyrazole with 3-methylazoalkene derivative was less efficient than the reaction with the 3-phenylazoalkene derivative, leading to BPM **4.28a** and **4.28b** in 57% and 77% yields, respectively (Table 4.1 entries 5 and 6).

**Table 4.1** Synthesis of Bis-(pyrazolyl)methane Oximes and Hydrazones.



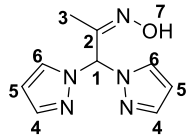

| Entry | R | X | Y | $R^1$ | Product, Yield (%) |
|---|---|---|---|---|---|
| 1 | *p*-$BrC_6H_4$ | Br | O | H | **4.27a** 67% |
| 2 | *p*-$ClC_6H_4$ | Cl | O | H | **4.27b** 53% |
| 3 | Me | Cl | O | H | **4.27c** 43% |
| 4 | *p*-$ClC_6H_4$ | Cl | O | $CO_2Et$ | **4.27d** 47% [a] |
| 5 | Me | Cl | $NCO_2{}^tBu$ | H | **4.28a** 57% |
| 6 | $C_6H_5$ | Cl | $NCO_2{}^tBu$ | H | **4.28a** 77% |

[a] Mixture of isomeric oximes

The unequivocal assignment of the structure of BPMs **4.27** and **4.28** was achieved by nuclear magnetic spectroscopy. The NMR spectra of the BPM **4.27c** are shown for exemplification purposes.

Table 4.2 shows the proton and carbon-13 assignments of the respective resonance signals based on the bi-dimensional NMR spectra (COSY, NOESY, HMQC, HMBC). The $^{1}H$ and $^{13}C$ NMR spectra of compound **4.27c**, are presented in figures 4.3 and 4.4, respectively.

**Table 4.2** $^{1}H$ and $^{13}C$ NMR signal assignments of BPM **4.27c**.




| Position | δ (ppm) $^{1}H$ NMR | δ (ppm) $^{13}C$ NMR |
|---|---|---|
| 3 | 1.73 (s, 3H) | 11.5 ($CH_3$) |
| 5 | 6.34 (*pseudo* t, *J* = 2.4 Hz, *J* = 1.6 Hz, 2H) | 106.2 (CH, Ar) |
| 1 | 7.46 (s, 1H) | 76.0 (CH *meso*) |
| 4 | 7.58 (d, *J* = 1.6 Hz, 2H) | 139.9 (CH, Ar) |
| 6 | 7.83 (d, *J* = 2.4 Hz, 2H) | 130.5 (CH, Ar) |
| 7 | 11.36 (s, 1H, OH) | - |
| 2 | - | 150.9 (C) |

In the $^{1}H$ NMR spectrum of BPM **4.27c** the typical signal of the oxime proton can be observed at 11.36 ppm (H-7) (Figure 4.3). The H-1 proton of the methylene bridge appears as a singlet at 7.46 ppm and the H-3 protons (methyl group) as a singlet at 1.72 ppm. In the $^{13}C$ NMR spectrum it is possible to observe the signal corresponding to C=N carbon of the oxime moiety at 150.9 ppm (C-2), the carbon of the methyl group at 11.5 ppm (C-3) and the methylene bridge carbon at 76.0 ppm (C-3) (Figure 4.4).

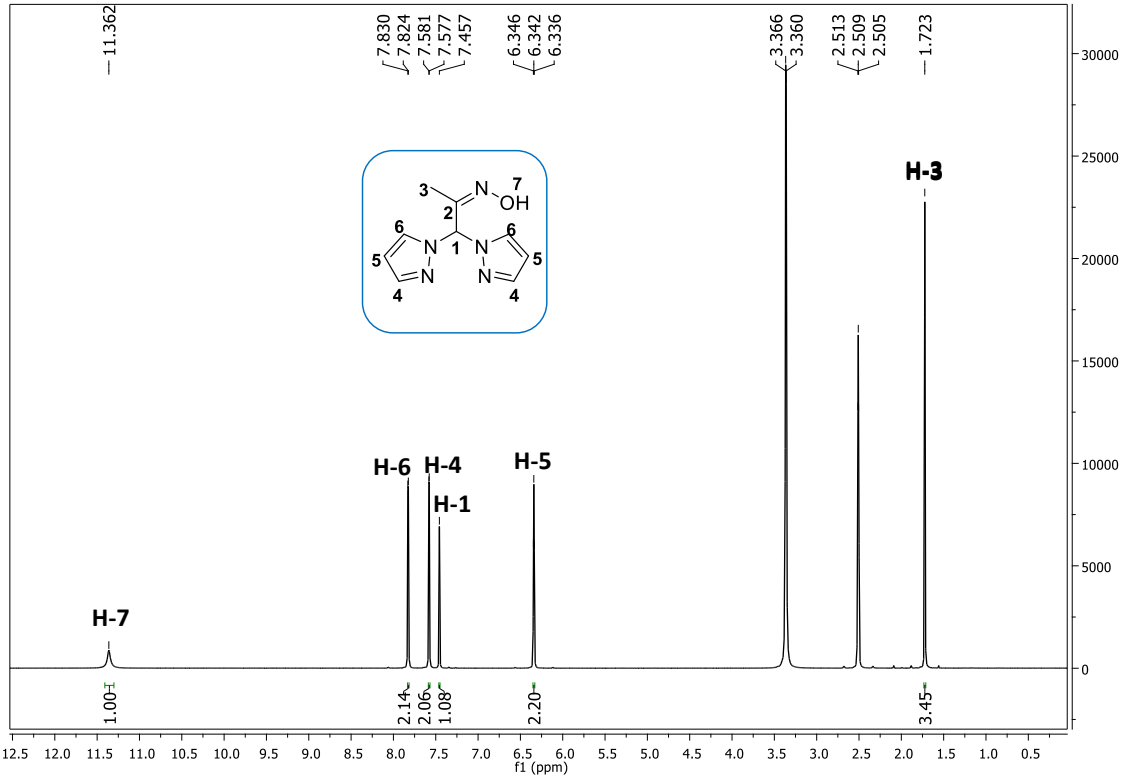



**Figure 4.3** $^{1}$H NMR spectrum of BPM **4.27c**.

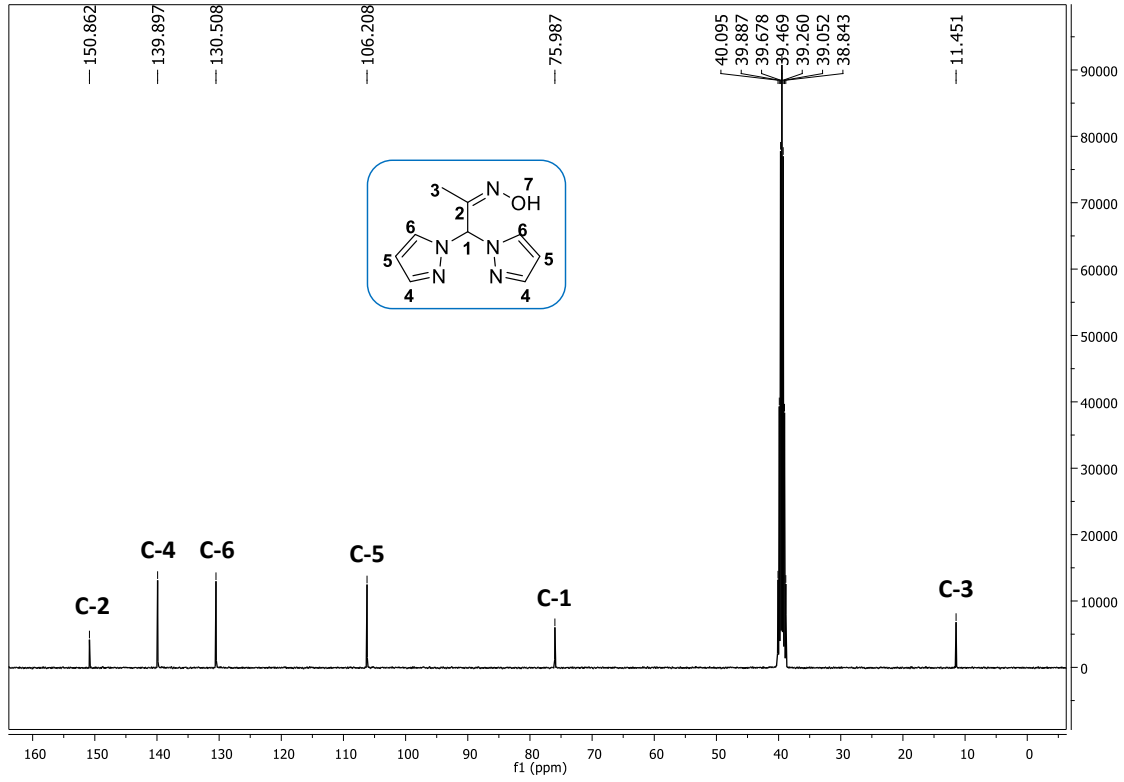



**Figure 4.4** $^{13}$C NMR spectrum of BPM **4.27c**.

The main interactions observed in the bi-dimensional NMR spectra are represented in Figure 4.5, allowing the assignment of the pyrazole's protons. In the COSY spectrum (Figure 4.6) it is possible to observe the correlation of the H-5 protons (*pseudo* triplet at 6.34 ppm) with H-6 protons (doublet at 7.86 ppm) and H-4 protons (doublet at 7.58 ppm). On the other hand, the H-6 protons (doublet at 7.83 ppm) were identified based on the NOESY spectrum in which it is observed the connectivity between these protons with the H-1 *meso* proton (singlet) at 7.16 ppm (Figure 4.7) .

The carbon assignments were made based on the HMQC and HMBC spectra analysis. The major connectivities observed in HMBC spectra are between the C-6 (130.5 ppm) and protons H-1 (7.46 ppm), H-5 (6.34 ppm) and H-4 (7.58 ppm), between C-5 (106.2 ppm) and protons H-6 (7.83 ppm) and H-4 and between C-4 (139.9 ppm) and H-5 and H-6 protons (Figure 4.9).

The geometry of the oxime moiety of BPM **4.27c** was confirmed by the analysis of the NOESY spectrum in which no connectivity was observed between the hydroxyl proton H-7 and the methyl group protons H-3. Therefore, this indicates that these substituents are in *trans* position. The same stereochemistry was assigned by analogy for all oximes **4.27** and hydrazones **4.28**.

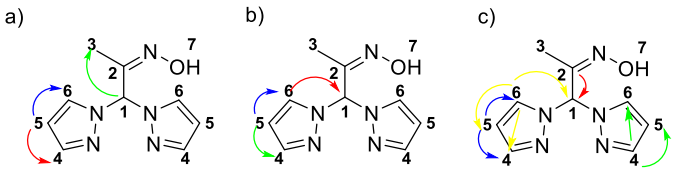



**Figure 4.5** Main connectivities observed in the COSY (a), NOESY (b) and HMBC (c) spectra of BPM **4.27c**.

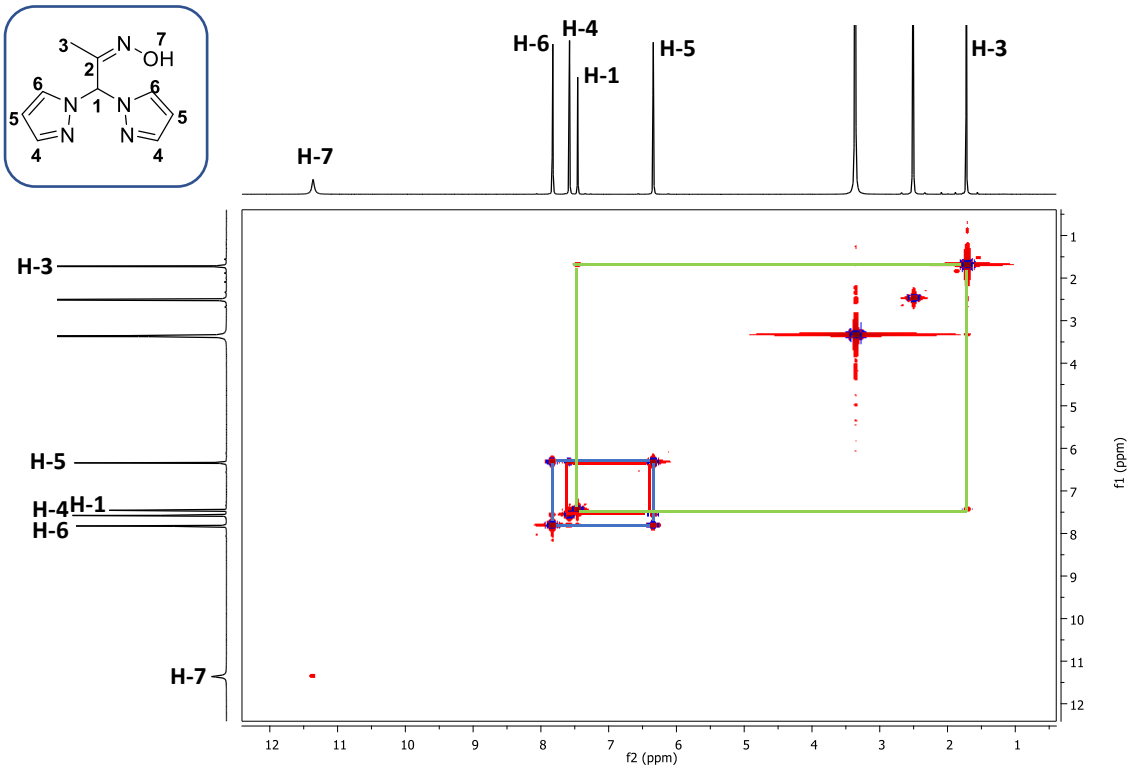



**Figure 4.6** COSY spectrum of BPM **4.27c**.

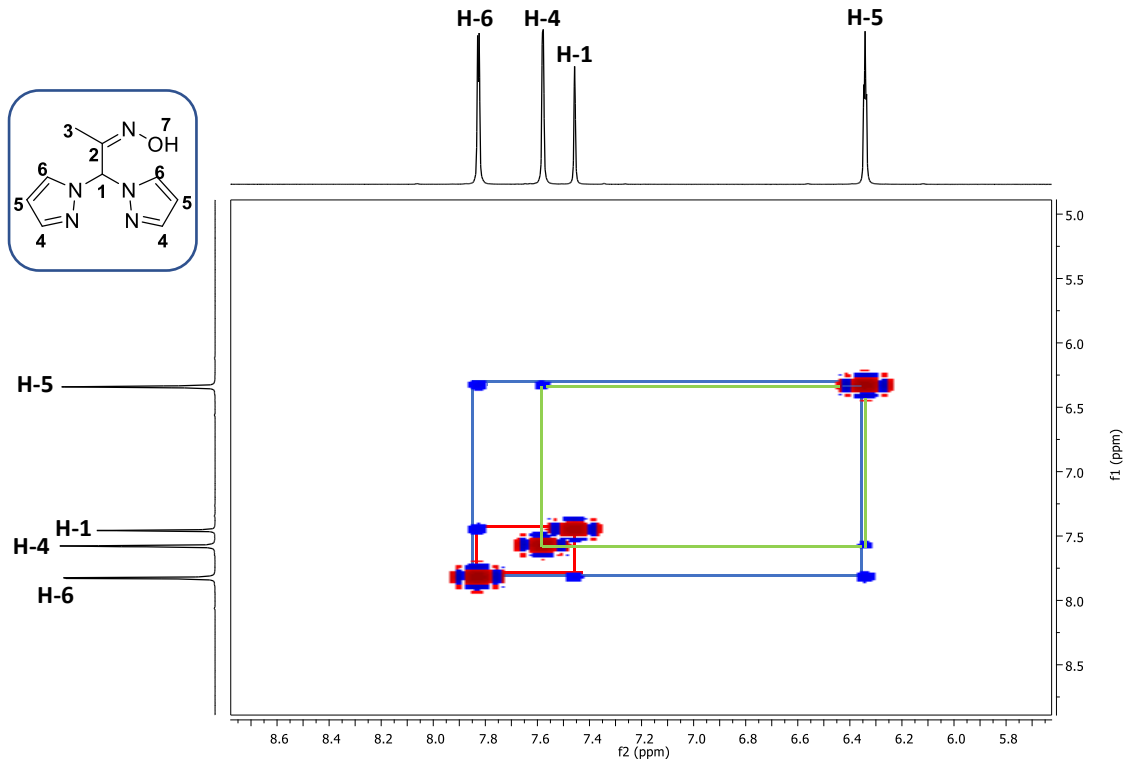



**Figure 4.7** NOESY spectrum of BPM **4.27c**.

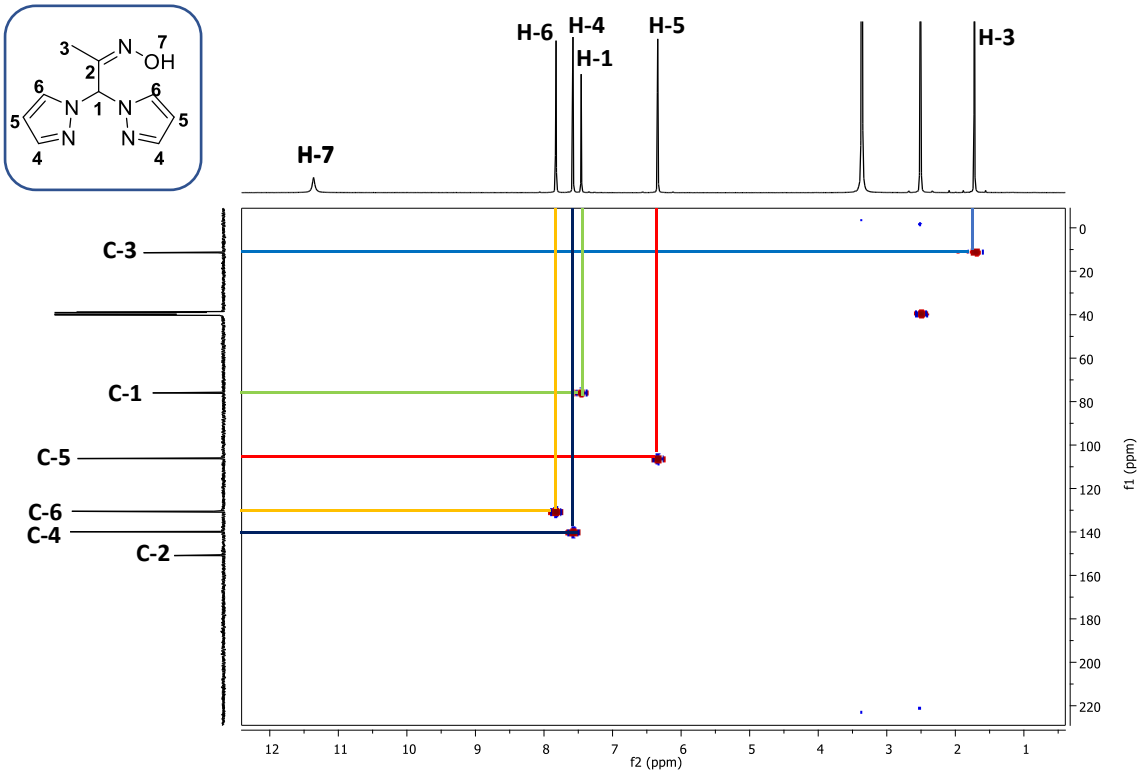



**Figure 4.8** HMQC spectrum of BPM **4.27c**.

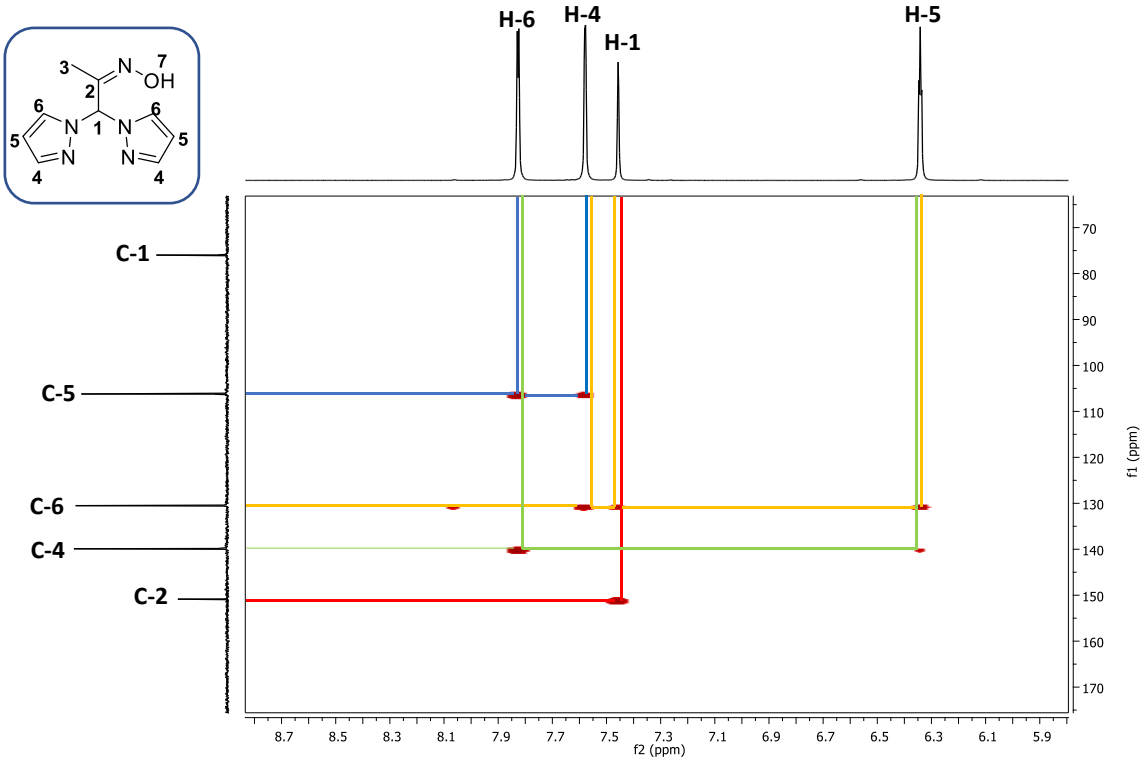
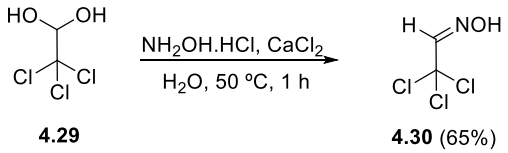



**Figure 4.9** HMBC spectrum of BPM **4.27c**.

## 4.5 Synthesis of tris(pyrazolyl)methane oximes

After these highly satisfactory results, we decided to try to extend this methodology to the synthesis of tris(pyrazolyl)methanes (TPM).

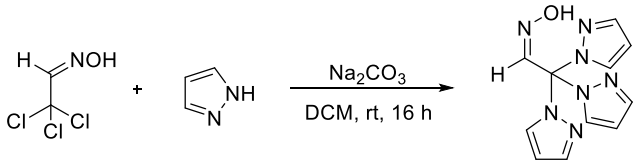
The experimental work started with the synthesis of the 2,2,2-trichloroacetaldehyde oxime **4.30** (Scheme 4.11). A mixture of chloral hydrate **4.29**, hydroxylamine hydrochloride ($NH_2OH.HCl$) and calcium chloride ($CaCl_2$) in water was stirred and heated to 50 ºC for one hour, leading to the target oxime in 65% yield (Scheme 4.11). [68]




**4.29** **4.30** (65%)

**Scheme 4.11** Synthesis of the 2,2,2-trichloroacetaldehyde oxime **4.30**.

The chloral oxime **4.30**, pyrazole **4.26a** and sodium carbonate in DCM, were stirred at room temperature during 16 h, giving rise to [(1-hydroxyimino)methyl]tris(1*H*-pyrazol-1-yl)methane (**4.31**) in excellent yield (87%) (Scheme 4.12).

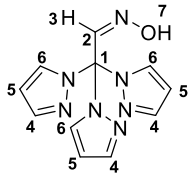



**4.30** **4.26a** **4.31** (87%)

**Scheme 4.12** Synthesis of [(1-hydroxyimino)methyl]tris(1*H*-pyrazol-1-yl)methane (**4.31**).

The structure of TPM **4.31** was also confirmed by nuclear magnetic resonance spectra. The proton and carbon-13 assignments were achieved based on the bi-dimensional NMRs (COSY, NOESY, HMQC, HMBC) (Table 4.3).

**Table 4.3** $^{1}$H and $^{13}$C NMR signal assignments of **4.31**.



| Position | δ(ppm) $^{1}$H NMR | δ(ppm) $^{13}$C NMR |
|---|---|---|
| 5 | 6.46 (*pseudo* t, *J* = 2.4 Hz, *J* = 2.0 Hz, 3H) | 107.1 (CH, Ar) |
| 4 | 7.40 (d, *J* = 2.4 Hz, 3H) | 130.8 (CH, Ar) |
| 6 | 7.71 (d, *J* = 1.6 Hz, 3H) | 141.0 (CH, H) |
| 3 | 8.64 (s, 1H) | - |
| 7 | 11.98 (s, 1H, OH)) | - |
| 1 | - | 88.3 (C *meso*) |
| 2 | - | 144.8 (C) |

In the $^{1}$H NMR spectrum (Figure 4.10) of TPM **4.31**, the signals corresponding to the oxime moiety proton (H-7) at 11.98 ppm and the H-3 protons at 8.64 ppm, both singlets, are easily identified. The $^{13}$C NMR spectrum (Figure 4.11) presents two signals corresponding to quaternary carbons at 88.3 ppm and 144.8 ppm, which were identified as the methylene bridge C-1 carbon and the oxime moiety C-2 carbon, respectively.

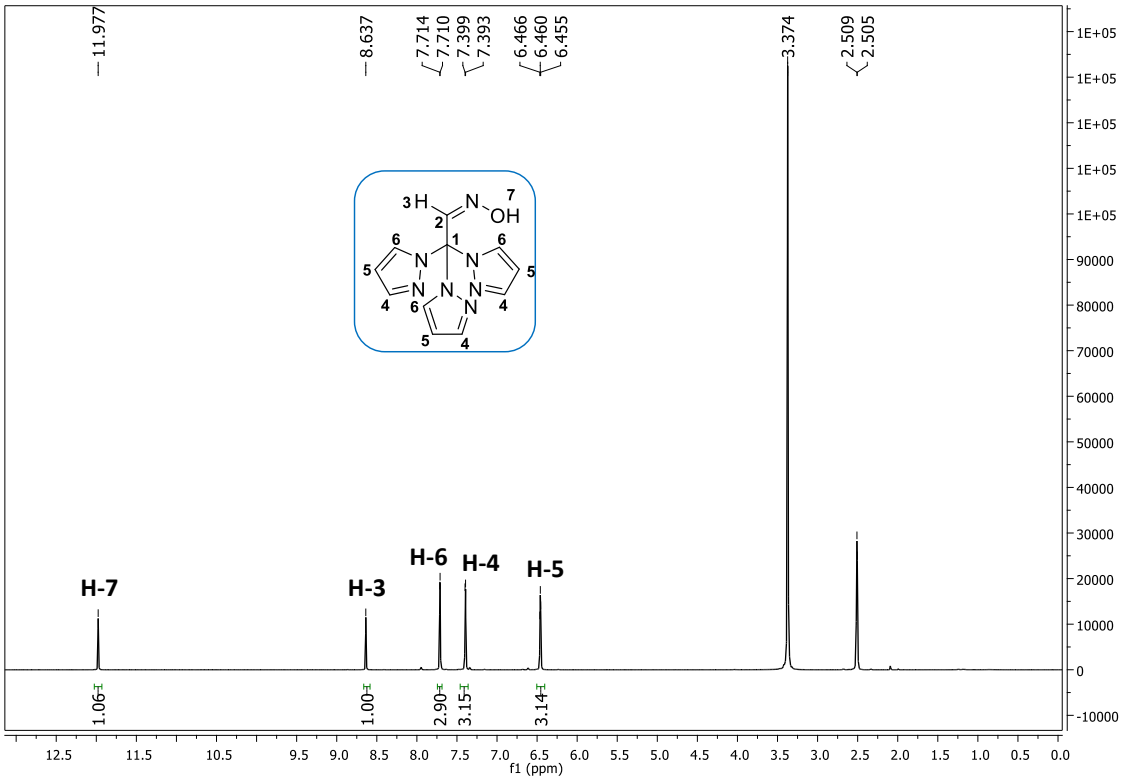



**Figure 4.10** $^{1}$H NMR spectrum of TPM **4.31**.

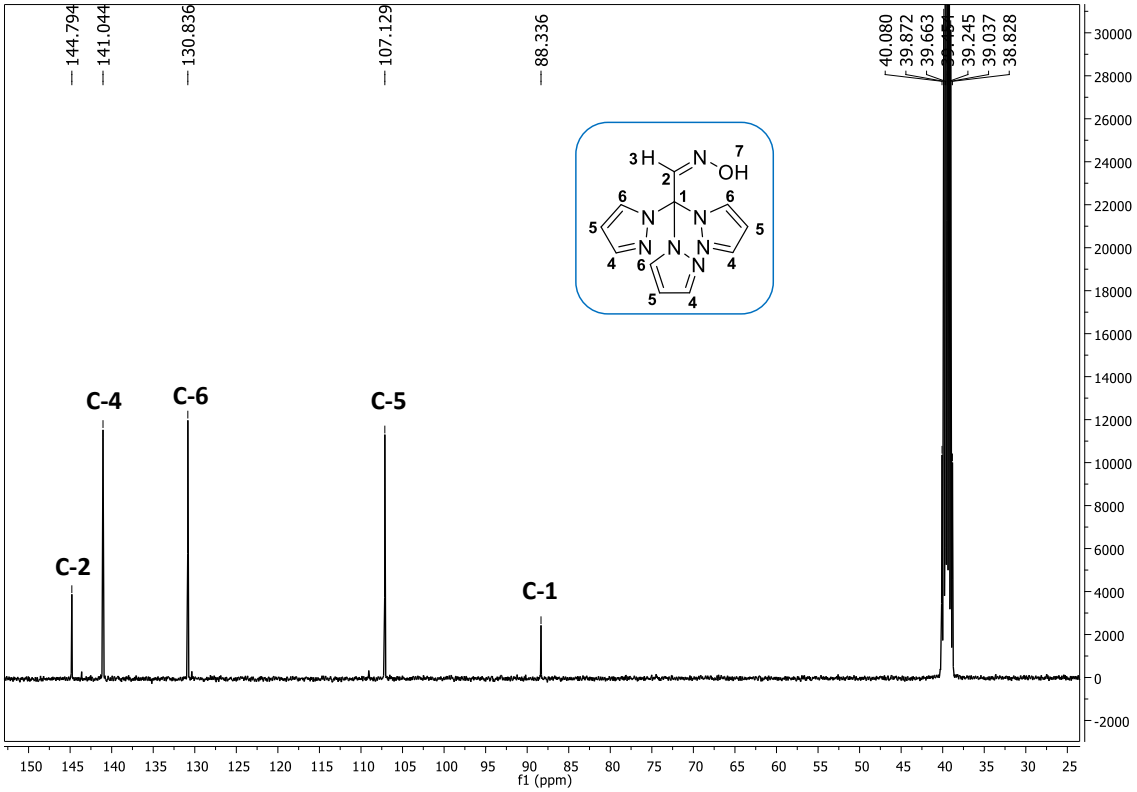



**Figure 4.11** $^{13}$C NMR spectrum of TPM **4.31**.

Similarly, to BPMs, the main interactions observed in the bi-dimensional NMR, represented in figure 4.12, allowed the assignment of the protons of the pyrazole nuclei. In the COSY spectrum (Figure 4.13) the correlation between the H-5 (6.46 ppm) and H-4 (7.40 ppm) and H-6 (7.71 ppm) protons is observed. In the NOESY is also observed the connectivity between the same protons (Figure 4.14). Analysing the HMQC and HMBC spectra, it was possible to assignment C-4 (130.8 ppm), C-5 (107.1 ppm) and C-6 (141.0 ppm) carbons and in the HMBC correlation between C-4 and H-5 as well as between C-6 and H-5 are observed (Figure 4.16).

Even though H-3 protons and C-1 carbons were easily assigned based on the chemical shifts observed in the $^{1}$H NMR and $^{13}$C NMR spectra, respectively, this was unequivocally confirmed through the analysis of the HMBC spectrum in which the correlation between them was observed. Correlation between C-2 carbon and H-7 proton was also observed (Figure 4.16).

The structure TPM **4.31** was assigned as being the (Z)-isomer, since no connectivity was observed between the hydroxyl proton H-7 and the methyl group protons H-3 in the NOESY spectrum (Figure 4.14). Nevertheless, this result was confirmed by x-ray

crystallography, carried out as part of a study where BPMs and TPMs were used as scorpionates ligands for the synthesis of new copper catalysts to be used in the azide-alkyne Huisgen cycloaddition reaction (click reaction). This work was not carried out within the scope of this PhD.

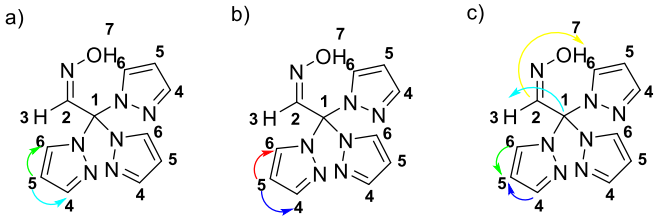



**Figure 4.12** Main observed connectivities in the COSY (a), NOESY (b) and HMBC (c) spectra of TPM **4.31c**.

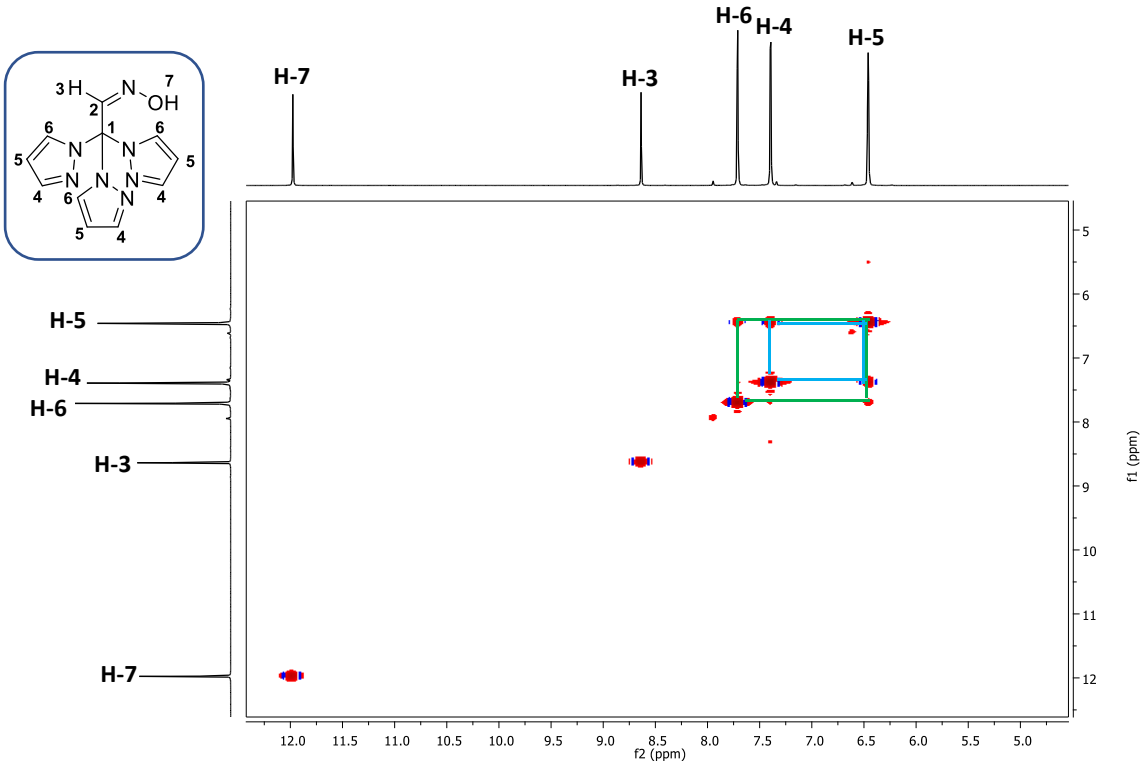



**Figure 4.13** Expansion of COSY spectrum of TPM **4.31**.

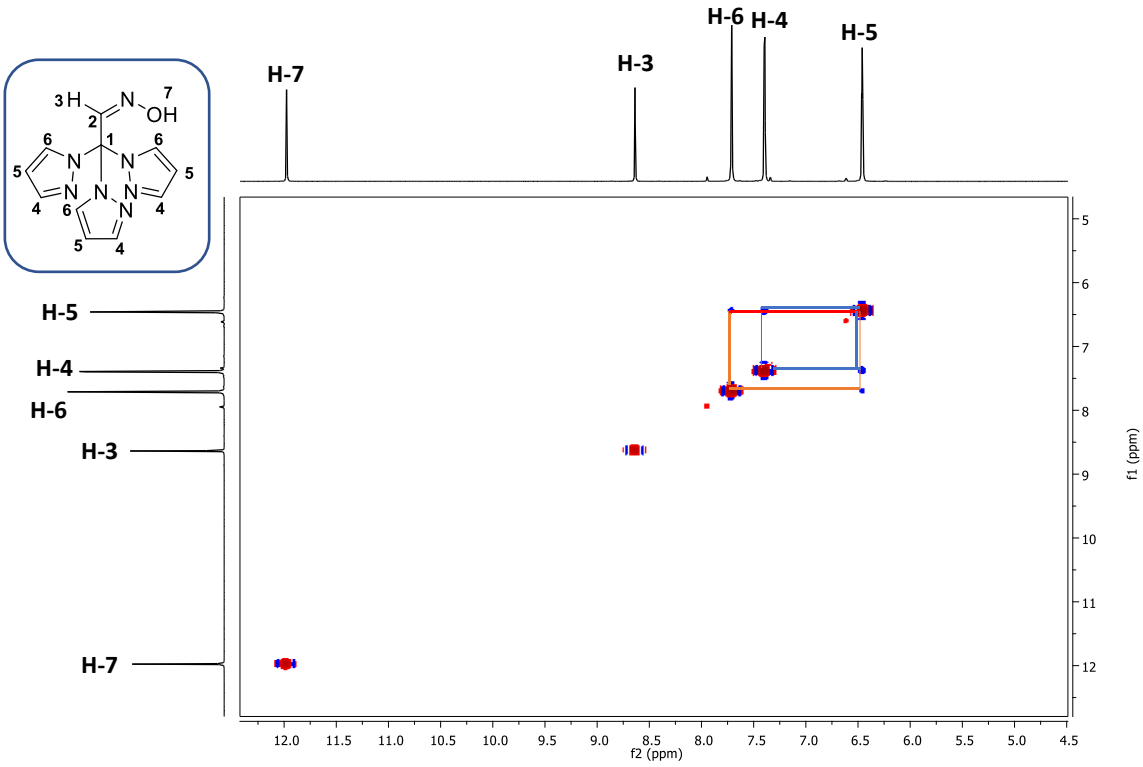



**Figure 4.14** Expansion of NOESY spectrum of TPM **4.31**.

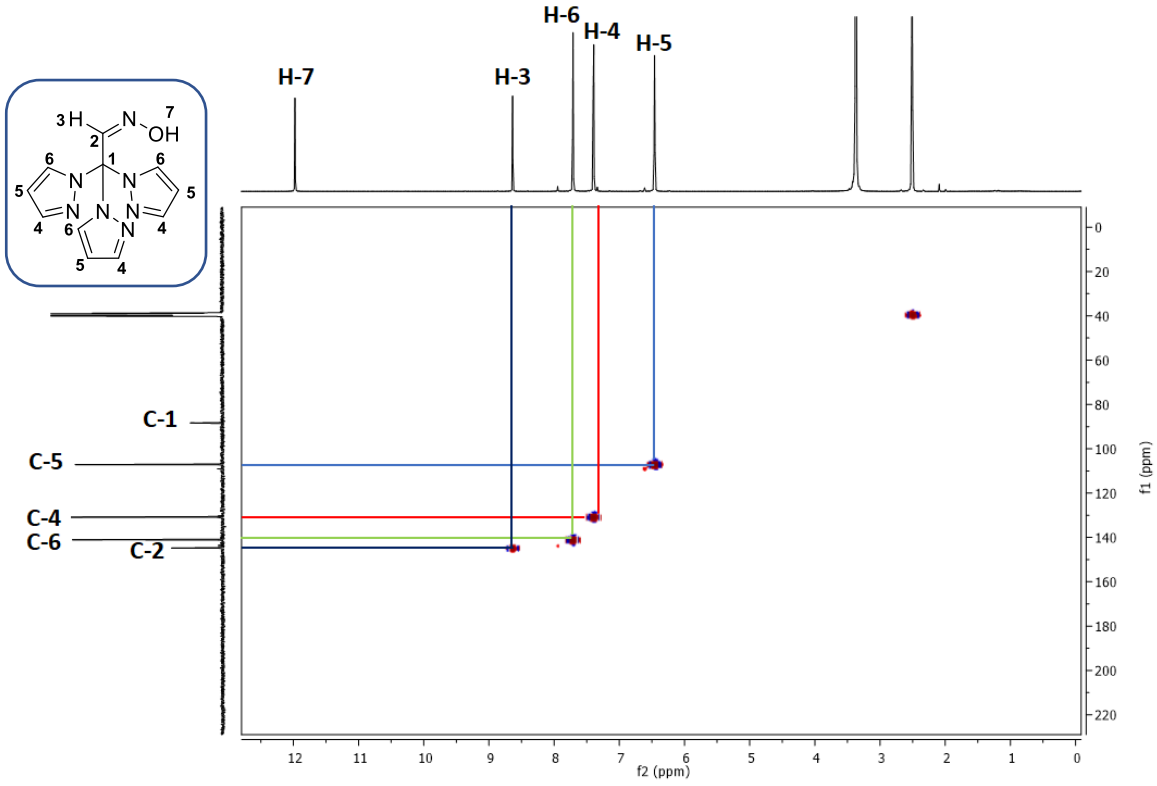



**Figure 4.15** HMQC spectrum of TPM **4.31**.

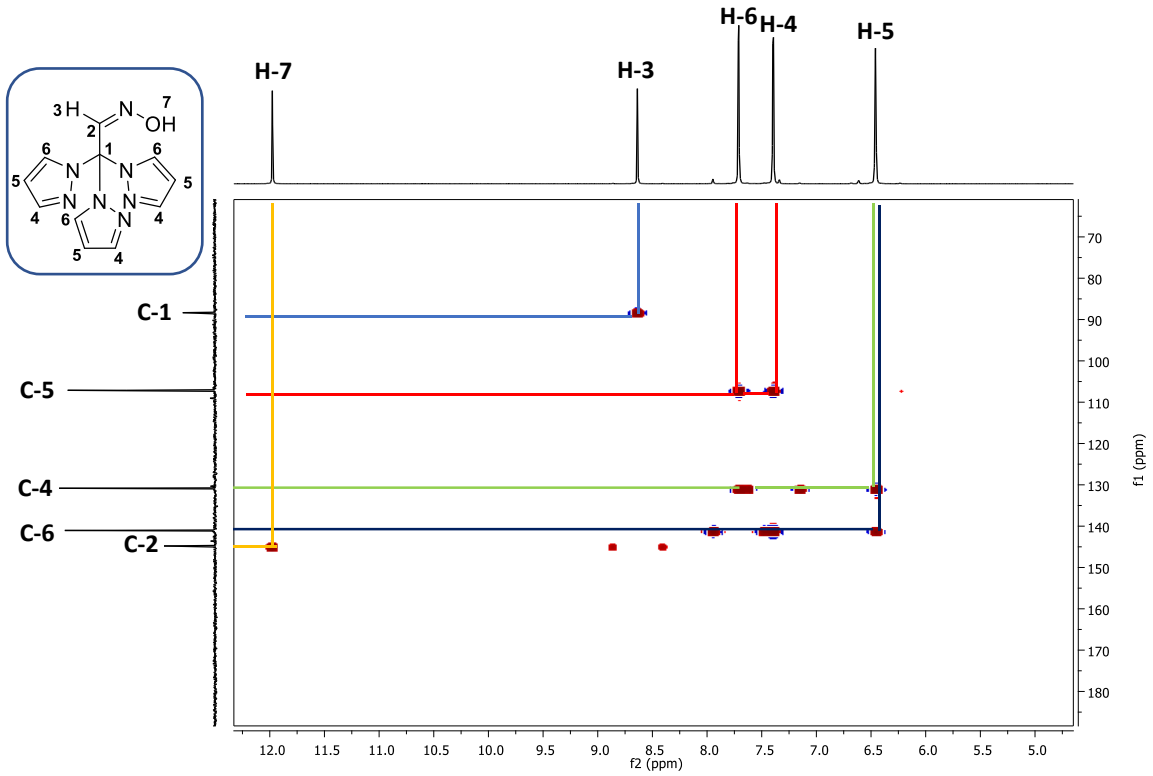
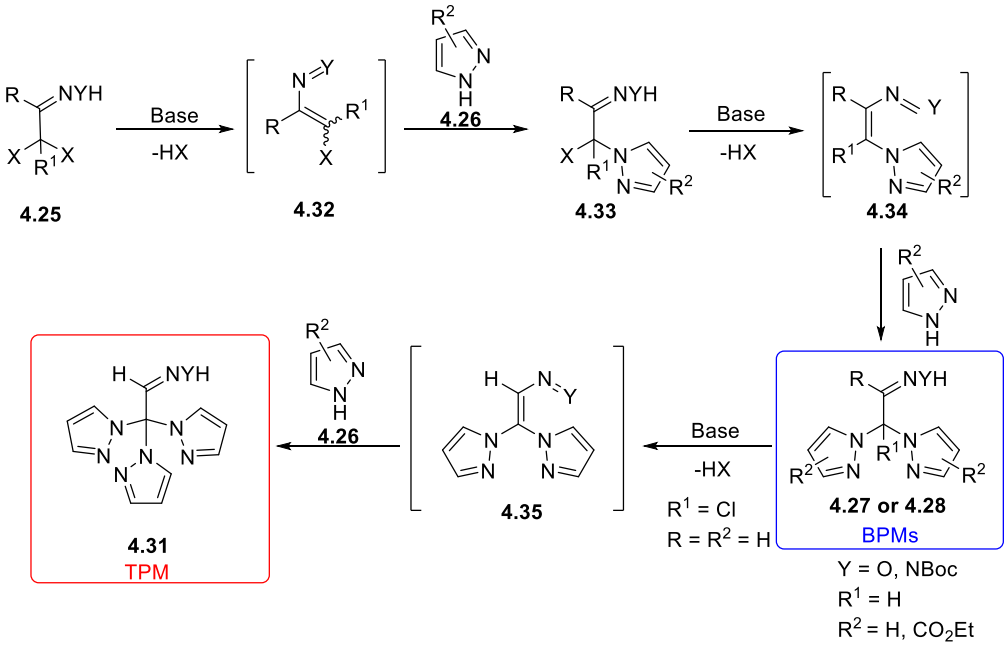



**Figure 4.16** Expansion of HMBC spectrum of TPM **4.31**.

## 4.6 Proposed mechanism

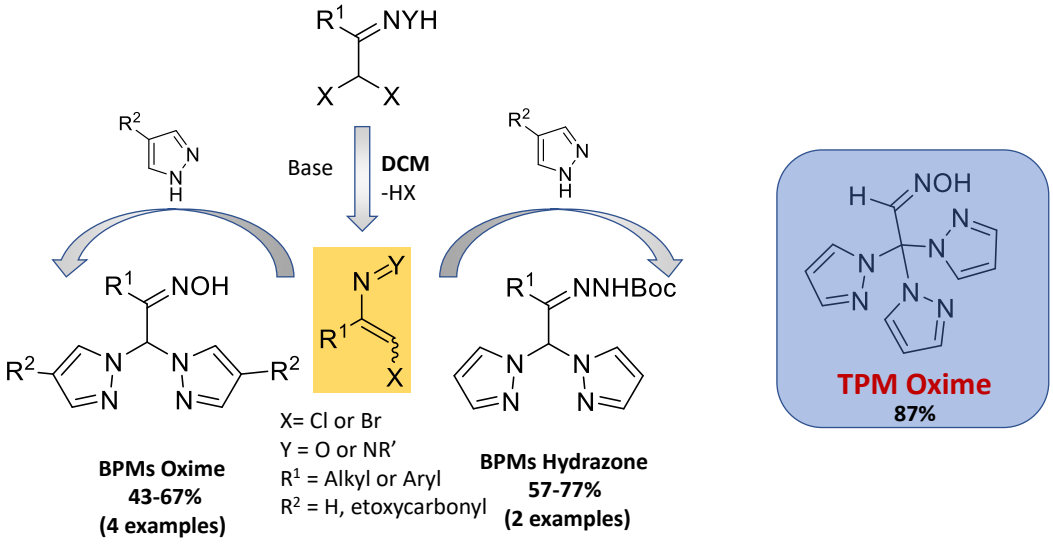
The proposed mechanism for these transformations is outlined in Scheme 4.13. The α,α'-dihaloximes or hydrazones **4.25**, in presence of base, were converted *in situ* into the corresponding transient and reactive nitroso- or azoalkenes **4.32**, which underwent conjugate addition by reacting with a first molecule of the appropriate pyrazole **4.26**, leading to functionalised pyrazoles **4.33**. Subsequent dehydrohalogenation of side-chain of **4.33** produces nitroso- or azoalkenes **4.34** which reacted with a second molecule of pyrazole **4.26** giving the target bis(pyrazol-1-yl)methanes **4.27** or **4.28**. Starting from α,α',α''-trichlorooxime ($R^1$ = Cl), an additional dehydrohalogenation can occur affording heterodyne **4.35** followed by its interception with a third molecule of pyrazole **4.26** leads to the target tris(pyrazolyl)oxime **4.31** in a one-pot procedure (Scheme 4.13)**.**




**Scheme 4.13** Proposed mechanism for the synthesis of BPMs and TPM.

## 4.7 Conclusions

A new and straightforward synthetic approach towards bis(pyrazolyl)methanes, bearing an oxime or hydrazone moiety at the methylene bridge was developed. The conjugate addition of pyrazoles to the *in situ* generated nitroso- and azoalkenes was carried out under mild and simple reaction conditions leading to BPMs in good yields. The ready access to a great range of starting oximes, hydrazones, and pyrazoles, allows an easy modulation of the bis(pyrazolyl)methanes structure, that would be very difficult to obtain by other methods. It was demonstrate that tris(pyrazolyl)methanes can also be obtained by this methodology.[69]



## 4.8 References

1. Suri, M.; Jousseaume, T.; Neumann, J. J.; Glorius, F., An efficient copper-catalyzed formation of highly substituted pyrazoles using molecular oxygen as the oxidant. *Green Chem.* **2012**, *14* (8), 2193–2196.

2. Abrigach, F.; Touzani, R., Pyrazole derivatives with NCN junction and their biological activity: A review. *Med. Chem.* **2016**, *06* (05), 292-298.

3. Küçükgüzel, Ş. G.; Şenkardeş, S., Recent advances in bioactive pyrazoles. *Eur. J. Med. Chem.* **2015**, *97*, 786-815.

4. Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y.; Al-aizari, F.; Ansar, M. h., Synthesis and pharmacological activities of pyrazole derivatives: A review. *Molecules* **2018**, *23* (1), 134-219.

5. Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J.-i., Synthesis and antibacterial activity of a novel series of potent DNA gyrase inhibitors. pyrazole derivatives. *J. Med. Chem.* **2004**, *47* (14), 3693-3696.

6. Venkat Ragavan, R.; Vijayakumar, V.; Suchetha Kumari, N., Synthesis of some novel bioactive 4-oxy/thio substituted-1*H*-pyrazol-5(4*H*)-ones via efficient cross-Claisen condensation. *Eur. J. Med. Chem.* **2009**, *44* (10), 3852-3857.

7. McDonald, E.; Jones, K.; Brough, P.; Drysdale, M.; Workman, P., Discovery and development of pyrazole-scaffold Hsp90 inhibitors. *Curr. Pain Headache Rep.* **2006**, *6* (11), 1193-1203.

8. Balbi, A.; Anzaldi, M.; Macciò, C.; Aiello, C.; Mazzei, M.; Gangemi, R.; Castagnola, P.; Miele, M.; Rosano, C.; Viale, M., Synthesis and biological evaluation of novel pyrazole derivatives with anticancer activity. *Eur. J. Med. Chem.* **2011**, *46* (11), 5293-5309.

9. Kumar, H.; Saini, D.; Jain, S.; Jain, N., Pyrazole scaffold: a remarkable tool in the development of anticancer agents. *Eur. J. Med. Chem.* **2013**, *70*, 248-258.

10. Mellini, P.; Marrocco, B.; Borovika, D.; Polletta, L.; Carnevale, I.; Saladini, S.; Stazi, G.; Zwergel, C.; Trapencieris, P.; Ferretti, E.; Tafani, M.; Valente, S.; Mai, A., Pyrazole-based inhibitors of enhancer of zeste homologue 2 induce apoptosis and autophagy in cancer cells. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **2018**, *373* (1748), 1-8.

11. Şener, N.; Özkinali, S.; Altunoglu, Y. C.; Yerlikaya, S.; Gökçe, H.; Zurnaci, M.; Gür, M.; Baloglu, M. C.; Şener, İ., Antiproliferative properties and structural analysis of newly synthesized Schiff bases bearing pyrazole derivatives and molecular docking studies. *J. Mol. Struct.* **2021**, *1241*, 1-11

12. Bailey, D. M.; Hansen, P. E.; Hlavac, A. G.; Baizman, E. R.; Pearl, J.; DeFelice, A. F.; Feigenson, M. E., 3,4-Diphenyl-1H-pyrazole-1-propanamine antidepressants. *J. Med. Chem.* **2002**, *28* (2), 256-260.

13. Tripathi, A. C.; Upadhyay, S.; Paliwal, S.; Saraf, S. K., Derivatives of 4,5-dihydro (1H) pyrazoles as possible MAO-A inhibitors in depression and anxiety disorders: synthesis, biological evaluation and molecular modeling studies. *Med. Chem. Res.* **2018**, *27* (5), 1485-1503.

14. Sridhar, R.; Perumal, P. T.; Etti, S.; Shanmugam, G.; Ponnuswamy, M. N.; Prabavathy, V. R.; Mathivanan, N., Design, synthesis and anti-microbial activity of 1H-pyrazole carboxylates. *Bioorg. Med. Chem. Lett.* **2004**, *14* (24), 6035-6040.

15. Puthran, D.; Poojary, B.; Purushotham, N.; Harikrishna, N.; Nayak, S. G.; Kamat, V., Synthesis of novel Schiff bases using 2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile and 1,3-Disubstituted pyrazole-4-carboxaldehydes derivatives and their antimicrobial activity. *Heliyon* **2019**, *5* (8), 1-6.

16. Madni, M.; Hameed, S.; Ahmed, M. N.; Tahir, M. N.; Al-Masoudi, N. A.; Pannecouque, C., Synthesis, crystal structure, anti-HIV, and antiproliferative activity of new pyrazolylthiazole derivatives. *Med. Chem. Res.* **2017**, *26* (10), 2653-2665.

17. Bekhit, A. A.; Abdel-Aziem, T., Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents. *Bioorg. Med. Chem.* **2004**, *12* (8), 1935-1945.

18. Sauzem, P. D.; Machado, P.; Rubin, M. A.; da S. Sant'Anna, G.; Faber, H. B.; de Souza, A. H.; Mello, C. F.; Beck, P.; Burrow, R. A.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. P., Design and microwave-assisted synthesis of 5-trifluoromethyl-4,5-dihydro-1H-pyrazoles: Novel agents with analgesic and anti-inflammatory properties. *Eur. J. Med. Chem.* **2008**, *43* (6), 1237-1247.

19. Bandgar, B. P.; Chavan, H. V.; Adsul, L. K.; Thakare, V. N.; Shringare, S. N.; Shaikh, R.; Gacche, R. N., Design, synthesis, characterization and biological evaluation of novel pyrazole integrated benzophenones. *Bioorg. Med. Chem. Lett.* **2013**, *23* (3), 912-916.

20. Mukarram, S.; Bandgar, B. P.; Shaikh, R. U.; Ganapure, S. D.; Chavan, H. V., Synthesis of novel α,α-difluoro-β-hydroxycarbonyl pyrazole derivatives as antioxidant, anti-inflammatory and anticancer agents. *Med. Chem. Res.* **2016**, *26* (1), 262-273.

21. Dourish, C. T.; Wilding, J. P. H.; Halford, J. C. G., Anti-obesity drugs: From animal models to clinical efficacy. In *Animal and Translational Models for CNS Drug Discovery*, 2008, 271-315.

22. Brent, J., Fomepizole for ethylene glycol and methanol poisoning. *N. Engl. J. Med.* **2009**, *360* (21), 2216-2223.

23. Goldstein, I.; Burnett, A. L.; Rosen, R. C.; Park, P. W.; Stecher, V. J., The Serendipitous Story of Sildenafil: An Unexpected Oral Therapy for Erectile Dysfunction. *Sex. Med. Rev.* **2019**, *7* (1), 115-128.

24. Ahmed, W.; Yan, X.; Hu, D.; Adnan, M.; Tang, R.-Y.; Cui, Z.-N., Synthesis and fungicidal activity of novel pyrazole derivatives containing 5-phenyl-2-furan. *Bioorg. Med. Chem.* **2019**, *27* (19), 115048-115056.

25. Song, H.; Liu, Y.; Xiong, L.; Li, Y.; Yang, N.; Wang, Q., Design, synthesis, and insecticidal evaluation of new pyrazole derivatives containing imine, oxime ether, oxime ester, and dihydroisoxazoline groups based on the inhibitor binding pocket of respiratory complex I. *J. Agric. Food Chem.* **2013**, *61* (37), 8730-8736.

26. Jani, D. H.; Patel, H. S.; Keharia, H.; Modi, C. K., Novel drug-based Fe(III) heterochelates: Synthetic, spectroscopic, thermal andin-vitroantibacterial significance. *Appl. Organomet. Chem.* **2009**, 99–111.

27. Gomes, P. M. O.; Silva, A. M. S.; Silva, V. L. M., Pyrazoles as key scaffolds for the development of fluorine-18-labeled radiotracers for positron emission tomography (PET). *Molecules* **2020**, *25* (7), 1722-1742.

28. Trofimenko, S., Geminal poly(1-pyrazolyl)alkanes and their coordination chemistry. *J. Am. Chem. Soc.* **1970**, *92* (17), 5118-5126.

29. Pettinari, C.; Pettinari, R., Metal derivatives of poly(pyrazolyl)alkanes☆II. Bis(pyrazolyl)alkanes and related systems. *Coord. Chem. Rev.* **2005**, *249* (5-6), 663-691.

30. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Tejeda, J.; Lara-Sánchez, A., Heteroscorpionate ligands based on bis(pyrazol-1-yl)methane: design and coordination chemistry. *Dalton Trans.* **2004**, (10), 1499-1510.

31. Otero, A.; Fernández‐Baeza, J.; Lara‐Sánchez, A.; Tejeda, J.; Sánchez‐Barba, L. F., Recent advances in the design and coordination chemistry of heteroscorpionate ligands bearing stereogenic centres. *Eur. J. Inorg. Chem.* **2008**, *2008* (34), 5309-5326.

32. Otero, A.; Fernández-Baeza, J.; Lara-Sánchez, A.; Sánchez-Barba, L. F., Metal complexes with heteroscorpionate ligands based on the bis(pyrazol-1-yl)methane moiety: Catalytic chemistry. *Coord. Chem. Rev.* **2013**, *257* (11-12), 1806-1868.

33. Bassanetti, I.; Marchiò, L., Structural variability in Ag(I) and Cu(I) coordination polymers with thioether-functionalized bis(pyrazolyl)methane ligands. *Inorg. Chem.* **2011**, *50* (21), 10786-10797.

34. Gardiner, J. R.; Hewage, J. S.; Lindeman, S. V., Isomer Dependence in the Assembly and Lability of Silver(I) Trifluoromethanesulfonate Complexes of the Heteroditopic Ligands, 2-, 3-, and 4-[Di(1H-pyrazolyl)methyl]phenyl(di-p-tolyl)phosphine. *Inorg. Chem.* **2014**, *53* (22), 12108-12121.

35. Keisers, K.; Hüppe, H. M.; Iffland-Mühlhaus, L.; Hoffmann, A.; Göbel, C.; Apfel, U.-P.; Weber, B.; Herres-Pawlis, S., Interplay of spin crossover and coordination-induced spin state switch for iron bis(pyrazolyl)methanes in solution. *Inorg. Chem.* **2020**, *59* (20), 15343-15354.

36. Godau, T.; Bleifuß, S. M.; Müller, A. L.; Roth, T.; Hoffmann, S.; Heinemann, F. W.; Burzlaff, N., Cu(i) catalysed cyclopropanation with enantiopure scorpionate type ligands derived from (+)-camphor or (−)-menthone. *Dalton Trans.* **2011**, *40* (24).

37. Bagnarelli, L.; Dolmella, A.; Santini, C.; Vallesi, R.; Giacomantonio, R.; Gabrielli, S.; Pellei, M., A new dimeric copper(II) complex of hexyl bis(pyrazolyl)acetate ligand as an efficient catalyst for allylic oxidations. *Molecules* **2021**, *26* (20).

38. Gabrielli, S.; Pellei, M.; Venditti, I.; Fratoddi, I.; Battocchio, C.; Iucci, G.; Schiesaro, I.; Meneghini, C.; Palmieri, A.; Marcantoni, E.; Bagnarelli, L.; Vallesi, R.; Santini, C., Development of new and efficient copper(ii) complexes of hexyl bis(pyrazolyl)acetate ligands as catalysts for allylic oxidation. *Dalton Trans.* **2020**, *49* (44), 15622-15632.

39. Thomas, F.; Steden, D.; Eith, A.; Hoffmann, A.; Herres-Pawlis, S., Chiral bis(pyrazolyl)methane copper(I) complexes and their application in nitrene transfer reactions. *Z. Naturforsch. B* **2021**, *76* (10-12), 835-847.

40. Carrión, M. C.; Durá, G.; Jalón, F. A.; Manzano, B. R.; Rodríguez, A. M., Polynuclear complexes containing ditopic bispyrazolylmethane ligands. Influence of metal geometry and supramolecular interactions. *Cryst. Growth Des.* **2012**, *12* (4), 1952-1969.

41. Durá, G.; Carrión, M. C.; Jalón, F. A.; Rodríguez, A. M.; Manzano, B. R., Metal supramolecular frameworks with silver and ditopic bis(pyrazolyl)methane ligands: Effect of the anions and ligand substitution. *Cryst. Growth Des.* **2014**, *14* (7), 3510-3529.

42. Durá, G.; Carrión, M. C.; Jalón, F. A.; Manzano, B. R.; Rodríguez, A. M., One- and two-step self-assembly processes in Zn(II) supramolecular frameworks with ditopic bis(pyrazolyl)methane ligands. Chiral recognition and formation of cyclic helicates. *Cryst. Growth Des.* **2015**, *15* (11), 5174-5182.

43. Bassanetti, I.; Atzeri, C.; Tinonin, D. A.; Marchiò, L., Silver(I) and thioether-bis(pyrazolyl)methane ligands: The correlation between ligand functionalization and coordination polymer architecture. *Cryst. Growth Des.* **2016**, *16* (6), 3543-3552.

44. Hoffmann, A.; Citek, C.; Binder, S.; Goos, A.; Rübhausen, M.; Troeppner, O.; Ivanović-Burmazović, I.; Wasinger, E. C.; Stack, T. D. P.; Herres-Pawlis, S., Catalytic phenol hydroxylation with dioxygen: extension of the tyrosinase mechanism beyond the protein matrix. *Angew. Chem., Int. Ed. Engl.* **2013**, *52* (20), 5398-5401.

45. Wilfer, C.; Liebhäuser, P.; Erdmann, H.; Hoffmann, A.; Herres‐Pawlis, S., Biomimetic hydroxylation catalysis through self‐assembly of a bis(pyrazolyl)methane copper–peroxo complex. *Eur. J. Inorg. Chem.* **2014**, *2015* (3), 494-502.

46. Wilfer, C.; Liebhäuser, P.; Hoffmann, A.; Erdmann, H.; Grossmann, O.; Runtsch, L.; Paffenholz, E.; Schepper, R.; Dick, R.; Bauer, M.; Dürr, M.; Ivanović-Burmazović, I.; Herres-Pawlis, S., Efficient biomimetic hydroxylation Catalysis with a Bis(pyrazolyl)imidazolylmethane copper peroxide complex. *Chem. Eur. J.* **2015**, *21* (49), 17639-17649.

47. Jayaweera, N. P.; Hall, A. E.; Wilson, A. A.; Konkle, M. E.; Wheeler, K. A.; Semeniuc, R. F., Tuning the catecholase activity of bis(pyrazolyl)methane-based copper(II) complexes by substitutions of the ligand core: unraveling a dual $O_2/H_2O_2$ oxidation mechanism. *Inorg. Chim. Acta* **2020**, *506*, 1-12.

48. Pellei, M.; Papini, G.; Trasatti, A.; Giorgetti, M.; Tonelli, D.; Minicucci, M.; Marzano, C.; Gandin, V.; Aquilanti, G.; Dolmella, A.; Santini, C., Nitroimidazole and

glucosamine conjugated heteroscorpionate ligands and related copper(ii) complexes. Syntheses, biological activity and XAS studies. *Dalton Trans.* **2011**, *40* (38), 9877-9888.

49. Santini, C.; Pellei, M.; Gandin, V.; Porchia, M.; Tisato, F.; Marzano, C., Advances in copper complexes as anticancer agents. *Chem. Rev.* **2013**, *114* (1), 815-862.

50. Pellei, M.; Bagnarelli, L.; Luciani, L.; Del Bello, F.; Giorgioni, G.; Piergentili, A.; Quaglia, W.; De Franco, M.; Gandin, V.; Marzano, C.; Santini, C., Synthesis and cytotoxic activity evaluation of new Cu(I) complexes of bis(pyrazol-1-yl) acetate ligands functionalized with an NMDA receptor antagonist. *Int. J. Mol. Sci.* **2020**, *21* (7), 2616-2635.

51. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Carrillo-Hermosilla, F.; Tejeda, J.; Lara-Sánchez, A.; Sánchez-Barba, L.; Fernández-López, M.; Rodríguez, A. M.; López-Solera, I., Preparation of new monoanionic "scorpionate" ligands: synthesis and structural characterization of titanium(IV) complexes bearing this class of ligand. *Inorg. Chem.* **2002**, *41* (20), 5193-5202.

52. Beck, A.; Weibert, B.; Burzlaff, N., Monoanionic *N*,*N*,*O*-scorpionate ligands and their iron(II) and zinc(II) complexes: Models for mononuclear active sites of non-heme iron oxidases and zinc enzymes. *Eur. J. Inorg. Chem.* **2001**, (2), 521-527.

53. Hegelmann, I.; Burzlaff, N., Direct synthesis of enantiopure tripod ligands from C2‐symmetric precursors. *Eur. J. Inorg. Chem.* **2003**, (3), 409-411.

54. Peters, L.; Burzlaff, N., Complexes bearing an enantiopure N,N,O scorpionate ligand derived from (−)-menthone. *Polyhedron* **2004**, *23* (2-3), 245-251.

55. Howe, R. G.; Tredget, C. S.; Lawrence, S. C.; Subongkoj, S.; Cowley, A. R.; Mountford, P., A novel transformation of a zirconium imido compound and the development of a new class of N3donor heteroscorpionate ligand. *Chem. Commun.* **2006**, (2), 223-225.

56. Kilpatrick, A. F. R.; Kulangara, S. V.; Cushion, M. G.; Duchateau, R.; Mountford, P., Synthesis and ethylene trimerisation capability of new chromium(ii) and chromium(iii) heteroscorpionate complexes. *Dalton Trans.* **2010**, *39* (15), 3653–3664.

57. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Tejeda, J.; Lara-Sánchez, A.; Sánchez-Barba, L. F.; López-Solera, I.; Rodríguez, A. M., Lithium, titanium, and zirconium complexes with novel amidinate scorpionate ligands. *Inorg. Chem.* **2007**, *46* (5), 1760-1770.

58. Otero, A.; Lara-Sánchez, A.; Fernández-Baeza, J.; Martínez-Caballero, E.; Márquez-Segovia, I.; Alonso-Moreno, C.; Sánchez-Barba, L. F.; Rodríguez, A. M.; López -Solera, I., New achiral and chiral NNE heteroscorpionate ligands. Synthesis of homoleptic lithium complexes as well as halide and alkyl scandium and yttrium complexes. *Dalton Trans.* **2010**, *39* (3), 930-940.

59. Tan, R.-Y.; Hong, J.; Du, M.; Tang, L.-F., New multidentate heteroscorpionate ligands: *N*-Phenyl-2,2-bis(pyrazol-1-yl)thioacetamide and ethyl 2,2-bis(pyrazol-1-yl)dithioacetate as well as their derivatives. *J. Organomet. Chem.* **2007**, *692* (8), 1708-1715.

60. Otero, A.; Fernández-Baeza, J.; Tejeda, J.; Lara-Sánchez, A. n.; Sánchez-Molina, M.; Franco, S.; López-Solera, I.; Rodríguez, A. M.; Sánchez-Barba, L. F.; Morante-Zarcero, S.; Garcés, A. s., On the search for NNO-donor enantiopure scorpionate ligands and their coordination to group 4 metals. *Inorg. Chem.* **2009**, *48* (12), 5540-5554.

61. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Tejeda, J.; Lara-Sánchez, A.; Sánchez-Barba, L. F.; Sánchez-Molina, M.; Franco, S.; López-Solera, M. I.; Rodríguez, A. M., Highly diastereoselective nucleophilic addition to myrtenal. Straightforward synthesis of an enantiopure scorpionate ligand. *Inorg. Chem.* **2007**, *46* (21), 8475-8477.

62. Hoffman, J. T.; Tran, B. L.; Carrano, C. J., Oxidation-state and metal-ion dependent stereoisomerization in oxo molybdenum and tungsten complexes of a bulky alkoxy heteroscorpionate ligand. *Dalton Trans.* **2006**, (31), 3822–3830.

63. Zhang, Z.; Cui, D., Heteroscorpionate rare-earth metal zwitterionic complexes: Syntheses, characterization, and heteroselective catalysis on the ring-opening polymerization of rac-lactide. *Chem. Eur. J.* **2011**, *17* (41), 11520-11526.

64. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Tejeda, J.; Lara-Sánchez, A.; Sánchez-Barba, L. F.; Sánchez-Molina, M.; Rodríguez, A. M.; Bo, C.; Urbano-Cuadrado, M., Expanding heteroscorpionates. Facile synthesis of new hybrid scorpionate/cyclopentadienyl ligands and their lithium and group 4 metal compounds: A combined experimental and density functional theory study. *Organometallics* **2007**, *26* (17), 4310-4320.

65. Otero, A.; Fernández-Baeza, J.; Antiñolo, A.; Lara-Sánchez, A.; Martínez-Caballero, E.; Tejeda, J.; Sánchez-Barba, L. F.; Alonso-Moreno, C.; López-Solera, I., Scandium and Yttrium Complexes Supported by NNCp Heteroscorpionate Ligands:

Synthesis, Structure, and Polymerization of ϵ-Caprolactone. *Organometallics* **2008**, *27* (5), 976-983.

66. Gilchrist, T. L.; Stretch, W.; Chrystal, E. J. T., Reaction of azoles with ethyl bromopyruvate oxime: alkylation by substitution and by elimination–addition. *J. Chem. Soc., Perkin Trans. 1* **1987**, (0), 2235-2239.

67. Pinho e Melo, T.; Lemos, A.; Pereira, N.; Lopes, S., On-Water synthesis of dipyrromethanes via bis-hetero-Diels–Alder reaction of azo- and nitrosoalkenes with pyrrole. *Synlett* **2013**, *25* (03), 423-427.

68. Brintzinger, H.; Titzmann, R., Notiz über einige halogenierte aliphatische Oxime. *Ber. Dtsch. Chem. Ges.* **2006**, *85* (4), 344-345.

69. Pinho e Melo, T.; Lemos, A.; Grosso, C., Conjugate addition of pyrazoles to halogenated nitroso- and azoalkenes: A new entry to novel bis(pyrazol-1-yl)methanes. *Synlett* **2014**, *25* (20), 2868-2872.

# Chapter 5

Synthesis of 3-(1*H*-tetrazol-5-yl)-indoles

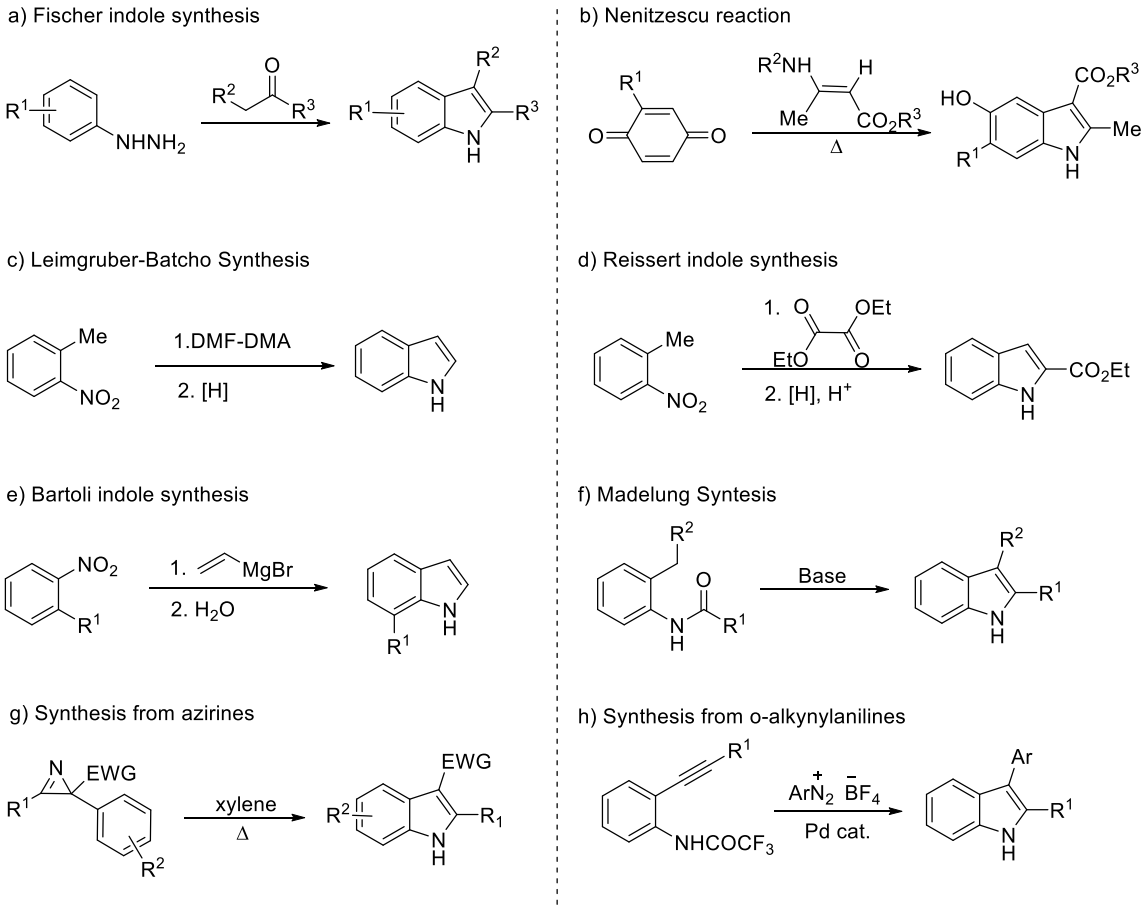

## Abstract

In this chapter, a synthetic approach to a new class of indole derivatives bearing a tetrazole moiety is presented. Arynes, generated *in situ* from 2-(trimethylsilyl)aryl triflates and KF, reacted smoothly with 2-(4-nitrobenzyltetrazolyl)-2*H*-azirines to give 3-(4-nitrobenzyltetrazolyl-indole derivatives with high selectivity and in good yields. Deprotection of the tetrazole moiety of indole gave 3-(1*H*-tetrazol-5-yl)-indole derivatives, bioisosters of 1*H*-indole-3-carboxylic acids.

# Chapter 5. Synthesis of 3-(1*H*-tetrazol-5-yl)-indoles

## 5.1 Indoles synthesis via aryne precursors

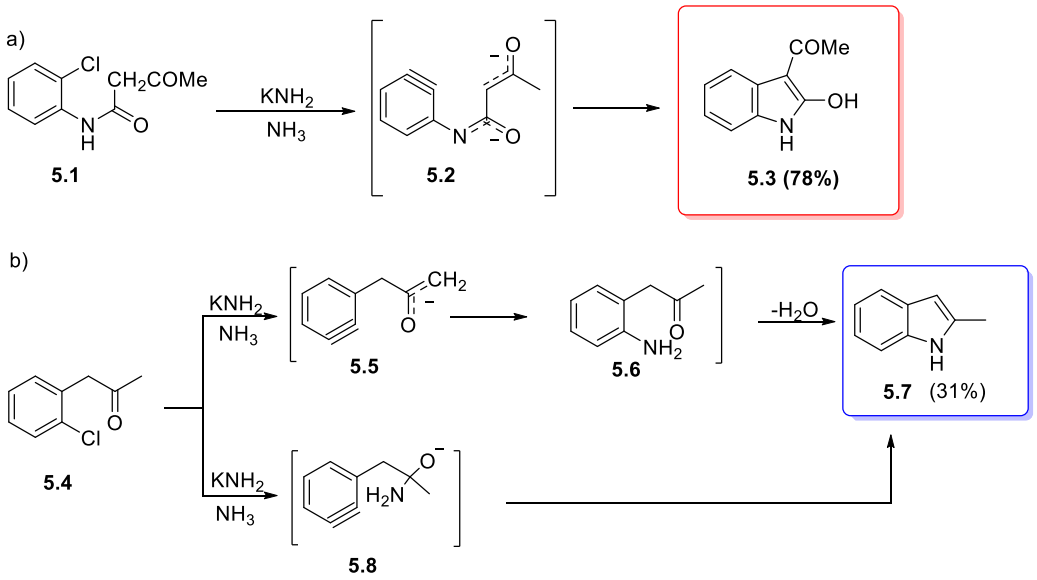
In the last decades, due to the significant applicability of molecules containing the indole core, impressive new synthetic routes as well as optimisation of classical methods, have been extensively reported.[1-8] The Fischer indole synthesis,[2, 5, 7, 8] Nenitzescu reaction,[2, 7, 8] Leimgruber-Batcho indole synthesis,[7, 8] Reissert indole synthesis,[7, 8] Bartoli indole synthesis,[7, 8] Madelung synthesis,[2, 7, 8] synthesis from azirines[7] and from *o*-alkynylanilines[1, 4, 6-8] are some of the synthetic routes to indoles and its derivatives (Scheme 5.1).




**Scheme 5.1** Examples of strategies to indole synthesis.

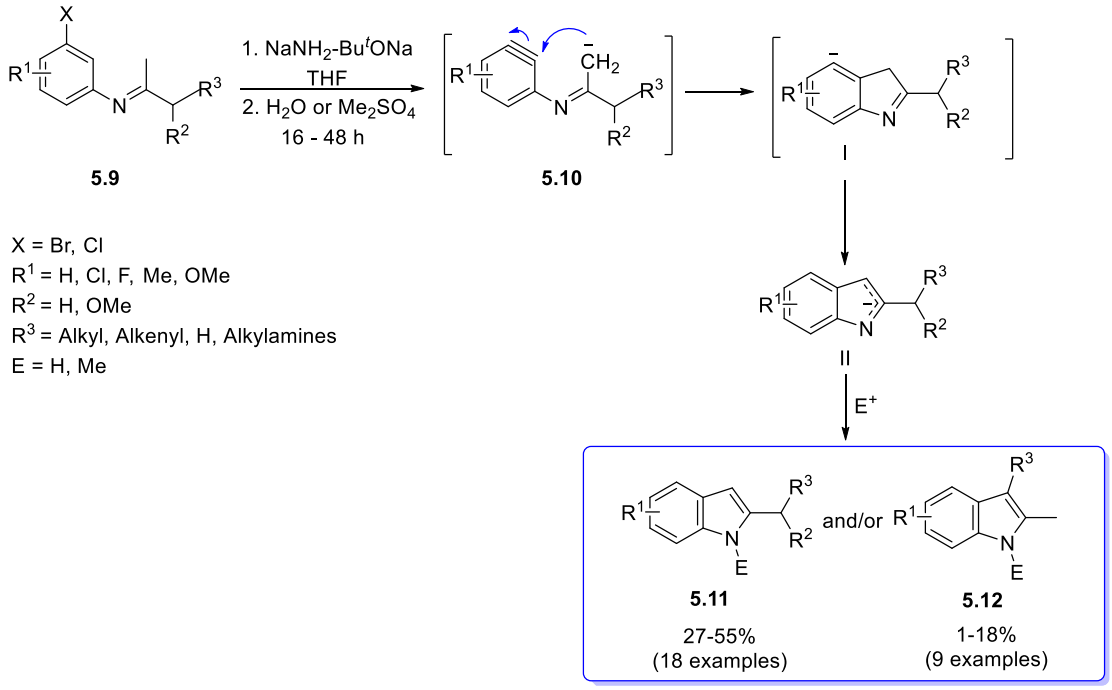
Arynes are a family of transient species being amongst the most reactive organic intermediates. [9, 10] The use of these very reactive species in organic synthesis has significantly increased in the last decades. Due to their synthetic versatility, arynes have been used in the synthesis of heterocycles, including indole and its derivatives.[9-12] In fact, aryne cyclization has proved to be an exceptional transition-metal-free synthesis for heterocyclic molecules.[7, 9, 12]

The concept of ring closure via aryne intermediates, as a general principle of synthesis, was first reported by Bunnett and Hrutfiord in 1961.[13] In this study, the preparation of a range of heterocyclic systems including indoles, was accomplished. The starting anilide **5.1**, after treatment with potassium amide, originated the aryne carbanion **5.2**. The subsequent intramolecular addition led to the 3-acetyloxindole **5.3** in 78% yield (Scheme 5.2a). In addition, they envisioned that treatment of *o*-chlorophenylacetone **5.4** with potassium amide would produce 2-indanone, but instead they obtained 2-methylindole **5.7** in low yield (31%)**.** To rationalize this unexpected outcome, the authors proposed two possible mechanisms. In the first one, *o*-chlrophenylacetone **5.4**, in the presence of the base, originates aryne **5.5** which in the presence of $NH_3$ leads to the amino ketone **5.6** followed by cyclization with elimination of water, leading to 2-methyl indole **5.7**. The second pathway involved the generation of aryne **5.8** which by intramolecular addition gave indole **5.7** (Scheme 5.2.b).



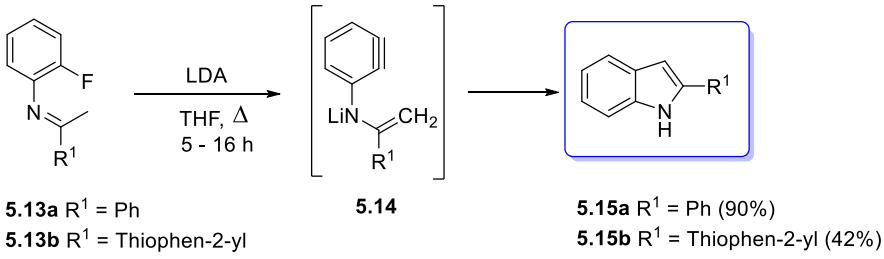

**Scheme 5.2** Synthesis of indoles via aryne intermediates.

Caubère and co-workers described the synthesis of indoles via aryne starting from imines **5.9** (Scheme 5.3).[14] In this work, a complex-base ($NaNH_2$-$Bu^tONa$) was used to generate aryne **5.10** which underwent intramolecular cyclization to intermediary **I** followed by the addition of the appropriate electrophile to give indoles **5.11**. The authors also reported the formation of indole **5.12**, as a result of the enolization of imine **5.9**.




**Scheme 5.3** Synthesis of indoles via aryne starting from imines.

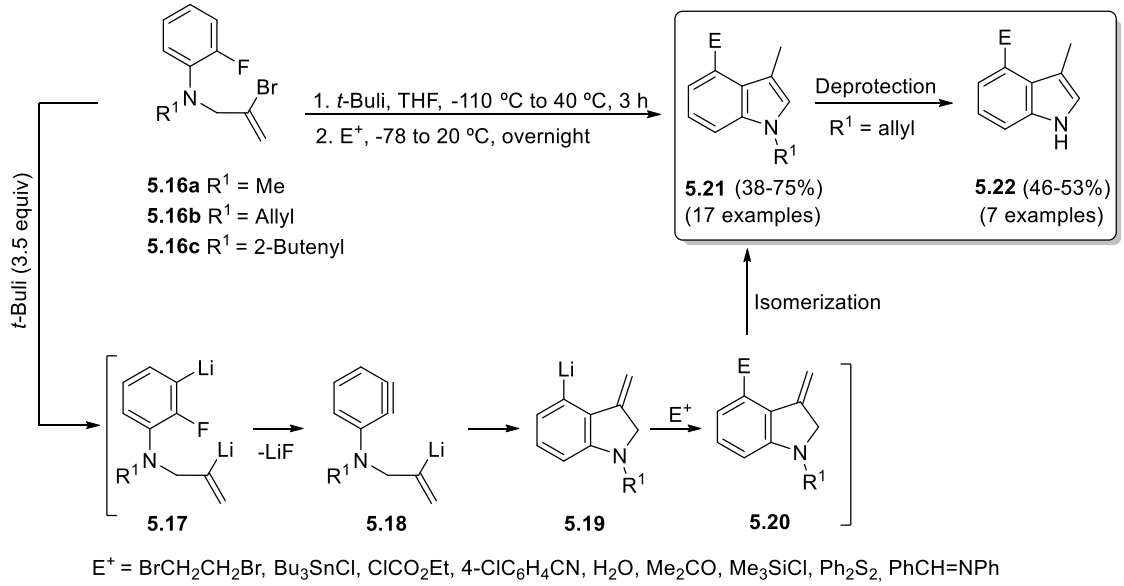
A similar strategy was followed by Kudzuma in 2003.[15] Starting from 2-fluorophenyl imines **5.13** and LDA, indoles **5.15** were obtained in moderate to excellent yields (Scheme 5.4). The need of excess of base led the authors to suggest that this transformation involved the generation of benzyne **5.14** rather than an $S_NAr$ mechanism, which was confirmed by deuterium labelling studies.




**Scheme 5.4** Synthesis of indoles starting from 2-fluorophenyl imines.

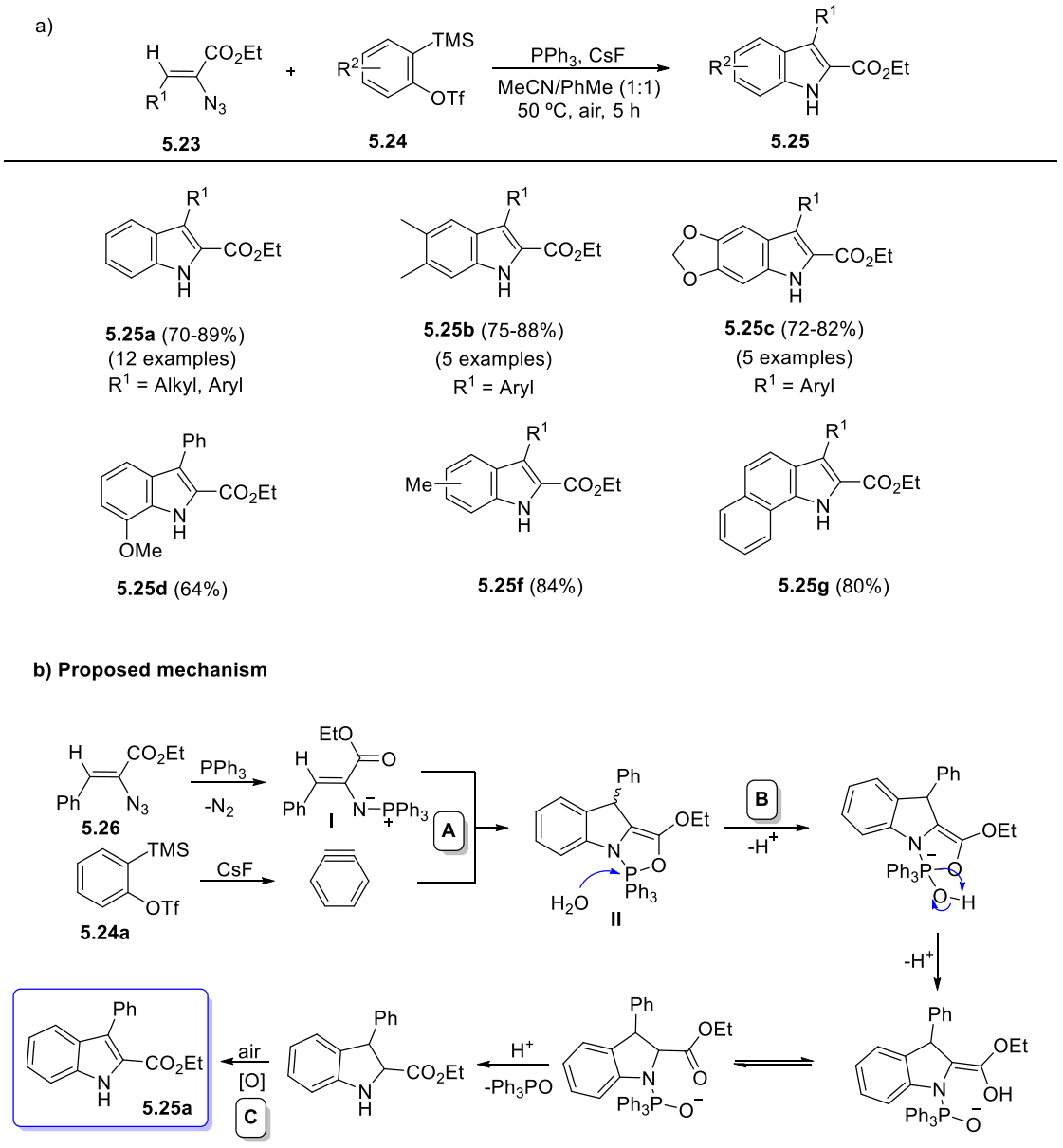
A novel methodology for the regioselective synthesis of functionalized indoles **5.21** from 2-fluoro-anilines, was described by Barluenga and collaborators.[16] The key step of this approach involves the generation of a benzyne-tethered organolithium compound **5.18**, which undergoes an intramolecular anionic cyclization to afford regiospecifically the metalated heterocyclic derivative **5.19**. The organolithium compound **5.19** reacts with a

selection of electrophiles giving 3-methyleneindolines derivatives **5.20**, which isomerize into 3,4-disubstituted indole derivatives **5.21** in moderate to good yields. The indole derivates **5.21** ($R^1$ = allyl), after treatment with DIBAL-H in the presence of a catalytic amount of [$NiCl_2$(dppp)] in toluene afforded the *N*-unsubstituted 3,4-disubstituted indole derivatives **5.22** in moderate yields (Scheme 5.5).



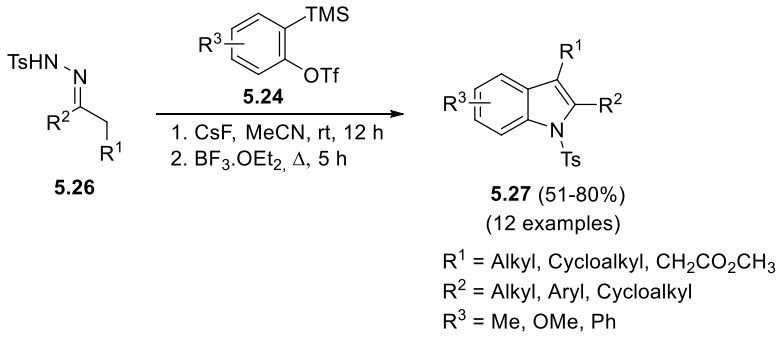

**Scheme 5.5** Synthesis of functionalized indoles from 2-fluoro-anilines.

Reactions between 2-azidoacrylates and triflates have also been explored for the synthesis of substituted indoles.[17] A mild and efficient strategy for the regioselective synthesis of 2,3-substituted indoles **5.25** via reaction between 2-azidoacrylates **5.23** and Kobayashi aryne precursors (*O*-silylaryl triflates) **5.24**, in the presence of $PPh_3$ and CsF, has been described (Scheme 5.6a).[17] The key step of the proposed mechanism involves the formation of nucleophilic iminophosphorane **I** from the reaction of the 2-azidoacrylate with $PPh_3$. **I** subsequently reacts with the in situ generated aryne intermediate to yield intermediate **II** by a nucleophilic double cyclization (**A**). After hydrolysis (**B**) and air-oxidation (**C**) cascade reactions, indoles **5.25** were formed (Scheme 5.6b).



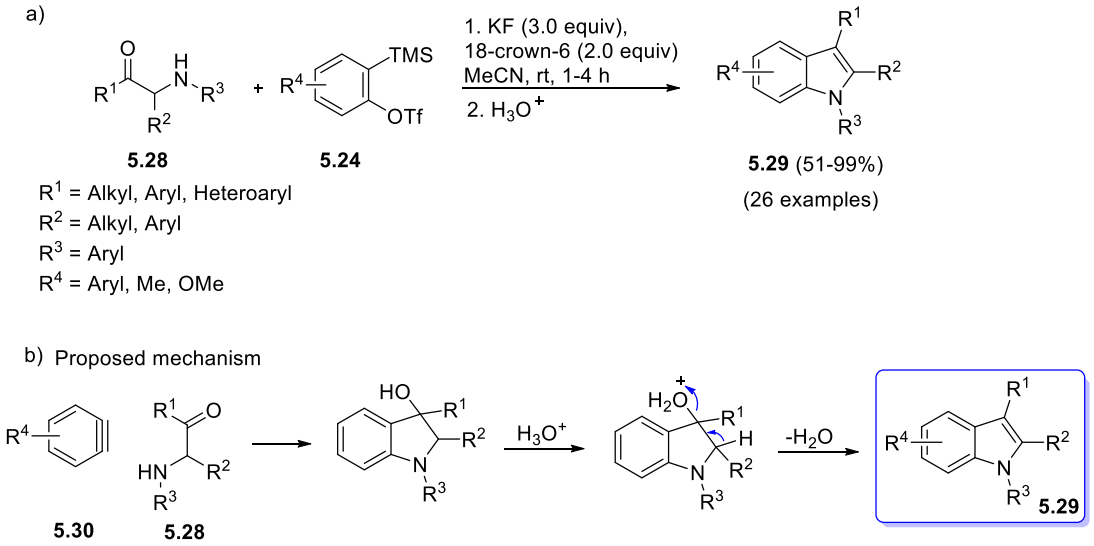

**Scheme 5.6** a) Synthesis of substituted indoles starting from 2-azidoacrylates. b) Proposed mechanism.

The Fischer-indole reaction remains as an important method by allowing a simple transition-metal-free preparation of indole derivatives, particularly 2- or 3-substituted-indoles. Thus, improved methodologies based on Fischer-indole reaction have been explored. One of those studies was the development of a Fischer-indole synthesis using benzyne chemistry.[18] The authors described the *N*-arylation of tosylhydrazones **5.26** with aryne precursors **5.24**, under mild conditions, followed by a Lewis acid-induced Fischer cyclization to give *N*-tosylindole derivatives **5.27** in moderate to very good yields (Scheme 5.7).



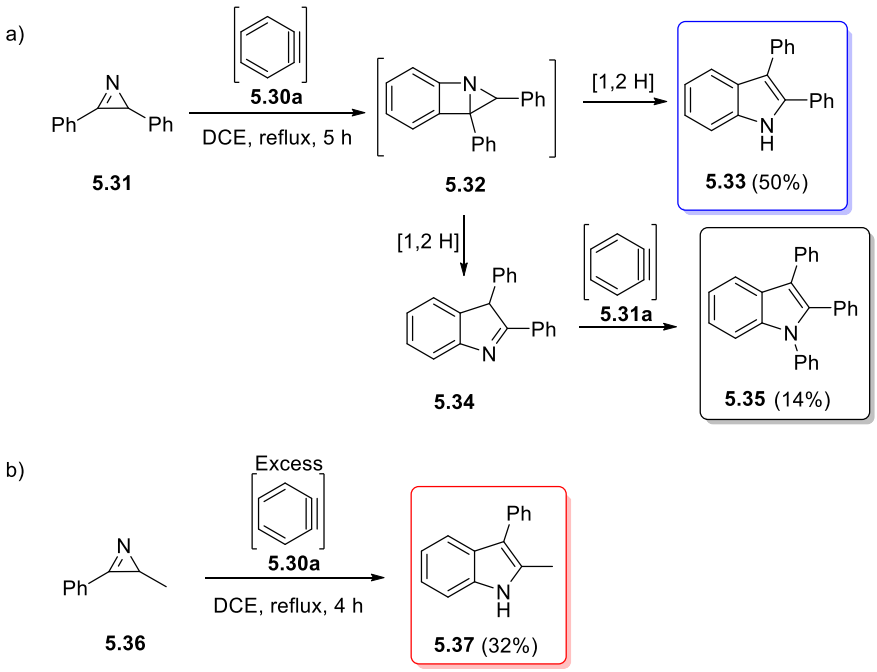

**Scheme 5.7** One-pot Fischer-indole synthesis using aryne chemistry.

More recently, a straightforward strategy for the synthesis of multi-substituted *N*-arylindoles **5.29** was reported.[19] The strategy involves a tandem reaction between α-aminoketones **5.28** and arynes **5.30** in the presence of KF and 18-crown-6 in MeCN at room temperature. This methodology proved to be adequate even when electron-withdrawing, electron-donating substituents, or heterocycles at different positions of α-aminoketones were used. When either symmetric or asymmetric substituted arynes were used *N*-arylindoles **5.29** were obtained in moderate to excellent yield (Scheme 5.8a). Interestingly, when asymmetric substituted aryne were used only one regioisomer was obtained. According to the proposed mechanism, the amino group of the α-aminoketone reacted with the aryne via an insertion reaction and subsequently, the resulting intermediate participated in a nucleophilic addition to the ketone moiety giving rise to the substituted indoline. Dehydration under acidic conditions, led to the desired multi-substituted *N*-arylindoles **5.29** (Scheme 5.8.b).




**Scheme 5.8** a) Tandem reaction between α-aminoketones and arynes. b) Proposed mechanism.

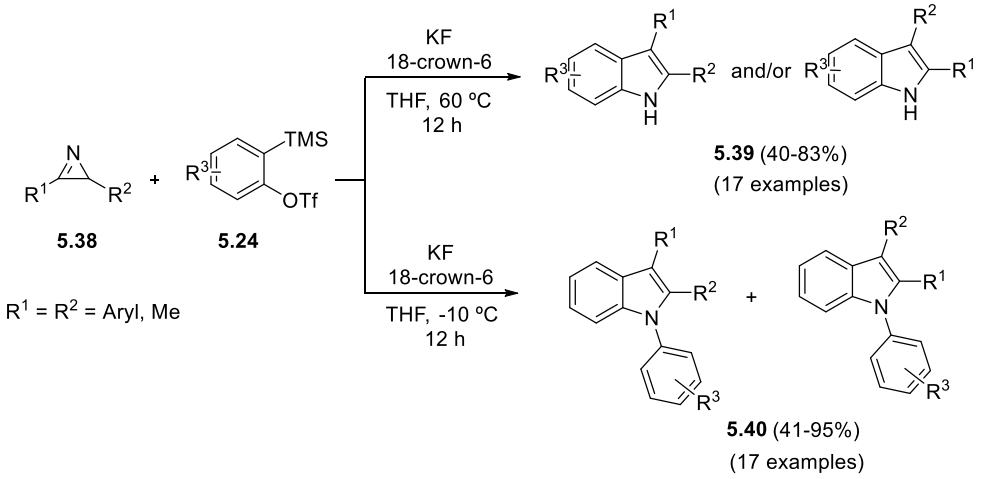
An alternative synthetic method towards indoles involving the reaction of di-phenylazirine **5.31** with benzyne (**5.30a**), generated *in situ* by thermal decomposition from benzenediazonium-2-carboxylate, was described by Nair and Kim.[20] The authors assumed that the reaction proceeded through a [2+2] cycloaddition of benzyne to the diphenylazirine leading to the formation of intermediate **5.32**. Then, two reaction pathways may occur. A 1,2-hidrogen shift to the nitrogen giving indole **5.33** in 50% yield, and a 1,2-hidrogen shift to the carbon leading to the intermediate **5.34** which can further react with another molecule of benzyne producing 1,2,3-triphenylindole **5.35** in 14 % yield (Scheme 5.9.a). Nevertheless, when 2-methyl-3-phenylazirine (**5.36**) was treated with excess of benzyne, the 3-phenyl-2-methyl-1*H*-indole **5.37** was obtained as single product (Scheme 5.9).

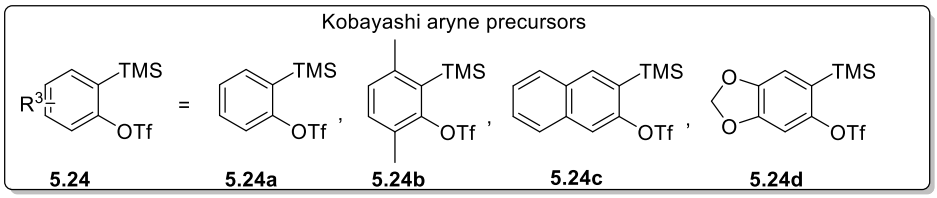



**Scheme 5.9** Indole synthesis starting from di-phenylazirine and 2-methyl-3-phenylazirine.

A highly selective reaction between arynes **5.24** and 2*H*-azirines **5.38** affording *N*-unsubstituted **5.39** and *N*-arylindoles **5.40** was recently described.[21] The selective outcome was strongly dependent on the reaction temperature, with 2,3-diarylindoles **5.39** being obtained with highly selectivity in moderate to excellent yields when the reaction was carried out at 60 ºC (Scheme 5.10). However, 2*H*-azirines with different substituents at C-2 and C-3 gave rise to a mixture of regioisomers. When the reaction was performed at -10 ºC, *N*-arylindoles **5.40** were obtained with very high selectivity and very good yields (Scheme 5.10).[21]

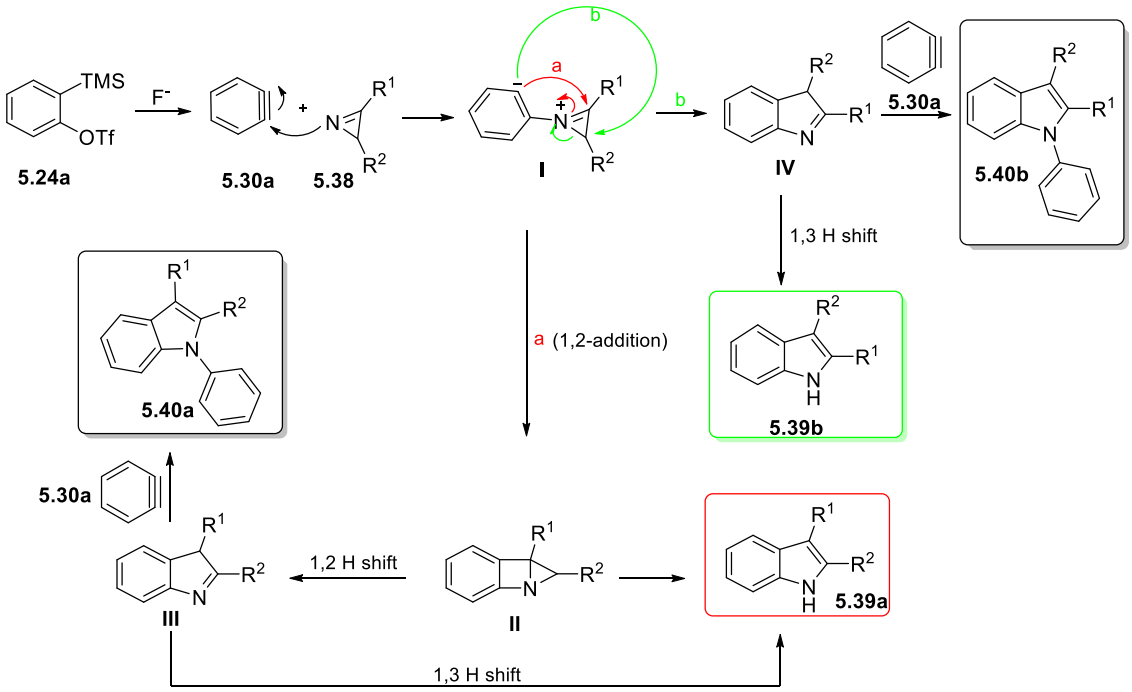
Additionally, they also observed that when symmetric azirines either with electron-withdrawing or -donating groups at the aryl group C-4 position were used, no significant differences were observed in regioselectivity or in the reaction efficacy. The use of the substituted arynes extended the range of the synthesised indoles without loss of regioselectivity.




**Scheme 5.10** Reaction between arynes and 2*H*-azirines affording *N*-unsubstituted and *N*-arylindoles.

Based on the reaction outcome, namely the formation of mixture of regioisomers, the authors proposed the reaction mechanism depicted in Scheme 5.11.[21] The nucleophilic addition of 2*H*-azirines **5.38** onto aryne **5.30a** generates the 1,4 zwitterionic intermediate **I**. This intermediate can cyclize by two different pathways. In path **a** (in red), following a 1,2-addition between aryne and the C=N bond leading to the intermediate **II**, a 1,2-hydrogen shift to nitrogen occurs resulting in the formation of *N*-unsubstituted indole **5.39a**. Alternatively, a 1,2-hydrogen shift to carbon produces intermediate **III**, which through subsequent 1,3-hydrogen shift gives rise to the same indole **5.39a**. On the other hand, an ene reaction between **5.30a** and **III** can occur giving the corresponding *N*-phenylindole **5.40a**. In path **b** (in green), an intramolecular nucleophilic attack of the aryl anion onto the 2*H*-azirines **5.38** C3 induces the C-N bond cleavage generating derivative **IV**, which leads to **5.39b** via a 1,3-hydrogen shift. As in the path **a**, an ene reaction of **IV** with another molecule of **5.30a** can occur resulting in the formation of **5.40b** (Scheme 5.11).[21]




**Scheme 5.11** Proposed mechanism for the synthesis of indoles.

## 5.2 Rationale and goals

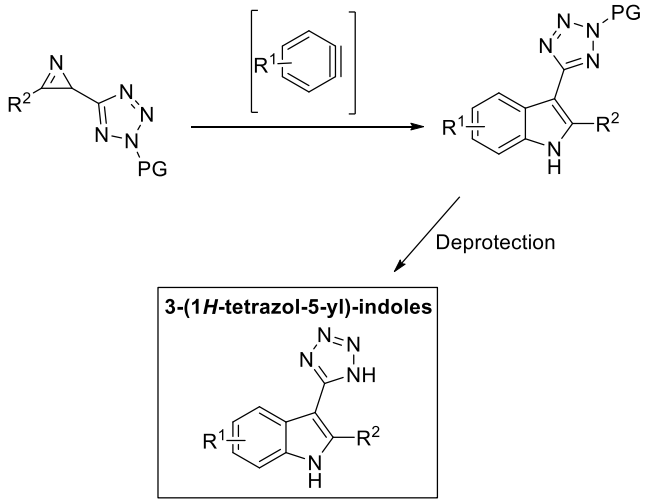
2*H*-Azirines represent a versatile class of heterocyclic system, that have been extensively used as powerful building blocks for the synthesis of a plethora of *N*-heterocycles[22-24], included ***N***-unsubstituted and *N*-arylindole derivatives.[20, 21] The contribution of Pinho e Melo´s research group included the Neber approach to novel 2-(tetrazol-5-yl)-2*H*-azirines bearing phenyl, furan-2-yl, thiophen-2-yl or 1*H*-pyrrol-2-yl substituents at C-3.[25, 26] Moreover, the reactivity of these interesting molecules towards imines in the presence of Lewis acids was also explored for the synthesis of 4-(tetrazol-5-yl)-1*H*-imidazoles.[27]

In addition, the chemistry of arynes has also been one of the group research interests. In fact, the synthesis of isoquinolines via cycloaddition of arynes with 1,2,4-triazines [28] as well as the synthesis of 1,3-dihydrothiazolo[3,4-b]indazoles via 1,3-dipolar cycloadditions of thiazolidine-derived sydnones with benzyne, was previously reported.[29]

Over the past decades, the use of bioisosteres in drug discovery to improve the efficiency, drug permeability and reduce or avoid toxicity has been extensively investigated.[30] It is well established that 5-substituted-1*H*-tetrazoles are effective biosisosteres of the carboxylic acid functionality.[31] In fact, they have similar acidity at physiological pH and both are planar. Nevertheless, tetrazoles present higher lipophilicity

than carboxylic acids, which is the key factor when crossing the cell membranes. Thus, a useful strategy to find new scaffolds is to explore the carboxylic acid/tetrazole bioisosterism. Taking in account these considerations, it is reasonable to consider 3-(1*H*-tetrazol-5-yl)-indole derivatives as bioisosteres of 1*H*-indole-3-carboxylic acid derivatives, the latter used as building blocks in the synthesis of indole derivatives with biological activities.[32] Therefore, constructing indoles bearing tetrazole moieties can also open the access for the development of more complex molecules with potential biological activities.

In this context, we decided to explore the reactivity of tetrazolyl-2*H*-azirines towards arynes in order to obtain a new class of indole derivatives bearing a tetrazole moiety. The approach for the synthesis of 3-(1*H*-tetrazol-5-yl)-indoles is outlined in Scheme 5.12.



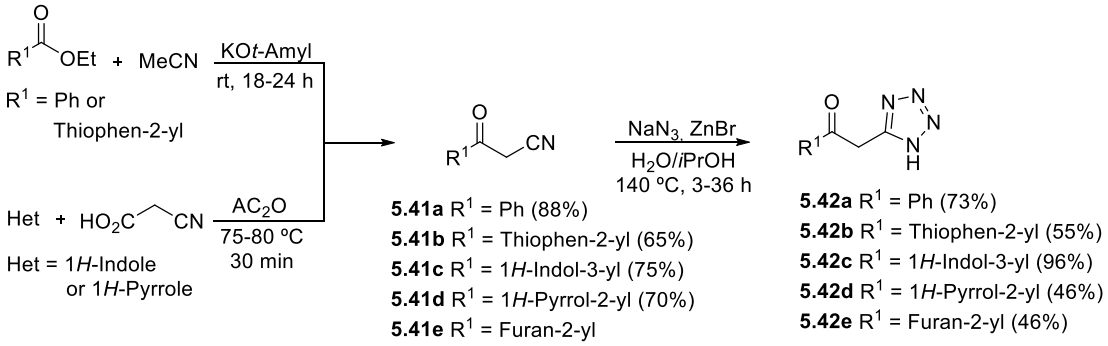

**Scheme 5.12** Synthetic strategy towards 3-(1*H*-tetrazol-5-yl)-indoles.

## 5.3 Synthesis of 3-tetrazolyl-indoles

### 5.3.1 Synthesis of tetrazolyl-2*H*-azirines

The present study was initiated with the synthesis of the tetrazolyl-2*H*-azirines. These heterocycles were synthesised following a literature procedure.[25] This synthetic route started with the synthesis of β-ketonitriles **5.41a**,**b**, based on the acylation of nitrile anions with the corresponding ethyl esters in presence of KO$^t$Amyl.[33] Cyanoacetylation in acetic anhydride was carried out for the synthesis of β-ketonitriles **5.41c** and **5.41d**. In both methods, nitriles were obtained in excellent yields (Scheme 5.13). Nitrile **5.41e** is commercially available. The conversion of **5.41** into the corresponding β-keto-1*H*-tetrazoles **5.42** following the remarkably simple general protocol reported by Sharpless *et*

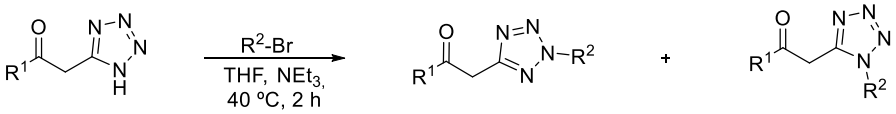
*al* in which nitriles reacted with sodium azide in water, using zinc bromide as catalyst, was accomplished leading to tetrazoles **5.42** in moderate to excellent yields (scheme 5.13)




**Scheme 5.13** Synthesis of β-keto-1*H*-tetrazoles.

The next step consisted in the tetrazoles protection with groups that could be easily removed.[25] Thus, the reaction was performed using *p*-nitrobenzyl bromide (PNB-Br) or benzyl bromide, in presence of triethylamine, in THF at 40 ºC for 2 h, 1*H*- and 2*H*-tetrazole derivatives **5.43** and **5.44** were obtained in good overall yield (Table 5.1).

**Table 5.1.** Alkylation of β-keto-tetrazoles **5.42**.

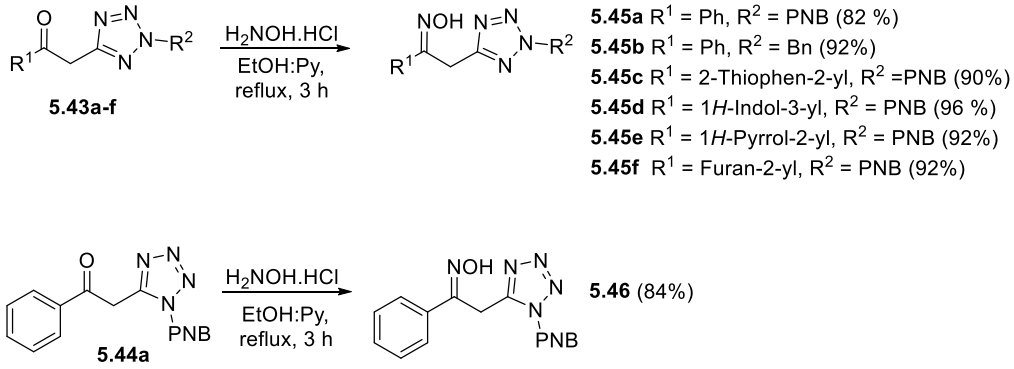



**a** $R^1$ = Ph, $R^2$ = PNB
**b** $R^1$ = Ph, $R^2$ = Bn
**c** $R^1$ = Thiophen-2-yl, $R^2$ = PNB
**d** $R^1$ = 1*H*-Indol-3-yl, $R^2$ = PNB
**e** $R^1$ = 1*H*-Pyrrol-2-yl, $R^2$ = PNB
**f** $R^1$ = Furan 2-yl, $R^2$ = PNB

| Entry | **5.42** | $R^2$ | Yield of **5.43** (%) | Yield of **5.44** (%) |
|---|---|---|---|---|
| 1 | **a** | PNB | 42 | 28 |
| 2 | **a** | Bn | 34* | 33* |
| 3 | **b** | PNB | 38 | 28 |
| 4 | **c** | PNB | 25 | 37 |
| 5 | **d** | PNB | 39 | 33 |
| 6 | **e** | PNB | 45 | 26 |

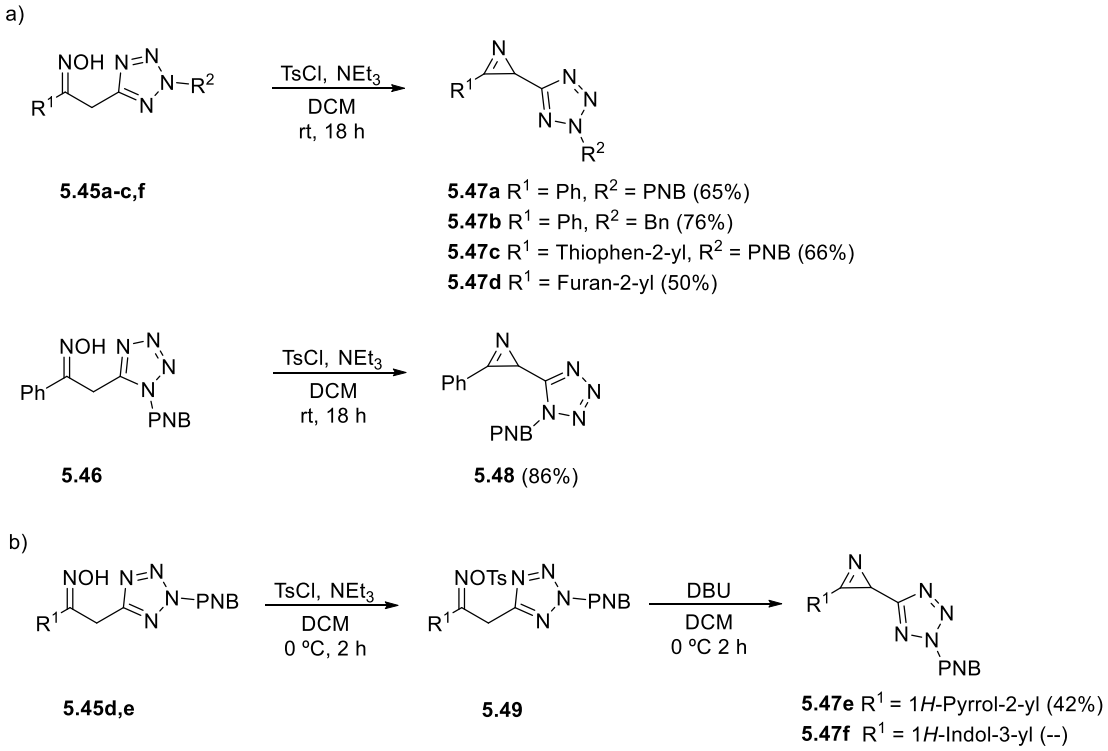
*Reaction time = 18 h

*β*-Keto-2*H*-tetrazoles **5.43-f** and *β*-keto-1*H*-tetrazoles **5.44a** were easily converted into the corresponding ketoximes **5.45a-f** and **5.46**, respectively, by the reaction with hydroxylamine hydrochloride in refluxing EtOH/pyridine for 3 h. The products were obtained as a mixture of *E* and *Z* isomers in excellent yields (82-96%) (Scheme 5.14).[25]




**Scheme 5.14** Synthesis of *β*-ketoxime-tetrazoles **5.45a-f** and **5.46**.

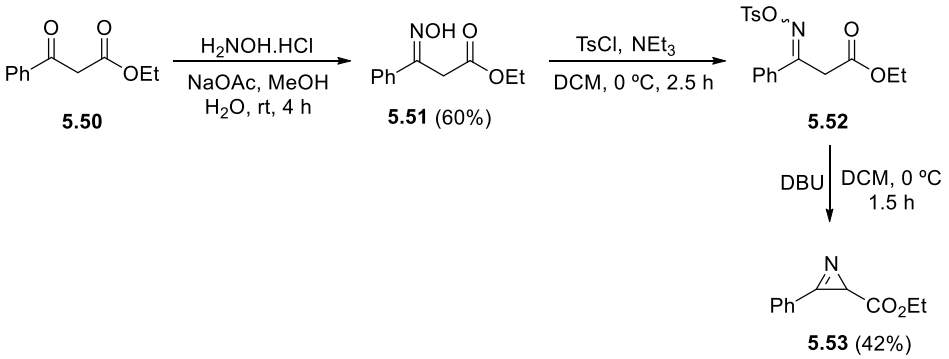
Finally, the last step was the conversion of the *β*-ketoxime-tetrazoles **5.45** and **5.46**, using the Neber approach, into the corresponding 2-(tetrazol-5-yl)-2*H*-azirines (**5.47** and **5.49**).[25] The *in situ* tosylation of *β*-ketoxime-tetrazoles **5.45a-c**,**f** and **5.46**, in presence of triethylamine, induced the formation of the corresponding 2-(tetrazol-5-yl)-2*H*-azirines **5.47a-d** and **5.48**, respectively, in good yields. However, when starting from oxime **5.47e** or **5.47d**, bearing 1*H*-pyrrol-2-yl or 1*H*-indol-3-yl at C-3, the Neber approach via *in situ* tosylation, did not lead to the target 2-(tetrazol-5-yl)-2*H*-azirines (Scheme 5.15a). Thus, an alternative procedure was explored based on a known general synthetic methodology.[34, 35] The first step consisted in the conversion of the oximes into the tosylates **5.49**, using triethylamine as base, in DCM, at 0 ºC for 2 h, which was used for the next step without purification. After the DBU-mediated Neber reaction of **5.49** the desired 2-(tetrazol-5-yl)-2*H*-azirine **5.47e** was finally obtained in moderate yield (42%). Unfortunately, no reaction was observed starting from the tosylated *β*-ketoxime-tetrazole **5.49** bearing the 1*H*-indol-3-yl substituent (Scheme 5.15b).




**Scheme 5.15** Synthesis of 2-(tetrazol-5-yl)-2*H*-azirines.

### 5.3.2 Synthesis of ethyl 3-phenyl-2*H*-azirine-2-carboxylate

In addition to tetrazolyl-2*H*-azirines, it was also decided to study the reactivity of the ethyl 3-phenyl-2*H*-azirine-2-carboxylate (**5.53**). This 2*H*-azirine was synthesised following a procedure very similar to the one reported previously for the synthesis of tetrazolyl-2*H*-azirine **5.45e.**[35] The starting *β*-ketoester **5.50** was easily converted into the corresponding oxime **5.51** by treatment with hydroxylamine hydrochloride in presence of sodium acetate (NaOAc), using the MeOH/$H_2O$ solvent system, at room temperature for 4 h. The desired oxime was obtained in 60% yield. Next, oxime **5.51** was transformed into the oxime tosylate **5.52**, using triethylamine as base, in DCM, at 0 ºC for 2.5 h, and was used in the next step without purification. The DBU-mediated Neber reaction of **5.52** afforded the desired ethyl 3-phenyl-2*H*-azirine-2-carboxylate (**5.53**) in moderate yield (42%) (Scheme 5.16).

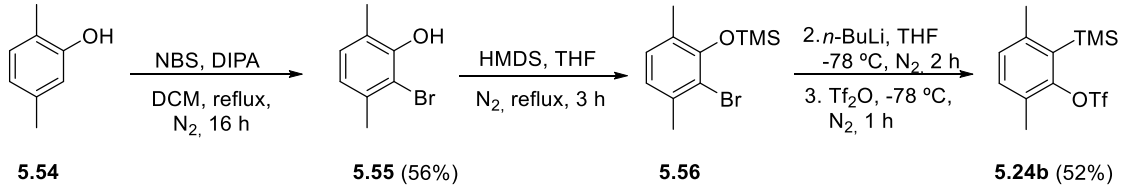



**Scheme 5.16** Synthesis of ethyl 3-phenyl-2*H*-azirine-2-carboxylate.

### 5.3.3 Synthesis of 3,6-dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate

As with most procedures for benzyne generation, Kobayashi's precursor also requires an *ortho*-difunctionalised arene. These are most commonly derived from 2-halophenols, which may in turn be obtained via electrophilic partial halogenation or similar routes. [9]

Compound **5.55** was obtained from 2,5-dimethylphenol **5.54** in presence of *N*-bromosuccinimide (NBS) and diisopropylamine (DIPA) in 56% yield.[36] 3,6-Dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (**5.24b**) was synthesised following the Peña *et al* protocol.[37] Starting from **5.55**, a sequential *O*-silylation with hexamethyldisilazane (HMDS), metal-halogen exchange with *n*-BuLi at low temperature, *O*- to *C*-silyl group migration, and triflation afforded functionalized Kobayashi aryne precursor **5.24b** in good yield (52%) (Scheme 5.17).




**Scheme 5.17** Synthesis of 3,6-dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate.

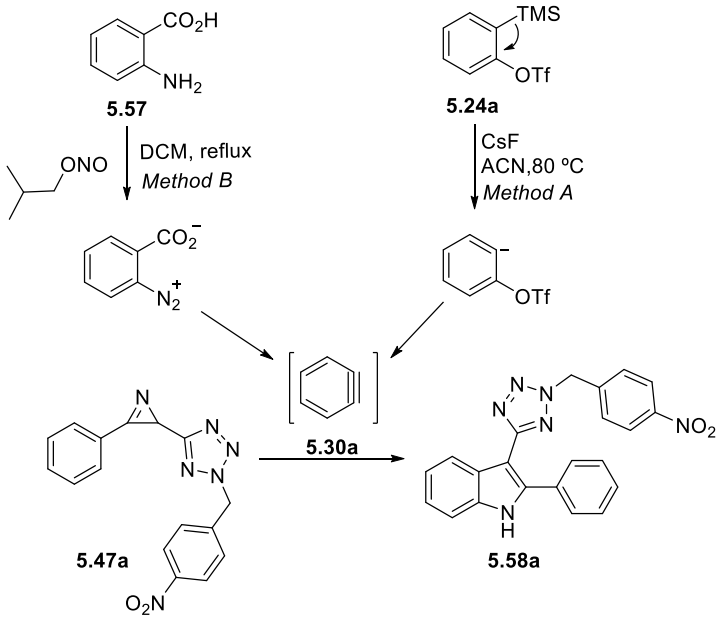
### 5.3.4 Optimization studies for the synthesis of 3-tetrazolyl-indoles

The reaction between 2-(tetrazol-5-yl)-2*H*-azirine **5.47a** ($R^1$ = Ph and $R^2$ = PNB) and aryne **5.30a** was the model reaction for the optimization studies. For this purpose, three different methods for aryne generation were tested.[9, 29, 38, 39] In the first approach (*method*

*A*), aryne **5.30a** was generated *in situ* from 2-(trimethylsilyl)aryl triflate **5.24a**, using cesium fluoride (CsF) as fluoride source in THF at 80 ºC for 24 h. Under these conditions and using 1.2 equiv. of **5.24a**, indole **5.58a** was obtained in 15% yield (Table 5.2, entry 1). Increasing the amount of triflate **5.24a** to 1.8 equiv. an improvement was observed, giving rise the desired indole in 44% yield (Table 5.2, entry 2). Attempts to further improve the efficiency of this reaction, by increasing the number of equivalents of the of aryne precursor **5.24a**, did not lead to the desired indole and only degradation products were obtained (Table 5.2, entry 3).

The generation of benzyne using anthranilic acid **5.57** was also investigated. In this method, the diazonium salt obtained from anthranilic acid in presence of isoamyl nitrite in DCM at reflux for 1 h (Method B), is decomposed to generate *in situ* the corresponding aryne.[39] However, this strategy did not lead the desired product (Table 5.2, entry 4).

**Table 5.2** Optimization of the synthesis of 3-tetrazolyl-indole **5.58a**.

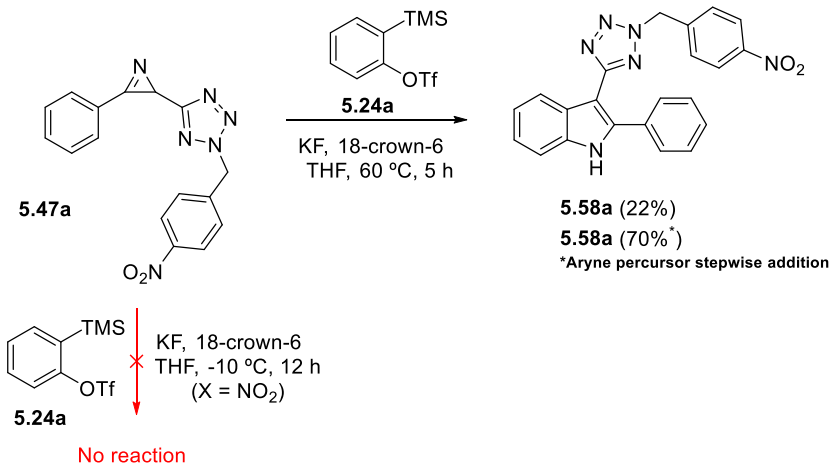



| Entry | Reaction conditions | Benzyne precursor (Equiv) | CsF (Equiv) | Time | 5.58a Yield (%) |
|---|---|---|---|---|---|
| 1 | *Method A* | **5.24a** (1.2**)** | 2.5 | 24 h | 15 |
| 2 | *Method A* | **5.24a** (1.8) | 2.5 | 24 h | 44 |
| 3 | *Method A* | **5.24a** (5)[a] | 8 | 24 h | --[b] |
| 4 | *Method B* | **5.57a** (1)[c] | -- | 1 h | ---[b] |

[a] Stepwise addition; [b] Azirine recovered; [c] Two equiv. of isoamyl nitrite were used.

In order to improve this transformation, the reaction between 2-(tetrazol-5-yl)-2*H*-azirine **5.47a** and benzyne **5.30a**, generated *in situ* from the 2-(trimethylsilyl)aryl triflate precursor **5.24a** was also carried out, using KF as fluoride source in presence of 18-crown-6 in THF at 60 ºC for 5 h, affording the desired product in 22% yield. To our delight, the stepwise addition of the aryne precursor **5.24a** led to the isolation of indole **5.58a** in 70%. (Scheme 5.18). The reaction was regioselective, affording *N*-unsubstituted 3-(tetrazol-5-yl)-indole **5.58a** as single product.

Thangaraj *et al*,[21] described a transition-metal-free and temperature-dependent highly selective reaction of arynes with 2*H*-azirines that allowed the synthesis of either *N*-unsubstituted or *N*-arylindoles. In this study when the reaction was carried out at −10 °C, the selectivity was switched to the formation of 1,2,3-triarylindoles in good yields (see Scheme 5.10). Therefore, we decided to investigate whether in the reaction between azirine **5.47a** and benzyne a similar reactivity/selectivity was observed. However, at -10 ºC, in THF during 12 h, no product was obtained (Scheme 5.18).




**Scheme 5.18.** Optimization of the synthesis of 3-tetrazolyl-indoles using KF/18-crown-6.

The $^{1}$H NMR spectrum of 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole **5.58a** is presented in Figure 5.1. The broad singlet at 9.11 ppm is easily identified as the typical signal for the indole NH proton (H-1 proton). The singlet at 5.82 ppm is assigned to the methylene protons of the *p*-nitrobenzyl group (H-6). The system AA´BB´ corresponding to the protons in the ortho position (H-8 and H-8') and the protons in the meta position (H-7 and H-7') regarding the nitro group, are observed at 8.24 and 7.53 ppm, respectively.

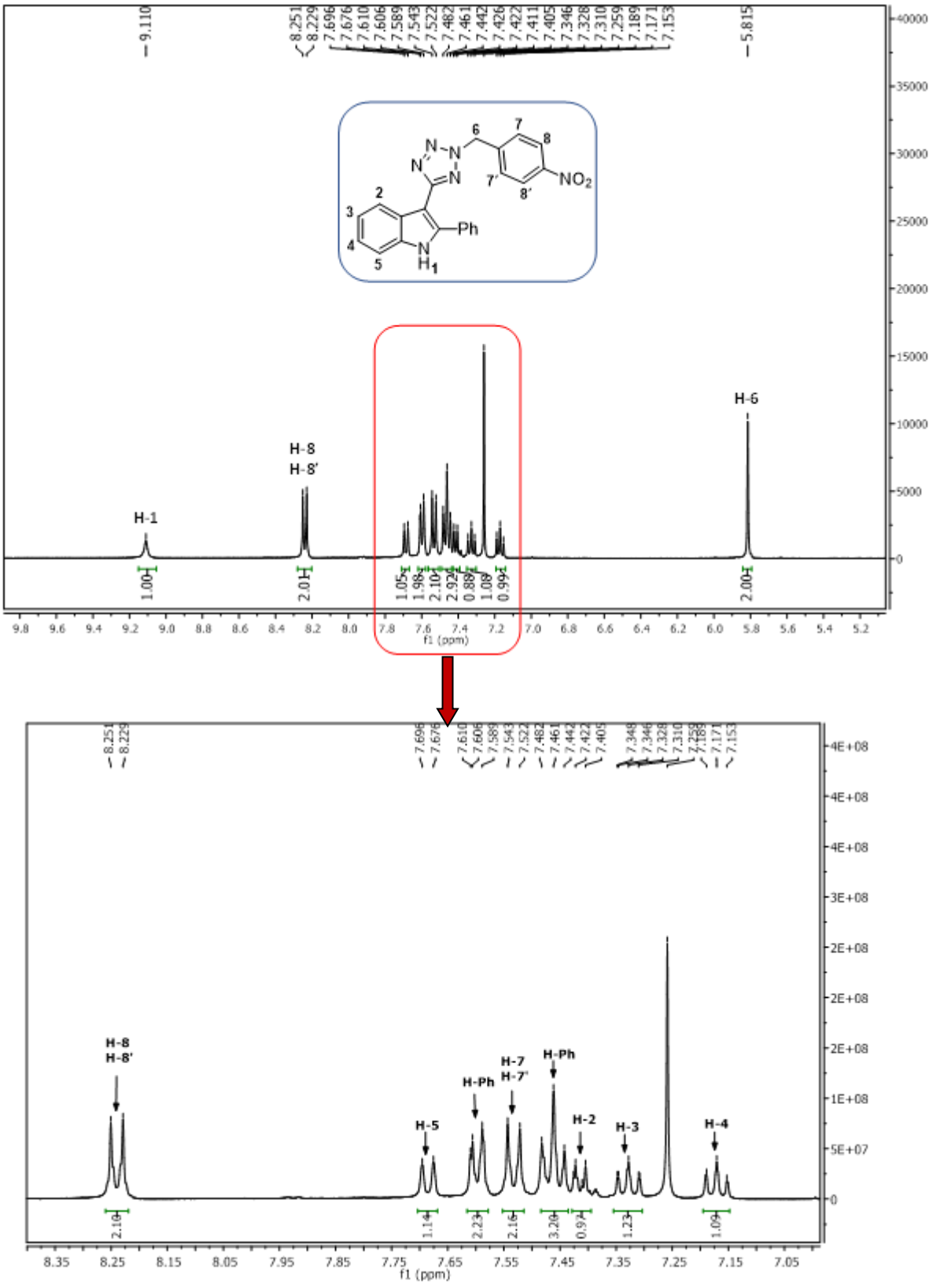



**Figure 5.1.** $^{1}$H NMR spectrum of 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole (**5.58a**).

The main observed interactions in the COSY spectrum are represented in figure **5.2**, allowing the assignment of protons from indole and the *p*-nitrobenzyl group. It was possible to observe the correlation between the H-5 (doublet at 7.69 ppm) and H-4 (*pseudo* triplet at 7.17 ppm) which in turn showed correlation with the H-3 protons (*pseudo* triplet at 7.33 ppm). Furthermore, is also possible to observe the correlation between the H-8 (doublet at 8.24 ppm) and H-7 (doublet at 7.53ppm) protons and the H-6 protons of the methylene bridge (singlet at 5.82 ppm).

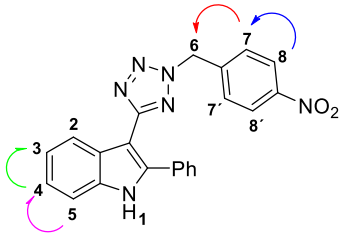
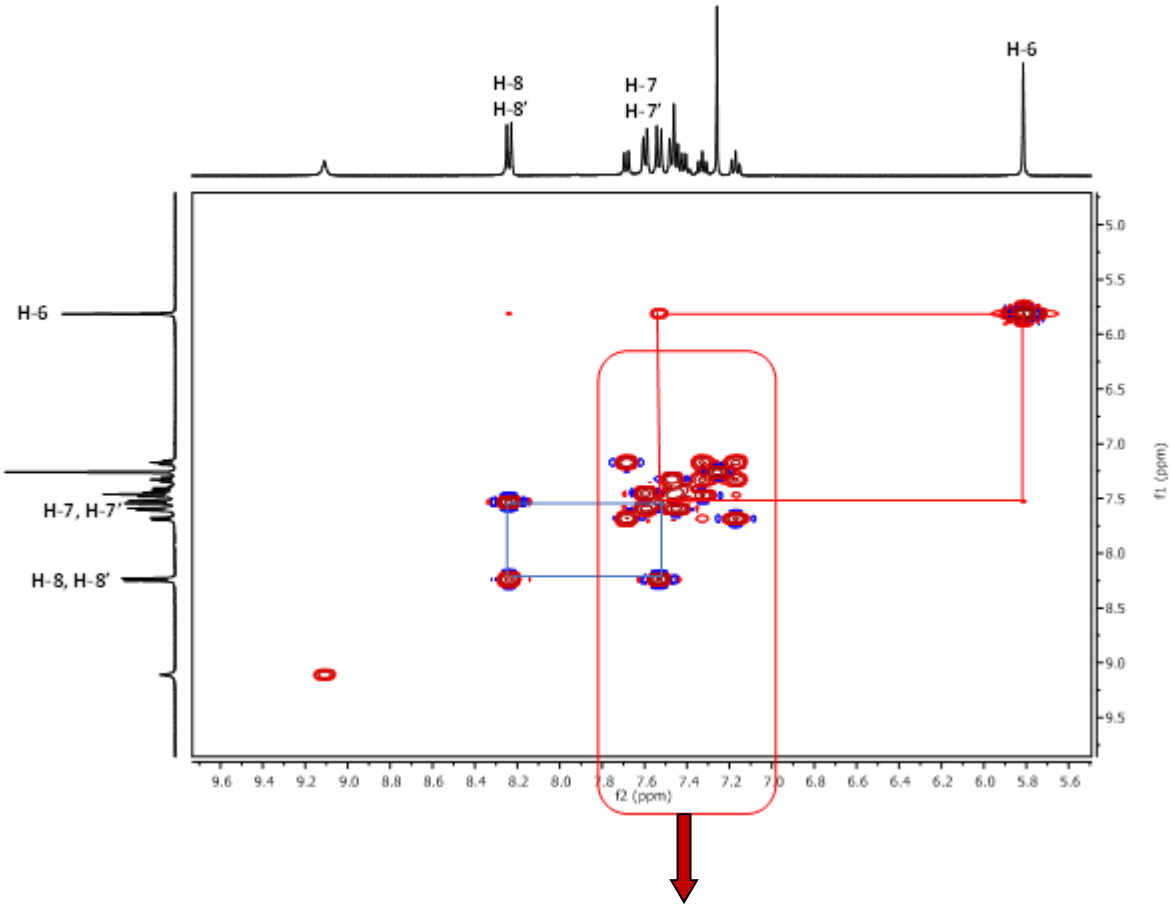



**Figure 5.2** Main observed connectivities in the COSY spectrum of compound **5.58a**.



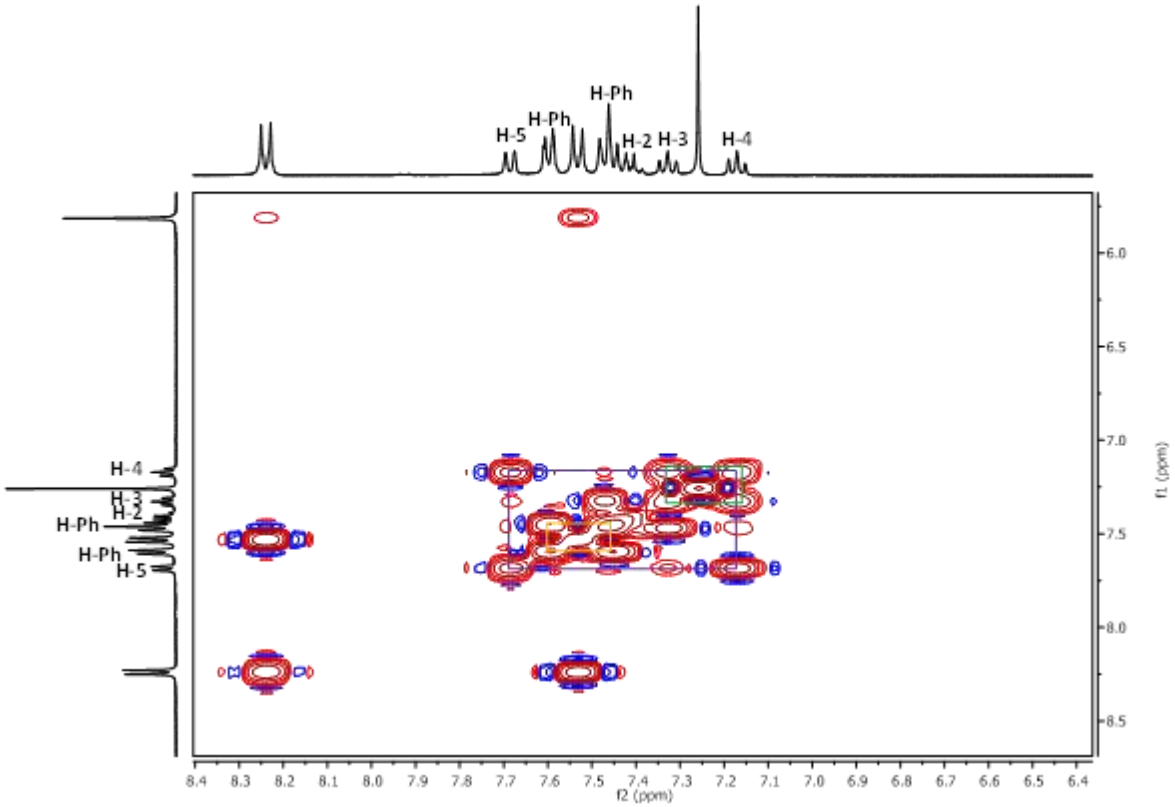



**Figure 5.3** Expansion of the COSY spectrum of 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole (**5.58a**).

The regiochemistry assignment of the 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole (**5.58a**) was confirmed by the bidimensional NOESY spectrum analysis in which it was possible to observe the connectivity between the indole´s NH proton (H-1) and the protons of phenyl group (H-Ph) and no connectivity between the 4-nitrobenzyl group protons and the NH indole´s proton was observed (Figure 5.4).

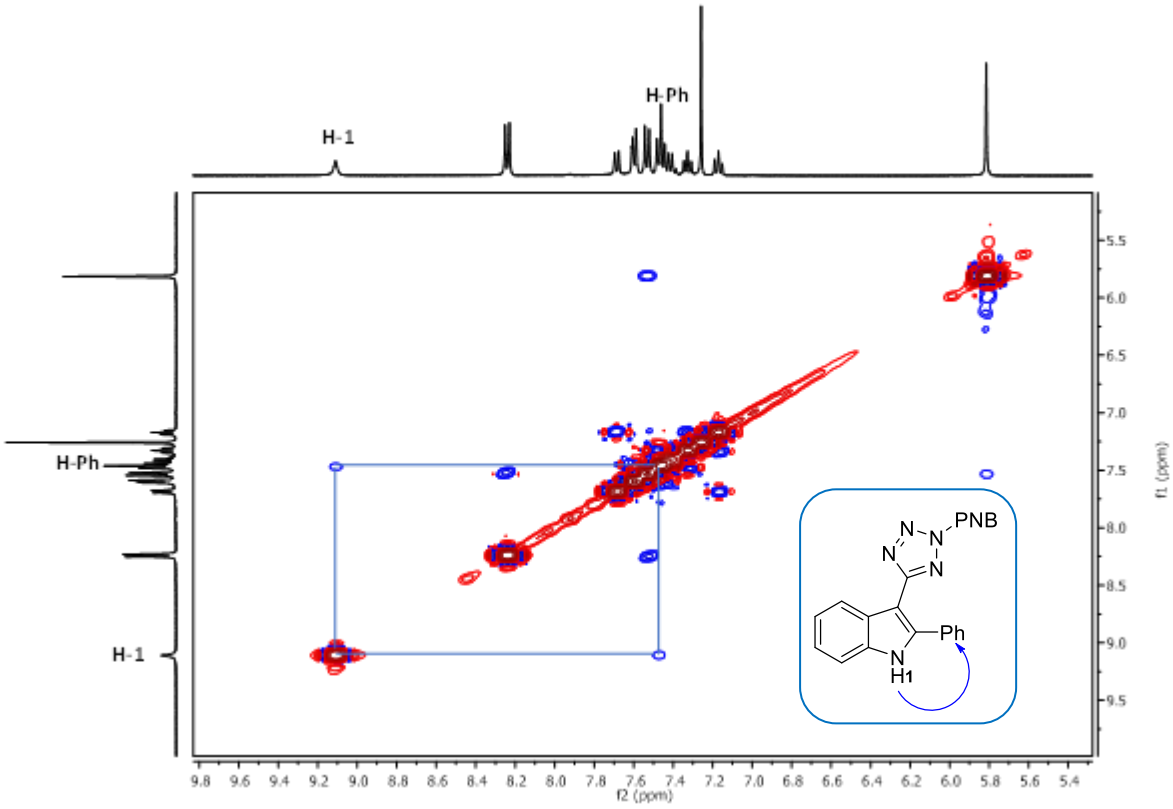
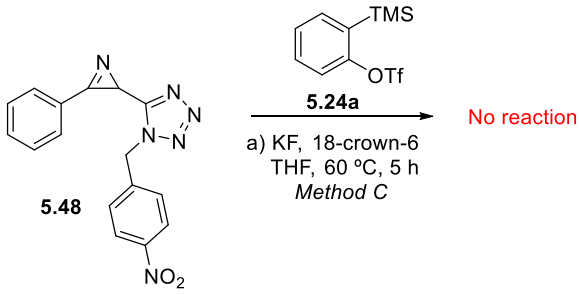



**Figure 5.4** Expansion of the NOESY spectrum of 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2- phenyl-1*H*-indole (**5.58a**).

Next, was decided to investigate the chemical behaviour of 2*H*-azirine ***5.48***, bearing a *p*-nitrobenzyl protecting group at N1 position, towards benzyne. Surprisingly, under the optimal conditions the reaction did not lead to the desired indole but to the recovery of the starting material ***5.48*** (Scheme 5.19).




**Scheme 5.19** Reaction between 2-(tetrazolyl-5-yl)-2*H*-azirine and 2-(trimethylsilyl)aryl triflate as benzyne precursor.

To explain the absence of reactivity of 2-tetrazolyl-2*H*-azirine **5.48**, quantum chemical calculations, at the DFT level of theory, to obtain the optimization of 2-(tetrazolyl-5-yl)-

2*H*-azirines **5.47a** (Figure 5.6a) and **5.48** (Figure 5.6b) geometries, were carried out. The results showed that the 2*H*-azirine moiety of **5.48** is blocked by the *p*-nitrobenzyl protecting group, which allowed to rationalize the lack of reactivity of 2*H*-azirine **5.48** due to steric hindrance.

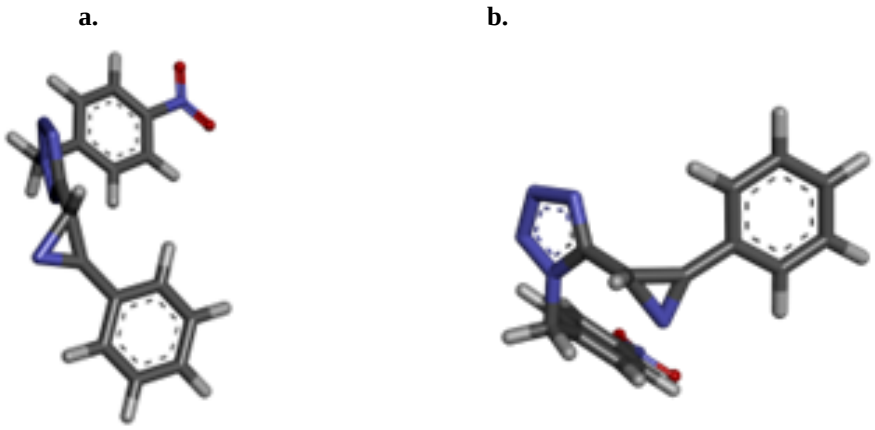
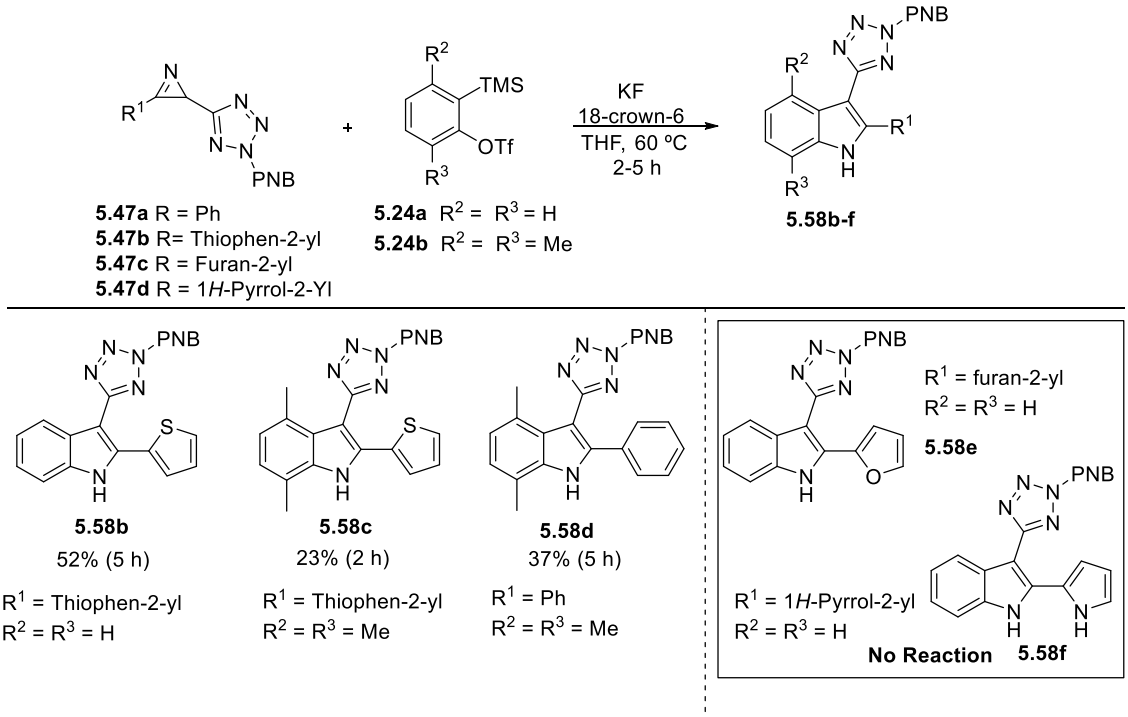



**Figure 5.6**. Optimized geometries (B3LYP/6-31G(d) level) of 2-(tetrazol-5-yl)-2*H*-azirines **5.47a** (a) and **5.48** (b).

### 5.3.5 Scope of the reaction

The study was extended to azirines **5.47b**, **5.47c** and **5.47d** bearing a thiophen-2-yl, furan-2-yl and 1*H*-pyrrol substituent at C3, respectively. However, upon performing the reactions with *in situ* generated benzyne under the optimal conditions, different outcomes were observed depending on the 2*H*-azirine heteroaromatic substituent. The reaction between azirine **5.47b** and triflate **5.54a** led to 2-thiophen-2-yl-3-tetrazolyl-1*H*-indole **5.58b** in 52% yield. In contrast with this result, the attempted reactions with 3-tetrazolyl-*2H*-azirine bearing furan-2-yl **5.47c** or 1*H*-pyrrol-2-yl **5.47d** substituents did not lead to the desired products.

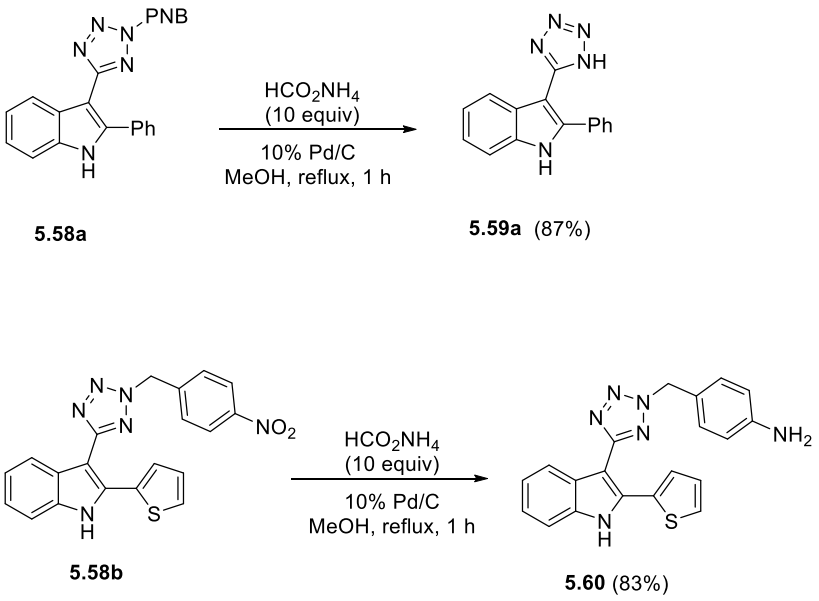
Next, was decided to extend this approach to 3-(1*H*-tetrazol-5-yl)-indoles to the use of other aryne precursor **5.24b** ($R^1 = R^2 = Me$). The reaction with 2*H*-azirine **5.47a** and **5.47b** led to the target indole derivatives **5.58c** and **5.58d**, although they were obtained with moderate yields (23% and 37%, respectively). These reactions were also regioselective, affording *N*-unsubstituted indoles **5.58** as single products (Scheme 5.20).




**Scheme 5.20** Scope of the reaction.

### 5.3.6 Deprotection of tetrazole moieties

In order to prepare the 3-tetrazolyl-1*H*-indoles, bioisosteres of 1*H*-indole-3-carboxylic acids, the tetrazole moiety in 2-[2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl]-1*H*-indoles **5.58** had to be deprotected. Previously in our research group, the deprotection of tetrazoles was successfully accomplished by using a general method reported to be efficient for the cleavage of the benzyl group of *N*-benzylamines and 1-benzyl-1*H*-imidazoles.[27, 40-42] This deprotection method was also previously successfully extended to the deprotection of 1*H*-tetrazoles. Thus, a suspension of indole **5.58a** and 10% Pd/C in methanol was treated with excess ammonium formate and heated at reflux for 1 hour affording the target **5.59a**, bearing the unprotected 1*H*-tetrazolyl group, in high yield (87%). Unfortunately, using the same reaction conditions indole **5.58b** was converted into derivative **5.60** in 83% yield, instead of the desired deprotection of the tetrazolyl group (Scheme 5.21).




**Scheme 5.21** Deprotection reaction of tetrazole moiety.

Beside the differences of polarity observed between compounds **5.58a** and **5.59a** confirmed by TLC, the deprotection of the tetrazole can also be clearly confirmed by the analysis of their corresponding $^{1}$H NMR spectra. The $^{1}$H NMR spectrum of the unprotected tetrazole **5.59a** is presented in Figure 5.7 ($^{1}$H NMR spectrum of the compound **5.58a** in Figure 5.1). No signals corresponding to the benzyl group were observed, only protons of the indole core and of the phenyl group are present.

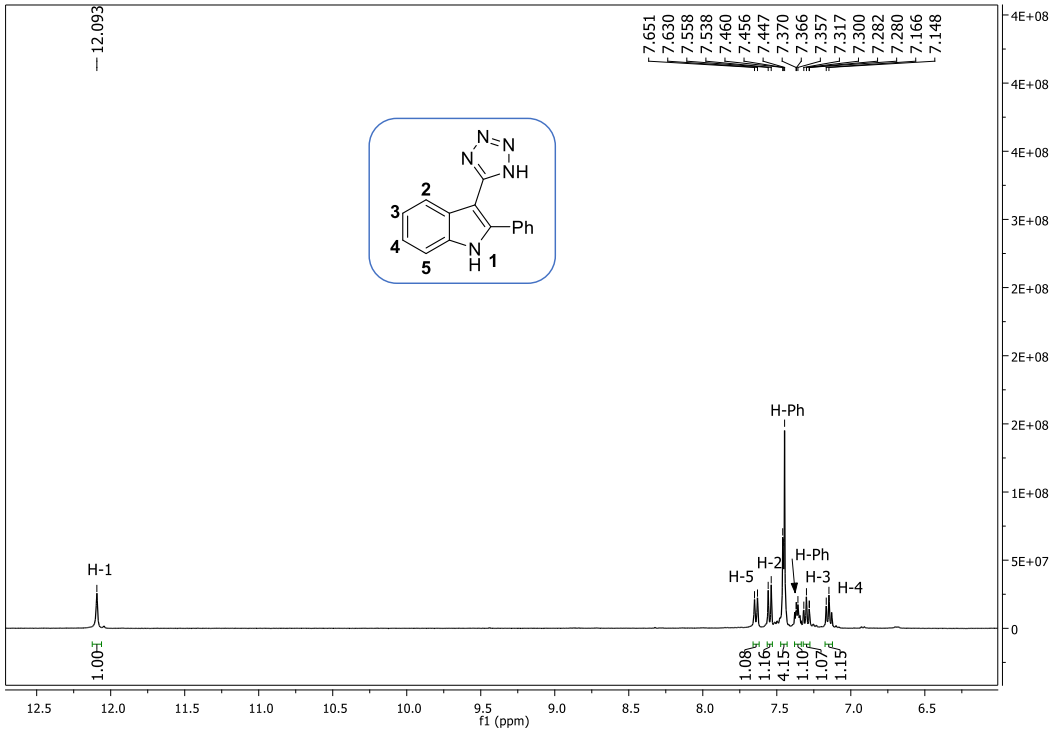
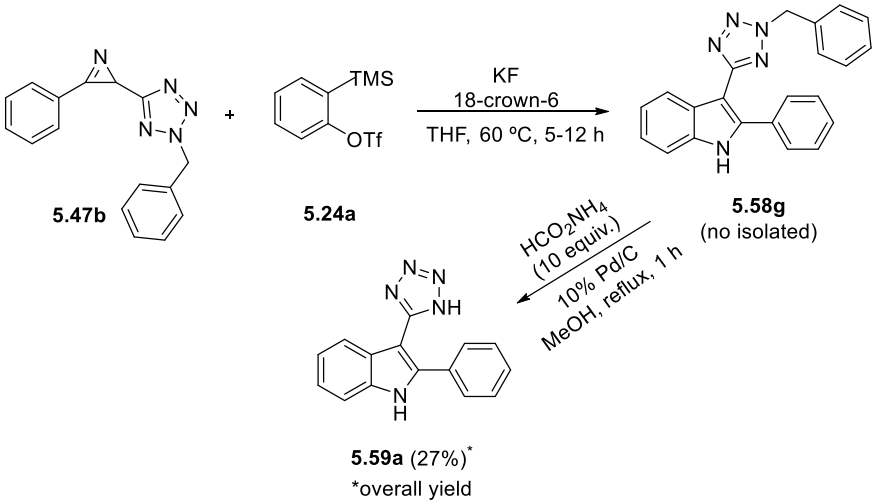



**Figura 5.7** Expansion of $^{1}$H NMR spectrum of compound **5.59a**.

3-Tetrazolyl-1*H*-indole **5.58g** could also be obtained via two-step one-pot procedure as outlined in Scheme 5.22 The reaction between azirine **5.47b** and 2-(trimethylsilyl)aryl triflate **5.24a** gave indole **5.58g** which was used without purification in the deprotection reaction to give corresponding 3-tetrazolyl-1*H*-indole **5.59a** in 27% overall yield.




**Scheme 5.22** Two-step one-pot procedure towards 3-tetrazolyl-1*H*-indole.

Finally, it was decided to explore the chemical behaviour of ethyl 3-phenyl-2*H*-azirine-2-carboxylate (**5.53**) and 2-(trimethylsilyl)aryl triflate **5.24a** under the same reaction conditions. Unfortunately, the desired indole was not formed and the azirine **5.53** was recovered, indicating the lack of reactivity towards benzyne even when the reaction time was increased to 12 h (Scheme 5.23).

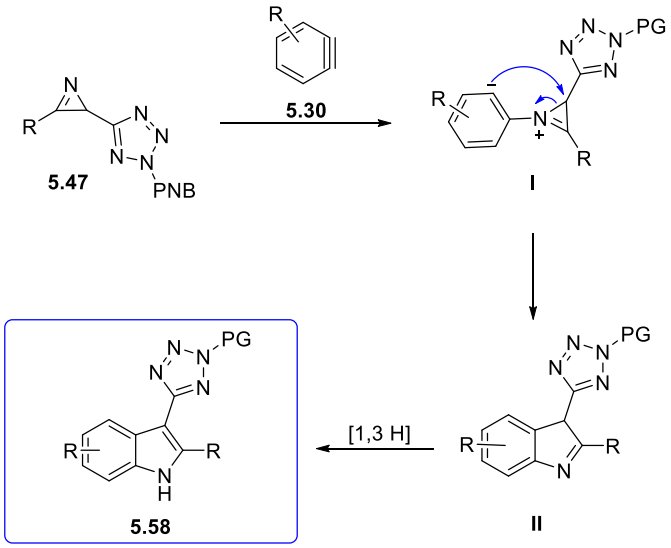

**5.53** **5.24a**

**Scheme 5.23** Reaction between ethyl 3-phenyl-2*H*-azirine-2-carboxylate and 2-(trimethylsilyl)aryl triflate.

### 5.3.7 Mechanistic considerations

Taking in account the aforementioned results the proposed mechanism for the reaction between 2-(tetrazolyl-5-yl)-2*H*-azirines **5.47** and arynes **5.24** is outlined in Scheme 5.24. Arynes undergo a nucleophilic addition on reacting with 2-(tetrazolyl-5-yl)-2*H*-azirines **5.47** to give zwitterionic intermediate **I**. Subsequent intramolecular nucleophilic attack of the aryl anion onto the azirine C-2 carbon bearing the tetrazolyl substituent induces the C-N bond cleavage generating 3*H*-indole derivative **II** which undergoes a 1,3-hydrogen shift to afford the indole **5.58**.

A formal [2+2] cycloaddition reaction between the aryne and the 2*H*-azirine imine bond must be ruled out since it would lead to 2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-indoles instead of the obtained 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-indoles **5.58** (Scheme 5.24).




**Scheme 5.24** Proposed mechanism for the synthesis of 3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-indoles.

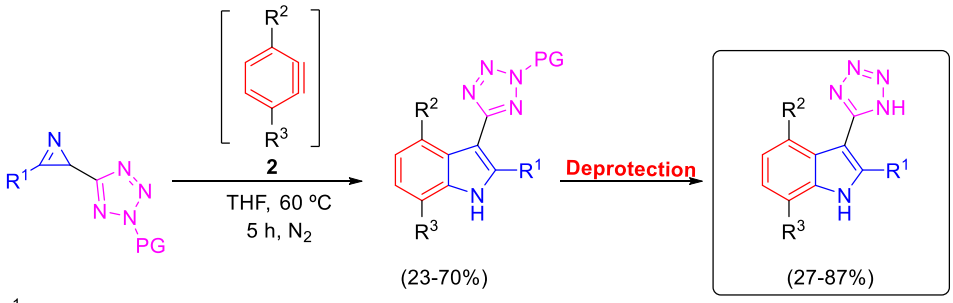
## 5.4 Conclusions

In this chapter, a selective approach to the synthesis 3-tetrazolyl-indoles involving the reaction of 2-[2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl]-2*H*-azirines with arynes was developed.

This methodology allows the synthesis of *N*-unsubstituted 3-tetrazolyl-indoles bearing phenyl and thiophen-2-yl substituents, in moderate to good yields. However, of *N*-unsubstituted 3-tetrazolyl-indoles bearing furan and pyrrole substituents did not lead to the desired products.

Computational studies were carried out allowing to rationalize the lack of reactivity of 2*H*-azirines having a 2-(4-nitrobenzyl)-1*H*-tetrazol-5-yl substituent due to steric hindrance.

The deprotection of the tetrazole moiety of indole **5.58a** was successfully achieved giving rise to the corresponding 3-tetrazolyl-indole, bioisoster of 1*H*-indole-3-carboxylic acid, in excellent yield.




$R^1$ = Ph, thiophen-2-yl
$R^2$ = $R^3$ = H. Me
PG = PNB or Bn

## 5.5 References

1. Vicente, R., Recent advances in indole syntheses: New routes for a classic target. *Org. Biomol. Chem.* **2011**, *9* (19), 6469–6480.

2. Taber, D. F.; Tirunahari, P. K., Indole synthesis: a review and proposed classification. *Tetrahedron* **2011**, *67* (38), 7195-7210.

3. Inman, M.; Moody, C. J., Indole synthesis – something old, something new. *Chem. Sci.* **2013**, *4* (1), 29-41.

4. Guo, T.; Huang, F.; Yu, L.; Yu, Z., Indole synthesis through transition metal-catalyzed C–H activation. *Tetrahedron Lett.* **2015**, *56* (2), 296-302.

5. Heravi, Majid M.; Rohani, S.; Zadsirjan, V.; Zahedi, N., Fischer indole synthesis applied to the total synthesis of natural products. *RSC Adv.* **2017**, *7* (83), 52852-52887.

6. Mancuso, R.; Dalpozzo, R., Recent progress in the transition metal catalyzed synthesis of indoles. *Catalysts* **2018**, *8* (10), 458-512.

7. Bugaenko, D. I.; Karchava, A. V.; Yurovskaya, M. A., Synthesis of indoles: Recent advances. *Russ. Chem. Rev.* **2019**, *88* (2), 99-159.

8. Neto, J. S. S.; Zeni, G., Recent advances in the synthesis of indoles from alkynes and nitrogen sources. *Org. Chem. Front.* **2020**, *7* (1), 155-210.

9. Shi, J.; Li, L.; Li, Y., o-Silylaryl triflates: A journey of kobayashi aryne precursors. *Chem. Rev.* **2021**, *121* (7), 3892-4044.

10. Dubrovskiy, A. V.; Markina, N. A.; Larock, R. C., Use of benzynes for the synthesis of heterocycles. *Org. Biomol. Chem.* **2013**, *11* (2), 191-218.

11. Miyabe, H., Synthesis of oxygen heterocycles via aromatic C-O bond formation using arynes. *Molecules* **2015**, *20* (7), 12558-12575.

12. Gribble, G. W., Indoles via arynes. In *Indole Ring Synthesis*, Ltd., J. W. S., Ed. © 2016 John Wiley & Sons Ltd.: 2016; pp 528-536.

13. Bunnett, J. F.; Hrutfiord, B. F., Ring closure via aryne Intermediates: A general principle of synthesis 1,2. *J. Am. Chem. Soc.* **1961**, *83* (7), 1691-1697.

14. Kuehm-Caubère, C.; Rodriguez, I.; Pfeiffer, B.; Renard, P.; Caubère, P., Efficient selective synthesis of 2-substituted indoles from complex-base-promoted arynic cyclisations. *J. Chem. Soc., Perkin trans.* **1997**, (19), 2857-2862.

15. Kudzma, L., Synthesis of substituted indolesand carbazoles from 2-fluorophenyl imines. *Synthesis* **2003**, (11), 1661-1666.

16. Barluenga, J.; Fañanás, F. J.; Sanz, R.; Fernández, Y., Synthesis of functionalized indole- and benzo-fused heterocyclic derivatives through anionic benzyne cyclization. *Chem. Eur. J.* **2002**, *8* (9), 2034-2046.

17. Hong, D.; Chen, Z.; Lin, X.; Wang, Y., Synthesis of substituted indoles from 2-azidoacrylates and ortho-silyl aryltriflates. *Org. Lett.* **2010**, *12* (20), 4608-4611.

18. McAusland, D.; Seo, S.; Pintori, D. G.; Finlayson, J.; Greaney, M. F., The Benzyne Fischer-indole reaction. *Org. Lett.* **2011**, *13* (14), 3667-3669.

19. He, L.; Pian, J.-X.; Shi, J.-F.; Du, G.-F.; Dai, B., Transition-metal-free synthesis of multisubstituted N-arylindoles via reaction of arynes and α-amino ketones. *Tetrahedron* **2014**, *70* (14), 2400-2405.

20. Nair, V.; Kim, K. H., Transformation of 1-azirines to 1*H*-indoles with benzyne. Evidence for the intermediacy of the 3*H*-indole system. *J. Org. Chem.* **1975**, *40* (25), 3784-3786.

21. Thangaraj, M.; Bhojgude, S. S.; Jain, S.; Gonnade, R. G.; Biju, A. T., Selective synthesis of *N*-unsubstituted and *N*-arylindoles by the reaction of arynes with azirines. *J. Org. Chem.* **2016**, *81* (18), 8604-8611.

22. Khlebnikov, A. F.; Novikov, M. S.; Rostovskii, N. V., Advances in 2*H*-azirine chemistry: A seven-year update. *Tetrahedron* **2019**, *75* (18), 2555-2624.

23. Tarannum, S.; Chauhan, N.; Ghorai, M. K., Aziridines and 2*H*-Azirines—Monocyclic. In *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, **2020**, 1-114.

24. Palacios, F.; de Retana, Ana María O.; de Marigorta, Eduardo M.; de los Santos, Jesús M., 2*H*-Azirines as Synthetic Tools in Organic Chemistry. *Eur. J. Org. Chem.* **2001**, (13), 2401-2414.

25. Cardoso, A. L.; Gimeno, L.; Lemos, A.; Palacios, F.; Melo, T. M. V. D. P. e., The Neber approach to 2-(tetrazol-5-yl)-2*H*-azirines. *J. Org. Chem.* **2013**, *78* (14), 6983-6991.

26. Alves, C.; Grosso, C.; Barrulas, P.; Paixão, J. A.; Cardoso, A. L.; Burke, A. J.; Lemos, A.; Pinho e Melo, T. M. V. D., Asymmetric Neber reaction in the synthesis of chiral 2-(tetrazol-5-yl)-2*H*-azirines. *Synlett* **2019**, *31* (06), 553-558.

27. Cardoso, A. L.; Lemos, A.; Pinho e Melo, T. M. V. D., Selective synthesis of tetrasubstituted 4-(tetrazol-5-yl)-1*H*-imidazoles from 2-(tetrazol-5-yl)-2*H*-azirines. *Eur. J. Org. Chem.* **2014**, (24), 5159-5165.

28. Gonsalves, A. M. d. A. R.; Pinho e Melo, T. M. V. D.; Gilchrist, T. L., Synthesis of isoquinolines by cycloaddition of arynes to 1,2,4-triazines. *Tetrahedron* **1992**, *48* (33), 6821-6826.

29. Soares, M. I. L.; Nunes, C. M.; Gomes, C. S. B.; Pinho e Melo, T. M. V. D., Thiazolo[3,4-b]indazole-2,2-dioxides as Masked Extended Dipoles: Pericyclic Reactions of Benzodiazafulvenium Methides. *J. Org. Chem.* **2012**, *78* (2), 628-637.

30. Meanwell, N. A., Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, *54* (8), 2529-2591.

31. Malik, M. A.; Wani, M. Y.; Al-Thabaiti, S. A.; Shiekh, R. A., Tetrazoles as carboxylic acid isosteres: chemistry and biology. *J. Incl. Phenom. Macrocycl. Chem.* **2013**, *78* (1-4), 15-37.

32. Melkonyan, F. S.; Karchava, A. V.; Yurovskaya, M. A., Synthesis of *N*-substituted indole-3-carboxylic acid derivatives via Cu(I)-catalyzed intramolecular amination of aryl bromides. *J. Org. Chem.* **2008**, *73* (11), 4275-4278.

33. Ji, Y.; Trenkle, W. C.; Vowles, J. V., A High-yielding preparation of β-ketonitriles. *Org. Lett.* **2006**, *8* (6), 1161-1163.

34. Taber, D. F.; Tian, W., The Neber route to substituted indoles. *J. Am. Chem. Soc.* **2006**, *128* (4), 1058-1059.

35. Wang, Y.; Lei, X.; Tang, Y., Rh(ii)-catalyzed cycloadditions of 1-tosyl 1,2,3-triazoles with 2H-azirines: switchable reactivity of Rh-azavinylcarbene as [2C]- or aza-[3C]-synthon. *Chem. Comm.* **2015**, *51* (21), 4507-4510.

36. Velder, J.; Robert, T.; Weidner, I.; Neudörfl, J.-M.; Lex, J.; Schmalz, H.-G., Modular synthesis of chiral phosphine-phosphite-ligands from phenolic precursors: A new approach to bidentate chelate ligands exploiting a P-O to P-C migration rearrangement. *Adv. Synth. Catal.* **2008**, *350* (9), 1309-1315.

37. Guitián, E.; Pérez, D.; Peña, D.; Cobas, A., An efficient procedure for the synthesis of ortho-trialkylsilylaryl triflates: Easy access to precursors of functionalized arynes. *Synthesis* **2002**, (10), 1454-1458.

38. Himeshima, Y.; Sonoda, T.; Kobayashi, H., Fluoride-Induced 1,2-Elimination Ofo-Trimethylsilylphenyl Triflate to Benzyne under Mild Conditions. *Chem. Lett.* **1983**, *12* (8), 1211-1214.

39. Friedman, L.; Logullo, F. M., Benzynes via aprotic diazotization of anthranilic acids: a convenient synthesis of triptycene and derivatives. *J. Am. Chem. Soc.* **1963**, *85* (10), 1549-1549.

40. Ram, S.; Spicer, L. D., Rapid debenzylation of *N*-benzylamino derivatives to amino-derivatives using ammonium formate as catalytic hydrogen transfer agent. *Tetrahedron Lett.* **1987**, *28* (5), 515-516.

41. Lopes, S. M. M.; Palacios, F.; Lemos, A.; Pinho e Melo, T. M. V. D., Diels–Alder reactions of 3-(1H-tetrazol-5-yl)-nitrosoalkenes: synthesis of functionalized 5-(substituted)-1H-tetrazoles. *Tetrahedron* **2011**, *67* (46), 8902-8909.

42. Lopes, S. M. M.; Brigas, A. F.; Palacios, F.; Lemos, A.; Pinho e Melo, T. M. V. D., [4+2] Cycloadditions of 3-tetrazolyl-1,2-diaza-1,3-butadienes: synthesis of 3-tetrazolyl-1,4,5,6-tetrahydropyridazines. *Eur. J. Org. Chem.* **2012**, *2012* (11), 2152-2160.

# Chapter 6

Experimental Section


## Abstract

In this chapter the experimental procedures used during this PhD project as well as structure characterization of all new compounds are described.

# Chapter 6. Experimental Section

## 6.1 Reagents

All reagents were purchased from commercial sources and used without any further purification.

## 6.2 Solvents

The solvents were purified following known procedures reported in the literature.[1]

## 6.3 Equipment

- **Melting point**

Melting points (m.p.) were determined with a Falc Melting Point heated plate microscope, with the use of open capillaries and are uncorrected.

- **Infrared spectroscopy**

Infrared spectra (IR) were obtained on an Agilent Technologies Cary 630 FTIR spectrometer through the Attenuated Total Reflectance method (ATR) or on a PerkinElmer 1720X FTIR spectrometer (samples were measured as KBr disks). The main bands are given in $cm^{-1}$.

- **Nuclear magnetic resonance spectroscopy**

Nuclear magnetic resonance spectra (NMR) were obtained on a Brucker Avance III spectrometer. $^{1}H$ NMR spectra were recorded with the instrument operating at 400 MHz or 500 MHz and $^{13}C$ NMR spectra at 100 MHz or 126 MHz. Deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-$d_6$) were used as solvents. Chemical shift values($\delta$) are given in parts per million (ppm) relative to tetramethyl silane (TMS), and the coupling constants (J) are expressed in Hz.

- **Mass spectroscopy**

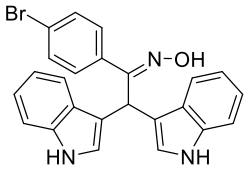
Mass spectra were recorded under electrospray ionization (ESI). HRMS spectra were recorded on a Finnigan MAT95 S instrument.

- **Chromatography**

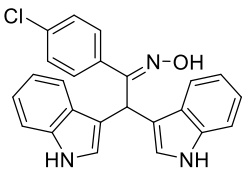
Thin-layer Chromatography (TLC) analysis was used to follow the evolution of the reactions and performed using precoated 60 $F_{254}$ silica gel plates. Flash chromatography was performed using silica gel 60 (0.040-0.063 mm) (supplied by Merck, Macharey-Nagel or Fluka) as stationary phase.

## 6.4 Index of new compounds

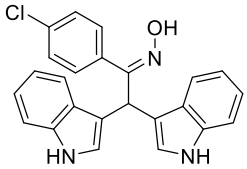
**Chapter 2.** Hetero-Diels-Alder approach to bis(indolyl)methanes



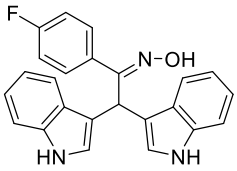

**2.82a**
p. 102



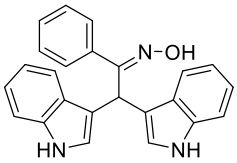

**2.82b**
p. 102



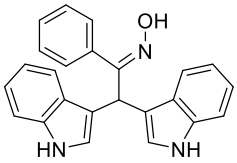

**2.83b**
p. 102



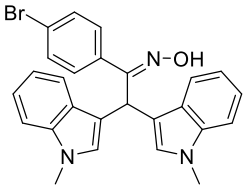

**2.82c**
p. 102



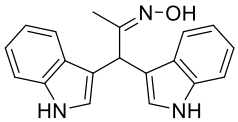

**2.82d**
p. 102



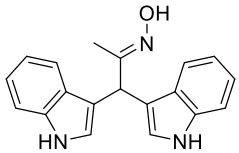

**2.83d**
p. 102



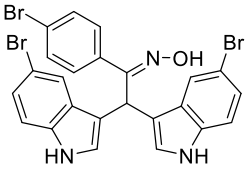

**2.82e**
p. 102



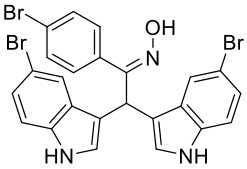

**2.82f**
p. 102



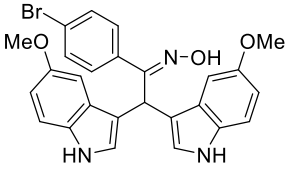

**2.83f**
p. 102



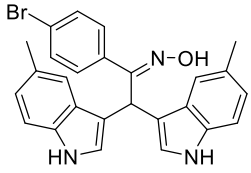

**2.82g**
p. 113



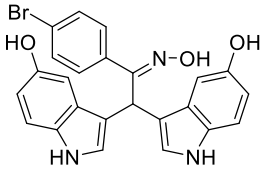

**2.83g**
p. 113



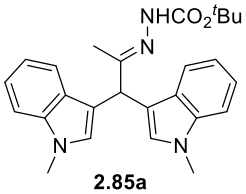

**2.83h**
p. 113



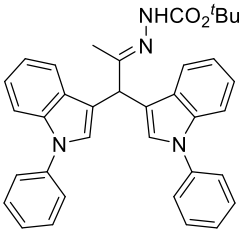
**2.82i**
p. 113



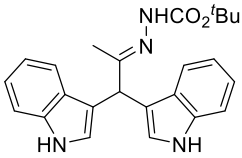
**2.82j**
p. 113



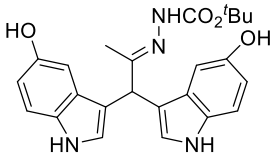
**2.85a**
p. 115



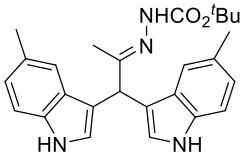
**2.85b**
p. 115



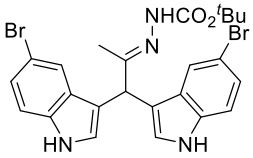
**2.85c**
p. 115



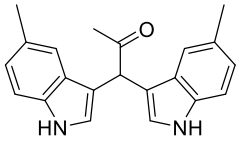
**2.85d**
p. 115



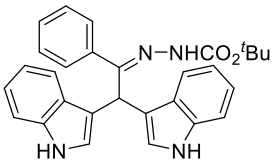
**2.85e**
p. 115



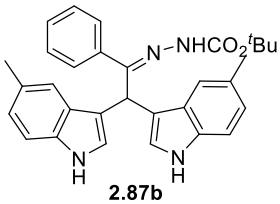
**2.85f**
p. 115



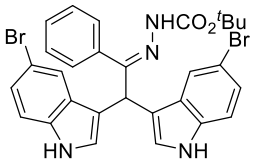
**2.86**
p. 116



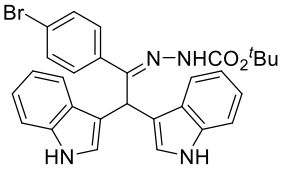
**2.87a**
p. 118



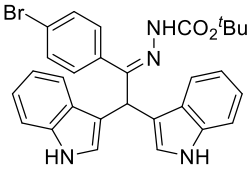
**2.87b**
p. 118



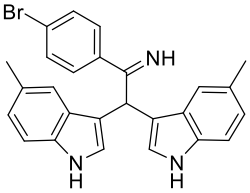
**2.87c**
p. 118



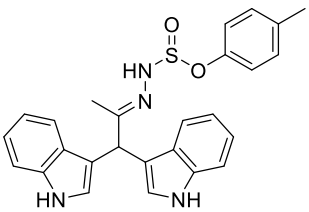
**2.87d**
p. 118



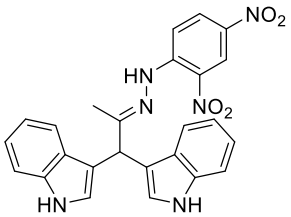
**2.88d**
p. 118



**2.87e**
p. 118

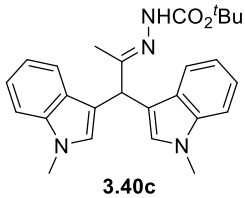


**2.89**
p. 120

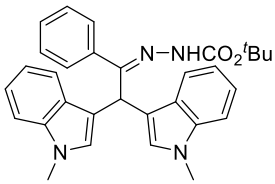


**2.90**
p. 120

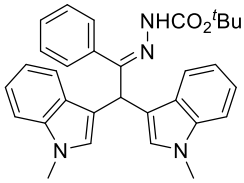
**Chapter 3.** Natural deep eutectic solvents in the hetero-Diels-Alder approach to bis(indolyl)methanes



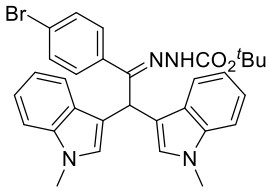
**3.40c**
p. 159



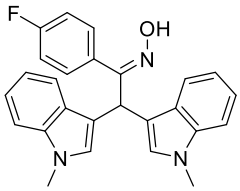
**3.42d**
p. 161



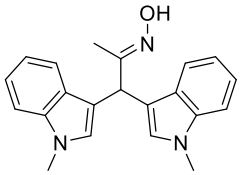
**3.43d**
p. 161



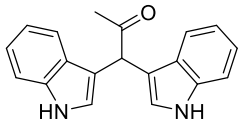
**3.42e/3.43d**
p. 161



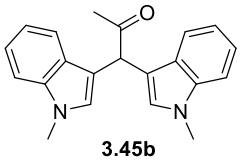
**3.44a**
p. 162



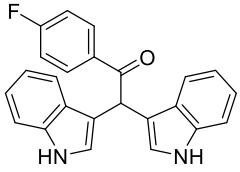
**3.41c**
p. 162



**3.45a**
p. 163

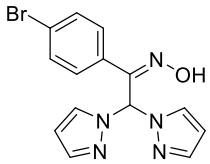


**3.45b**
p. 163

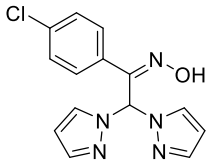


**3.45d**
p. 168

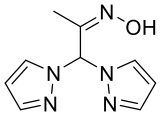
**Chapter 4.** Conjugate addition of pyrazoles to halogenated nitroso- and azoalkenes: a new entry to novel bis(pyrazol-1-yl)methanes



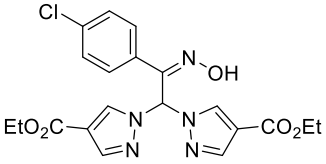
**4.27a**
p. 191



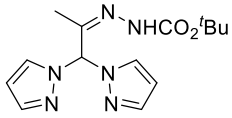
**4.27b**
p. 191



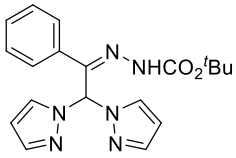
**4.27c**
p. 191



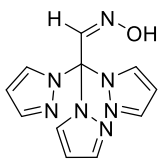
**4.27d**
p. 191



**4.28a**
p. 191

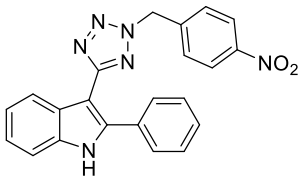


**4.28b**
p. 191

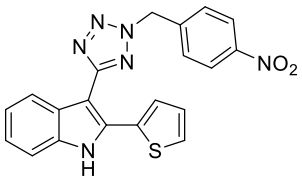


**4.31**
p. 197

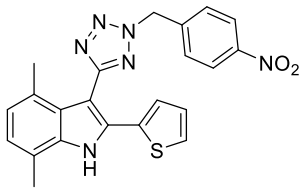
**Chapter 5.** Synthesis of 3-(1*H*-tetrazol-5-yl)-indoles



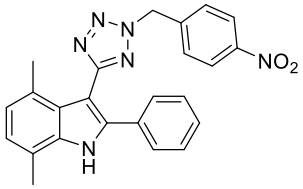
**5.58a**
p. 232



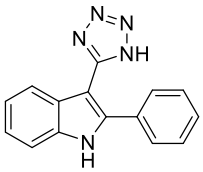
**5.58b**
p. 239



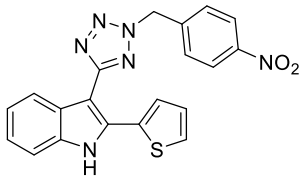
**5.58c**
p. 239



**5.58d**
p. 239



**5.59a**
p. 240



**5.60**
p. 240

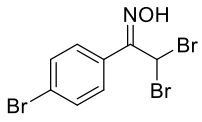
## 6.5 Synthesis related to Chapter 2

### 6.5.1 Synthesis of *α,α*'-dihalooximes and hydrazones

- **General procedure for the synthesis of *α,α*'-dihalooximes 2.73**[2]

*α,α*'-Dihalooximes **2.73** were prepared following known procedures with slight modifications [2]. To a solution of the appropriate α,α'-haloketone **2.72** (12 mmol) in ethanol (30 mL) hydroxylamine hydrochloride (36 mmol) was added. The reaction mixture was stirred at room temperature during 48 h. After, the solvent was evaporated under reduced pressure. To the reaction crude cold water (30 mL) was added giving white solids, which were filtered, washed with water, and recrystallized.

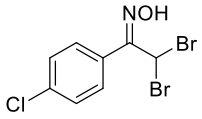
**2,2-Dibromo-1-(4-bromophenyl)ethan-1-one oxime**[2] **(2.73a)**



According to the general procedure starting from 2,2-dibromo-1-(4-bromophenyl)ethan-1-one. Purification of the product by crystallization in carbon tetrachloride ($CCl_4$).

Yield: 72%; white solid; m.p. 101.0-103.0 ºC (from $CCl_4$) (lit. 100.0-101.0 ºC)[2]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 7.51 (s, 1H, CH), 7.68 (d, $J$ = 8.5 Hz, 2H, Ar), 7.75 (d, $J$ = 8.5 Hz, 2H, Ar), 12.8 (br s, 1H, OH) ppm.

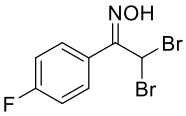
**2,2-Dibromo-1-(4-chlorophenyl)ethan-1-one oxime[2] (2.73b)**




According to the general procedure starting from 2,2-dibromo-1-(4-chlorophenyl)ethan-1-one. Purification of the product by crystallization in ethanol (EtOH).

Yield: 78%; white solid; m.p. 107.2-108.5 ºC (from ethanol) (lit. 108.0-109.0 ºC)[2]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.39 (s, 1H, CH), 7.49 (d, $J$ = 8.0 Hz, 2H, Ar), 7.95 (d, $J$ = 8.0 Hz, 2H, Ar), 11.21 (br s, 1H, OH) ppm.

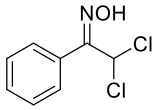
**2,2-Dibromo-1-(4-fluorophenyl)ethan-1-one oxime[2] (2.73c)**




According to the general procedure starting from 2,2-dibromo-1-(4-fluorophenyl)ethan-1-one. Purification of the product by crystallization in $CCl_4$

Yield: 75%; light yellow liquid; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.13 (t, $J$ = 8.4 Hz, 2H, Ar), 7.32 (s, 1H, CH), 7.83-7.87 (m, 2H, Ar), 8.93 (br s, 1H, OH) ppm.

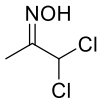
**2,2-Dichloro-1-phenylethan-1-one oxime[2] (2.73d)**




According to the general procedure starting from 2,2-dichloro-1-phenylethan-1-one. Purification of the product by crystallization in chloroform ($CCl_3$).

Yield: 75%; light yellow liquid; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.73 (s, 1H, CH), 7.52-7.55 (m, 3H, Ar), 7.94-7.96 (m, 2H, Ar), 11.19 (br s, 1H, OH) ppm.

**1,1-Dichloropropan-2-one oxime[3] (2.73e)**



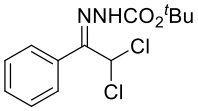

According to the general procedure starting from 1,1-dichloropropan-2-one with slight modifications. The reaction was performed with ethanol (90 mL) as solvent. After completion, the solvent was evaporated, and water was added (90 mL). The product was extracted with dichloromethane (2 x 90 mL), dried over anhydrous $Na_2SO_4$ and filtered. Evaporation of the solvent originates a light-yellow oil which solidifies at low temperature. Purification of the product by crystallization in *n*-hexane.

Yield: 55%; white crystals; m.p. 37.2-38.5 ºC (from hexane) (lit. 37.0-39.0 ºC)[3]; IR (KBr): $\tilde{\nu}$ = 3332, 1705, 1373, 739 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.96 (s, 3H, $CH_3$), 6.88 (s, 1H, CH), 11.58 (br s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$ ): $\delta$ = 8.4, 72.3, 152.5 ppm.

- **General procedure for the synthesis of *α,α*'-dihalohydrazones 2.74**

*α,α*'-Dihalohydrazones **2.74** were prepared following known procedures with slight modifications.[4] To a solution of the appropriate α,α'-haloketone (14.3 mmol) in diethyl ether (60 mL) *tert*-butylhydrazinecarboxylate (16.5 mmol) was added. The reaction mixture was stirred at room temperature during 16 h. The precipitated product was filtered, washed with diethyl ether and dried. Purification by crystallization.

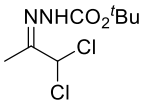
***tert*-Butyl 2-(2,2-dichloro-1-phenylethylidene)hydrazine-1-carboxylate[4] (2.74a)**




According to the general procedure starting from 2,2-dichloro-1-phenylethan-1-one. Purification of the product by crystallization in diethyl ether/*n*-hexane.

Yield: 76%; white solid; m.p. 143.1-144.6 ºC (from diethyl ether/*n*-hexane) (lit. 143.0-144.0 ºC)[3]; IR (KBr): $\tilde{\nu}$ = 3185, 2977, 1724, 1513, 1367, 1295, 1240, 1153, 1099, 744 $cm^{-1}$, $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.41 (s, 9H, $CH_3$), 7.12 (br s, 1H, CH), 7.36-7.38 (m, 2H, Ar), 7.54-7.55 (m, 3H, Ar), 9.32 (br s, 1H, NH) ppm.

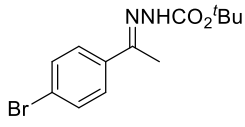
***tert*-Butyl 2-(1,1-dichloropropan-2-ylidene)hydrazine-1-carboxylate[4] (2.74b)**




According to the general procedure starting from 1,1-dichloropropan-2-one. Purification of the product by crystallization in diethyl ether.

Yield: 63%; white solid; m.p. 131.0-132.2 ºC (from diethyl ether) (lit. 130.1-132.3 ºC)[4]; IR (KBr): $\tilde{\nu}$ = 3197, 2983, 1708, 1537, 1253, 1144, 729 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.52 (s, 9H, $CH_3$), 2.04 (s, 3H, Me), 6.39 (s, 1H, CH), 7.64 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 9.4, 28.2, 73.4, 82.3, 145.9, 152.0 ppm.

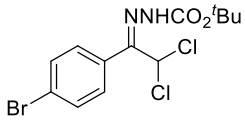
***tert*-Butyl-2-[1-(4-bromophenyl)ethylidene]hydrazine-1-carboxylate[5] (2.76)**




According to a known procedure.[5] To a suspension of *tert*-butylcarbazate (5 mmol) in hexane (10 mL) was added slowly 1-(4-bromophenyl)ethan-1-one (7.50 mmol). The reaction mixture was stirred and heated at reflux during 8 h. After cooling to room temperature, the product precipitated and was filtered. Purification of the product by crystallization in ethanol.

Yield: 90%; white solid; m.p. 166.8-167.5 ºC (from ethanol) (lit. 107.0-108.0 ºC)[5]; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.54 (s, 9H, $CH_3$), 2.16 (s, 3H, $CH_3$), 7.48 (d, $J$ = 8.7 Hz, 2H, Ar), 7.66 (d, $J$ = 8.7 Hz, 2H), 7.70 (s, 1H, NH) ppm.

**_tert_-Butyl-2-[1-(4-bromophenyl)-2,2-dichloroethylidene]hydrazine-1-carboxylate[6] (2.74c)**

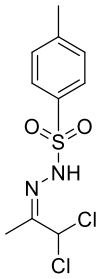



According to a known procedure with slight modifications.[6]

To a solution of hydrazone **2.76** (3.19 mmol) in carbon tetrachloride (50 mL) was added *N*-Chlorosuccinimide (7.02 mmol) and benzoyl peroxide (5 mg). The reaction mixture was stirred, and carefully heated at reflux (isothermal reaction) during 2 h. After cooling to room temperature, the succinimide was filtered. Evaporation of the filtrate affords the product as a light-yellow oil which crystallise in diethyl ether/*n*-hexane.

Yield: 80%; white solid; m.p. 162.2-163.9 ºC (from diethyl ether / hexane) (lit. 162.0-164.0 ºC)[3]; IR (KBr): $\tilde{\nu}$ = 3214, 2977, 1729, 1508, 1365, 1236, 1151, 1095, 1012, 750 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.41 (s, 9H, $CH_3$), 7.12 (s, 1H, CH), 7.30 (d, $J$ = 8.4 Hz, 2H, Ar), 7.73 (d, $J$ = 8.4 Hz, 2H, Ar), 9.80 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 27.8, 73.0, 80.4, 123.7, 128.0, 131.6, 131.9, 145.1, 152.2 ppm.

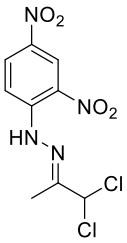
**_N_'-(1,1-Dichloropropan-2-ylidene)-4-methylbenzenesulfonohydrazide[7] (2.78a)**




According to a known procedure.[7] To a solution of *p*-toluenesulfonyl hydrazide (36 mmol) in propionic acid (30 mL), the 1,1-dichloropropan-2-one (39 mmol) was added. The reaction mixture was stirred at room temperature during 4 h. After completion, cyclohexane (30 mL) was added and the rection mixture was cooled in an ice bath. The product precipitates, is filtered and washed with cyclohexane.

Yield: 83%; white solid; m.p. > 136.8 ºC (decomposition from cyclohexane) (lit. 137.0-138.0 ºC, decomp.)[7]; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 2.00 (s, 3H, $CH_3$), 2.44 (s, 3H, $CH_3$), 6.18 (br s, 1H, CH), 7.34 (d, $J$ = 8.0 Hz, 2H, Ar), 7.82 ( d, $J$ = 8.4 Hz, 2H), 8.07 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 10.0, 21.7, 72.3, 127.9, 129.8, 134.8, 144.8, 150.4 ppm.

**(*E*)-1-(1,1-Dichloropropan-2-ylidene)-2-(2,4-dinitrophenyl)hydrazine[8] (2.78b)**

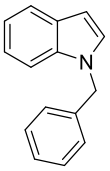


According to a known procedure.[8] To a solution of 1,1-dichloropropan-2-one (3.75 mmol) in the minimum volume of diethyl phosphonate a solution of 2,4-dinitrofenilhidrazina (25% $H_2O$) (5 mmol) in diethyl phosphonate (20 mL) was added. The reaction mixture was stirred at room temperature during 4 h. After completion, an equal volume of water was added and the product precipitated, which was filtered and washed with petroleum ether. Purification by crystallization in petroleum ether.

Yield: 95%; orange solid; m.p. 108.3-108.8 ºC (from petroleum ether); IR (KBr): $\tilde{\nu}$ = 3317, 3094, 1613, 1283, 1101, 731 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 2.30 (s, 3H, $CH_3$), 6.42 (s, 1H, CH), 7.95 (d, *J* = 9.5, 1H, Ar), 8.38 (dd, *J* = 9.5 Hz, 2.4 Hz, 1H, Ar), 9.14 (d, *J* = 2.5 Hz, 1H, Ar), 11.0 (s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 10.4, 72.5, 116.8, 123.2, 130.2, 130.6, 139.3, 144.5, 150.3 ppm.

### 6.5.2 Indole alkylation

**1-Benzyl-1*H*-indole[9] (2.81g)**



According to a known procedure.[9] To a solution of NaOH [50% (m/m) 107 mL)] the indole **2.81a** (0.04 mmol), tetrapropylammonium bromide (0,04 mmol) and benzyl bromide (0.05 mmol) were added. The reaction mixture was stirred at room temperature during 2 h. The mixture was extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and the solvent evaporated off. The product was precipitated with dichloromethane/*n*- hexane, filtered, and washed with *n*-hexane.

Yield: 78%; white solid; m.p. 43.2-44.1 ºC ( from *n*-hexane) (lit. 43.0-44.0 ºC)[9]; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 5.27 (s, 2H, $CH_2$), 6.54 (d, *J* = 3.2 Hz, 1H, Ar), 7.06-7.09 (m, 4H, Ar), 7.11-7.17 (m, 1H, Ar), 7.22-7.28 (m, 4H, Ar), 7.64 (d, *J* = 7.6 Hz, 1H, Ar) ppm.

### 6.5.3 Synthesis of novel bis(indolyl)methanes (BIMs) via bis-hetero-Diels-Alder reaction of nitroso and azoalkenes with indoles

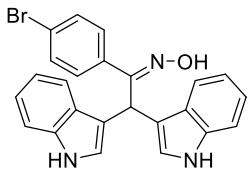
- **General procedures for the synthesis of bis(indolyl)methane oximes 2.82 and 2.83 and hydrazones 2.85, 2.87 and 2.88**

**Method A** (*in dichloromethane*): The appropriate oxime **2.73** (1 equiv.) or hydrazone **2.74** (1 equiv.) and the appropriate indole **2.81** (4 equiv.) were added to a suspension of $Na_2CO_3$ (10 equiv.) in dichloromethane (30 mL). The reaction mixture was stirred for the appropriate time at room temperature. Upon completion, the reaction mixture was filtered through a Celite pad, which was washed with dichloromethane. The solvent was evaporated to give the crude product, which was purified by flash chromatography.

**Method B** (*in water with dichloromethane as co-solvent*): The appropriate indole **2.81** (4 equiv.) was added to a stirred solution of $Na_2CO_3$ (10 equiv.) in water (9 mL). The appropriate oxime **2.73** (1 equiv.) or hydrazone **2.74** (1 equiv.) in dichloromethane (1.5 mL) was added. The reaction mixture was stirred for the appropriate time at room temperature. Upon completion, the mixture was extracted with dichloromethane (3 x 20 mL), and the combined organic layers were dried with anhydrous $Na_2SO_4$. After filtration, the solvent was evaporated, and the obtained crude was purified by flash chromatography.

Method C (*in water*): 1-Methyl-1*H*-indole (**2.81b**) (4 equiv.) was added to a stirred solution of $Na_2CO_3$ (10 equiv.) in water (9 mL). The hydrazone **2.74a** (1 equiv.) (0.83 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. Upon completion the mixture was extracted with dichloromethane (3 x 20 mL), and the combined organic layers were dried with anhydrous $Na_2SO_4$ After filtration the solvent was evaporated, and the target BIM hydrazone was obtained by crystallization.

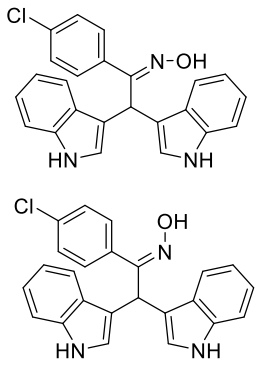
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(1*H*-indol-3-yl)methane (2.82a)**




According to the general procedures: 0.201 g (0.54 mmol) of oxime **2.73a,** 0.253 g (2.16 mmol) of indole **2.81a** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 55% (0.132 g, Method A), 34 % (0.082 g, Method B); light yellow solid; m.p. >175.0 ºC (decomp. dichloromethane); IR (KBr): $\tilde{\nu}$ = 3401, 1486, 1455, 1010, 922 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.89 (s, 1H, CH *meso*), 6.90-6.96 (m, 4H, Ar), 7.08 (t, *J* = 8.0 Hz, 2H, Ar), 7.33-7.39 (m, 6H, Ar), 7.42 (d, *J* = 8.0 Hz, 2H, Ar), 10.88 (br s, 2H, NH), 11.75 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.2, 111.5, 113.1, 118.4, 118.6, 121.1, 121.4, 123.9, 126.8, 129.6, 130.4, 135.6, 136.2, 156.4 ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{19}BrN_3O$ $[M + H]^+$ 444.0706, found 444.0713.

**(*E*)-1-[(4-Chlorophenyl)-1-hydroxyiminomethyl]bis(1*H*-indol-3-yl)methane (2.82b) and (*Z*)-1-[(4-Chlorophenyl)-1-hydroxyiminomethyl]bis(1*H*-indol-3-yl)methane (2.83b)**



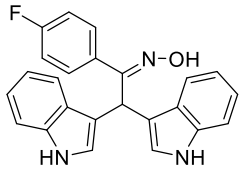

According to the general procedures: 0.177 g (0.54 mmol) of oxime **2.73b**, 0.253 g (2.16 mmol) of indole **2.81a** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] afforded in order of elution, compound **2.82b** and compound **2.83b**.

Data for **2.82b**:Yield: 80% (0.173 g, Method A), 64% (0.138 g, Method B); light brown solid; m.p. 177.7-180.9 ºC (from dichloromethane); IR (KBr): $\tilde{\nu}$ = 3401, 1486, 1455, 1010, 922 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.81 (s, 1H, CH *meso*), 6.92 (br s, 2H, Ar), 7.08 (t, *J* = 8.0 Hz, 2H, Ar), 7.20 (d, *J* = 8.0 Hz, 2H, Ar), 7.37 (d, *J* = 8.0 Hz, 2H, Ar), 7.43-7.46 (m, 4H, Ar), 10.88 (br s, 2H, NH), 11.74 (br s, 1H, OH); $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.2, 115.5, 113.1, 118.4, 121.1, 123.9, 126.8, 127.5, 129.3,132.6, 135.2, 136.2, 156.3 ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{19}ClN_3O$ $[M + H]^+$ 400.1208 found 400.1211.

Data for **2.83b**: Yield: 9% (0.019 g, Method B); light yellow solid; m.p. 159.3-161.5 ºC (from dichloromethane); IR (KBr): $\tilde{\nu}$ = 3401, 1486, 1455, 1010, 922 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.74 (s, 1H, CH *meso*), 6.94 (d, *J* = 2.4 Hz, 2H, Ar ), 6.97 (d, *J* = 7.6 Hz, 2H, Ar), 7.07 (t, *J* = 7.2 Hz, 2H, Ar), 7.27 (d, *J* = 8.0 Hz, 2H, Ar), 7.33-7.36 (m, 4H, Ar), 7.59 (d, *J* = 8.0 Hz, 2H, Ar), 10.81 (br s, 3H) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 40.1, 111.4, 114.4, 118.3, 119.1, 120.9, 123.9,

126.8, 127.6,130.0, 132.4, 133.7, 136.4, 155.7 ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{19}ClN_3O$ $[M + H]^+$ 400.1208 found 400.1211.

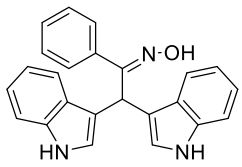
**(*E*)-1-[(4-Fluorophenyl)-1-hydroxyiminomethyl]bis(1*H*-indol-3-yl)methane (2.82c)**

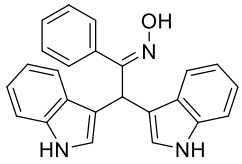



According to the general procedures: 0.168 g (0.54 mmol) of oxime **2.73c** 0.253 g (2.16 mmol) of indole **2.81a** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate *n*-hexane from (1:2) to (1:1)].

Yield: 33% (0.068 g, Method A), 71% (0.147 g, Method B); light brown solid; m.p. 181.3-182.5 ºC (from *n*-hexane); IR (KBr): $\tilde{\nu}$ = 3410, 1698, 1600, 1509, 1456, 744 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.83 (s, 1H, CH *meso*), 6.91 (s, 2H, Ar), 6.94-7.00 (m, 4H), 7.09 (pseudo t, $J$ = 8.0 Hz, 2H, Ar), 7.38 (d, $J$ = *8.4* Hz, 2H, Ar), 7.43-7.47 (m, 4H, Ar), 10.88 (s, 2H, NH), 11.64 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.3, 111.5, 113.2, 114.3 (d, $_{CF}J$ = 21.2 Hz), 118.4 , 118.4, 118.7, 121.1, 123.9, 126.9, 129.7, (d, $_{CF}J$ = 8.1 Hz), 132.9 (d, $_{CF}J$ = 2.8 Hz), 136.2, 156.4, 161.8 (d, $_{CF}J$ = 244 Hz) ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{19}FN_3O$ $[M + H]^+$ 384.1503 found 384.1507).

**(*E*)-1-(1-Hydroxyimino-phenylmethyl)bis(1*H*-indol-3-yl)methane (2.82d) (*Z*)-1-(1-Hydroxyimino-phenylmethyl)bis(1*H*-indol-3-yl)methane (2.83d)**



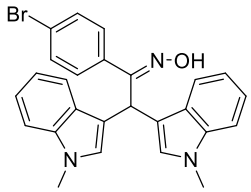

According to the general procedures: 0.110 g (0.54 mmol) of oxime **2.73d**, 0.253 g (2.16 mmol) of indole **2.81a** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate: *n*-hexane from (1:2) to (1:1)] afforded in order of elution, compound **2.82d** and compound **2.83d**.

Data for **2.82d**: Yield: 44% (0.087 g, Method A), 42% (0.083 g, Method B); White solid; m.p. 183.4-184.8 ºC (from dichloromethane); IR (KBr): $\tilde{\nu}$ = 3410, 1724, 1710, 1458, 1373, 1249, 1045, 744, 696 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.82 (s, 1H, CH *meso*), 6.88 (d, $J$ = 1.8 Hz, 2H, Ar), 6.94 (t, $J$ = 7.2 Hz, 2H, Ar), 7.08 (t, $J$ = 7.2 Hz, 2H, Ar), 7.14-7.19 (m, 3H, Ar), 7.37 (d, $J$ = 8.0 Hz, 2H, Ar), 7.40-7.42 (m, 2H, Ar), 7.45 (d, $J$ = 8.0 Hz, 2H, Ar), 10.85 (s, 2H, NH),

11.59 (br s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.3, 115.5, 113.4, 118.3, 118.7, 121.1, 123.9, 126.9, 127.5, 127.6, 127.9, 136.1, 136.6, 157.2 ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{20}N_3O$ $[M + H]^+$ 366.1601 found 366.1592.

Data for **2.83d**: Yield: 12% (0.024 g) (Method B); white solid; m.p. 202.3-203.4 ºC (from dichloromethane); IR (KBr): $\tilde{\nu}$ = 3410, 1724, 1710, 1458, 1373, 1249, 1045, 744, 696 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.77 (s, 1H), 6.96 (d, $J$ = 2.0 Hz, 2H), 7.00 (t, $J$ = 7.2 Hz, 2H), 7.11 (t, $J$ = 7.2 Hz, 2H), 7.29-7.32 (m, 5H), 7.39 (d, $J$ = 8.0 Hz, 2H), 7.63 (d, $J$ = 8.0 Hz, 2H), 10.69 (s, 1H), 10.83 (br s, 2H) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ = 40.2, 111.4, 114.8, 118.2, 119.1, 120.8, 123.9, 126.9, 127.5, 127.7, 128.1, 135.1, 136.4, 156.6 ppm; HRMS (ESI): calcd. for m/z $C_{24}H_{20}N_3O$ $[M + H]^+$ 366.1601, found 366.1592.

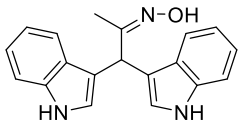
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(1-methyl-1*H*-indol-3-yl) methane (2.82e)**

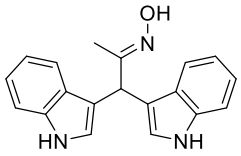



According to the general procedures: 0.201 g (0.54 mmol) of oxime **2.73a**, 0.283 g (2.16 mmol) of indole **2.81b** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 63% (0.161 g) (Method A), 70% (0.179 g) (Method B); light pink solid; m.p. 161.3-162.7 ºC (from dichloromethane); IR (KBr): $\tilde{\nu}$ = 3276, 3224, 1485, 1331, 1011, 939 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 3.70 (s, 3H, $CH_3$), 6.78 (s, 1H, CH *meso*), 6.96-7.00 (m, 4H, Ar), 7.14 (t, $J$ = 7.2 Hz, 2H, Ar), 7.34-7.45 (m, 9H, Ar), 11.76 (br s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.1, 32.4, 109.7, 112.3, 118.6, 118.7, 121.3, 121.4, 127.2, 128.2, 129.5, 130.5, 135.4, 136.6, 156.1 ppm; HRMS (ESI): calcd. for *m/z* $C_{26}H_{23}BrN_3O$ $[M + H]^+$ 471.0946 found 471.0953.

**(*Z*)-1-(1-Hydroxyiminomethyl)bis(1*H*-indol-3-yl)methane (2.82f) and (*E*)-1-(1-Hydroxyiminomethyl)bis(1*H*-indol-3-yl)methane (2.83f)**



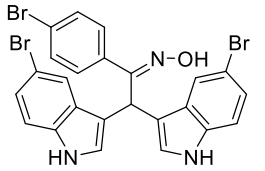



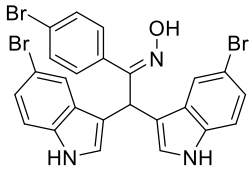

According to the general procedures: 0.077 g (0.54 mmol) of oxime **2.73e**, 0.253 g (2.16 mmol) of indole **2.81a** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] afforded in order of elution, compound **2.82f** and compound **2.83f**.

Data for **2.82f**: Yield: 9% (0.015 g, Method A); white solid; m.p. 68.2-69.6 ºC (from dichloromethane); IR: $\tilde{\nu}$ = 3426, 1707, 1455 1430, 1373, 1239, 1041 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.67 (s, 3H, $CH_3$), 6.39 (s, 1H, CH *meso*), 6.94 (t, *J* = 7.2 Hz, 2H, Ar), 7.08 (t, *J* = 7.2 Hz, 2H, Ar), 7.13 (d, *J* = 1.6 Hz, 2H, Ar), 7.37 (d, *J* = 8.0 Hz, 2H, Ar), 7.43 (d, *J* = 8.0 Hz, 2H, Ar), 10.61 (s, 1H, OH), 10.91 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 17.1, 30.5, 111.4, 113.3, 118.3, 118.6, 121.0, 123.4, 126.9, 136.2, 156.1 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{20}N_3O$ $[M + H]^+$ 304.1442 found 304.1444.

Data for **2.83f**: Yield: 19% (0.031 g, Method A), 57% (0.093 g, Method B); white solid; m.p > 120.0 ºC (decomp, diethyl ether); IR: $\tilde{\nu}$ = 3416, 1705, 1458, 1425, 1370, 1247, 1043 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.78 (s, 3H, $CH_3$), 5.38 (s, 1H, CH *meso*), 6.94 (pseudo t, *J* = 7.4 Hz, 7.2 Hz 2H, Ar), 7.07 (pseudo t, *J* = 7.2 Hz, 6.4 Hz, 2H, Ar), 7.12 (d, *J* = 2.0 Hz, 2H, Ar), 7.37 (d, *J* = 8.4 Hz, 2H, Ar), 7.46 (d, *J* = 8.0 Hz, 2H, Ar), 10.41 (s, 1H, OH), 10.90 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 12.2, 111.4, 114.2, 118.2, 118.9, 120.9, 123.4, 126.8, 136.3, 156.9 ppm; HMRS (ESI): calcd, for *m/z* $C_{24}H_{20}N_3O$ $[M + H]^+$ 304.1442 found 304.1444.

**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-bromo-1*H*-indol-3-yl) methane (2.82g) and (*Z*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-bromo-1*H*-indol-3-yl)methane (2.83g)**



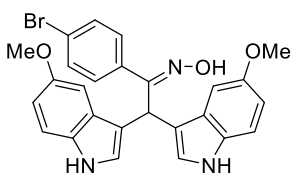




According to the general procedure: 0.201 g (0.54 mmol) of oxime **2.73a**, 0.423 g (2.16 mmol) of indole **2.81c** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] afforded in order of elution, compound **2.82g** and compound **2.83g**.

Data for **2.82g**: Yield: 41% (0.133 g, Method B); light brown solid; m.p. > 161.2 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3423, 2918, 1587, 1458, 1103, 1072, 1022, 1011, 885, 798 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.71 (s, 1H, CH *meso*), 7.04 (d, *J* = 2.0 Hz, 2H, Ar), 7.19 (d, *J* = 2.0 Hz, 2H, Ar), 7.21 (d, *J* = 2.0 Hz, 1H, Ar), 7.29-7.30 (m, 1H, Ar), 7.31-7.32 (m, 1H, Ar), 7.34-7.38 (m, 4H, Ar), 7.54 (d, *J* = 2.0 Hz, 2H, Ar), 11.16 ( d, *J* = 2.4 Hz, 2H, NH), 11.90 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.0, 111.1, 112.3, 113.7, 120.7, 121.6, 123.7, 125.6, 128.5, 129.5, 130.6, 134.9, 135.2, 155.8 ppm; HMRS (ESI): calcd for *m/z* $C_{24}H_{17}Br_3N_3O$ $[M + H]^+$ 599.89163 found 599.89128.

Data for **2.83g**: Yield: 10% (0.033 g, Method B); m.p. > 155.7 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3431, 2922, 1578, 1463, 1143, 1085, 1043, 1023, 910 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.78 (s, 1H, CH *meso*), 7.09 (d, *J* = 2.4 Hz, 2H, Ar), 7.16 (dd, *J* = 1.6 Hz, 8.8 Hz, 2H, Ar), 7.27 (d, *J* = 8.4 Hz, 2H, Ar), 7.31 (d, *J* = 8.8 Hz, 2H, Ar), 7.49 (d, *J* = 8.4 Hz, 2H, Ar), 7.70 ( d, *J* = 1.6 Hz, 2H, Ar), 10.91 (s, 1H, OH), 11.03 (br s, 2H, NH) ppm; HMRS (ESI): calcd for *m/z* $C_{24}H_{17}Br_3N_3O$ $[M + H]^+$ 599.8916 found 599.8913.

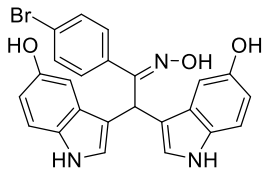
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-methoxy-*1H*-indol-3-yl) methane (2.82h)**




According to the general procedures: 0.201 g (0.54 mmol) of oxime **2.73a**, 0.318 g (2.16 mmol) of indole **2.81d** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 71% (0.193 g, Method A), 78% (0.062 g, Method B); light brown solid; m.p. > 148.7 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3394, 1587, 1487, 1215, 1173 1038, 930 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 3.66 (s, 6H, $CH_3$), 6.68 (br s, 1H, CH *meso*), 6.73 (dd, *J* = 2.4 Hz, 8.8 Hz, 2H, Ar), 6.91 (dd, *J* = 2.4 Hz, 6.0 Hz, 4H, Ar), 7.26 (d, *J* = 8.8 Hz, 2H, Ar), 7.31 (d, *J* = 8.8 Hz, 2H, Ar), 7.34 (d, *J* = 8.8 Hz, 2H, Ar), 10.72 (br s, 2H, NH), 11.78 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.3, 55.2, 100.8, 111.0, 112.1, 112.5, 121.4, 124.5, 127.2, 129.6, 130.4, 131.3, 135.7, 152.9, 156.3 ppm; HMRS (ESI): calcd. for *m/z* $C_{26}H_{23}BrN_3O_3$ $[M + H]^+$ 504.0917 found 504.0912).

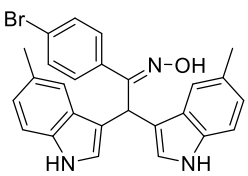
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-hydroxy-1*H*-indol-3-yl) methane (2.82i)**




According to the general procedures: 0.201 g (0.54 mmol) of oxime **2.73a**, 0.288 g (2.16 mmol) of indole **2.81e** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 23% (0.059 g, Method A), 78% (0.200 g, Method B); light brown solid; m.p. > 188.2 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3392, 3298, 1705, 1585, 1487, 1458, 1196, 1184 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.53 (br s, 1H, CH *meso*), 6.59 (dd, *J* = 2.4 Hz, 8.8 Hz, 2H, Ar), 6.73 (br s, 4H, Ar), 7.14 (d, *J* = 8.8 Hz, 2H, Ar), 7.34 (d, *J* = 8.8 Hz, 2H, Ar), 7.42 (d, *J* = 8.8 Hz, 2H, Ar), 8.58 (br s, 2H, Ar), 10.52 (d, *J* = 2.0 Hz, 2H, NH), 11.64 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 30.3, 102.7, 111.4, 111.7, 112.2, 121.3, 124.2, 127.6, 129.6, 130.3, 130.7, 135.6, 150.1, 156.3 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{19}BrN_3O_3$ $[M + H]^+$ 476.0604 found 476.0598.

**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-methyl-1*H*-indol-3-yl) methane (2.82j)**

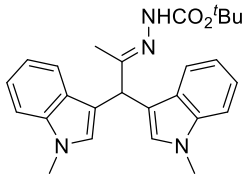



According to the general procedures: 0.201 g (0.54 mmol) of oxime **2.73a**, 0.283 g (2.16 mmol) of indole **2.81f** and 0.572 g (5.4 mmol) of $Na_2CO_3$. Reaction time 36 h (Method A) or 3 h (Method B).

Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 20% (0.051 g, Method A), 55% (0.140 g, Method B); light brown solid; m.p. > 171.3 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3292, 2979, 1724, 1697, 1502, 1458, 1369, 1263, 1246, 1159 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.31 (s, 6H, $CH_3$). 6.71 (s, 1H, CH *meso*), 6.79 (br s, 2H, Ar), 6.90 (d, *J* = 8.4 Hz, 2H, Ar), 7.18 (br s, 2H, Ar), 7.24 (d, *J* = 8.4 Hz, 2H, Ar), 7.34 (br s, 4H, Ar), 10.71 (br s, 2H, NH), 11.68 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 21.3, 30.1, 111.2, 112.7, 118.2, 121.4, 122.8, 123.9, 126.7, 127.0, 129.7, 130.4, 134.6, 135.7, 156.5 ppm; HRMS (ESI):calcd. for *m/z* $C_{26}H_{23}BrN_3O$ $[M + H]^+$ 472.1019 found 472.1015.

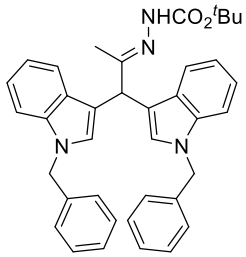
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1-methyl-1*H*-indol-3-yl) methane (2.85a)**




According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.435 g (3.32 mmol) of indole **2.81b** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 24 h (Method A), 3 h (Method B) or 2 h (Method C). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)] (Method A and B) or by crystallization in diethyl ether (Method C).

Yield: 29% (0.104 g, Method A), 27% (0.096 g, Method B), 49% (0.175 g, (Method C); white solid; m.p. > 177.6 ºC (decomp., Hexanes); IR: $\tilde{\nu}$ = 3251, 2979, 1721, 1699, 1524, 1474, 1369, 1330, 1284, 1245, 1158 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.52 (s, 9H, $CH_3$), 1.85 (s, 3H, $CH_3$), 3.72 (s, 6H, $CH_3$), 5.66 (s, 1H, CH *meso*) 6.87 (br s, 2H, Ar), 7.05 (t, *J* = 8.0 Hz, 2H, Ar), 7.19-7.29 (m, 4H, Ar), 7.42 (br s, 1H, NH), 7.59 (d, *J* = 8.0 Hz, 2H, Ar) ppm; NMR $^{13}C$ (100 MHz, $CDCl_3$): $\delta$ = 13.4, 28.4, 32.8, 43.6, 80.8, 109.1, 114.1, 118.9, 120.3, 121.6, 127.5, 127.7, 137.2, 152.8, 153.7 ppm; HMRS (ESI) calcd. for *m/z* $C_{26}H_{31}N_4O_2$ $[M + H]^+$ 431.2442 found 431.2442.

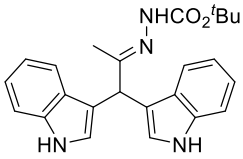
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1-benzyl-1*H*-indol-3-yl)methane (2.85b)**




According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.688 g (3.32 mmol) of indole **2.81g** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 24 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 31% (0.150 g, Method A), 29% (0.140 g, Method B); light brown solid; m.p. 180.6-181.4 ºC (from hexanes); IR: $\tilde{\nu}$ = 3238, 1699, 1468, 1454, 1402, 1363, 1331, 1252, 1157, 739 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.52 (s, 9H, $CH_3$), 1.83 (s, 3H, $CH_3$), 5.25 (s, 4H, $CH_2$), 5.73 (s, 1H, CH *meso*), 6.95 (br s, 2H, Ar), 7.01-7.06 (m, 5H, Ar), 7.12-7.16 (m, 2H, Ar), 7.21-7.28 (m, 9H, Ar), 7.43 (br s, 1H, NH), 7.61 (d, *J* = 8.0 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 13.2, 28.4, 43.8, 50.0, 80.9, 109.6, 114.6, 119.2, 120.6, 121.9, 126.6, 127.2, 127.5, 127.8, 128.7, 136.9, 137.7, 152.8, 153.2 ppm; HMRS (ESI): calcd. for *m/z* $C_{38}H_{39}N_4O_2$ $[M + H]^+$ 583.3068 found 583.3059).

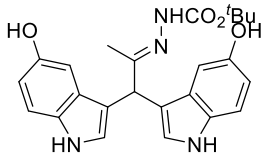
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (2.85c)**




According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.389 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 24 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 22% (0.73 g, Method A), 55% (0.184 g, Method B); white solid; m.p. > 179.8 ºC (decomp, ethyl acetate); IR: $\tilde{\nu}$ = 3435, 3263, 1714, 1498, 1456, 1433, 1365, 1248, 1161, 1124, 750, 742 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.47 (s, 9H, $CH_3$), 1.83 (s, 3H, $CH_3$), 5.38 (s, 1H, CH *meso*), 6.93 (t, *J* = 7.2 Hz, 2H, Ar), 7.06 (t, *J* = 7.2 Hz, 2H, Ar), 7.16 (d, *J* = 1.6 Hz, 2H, Ar), 7.36 (d, *J* = 8.0 Hz, 2H, Ar), 7.44 (d, *J* = 8.0 Hz, 2H, Ar), 9.35 (br s, 1H, NH), 10.91 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 14.4, 28.1, 43.0, 78.9, 111.4, 114.1, 118.3, 118.9, 120.9, 123.4, 126.8, 136.2, 153.3, 155.4 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{27}N_4O_2$ $[M + H]^+$ 403.21285 found 403.21232.

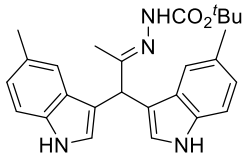
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-hydroxy-1*H*-indol-3-yl) methane (2.85d)**




According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.450 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 24 h (Method A) or 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 21% (0.076 g, Method A), 55% (0.198 g, Method B); light brown solid; m.p. > 192.3 ºC (decomp., dichloromethane); IR: $\tilde{\nu}$ = 3400, 3330, 1716, 1703, 1495, 1468, 1458, 1433, 1392, 1385, 1369, 1246, 1219, 1188, 1159 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.46 (s, 9H, $CH_3$), 1.81 (s, 3H, $CH_3$), 5.12 (s, 1H, CH *meso*), 6.58 (dd, *J* = 2.0 Hz, 8.8 Hz, 2H, Ar), 6.71 (d, *J* = 2.0 Hz, 2H, Ar), 7.14 (d, *J* = 8.8 Hz, 2H, Ar), 8.57 (s, 2H, Ar), 9.31 (br s, 1H, NH), 10.58 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 14.2, 28.2, 43.2, 78.8, 102.9, 111.3, 111.6, 113.2, 123.8, 127.5, 130.8, 150.1, 150.4 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{27}N_4O_4$ $[M + H]^+$ 435.2027 found 435.2019.

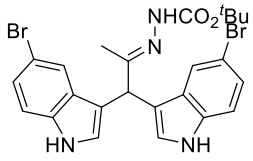
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3-yl) methane (2.85e)**




According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.435 g (3.32 mmol) of indole **2.81e** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 43% (0.154 g, Method B); light brown solid; m.p. > 186.4 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3411, 3317, 1722, 1703, 1504, 1367, 1244, 1161 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.47 (s, 9H, $CH_3$), 1.82 (s, 3H, $CH_3$), 2.33 (s, 6H, $CH_3$), 5.31 (s, 1H, CH *meso*), 6.89 (d, *J* = 8.4 Hz, 2H, Ar), 7.08 (br s, 2H Ar), 7.24-7.26 (m, 4H, Ar), 9.34 (br s, 1H, NH), 10.77 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 14.3, 21.3, 28.1, 42.9, 78.8, 111.1, 113.6, 118.5, 122.5, 123.5, 126.6, 127.0, 134.6, 153.2, 155.5 ppm; HMRS (ESI): calcd. for *m/z* $C_{26}H_{31}N_4O_2$ $[M + H]^+$ 431.2442 found 431.2434.

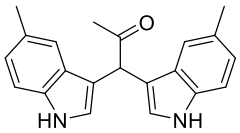
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-bromo-1*H*-indol-3-yl)methane (2.85f)**




According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.651 g (3.32 mmol) of indole **2.81c** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 63% (0.293 g) (Method B); pink solid; m.p. > 189.7 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3421, 3292, 2979, 1724, 1697, 1502, 1458, 1433, 1392, 1369, 1263, 1246, 1159, 1049, 885, 793 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.47 (s, 9H, $CH_3$), 1.83 (s, 3H, $CH_3$), 5.32 (s, 1H, CH *meso*), 7.16 (dd, *J* = 2.0 Hz, 8.8 Hz, 2H, Ar), 7.31 (br s, 2H, Ar), 7.33 (d, *J* = 8.8 Hz, 2H, Ar), 7.60 (br s, 2H, Ar), 9.40 (s, 1H, NH), 11.15 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 14.7, 28.2, 42.6, 79.0, 111.0, 113.4, 113.9, 121.3, 123.4, 125.3, 128.6, 135.1, 153.3, 154.3 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{25}N_4O_2$ $[M + H]^+$ 559.0339 found 559.0330.

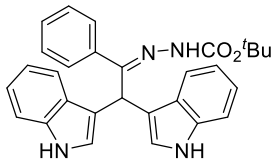
**1-Acetyl-1-bis(5-methyl-1*H*-indol-3-yl) (2.86)**




According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **2.74a**, 0.435 g (3.32 mmol) of indole **2.81e** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 3 h. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 17 % (0.065 g, Method B); brown solid; m.p. 94.8-95.6 ºC (from hexanes); IR: $\tilde{\nu}$ = 3400, 2920, 1705, 1485, 1423, 1246, 1159, 796 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 2.32 (s, 3H, $CH_3$), 2.42 (s, 6H, $CH_3$), 5.49 (br s, 1H, CH *meso*), 6.96 (d, *J* = 2.8 Hz, 2H, Ar), 7.02 (d, *J* = 8.0 Hz, 2H, Ar), 7.23 (s, 2H, Ar), 7.25 (s, 1H, Ar), 7.32 (s, 2H, Ar), 8.07 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 21.5, 28.8, 48.2, 111.0, 112.9, 118.7, 123.6, 123.9, 127.1, 129.0, 134.8, 207.6 ppm; HMRS (ESI): calcd. for *m/z* $C_{21}H_{21}N_2O_2$ $[M + H]^+$ 317.1648 found 317.1649.

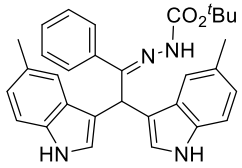
**(*E*)-1-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (2.87a)**




According to the general procedure: 0.252 g (0.83 mmol) of hydrazone **2.74b**, 0.389 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 12 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 29% (0.112 g, Method B); light brown solid; m.p. > 175.6 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3413, 3357, 3321, 1726, 1483, 1456, 1367, 1236, 1095, 742 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.48 (s, 9H, $CH_3$), 5.73 (s, 1H, CH *meso*), 6.75 (br s, 2H, Ar), 6.99-7.03 (m, 4H, Ar), 7.14 (t, *J* = 7.6 Hz, 2H, Ar), 7.28 (d, *J* = 8.0 Hz, 2H, Ar), 7.34-7.37 (m, 3H, Ar), 7.51 (d, *J* = 7.2 Hz, 2H, Ar), 7.66 (br s, 1H, NH), 8.20 (br s, 2H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{29}H_{29}N_4O_2$ $[M + H]^+$ 465.2285 found 465.2276.

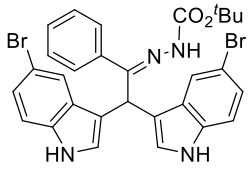
**(*E*)-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3-yl) methane (2.87b)**




According to the general procedure: 0.252 g (0.83 mmol) of hydrazone , 0.435 g (3.32 mmol) of indole **2.81e** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 12 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 7% (0.029 g, Method B); light brown solid; m.p. > 168.6 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3417, 3359, 1728, 1483, 1367, 1236, 1157, 795 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.39 (s, 9H, $CH_3$), 2.33 (s, 6H, $CH_3$), 5.59 (s, 1H, CH *meso*), 6.87-6.91 (m, 4H, Ar), 6.94 (br s, 2H, Ar), 7.22 (d, *J* = 8.4 Hz, 2H, Ar), 7.36 (br s, 5H, Ar), 8.21 (br s, 1H, NH), 10.67 (br s, 2H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{31}H_{33}N_4O_2$ $[M + H]^+$ 493.25980 found 493.25905.

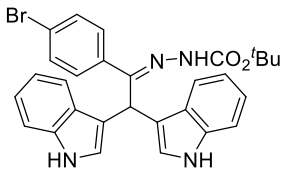
**(*E*)-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(5-bromo-1*H*-indol-3-yl) methane (2.87c)**

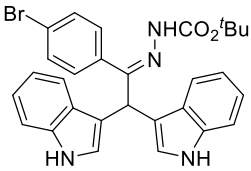



According to the general procedure: 0.252 g (0.83 mmol) of hydrazone **2.74a**, 0.651 g (3.32 mmol) of indole **2.81c** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 12 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 11% (0.057 g, Method B); light yellow solid; m.p. > 183.4 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3413, 3356, 1730, 1483, 1456, 1369, 1157 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.49 (s, 9H, M $CH_3$), 5.56 (s, 1H, CH *meso*), 6.84 (br s, 2H, Ar), 7.00 (br s, 2H, Ar), 7.17-7.23 (m, 4H, Ar), 7.28 (s, 1H, Ar), 7.37-7.39 (m, 3H, Ar), 7.54 (br s, 2H, Ar), 7.72 (br s, 1H, NH), 8.35 (br s, 2H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{29}H_{27}Br_2N_4O_2$ $[M + H]^+$ 621.04578 found 621.04881.

**(*E*)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl) methane (2.87d) and (Z)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (2.88d)**



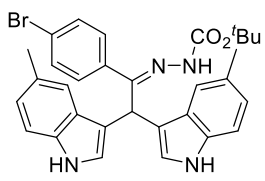

According to the general procedure: 0.317 g (0.83 mmol) of hydrazone **2.74c**, 0.389 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 12 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)] afforded in order of elution, compound **2.87d** and compound **2.88d**.

Data for **2.87d**: Yield: 17% (0.077 g) (Method B); white solid; m.p. > 164.3 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3411, 3361, 1726, 1483, 1456, 1238, 1157, 1092, 1011, 744 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.32 (s, 9H, $CH_3$), 6.07 (s, 1H, CH *meso*), 6.95 (br s, 2H, Ar), 7.6-7.10 (m, 2H, Ar), 7.23-7.25 (m, 2H,Ar), 7.37-7.42 (m, 6H, Ar), 7.71 (d, *J* = 8.8 Hz, 2H, Ar), 8.19 (br s, 2H, NH), 8.91(br s, 1H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{29}H_{28}BrN_4O_2$ $[M + H]^+$ 543.13902 found 543.13847.

Data for **2.88d**: Yield: 12% (0.054 g) (Method B); white solid; m.p. > 169.8 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3408, 3361, 2964, 1728, 1483, 1261, 1093, 1012, 800 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.32 (s, 9H, $CH_3$), 6.08 (s, 1H, CH *meso*), 6.97 (br s, 2H, Ar), 6.71 (t, *J* = 7.2 Hz, 2H, Ar), 7.24 (d, *J* = 7.2 Hz, 2H, Ar), 7.39

(d, *J* = 8.4 Hz, 2H, Ar), 7.42 (d, *J* = 8.0 Hz, 2H, Ar), 7.72 (d, *J* = 8.8 Hz, 2H, Ar), 8.18 (br s, 2H, NH), 8.91 (br s, 1H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{29}H_{28}BrN_4O_2$ $[M + H]^+$ 543.1390 found 543.1385.

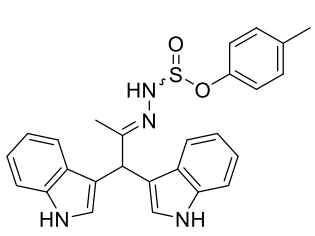
**(*E*)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3-yl)methane (2.87e)**




According to the general procedure: 0.317 g (0.83 mmol) of hydrazone **2.74c**, 0.435 g (3.32 mmol) of indole **2.81e** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time 12 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 13% (0.062 g, Method B); light brown solid; m.p. > 184.6 ºC (decomp., hexanes); IR: $\tilde{\nu}$ = 3408, 3363, 2978, 2920, 1732, 1483, 1367, 1238, 1159, 1092, 1011 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.48 (s, 9H, $CH_3$), 2.37 (s, 6H, $CH_3$), 5.74 (s, 1H, CH *meso*), 6.72 (d, *J* = 8.0 Hz, 2H, Ar), 6.75 (d, *J* = 4.8 Hz, 2H, Ar), 6.97 (d, *J* = 8.0 Hz, 2H, Ar), 7.19 (d, *J* = 8.0 Hz, 2H, Ar), 7.30 (br s, 2H, Ar), 7.40 (d, *J* = 8.0 Hz, 2H, Ar), 7.46 (br s, 1H, NH), 8.05 (br s, 2H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{31}H_{32}BrN_4O_2$ $[M + H]^+$ 571.1703 found 571.1702.

**(*E*)-1-[1-(*p*-Toluenesulphonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (2.89a)**

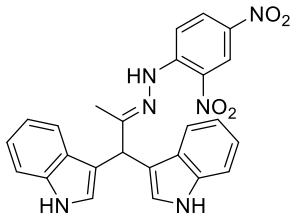



According to the general procedures: 0.245 g (0.83 mmol) of hydrazone **2.78a**, 0.389 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time: 24 h (Method A), 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 19% (0.072 g, Method A), 41% (0.155 g, Method B); light orange solid; m.p. 159.2-162.3 ºC (from diethyl ether); IR: $\tilde{\nu}$ = 3408, 3221, 1458, 1336, 1165, 1092, 742, 677, 669 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.77 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 5.27 (s, 1H, CH *meso*), 6.78 (t, *J* = 7.2 Hz, 2H, Ar), 7.04(t, *J* = 7.2 Hz, 2H, Ar), 7.13 (d, *J* = 2.0 Hz, 2H, Ar), 7.15 (d, *J* = 8.0 Hz, 2H, Ar), 7.33 (d, *J* = 4.0 Hz, 2H, Ar), 7.40 (d, *J* = 8.0 Hz, 2H, R), 7.78 (d, *J* = 8.4 Hz, 2H, Ar), 9.99 (br s, 1H, NH), 10.91 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ = 14.5, 21.2, 43.0, 111.3, 113.2, 118.3, 118.8, 120.9, 123.4, 126.6,

127.6, 129.3, 136.2, 136.3, 142.9, 159.3 ppm; HMRS (ESI): calcd. for *m/z* $C_{26}H_{25}N_4O_2S$ $[M + H]^+$ 457.1693 found 457.1690.

**(*E*)-1-[1-(2,4-Dinitrophenylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (2.89b)**



According to the general procedure: 0.255 g (0.83 mmol) of hydrazone **2.78b**, 0.389 g (3.32 mmol) of indole **2.81a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Reaction time: 24 h (Method A), 3 h (Method B). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)].

Yield: 63% (0.245 g, Method A), 87% (0.338 g, Method B); orange solid; m.p. > 173.5 ºC ( decomp., hexanes); IR: $\tilde{\nu}$ = 3413, 3320, 1678, 1590, 1516, 1421, 1334, 1312, 1280, 1088 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ =2.13 (s, 3H, $CH_3$), 5.63 (s, 1H, CH *meso*), 6.92-6.97 (m, 2H, Ar), 7.05-7.10 (m, 3H, Ar), 7.25 (br s, 1H, Ar), 7.39 (t, $J$ = 5.2 Hz, 2H, Ar), 7.49 (t, $J$ = 5.2 Hz, 2H, Ar), 7.89-7.93 (m, 1H, Ar), 8.34-8.37 (m, 1H, Ar), 8.86-8.88 (m, 1H, Ar), 10.88 (br s, 1H, NH), 11.01 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ = 14.4, 43.2, 111.5, 113.2, 116.1, 118.5,118.8, 121.1, 123.0, 123.8, 126.7, 129.2, 130.1, 136.3, 136.8, 144.9, 160.5 ppm; HMRS (ESI): calcd. for *m/z* $C_{25}H_{21}N_6O_4$ $[M + H]^+$ 469.1619 found 469.1619.

## 6.6 Synthesis related to Chapter 3

### 6.6.1 Synthesis of BIMs using NADESs as solvents

- **General procedures for the synthesis of BIM hydrazones 3.40, 3.42 and 3.43 or oximes 3.41 and 3.44 using NADES**

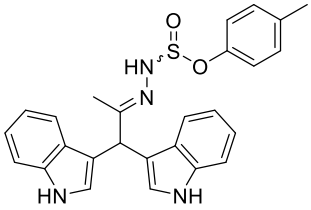
**Method D (***in pure NADES*): to a suspension of $Na_2CO_3$ (10 equiv.) in 5 mL of NADES were added the appropriate indole **3.2** (4 equiv.) and the appropriate hydrazone **3.38** (1 equiv.) or oxime **3.39** (1 equiv.). The reaction mixture was stirred for 3 h at room temperature and then diluted in water. The precipitate was filtered and washed with $H_2O$. The target BIMs were purified by crystallization.

**Method E** (*in $H_2O$/NADES (3:1)*)**:** to a suspension of $Na_2CO_3$ (10 equiv.) in $H_2O$/NADES (6:2 mL) the appropriate indole **3.2** (4 equiv.), hydrazone **3.38** (1 equiv.) or oxime **3.39** (1 equiv.) was added. The reaction mixture was stirred for the appropriate time. After completion, the reaction mixture was extracted with dichloromethane (3 × 20 mL) and the

combined organic layers dried over anhydrous $Na_2SO_4$. The solvent was evaporated, and the crude purified by flash chromatography.

**Method F** (*in $H_2O$/NADES (1:3)*)**:** to a suspension of $Na_2CO_3$ (10 equiv.) in $H_2O$/NADES (2:6 mL) the appropriate indole **3.2** (4 equiv.), hydrazone **3.38** (1 equiv.) and oxime **3.39** (1 equiv., 0.54 mmol) was added. The reaction mixture was stirred for the time indicated in each case. The product precipitated in the reaction medium, was isolated by filtration, washed with $H_2O$, and purified by flash chromatography or by crystallization.

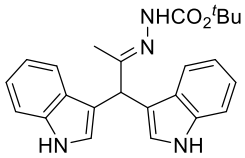
**(*E*)-1-[1-(*p*-Toluenesulphonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (3.40a)**



According to the general procedures: 0.245 g (0.83 mmol) of hydrazone **3.38a**, 0.389 g (3.32 mmol) of indole **3.2a** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using NADES based on CC/Gly (2:1) (Method D) and $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 3 h (Method D), 1 h (Method E) or 30 min. (Method F). Purification of the product by crystallization in diethyl ether (Method D and F) and by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] (Method E).

Yield: 24% (0.091 g, Method D), 80% (0.303 g, Method E), 70% (0.262 g, Method F). The characterization is in agreement with those previously reported for compound **2.89a** (see synthesis related to Chapter 2).

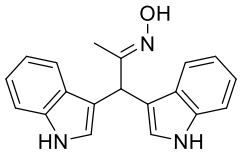
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (3.40b)**



According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **3.38b**, 0.389 g (3.32 mmol) of indole **3.2a** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using NADES based on CC/Gly (2:1) (Method D) and $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 3 h (Method D), 1 h (Method E) or 30 min. (Method F). Purification of the product by crystallization in diethyl ether (Method D and F) and by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] (Method E).

Yield: 22% (0.073 g, Method D), 54% (0.180 g, Method E), 50% (0.167 g, Method F). The characterization is in agreement with those previously reported for compound **2.85c** (see synthesis related to Chapter 2).

**(*E*)-1-(1-Hydroxyiminomethyl)bis(1*H*-indol-3-yl)methane (3.41a)**

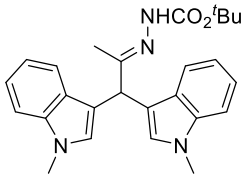


According to the general procedure: 0.077 g (0.54 mmol) of oxime **3.39a**, 0.253 g (2.16 mmol) of indole **3.2a** and 0.880 g (5.4 mmol) of $Na_2CO_3$ using NADEs based on CC/Gly (2:1) (Method D) and $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 3 h (Method D), 1 h (Method E) or 10 min. (Method F). Purification of the product by crystallization in diethyl ether (Method D and F) or by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] (Method E).

Yield: 28% (0.046 g, Method D), 45% (0.074 g, Method E) and 49% (0.080 g, Method F).

The characterization is in agreement with those previously reported for compound **3.82f** (see synthesis related to Chapter 2).

**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1-methyl-1*H*-indol-3-yl) methane(3.40c)**

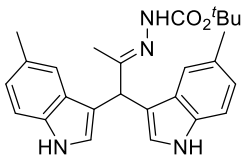


According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **3.38b**, 0.435 g (3.32 mmol) of indole **3.2b** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 1 h (Method E) or 10 min. (Method F). Purification of the product by flash chromatography [eluting with gradient ethyl ace acetate:*n*-hexane from (1:3) to (1:2)] (Method E) and by crystallization in diethyl ether (Method F).

Yield: 56% (0.200 g, Method E) and 50% (0.179 g, Method F).

The characterization is in agreement with those previously reported for compound **2.85a** (see synthesis related to Chapter 2).

**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3-yl) methane (3.40d)**

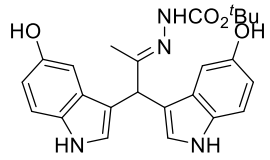


According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **3.38b**, 0.435 g (3.32 mmol) of indole **3.2c** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 1 h (Method E) or 10 min. (Method F). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)] (Method E) and by crystallization in diethyl ether (Method F).

Yield: 53% (0.189 g, Method E), 55% (0.189 g, Method F).

The characterization is in agreement with those previously reported for compound **2.85e** (see synthesis related to Chapter 2).

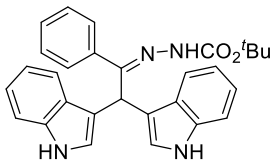
**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-hydroxy-1*H*-indol-3-yl) methane (3.40e)**




According to the general procedures: 0.200 g (0.83 mmol) of hydrazone **3.38b**, 0.253 g (3.32 mmol) of indole **3.2d** and 0.572 g (8.3 mmol) of $Na_2CO_3$ $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time 1 h (Method E) or 10 min. (Method F). Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:2)] (Method E) or by crystallization with diethyl ether (Method F).

Yield: 71% (0.256 g, Method E), 64% (0.239 g, Method F). The characterization is in agreement with those previously reported for compound **3.85d** (see synthesis related to Chapter 2).

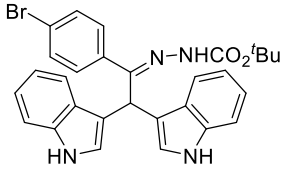
**(*E*)-1-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)methyl]bis(1*H*-indol-3-yl)methane (3.42a)**




According to the general procedures: 0.252 g (0.83 mmol) of hydrazone **3.38c**, 0.389g (3.32 mmol) of indole **3.2a** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time: 1 h (Method E) or 30 min (Method F); Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)].

Yield: 44% (0.170 g, Method E), 25% (0.096 g, Method F). The characterization agreed with those previously reported for compound **2.87a** (see synthesis related to Chapter 2).

**(*E*)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)methyl]bis(1*H*-indol-3-yl) methane (3.42b)**

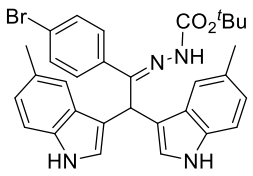


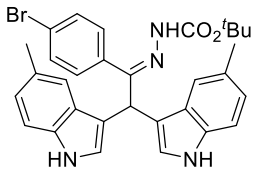

According to the general procedures: 0.317 g (0.83 mmol) of hydrazone **3.38d**, 0.389g (3.32 mmol) of indole **3.2a** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent . Reaction time: 1 h (Method E) or 30 min. (Method F). Purification

of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)].

Yield: 48% (0.217 g, Method E), 43% (0.194 g, Method F). The characterization is in agreement with those previously reported to compound **2.87d** (see synthesis related to Chapter 2).

**(*E*)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3-yl)methane (3.42c) and (Z)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)methyl]bis(5-methyl-1*H*-indol-3-yl)methane (3.43b)**



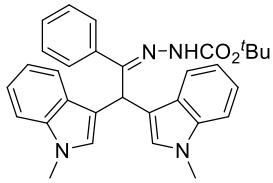




According to the general procedures: 0.317 g (0.83 mmol) of hydrazone **3.38d**, 0.435 g (3.32 mmol) of indole **3.2c** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent (Method E and F). Reaction time: 1 h (Method E) or 30 min. (Method F). Purification of the product by flash chromatography [eluting with gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)] afforded in order of elution, compound **3.42c** and compound **3.43b**.

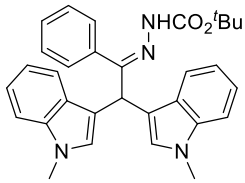
Data for **3.42c**: Yield: 42% (0.189 g) (Method E), 23% (0.104 g) (Method F). The characterization is in agreement with those previously reported for compound **2.87e** (see synthesis related to Chapter 2).

Data for **3.43b**: Yield: 8% (0.036 g, Method E), 10% (0.047 g, Method F); light Brown solid; m.p. 204.1-205.0 ºC (from *n*-hexane); IR: $\tilde{\nu}$ = 3424, 3304, 1734, 1477, 1234, 1156, 1011, 796 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.32 (s, 9H, $CH_3$), 2.36 (s, 6H, $CH_3$), 5.98 (s, 1H, CH *meso*), 6.87 (br s, 2H, Ar), 7.06 (dd, *J* = 7.2 Hz, 8.4 Hz, 2H, Ar), 7.15 (br s, 2H, Ar), 7.29 (d, *J* = 8.4 Hz, 2H, Ar), 7.39 (d, *J* = 8.4 Hz, 2H, Ar), 7.69 (d, *J* = 8.8 Hz, 2H, Ar), 8.14 (br s, 2H, NH), 8.99 (br s, 1H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{31}H_{32}BrN_4O_2$ $[M + H]^+$ 571.1703 found 571.1698.

**(*E*)-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)methyl]bis(1-methyl-1*H*-indol-3-yl)methane (3.42d) and (*Z*)-[Phenyl-1-(*tert*-butoxycarbonylhydrazono)methyl]bis(1-methyl-1*H*-indol-3-yl)methane (3.43c)**



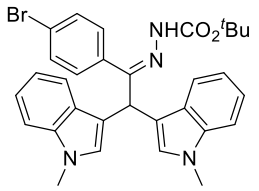

According to the general procedures: 0.252 g (0.83 mmol) of hydrazone **3.38c**, 0.435 g (3.32 mmol) of indole **3.2b** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)]. afforded in order of elution, compound **3.42d** and compound **3.43c**

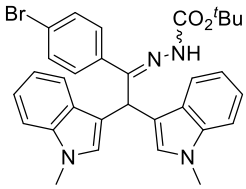



Data for **3.42d**: Yield: 60% (0.245 g, Method E), 44% (0.189 g, Method F); light yellow solid; m.p. > 177.3 ºC (decomp. from hexanes); IR: $\tilde{\nu}$ = 3360, 1741, 1476, 1367, 1329, 1226, 1154, 739 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.30 (s, 9H, $CH_3$), 3.72 (s, 6H, $CH_3$), 6.16 (s, 2H, Ar), 6.16 (s, 1H, CH), 6.86 (s, 2H, Ar), 7.07 (t, *J* = 7.6 Hz, 2H, Ar), 7.28 (d, *J* = 7.2 Hz, 2H, Ar), 7.31-7.36 (m, 5H, Ar), 7.42 (d, *J* = 8.0 Hz, 2H, Ar), 9.03 (br s, 1H, NH) ppm; HMRS (ESI): *m/z* calcd. For $C_{13}H_{33}N_4O_2$ $[M + H]^+$ 493.25980, found 493.25854.

Data for **3.43c**: Yield: 18% (0.074 g) (Method E) and 21% (0.086 g) (Method F); white solid; m.p. > 172.9 ºC (decomp. from hexanes); IR: $\tilde{\nu}$ = 3361, 1743, 1482, 1368, 1227, 1154, 739 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.47 (s, 9H, $CH_3$), 3.68 (s, 6H, $CH_3$), 5.84 (br s, 1H, CH), 6.87 (d, *J* = 4.4 Hz, 2H. Ar), 7.08 (t, *J* = 7.2 Hz, 2H, Ar), 7.19 (t, *J* = 8.4 Hz, 2H, Ar), 7.27-7.28 (m, 3H, Ar), 7.30 (br s, 1H, Ar), 7.34 (d, *J* = 7.2 Hz, 1H, Ar), 7.48 (t, *J* = 7.6 Hz, 2H, Ar), 7.60 (d, *J* = 7.2 Hz, 2H, Ar), 7.81 (d, *J* = 7.2 Hz, 1H, NH); ppm; HMRS (ESI): *m/z* calcd. For $C_{13}H_{33}N_4O_2$ $[M + H]^+$ 493.2598, found 493.2586.

**(*Z*)-[4-Bromophenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl]bis(1-methyl-1*H*-indol-3-yl)methane (3.42e)** and **(*E*)-(4-Bromo-phenyl-1-(*tert*-butoxycarbonylhydrazono)ethyl)bis(1-methyl-1*H*-indol-3-yl)methane (3.43d)**

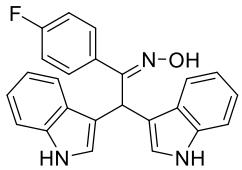


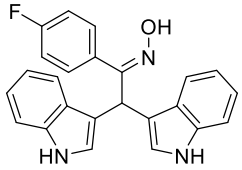


According to the general procedures: 0.317 g (0.83 mmol) of hydrazone **3.38d**, 0.435 g (3.32 mmol) of indole **3.2b** and 0.880 g (8.3 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)]. The obtained product was identified as a mixture of *E* and *Z* isomers which could not be separated by flash chromatography.

Yield: 80% (0.379, Method E), 48% (0.228g, Method F); light pink solid; m.p. 198.4-199.6 ºC (from *n*-hexane); IR: ṽ =3331, 1702, 1686, 1466, 1442, 1357, 1331, 1152, 1007, 740 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 1.30 (s, 9H, $CH_3$), 3.70 (minor) and 3.72 (major) (s, 6H, $CH_3$), 6.08 (major) and 6.43 (minor) (br s, 1H, CH *meso*), 6.83 (major) and 6.86 (minor) (br s, 2H, Ar), 7.07-7.10 (major) (m, 2H, Ar), 7.21 (minor) (d, *J =8.2* Hz, 2H, Ar), 7.25-7.29 (major) (m, 2H, Ar), 7.34-7.44 (m, 6H, Ar), 7.53 (minor) (d, *J* = 8.0 Hz, 3H, Ar), 7.73 (major) (d, *J* = 8.8 Hz, 2H, Ar), 7.96 (minor) (d, *J* = 8.8 Hz, 2H, Ar), 9.00 (major) (br s, 1H, NH) ppm; HMRS (ESI): calcd. for *m/z* $C_{31}H_{32}BrN_4O_2$ $[M + H]^+$ 571.1703 found 571.1694.

**(*E*)-1-[(4-Fluorophenyl)-1-hydroxyiminoethyl]bis(1*H*-indol-3-yl)methane (3.41b) and (Z)-1-[(4-Fluorophenyl)-1-hydroxyiminoethyl]bis(1*H*-indol-3-yl)methane (3.44a)**





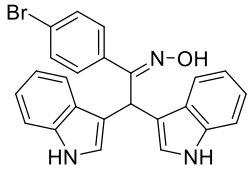
According to the general procedures: 0.200 g (0.54 mmol) of hydrazone **3.39b**, 0.253 g (2.16 mmol) of indole **3.2a** and 0.572 g (5.4 mmol) of $Na_2CO_3$ $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time: 1 h (Method E) or 30 min. (Method F); Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)] afforded in order of elution, compound **3.41b** and compound **3.44a**.

Data for **3.41b**: Yield: 60% (0.124 g, Method E and F). The characterization is in agreement with those previously reported for compound **2.81c** (see synthesis related to Chapter 2).

Data for **3.44a**: Yield: 15% (0.031 g, Method E), 22% (0.046 g, Method F); white solid; m.p. 218.7-219.6 ºC (from diethyl ether); IR: $\tilde{\nu}$ = 3403, 1508, 1454, 1222, 979 $cm^{-1}$; $^1H$

NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.78 (s, 1H, CH *meso*), 6.97-7.02 (m, 4H, Ar), 7.09-7.12 (m, 4H, Ar), 7.34-7.40 (m ,4H, Ar), 7.63 (d, $J$ = 8.0 Hz, 2H, Ar), 10.79 (br s, 1H, OH), 10.84 (d, $J$ = 1.6 Hz, 2H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 111.3, 114.3 114.5, 114.6 (d, $_{CF}J$ = 9.0 Hz), 118.3, 119.1, 120.8, 123.9, 126.8, 130.3 (d, $_{CF}J$ = 8.0 Hz), 131.2, 136.4, 155.7, 160.2, 162.3 (d, $_{CF}J$ = 243 Hz) ppm; HRMS (ESI): calcd. for *m/z* $C_{24}H_{19}FN_3O$ $[M + H]^+$ 384.1506 found 384.1500.

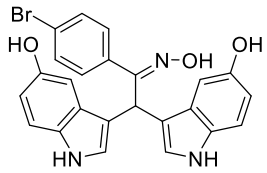
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(1*H*-indol-3-yl)methane (3.44b)**




According to the general procedures: 0.201 g (0.54 mmol) of hydrazone **3.39c**, 0.253g (2.16 mmol) of indole **3.2a** and 0.572 g (5.4 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time: 1 h (Method E) or 30 min. (Method F); Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)] (Method E).

Yield: 55% (0.132 g, Method E), 52% (0.125 g, Method F). The characterization is in agreement with those previously reported for compound **2.82a** (see synthesis related to Chapter 2).

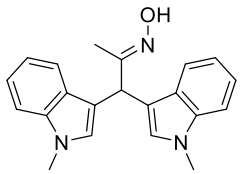
**(*E*)-1-[(4-Bromophenyl)-1-hydroxyiminomethyl]bis(5-hydroxy-1*H*-indol-3-yl)methane (3.44c)**




According to the general procedures: 0.201 g (0.54 mmol) of oxime **3.39c**, 0.286 g (2.16 mmol) of indole **3.2d** and 0.572 g (5.4 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time: 1 h (Method E) or 30 min. (Method F); Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to (1:1)].

Yield: 83% (0.213 g, Method E), 71% (0.190 g, Method F). The characterization is in agreement with those previously reported to compound **(2.82i)** (see synthesis related to Chapter 2).

**(*E*)-1-(1-Hydroxyiminomethyl)bis(1*H*-indol-3-yl)methane (3.41c)**



According to the general procedures: 0.077 g (0.54 mmol) of hydrazone **3.39a**, 0.283g (2.16 mmol) of indole **3.2b** and 0.572 g (5.4 mmol) of $Na_2CO_3$ using $H_2O$/[CC/Gly (2:1)] as solvent. Reaction time: 1 h (Method E) or 10 min. (Method F); Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)] (Method E) or by crystallization with diethyl ether ( Method F).

Yield: 56% (0.100 g, Method E) and 52% (0.093 g, Method F); white solid; m.p. 120.1–120.7 °C (from *n*-hexane); IR: $\tilde{\nu}$ = 3050, 1472, 1329, 1011, 945, 738 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.78 (s, 3H, $CH_3$), 3.74 (s, 6H. $CH_3$), 5.38 (br s, 1H, CH), 6.99 (t, $J$ = 7.6 Hz, 2H, Ar), 7.12–7.16 (m, 4H, Ar), 7.40 (d, $J$ = 8.0 Hz, 2H, Ar), 7.48 (d, $J$ = 8.0 Hz, 2H, Ar), 10.44 (br s, 1H, OH), ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 12.3, 32.3, 109.6, 113.4, 118.4, 119.0, 121.1, 127.1, 127.8, 136.7, 156.7 ppm;; HRMS (ESI): m/z calcd. for $C_{21}H_{21}N_3NaO$ $[M + Na]^+$ 354.157, found 354.1591.

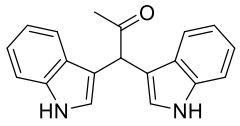
### 6.6.2 Hydrolysis reactions of bisindolylmethanes

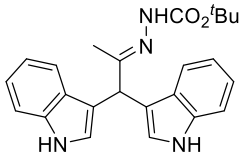
- **General procedure for the hydrolysis reactions of BIMs with the solvent system $H_2O$/[CC/Gly (2:1)]**

The appropriate indole **(3.2**, 4 equiv.**)** was added to a stirred solution of $Na_2CO_3$ (10 equiv.) in the solvent system $H_2O$/[CC/Gly (2:1)] (6:2 mL). The appropriate hydrazone **(3.38**, 1 equiv.**)** was added, and the reaction mixture was stirred for 6 h at room temperature. The reaction mixture was extracted with dichloromethane (3 × 20 mL) and the combined organic layers were dried over anhydrous $Na_2SO_4$. After filtration, the solvent was evaporated, and the crude product purified by flash chromatography (e gradient ethyl acetate:*n*-hexane).

**1-Acetyl-1,1-bis(1*H*-indol-3-yl)methane[10, 11] (3.45a)** and

**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1*H*-indol-3-yl)methane (3.40b)**





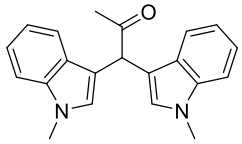
According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **3.38b** and 0.389 g (3.32 mmol) of indole **3.2a** and 0.880 g (8.3 mmol) of $Na_2CO_3$. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane (1:2) to (1:1)] afforded in order of elution, compound **3.45a** and compound **3.40b**.

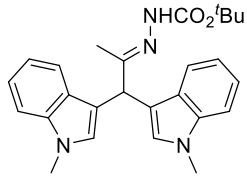
Data for **3.45a**: Yield: 40% (0.096 g); white solid; m.p 108.3-109.6 ºC (from diethyl ether); IR: ṽ = 3400, 1702, 1457, 1338, 1096, 1010, 739 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 2.20 (s, 3H, $CH_3$), 5.58 (br s, 1H, CH *meso*), 6.96 (t, $J$ = 8.0 Hz, 2H, Ar), 7.07 (t, $J$ = 8.0 Hz, 2H, Ar), 7.23 (d, $J$ = 2.4 Hz, 2H, Ar), 7.36 (d, $J$ = 8.4 Hz, 2H), 7.48 (d, $J$ = 8.0 Hz, 2H, Ar), 10.97 (br s, 2H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ = 28.2, 47.5, 111.5, 112.2, 118.4, 118.8, 121.0, 123.9, 126.6, 136.2, 206.4 ppm; HMRS (ESI): calcd. for *m/z* $C_{19}H_{17}N_2NaO$ $[M + H]^+$ 289.1335 found 289.1331.

Data for **3.40b**: Yield: 21% (0.070 g). The characterization is in agreement with those previously reported to compound **2.85a** (see synthesis related to Chapter 2).

**1-Acetyl-1,1-bis(1- methyl-1*H*-indol-3-yl)methane (3.45b) and**

**(*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1-methyl-1*H*-indol-3yl)methane (3.40c)**



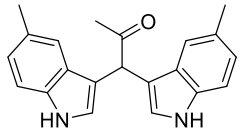


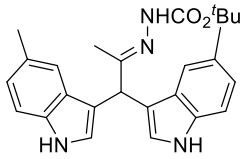
According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **3.38b** and 0.435 g (3.32 mmol) of indole **3.2b**. Purification of the products by flash chromatography {gradient ethyl acetate:*n*-hexane (1:2) to (1:1)] afforded in order of elution compound **3.45b** and compound **3.40c**.

Data for **3.45b**: Yield: 22% (0.058 g); light brown solid; m.p. 141.7-142.6 ºC (from hexanes); IR: ṽ = 1711, 1461, 1348, 1326, 1147, 742 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 2.31 (s, 3H, $CH_3$), 3.72 (s, 6H, $CH_3$), 5.54 (br s, 1H, CH *meso*), 6.95 (br s, 2H, Ar), 7.09 (t, $J$ = 8.0 Hz, 2H, Ar), 7.20-7.24 (m, 2H, Ar), 7.30 (d, $J$ = 8.0 Hz, 2H, Ar), 7.54 (d, $J$ = 7.6 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ = 29.0, 32.8, 109.4, 112.1, 119.2, 121.8, 127.3, 128.1, 137.2, 207.3 ppm; HMRS (ESI): calcd. for *m/z* $C_{21}H_{20}N_2NaO$ $[M+Na]^+$ 339.1468 found 339.1459.

Data for **3.40c**: Yield: 42% (0.110 g). The characterization is in agreement with those previously reported to compound **2.85a** (see synthesis related to Chapter 2).

**1-Acetyl-1,1-bis(5-methyl-1*H*-indol-3-yl)methane (3.45c) and (*E*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(5-methyl-1*H*-indol-3yl)methane (3.40d)**



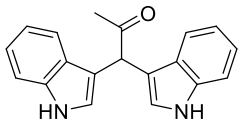

According to the general procedure: 0.200 g (0.83 mmol) of hydrazone **3.38b** and 0.389 g (3.32 mmol) of indole **3.2a**. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane (1:3) to (1:1)] afforded in order of elution, 19% (0.050 g) **3.45c** and 36% (0.129 g) **3.40d**.

The characterization is in agreement with those previously reported for compounds **2.86** and **2.85e** (see synthesis related to Chapter 2).

- **General procedure for the hydrolysis reactions of BIMs with sodium bisulfite**

The reactions were performed according to a known procedure with some adaptations.[12] A solution of the appropriate BIM oxime **3.41** (0.22 mmol, 1 equiv.) and sodium bisulfite (0.77 mmol, 3.5 equiv.) in 50% aqueous ethanol (10 mL) was stirred and refluxed for 2 h. After completion, the ethanol was evaporated. Dichloromethane (15 mL) and an excess of dilute hydrochloric acid were added to the crude and the reaction mixture was stirred during up to 30 min until two clear layers were obtained. The product was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were dried over anhydrous $Na_2SO_4$. The solvent was evaporated, and the product purified by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:3) to (1:1)].

**1-Acetyl-1,1-bis(1*H*-indol-3-yl)methane[10, 11] (3.45a)**

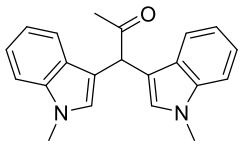



According to the general procedure: 0.067 g (0.22 mmol) of BIM **3.41a** and 0.080 g (0.77 mmol) of sodium bisulfite.

Yield: 50% (0.063 g).

The characterization is in agreement with those previously reported for compound **3.45a**.

**1-Acetyl-1,1-bis(1-methyl-1*H*-indol-3-yl)methane (3.45b)**

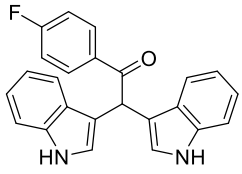


According to the general procedure: 0.073 g (0.22 mmol) of BIM **3.41c** and 0.080 g (0.77 mmol) of sodium bisulfite.

Yield: 32% (0.022 g).

The characterization is in agreement with those previously reported for compound **3.45b**.

**1-(4-Fluorophenyl)-2,2-di(1*H*-indol-3-yl)ethan-1-one (3.45d)**



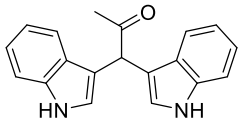
According to the general procedure: 0.084 g (0.22 mmol) of BIM **3.41b** and 0.080 g (0,77 mmol) of sodium bisulfite.

Yield: 38% (0.031 g); light Brown solid; m.p. 103.1-104.2 ºC (from diethyl ether); IR: $\tilde{\nu}$ = 1675, 1595, 1505, 1456, 1216, 1155, 740 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 6.46 (br s, 1H, CH *meso*), 6.99 (d, $J$ = 2.2 Hz, 2H, Ar), 7.05-7.11 (m, 4H), 7.20 (t, $J$ = 7.2 Hz, 2H, Ar), 7.36 (d, $J$ = 8.2 Hz, 2H, Ar), 7.55 (d, $J$ = 7.9 Hz, 2H, Ar), 8.05 (s, 2H, NH), 8.13 (dd, $J$ = 8.8 Hz, 5.4 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ = 42.1, 111.4, 114.2, 115.7 (d, $_{CF}J$ = 21.7 Hz), 118.9, 119.8, 122.3, 123.9, 126.6, 128.3, 130.1, 131.4 (d, $_{CF}J$ = 9.3 Hz), 136.5, 196.9 ppm; HMRS (ESI): calcd. for *m/z* $C_{24}H_{18}FN_2O$ $[M+Na]^+$ 369.1398 found 369.1389.

- **General procedure for the hydrolysis reactions of BIMs with amberlyst-15 and paraformaldehyde**

The reactions were performed according to a known procedure with some adaptations.[13] Paraformaldehyde (1.76 mmol, 8 equiv.) was added to a stirred solution of the appropriate BIM hydrazone **3.40** (0.22 mmol, 1 equiv.) in the solvent system acetone/water (7 mL / 7 mL). To the resulting suspension, 22.5 mg amberlyst-15 ionexchange resin was added and the reaction mixture was further stirred at room temperature for 48 h. Then it was filtered through a celite pad, which was washed with dichloromethane. After filtration, the solvent was evaporated, and the product was purified by lash chromatography [gradient ethyl acetate:hexane from (1:3) to (1:1)].

**1-Acetyl-1,1-bis(1*H*-indol-3-yl)methane[10, 11] (3.45a)**

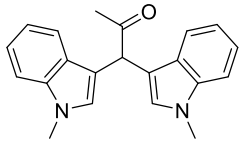


According to the general procedure: 0.088 g (0.22 mmol) of BIM **3.40b** and 0.053 g of paraformaldehyde.

Yield: 53% (0.033 g).

The characterization is in agreement with those previously reported for compound **3.45a**.

**1-Acetyl-1,1-bis(1-methyl-1*H*-indol-3-yl)methane (3.45b)**



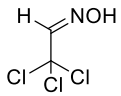
According to the general procedure: 0.070 g (0.22 mmol) of BIM **3.40c** and 0.053 g of paraformaldehyde.

Yield: 48% (0.033 g).

The characterization is in agreement with those previously reported for compound **3.45b.**

## 6.7 Synthesis related to Chapter 4

**Synthesis of Oxime 2,2,2-trichloroacetaldehyde oxime[14] (4.30)**



The reaction was performed according to a known procedure.[14]

To a mixture of 6.95 g ( 100 mmol) of hydroxylamine hydrochloride and 4.38g (200 mmol) of calcium chloride in water was added 16.5 g (100 mmol) of choral hydrate. The reaction mixture was stirred for 1 h at 50 ºC. After precipitation of the product, this is filtered and washed with diethyl ether.

Yield: 55% (17.8 g); yellow solid; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ = 6.57 (s, 1H), 11.03 (s, 1H, OH) ppm.

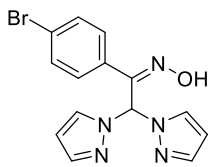
### 6.7.1 Synthesis of novel bis and tris(pyrazol-1-yl))methanes via conjugate addition reaction of nitroso- and azoalkenes with pyrazoles

- **General procedure for the synthesis of bis and tris(pyrazol-1-yl)methane oximes 4.27 or hydrazones 4.28**

The appropriate oxime or hydrazone **4.25** (1 equiv.) and pyrazole **5.26** (3 equiv.) were added to a suspension of $Na_2CO_3$ (5 equiv.) in dichloromethane (35 mL). The reaction mixture was stirred for 16 h at room temperature. Upon completion, the reaction mixture was filtered through a Celite pad and washed with dichloromethane. The solvent was

evaporated to give the crude product, which was purified by flash chromatography affording the bis(pyrazolyl)methanes (BPMs) or tris(pyrazolyl)methanes (TPMs).

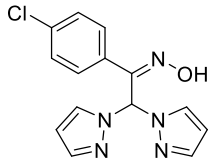
**(*Z*)-1-[(4-Bromophenyl)-hydroxyiminomethyl]bis(1*H*-pyrazol-1-yl)methane (4.27a)**



According to the general procedure: 1.30 g (3.5 mmol) of oxime **4.25a**, 0.715 g (10.5 mmol) of pyrazole **5.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:1) to ethyl acetate].

Yield: 67% ( 0.812 g), white solid; m.p. 159.1-160.7 ºC ( from hexanes); IR (KBr): ṽ = 3209, 2945, 1724, 1391, 1005, 771, 752 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.27 (s, 2H, Ar), 7.38 (d, $J$ = 8.4 Hz, 2H, Ar), 7.52 (m, 4H, Ar), 7.89 (d, $J$ = 2.5 Hz, 2H, Ar), 8.05 (s, 1H, CH *meso*), 11.75 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 75.1, 106.3, 122.2, 129.9, 130.3, 130.7, 130.8, 139.7, 149.5 ppm; HMRS (ESI): calcd. for m/z $C_{14}H_{12}BrN_5NaO$ $[M+Na]^+$ 368.01163 found 368.01174.

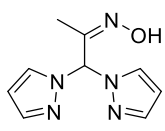
**(*Z*)-1-[(4-Chlorophenyl)-hydroxyiminomethyl]bis(1*H*-pyrazol-1-yl)methane (4.27b)**



According to the general procedure: 0.782 g (3.5 mmol) of oxime **4.25b**, 0.715 g (10.5 mmol) of pyrazole **4.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:1) to ethyl acetate].

Yield: 53% (0.560 g), light brown solid; m.p. 159.3-160.7 ºC ( from hexanes); IR (KBr): ṽ = 3179, 2862, 1734, 1648, 1595, 1516, 1492, 1426, 1394, 1323, 1262, 1093, 1055, 793 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 6.27 (t, $J$ = 2.1 Hz, 2H, Ar), 7.39 (d, $J$ = 8.4 Hz, 2H, Ar), 7.45 (d, $J$ = 8.4 Hz, 2H, Ar), 7.52 (d, $J$ = 1.8 Hz, 2H, Ar), 7.89 (d, $J$ = 2.5 Hz, 2H, Ar), 8.06 (s, 1H, CH *meso*), 11.75 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 75.1, 106.2, 127.9, 129.5, 130.7, 133.4, 139.7, 149.4 ppm; HMRS (ESI): calcd. for m/z $C_{14}H_{12}ClN_5NaO$ $[M + Na]^+$ 324.0628 found 324.0627.

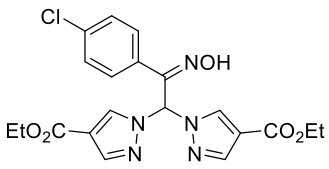
**(*Z*)-1-[(Methyl)-hydroxyiminomethyl]bis(1*H*-pyrazol-1-yl)methane (4.27c)**



According to the general procedure: 0.497 g (3.5 mmol) of oxime **4.25c**, 0.715 g (10.5 mmol) of pyrazole **4.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to ethyl acetate].

Yield: 43% (0.309 g); white solid; m.p. 128.3-129.2 ºC (from hexanes); IR: ṽ = 3326, 1732, 1519, 1433, 1393, 1091, 1042 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 1.72 (s, 3H, $CH_3$), 6.34 (*pseudo* t, $J$ = 2.4 Hz, $J$ = 1.6 Hz, 2H, Ar), 7.46 (s, 1H, CH *meso*), 7.58 (d, $J$ = 1.6 Hz, 2H, Ar), 7.83 (d, $J$ = 2.4 Hz, 2H, Ar), 11.36 (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 11.5, 76.0, 106.2, 130.5, 139.9, 150.9 ppm; HMRS (ESI): calcd. for m/z $C_9H_{11}N_5NaO$ $[M + Na]^+$ 228.0855 found 228.0856.

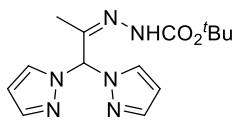
**1-[(4-Chlorophenyl)-1-hydroxyiminomethyl]bis(4-ethoxycarbonyl-1*H*-pyrazol-1-yl)methane (4.27d)**




According to the general procedures: 1.15 g (3.5 mmol) of oxime **4.25b**, 1.47 g (10.5 mmol) of pyrazole **4.26b** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to ethyl acetate]. The obtained product was identified as a mixture of *E* and *Z* isomers which could not be separated by flash chromatography.

Yield: 47% (0,733 g); white solid; m.p. 93.7-95.2 ºC (from hexanes); IR (KBr) ṽ = 3317, 2983, 1720, 1558, 1493, 1412, 1241, 1024, 884, 847, 794 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ = 1.26 (t, $J$ = 7.2 Hz, 6H, $CH_3$), 4.20 (q, $J$ = 7.2 Hz, 2H, $CH_2$), 7.44 (major) (d, $J$ = 8.4 Hz, 2H, Ar) , 7.53 (major) ( d, $J$ = 8.4 Hz, 2H, Ar), 7.74 (minor) (d, $J$ = 8.4 Hz, 2H, Ar), 7.79 (minor) (s, 1H, CH, *meso*), 7.97 (s, 2H, Ar), 8.30 (major) (s, 1H, CH *meso*), 8.55 (s, 2H, Ar), 12.00 (major) and 12.07 (minor) (s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$ = 14.0 (major), 20.4 (minor) , 44.7 (minor), 54.7 (minor) , 59.8 (major), 75.1 (major), 113.6 (minor), 115.1 (major), 127.8 (major), 128.0 (major), 128.2 (major), 128.5 (major), 129.9 (major), 133.1 (minor), 133.5 (minor), 133.72 (major), 134.4 (major), 140.2 (minor), 140.9 (major), 147.8 (major), 150.4 (minor), 161.5 (major), 161.9 (minor) ppm; HRMS (ESI): calcd. for $C_{20}H_{21}ClN_5O_5$ $[M + H]^+$ 446.1226 found 446.1226.

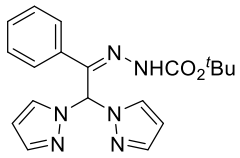
**(*Z*)-1-[1-(*tert*-Butoxycarbonylhydrazono)ethyl]bis(1*H*-pyrazol-1-yl)methane (4.28a)**




According to the general procedure: 0.844 g (3.5 mmol) of hydrazone **4.25d**, 0.715 g (10.5 mmol) of pyrazole **4.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to ethyl acetate].

Yield: 57% (0.607 g); light brown solid; m.p. 147.2-148.9 ºC (from hexanes); IR (KBr) ṽ = 3435, 3215, 2974, 1734, 1713, 1539, 1393, 1369, 1290, 1242, 1177, 1150, 1055, 847, 754 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ = 1.49 (s, 9H, $CH_3$), 1.93 (s, 3H, $CH_3$), 6.32 (dd, *J* = 2.5 and 1.8 Hz, 2H, Ar), 6.35 (t, *J* = 2.1 Hz, 1H), 7.19 (s, 1H, CH *meso*), 7.52 (br s, 1H, NH), 7.60 (dd, *J* = 2.1, 0.6 Hz, 2H), 7.62 (d, *J* = 2.1 Hz, 1H) ppm; $^{13}C$ NMR (126 MHz, $CDCl_3$) $\delta$ = 12.8, 28.2, 76.8, 77.0, 77.3, 78.6, 81.8, 105.0, 106.7, 130.1, 140.9 ppm; HRMS (ESI): calcd. for $C_{14}H_{20}N_6NaO_2$ $[M + Na]^+$ 327.1539 found 327.1540.

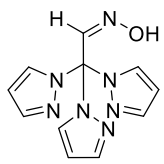
**(Z)-1-[Phenyl-1-(tert-butoxycarbonylhydrazono)ethyl]bis(1*H*-pyrazol-1-yl)methane (4.28b)**




According to the general procedure: 0.661 g (3.5 mmol) of hydrazone **4.25e**, 0.715 g (10.5 mmol) of pyrazole **4.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification of the product by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:2) to ethyl acetate].

Yield: 77% (0.988 g); light brown solid; m.p. 165.5-166.0 ºC (from hexane); IR (KBr) ṽ = 3360, 3213, 2976, 1753, 1485, 1394, 1225, 1177, 1153, 1092, 1042, 754 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ = 1.38 (s, 9H, $CH_3$), 6.24 (t, *J* = 2.1 Hz, 2H, Ar), 7.15–7.31 (m, 2H, Ar), 7.37–7.39 (m, 3H, Ar), 7.51 (s, 2 H), 7.80 (s, 1H, Ar), 7.92 (d, *J* = 2.4 Hz, 2H, Ar), 9.09 (s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ = 13.7, 17.2, 50.0, 100.4, 125.8, 126.0, 126.1, 127.2, 127.5, 127.9, 128.1, 128.2, 128.3, 128.6, 137.1, 150.8 ppm; HRMS (ESI): calcd. for $C_{19}H_{22}N_6NaO_2$ $[M + Na]^+$ 389.1695 found 389.1696.

**[(1-Hydroxyimino)methyl]tris(1*H*- pyrazol-1-yl)methane (4.31)**




According to the general procedure: 0.568 g (3.5 mmol) of oxime **4.30**, 1.47 g (10.5 mmol) of pyrazole **4.26a** and 1.85 g (17.5 mmol) of $Na_2CO_3$. Purification by recrystallization in DCM.

Yield: 87% (0.783 g); white solid; m.p. 135.8-136.4 ºC (from dichloromethane); IR (KBr) $\tilde{\nu}$ = 3297, 3129, 1515, 1424, 1386, 1323, 1270, 1247, 1200, 1101, 1085, 1045, 1016, 950, 907, 864 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ = 6.46 (*pseudo* t, *J* = 2.4 Hz, 2.0 Hz, 3H, Ar), 7.40 (d, *J* = 2.4 Hz, 3H, Ar), 7.71 (d, *J* = 1.6 Hz, 3H, Ar), 8.64 (s, 1H, CH), 11.98 (s, 1H, OH) ppm ; $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ = 88.3, 107.1, 141.0, 144.8 ppm; HRMS (ESI): calcd. for $C_{11}H_{11}N_7NaO$ $[M + Na]^+$ 280.0916 found 280.0917.

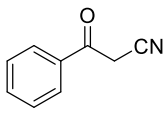
## 6.8 Synthesis related to Chapter 5

### 6.8.1 Synthesis of *β*-ketonitriles

- **General procedure for the synthesis of *β*-ketonitriles 5.41**

The reactions were performed according to a known procedure with a slight modification.[15] To a solution of dry acetonitrile (1.0 equiv.) in dry THF, stirred at room temperature and under inert atmosphere ($N_2$), was added dropwise a solution of KO*t*-amyl (3.0 equiv., 1.7 M in PhMe) followed by dropwise addition of the appropriated ethyl esters (4.0 equiv.). After 24 h, the reaction mixture was diluted with 0.25 M HCl (120 mL) and ethyl acetate (120 mL). The layers were separated, and the organic layer was washed with $H_2O$ (2 x 50 mL) and brine (2 x 50 mL), dried with anhydrous $Na_2SO_4$, and filtered. The solvent was evaporated, affording the corresponding nitrile. Purification by recrystallization in ethyl acetate:*n*-hexane.

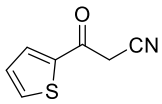
**3-Oxo-3-phenylpropanenitrile[15] (5.41a)**




According to the general procedure: 1.1 mL (20.8 mmol) of acetonitrile, 36.2 mL (62.4 mmol) of KO*t*-amyl and 12 mL (83 mmol) of ethyl benzoate in 72 mL of dry THF.

Yield: 88% (2.66 g), light yellow solid; m.p. 80.8-81.2 (from ethyl acetate:hexane) (lit. 81.0 ºC) [15]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 4.12 (s, 2H, $CH_2$), 7.51-7.54 (m, 2H, Ar), 7.64-7.68 (m, 1H. Ar), 7.91-7.92 (m, 2H, Ar) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ = 29.4, 113.9, 128.5, 129.2, 134.3, 134.8, 187.2 ppm.

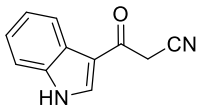
**3-Oxo-3-(thiophen-2-yl)propanenitrile[16] (5.41b)**




According to the general procedure: 0.37 mL (7.0 mmol) of acetonitrile, 12.1 mL (21 mmol) of KO*t*-amyl and 12 mL (21 mmol) of ethyl benzoate in 24 mL of dry THF.

Yield: 65% (0.688 g); light yellow solid; m.p. 131.1-132.8 ºC (from ethyl acetate:hexane)(lit. 131.0-133.0 ºC)[16]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 4.71 (s, 2H, $CH_2$), 7.30 (t, *J* = 3.6 Hz, 1H, Ar), 8.14 (d, *J* = 4.8 Hz, 1H, Ar) ppm; $^{13}$C NMR (DMSO-$d_6$): $\delta$ = 29.7, 115.4, 129.0, 135.2, 136.6, 140.9, 182.3 ppm.

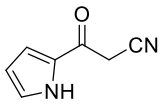
**3-(1*H*-indol-3-yl)-3-oxopropanenitrile [17] (5.41c)**




According to a known procedure: indole (5.85g, 50 mmol) was added to a solution of cyanoacetic acid (5.0 g, 50 mmol) in $Ac_2O$ (50 mL) at 50 °C. The solution was heated at 85 °C for 5 min. During that period the product started to crystallize. After 5 min, the mixture was allowed to cool and the solid was filtered, washed with MeOH, and dried.

Yield: 75% ( 6.91 g); white solid; m.p. 240.5 ºC ( from methanol) (lit 241.0 ºC) [17]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 4.50 (s, 2H, $CH_2$), 7.21-7.28 (m, 2H, Ar), 7.50-7.52 (m, 1H, Ar), 8.13-8.15 (m, 1H, Ar), 8.38 (d, $J$ = 3.6 Hz, 1H, Ar), 12.19 (s, 1H, NH) ppm.

**3-Oxo-3-(1*H*-pyrrol-2-yl)propanenitrile[17] (5.41d)**




According to a known procedure: pyrrole (6 g, 89.4 mmol) was added to a solution of cyanoacetic acid (7.68 g 89.4 mmol) in $Ac_2O$ (45 mL) and heated at 75 °C for 35 min. The mixture was allowed to cool and poured on ice. The product precipitates, is filtered , washed with methanol, and dried.

Yield: 70% (8.4 g); white solid; m.p. 78.5-79.7 ºC (from methanol) (lit 78.0 ºC) [17]; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ = 4.41 (s, 2H, $CH_2$), 6.24-6.26 (m, 1H, Ar), 7.07-7.09 (m, 1H, Ar), 12.11 (s, 1H, NH) ppm; $^{13}$C NMR (DMSO-$d_6$): $\delta$ = 28.2, 110.4, 116.1, 118.7, 127.2, 129.4, 177.7 ppm.

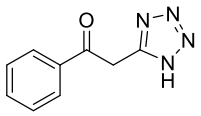
### 6.8.2 Synthesis of 5-substituted-1*H*-tetrazoles

- **General procedure for the synthesis of 5-substituted-1*H*-tetrazoles 5.42**

Tetrazoles were prepared following a known procedure with slight modifications.[16, 18] To a pressure tube the appropriate nitrile **5.41** (5.17 mmol), sodium azide (0.370 g, 5.69 mmol), zinc bromide (1.16 g, 5.17 mmol), water (12 mL), and $^i$PrOH (1.5 mL) were added. The sealed tube was immersed in an oil bath at 140 °C and stirred vigorously for the appropriate time. After cooling the tube to room temperature, HCl (3 N, 8 mL) and ethyl acetate (25 mL) were added, and vigorous stirring was continued until no solid was present. The organic layer was isolated, and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The combined organic layers were evaporated, 50 mL of 0.25 N NaOH was added, and the mixture was stirred for 30 min, until the original precipitate was dissolved, and a suspension of zinc hydroxide was formed. The suspension was filtered through Celite, and the Celite pad was washed with 5 mL of 1 N NaOH. To the filtrate was added

10 mL of 3 N HCl with vigorous stirring, causing the tetrazole to precipitate. The tetrazole was isolated by filtration and dried.

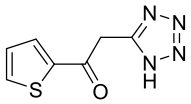
**1-Phenyl-2-(1*H*-tetrazol-5-yl)ethanone[19] (5.42)**



According to general procedure: 0.750g of nitrile **5.41a**.

Yield: 73% (0.710); light yellow solid; m.p. 186.0-187.5 ºC (lit. 187.0-188.0 ºC) [19]; IR ṽ = 3028, 2951, 2895, 1684, 1597, 1568, 1450, 1385 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.96 (s, 2H, $CH_2$), 7.60 (*pseudo* t, *J* = 7.6 Hz, 2H, Ar), 7.71−7.73 (m, 1H, Ar), 8.10 (d, *J* = 7.6 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 34.0, 128.4, 128.9, 134.0, 135.4, 150.8, 193.9 ppm.

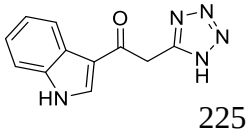
**2-(1*H*-Tetrazol-5-yl)-1-(thiophen-2-yl)ethanone[16] (5.42b)**



According to the general procedure: 0.782 g of the nitrile **5.41b**.

Yield: 55% (0.552 g); light brown solid; m.p. 166.2-167.9 ºC (lit. 166.0-168.0 ºC) [16]; IR ṽ = 3099, 3007, 2891, 1655, 1560, 1524, 1417, 1328 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.89 (s, 2H, $CH_2$), 7.33 (*pseudo* t, *J* = 4.4 Hz, 1H, Ar), 8.12 (d, *J* = 4.8 Hz, 1H, Ar), 8.19 (d, *J* = 3.6 Hz, 1H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 34.1, 129.0, 135.0, 136.2, 142.2, 150.5, 186.7 ppm.

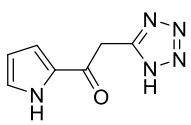
**1-(1*H*-Indol-3-yl)-2-(1*H*-tetrazol-5-yl)ethanone[16] (5.42c)**



According to the general procedure: 0.952 g of nitrile **5.41c**.

Yield: 96% (1.13 g); light pink solid; m.p. 222.8-224.7 ºC (lit.223.0-225.0 ºC)[16]; IR ṽ = 3254, 3227, 1624, 1519, 1434, 1413, 1377, 1233 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.71 (s, 2H, $CH_2$), 7.19-7.27 (m, 1H, Ar), 8.14 (d, *J* = 7.6 Hz, 1H, Ar), 8.44 (d, *J* = 7.2 Hz, 1H, Ar), 8.55 ( d, *J* = 3.2 Hz, 1H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 34.0, 112.3, 115.2, 121.0, 122.2, 125.2, 135.3, 136.6, 150.9, 187.7 ppm.

**1-(1*H*-Pyrrol-2-yl)-2-(1*H*-tetrazol-5-yl)ethanone[16] (5.42d)**

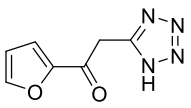


According to the general procedure: 0.694 g of nitrile **5.41d**.

Yield: 46% (0.421 g); brown solid; m.p. 235.4-237.0 ºC (lit. 235.0-237.0 ºC)[16]; IR ṽ = 3307, 2870, 2713, 1651, 1545, 1443, 1402 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.60 (s, 2H, $CH_2$), 6.26-6.28 (m, 1H, Ar), 7.21 (d, *J* = 12 Hz,

2H, Ar), 12.0 (s, 1H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 32.8, 110.3, 118.3, 126.8, 130.4, 182.2 ppm.

**1-(Furan-2-yl)-2-(1*H*-tetrazol-5-yl)ethanone[16] (5.42e)**



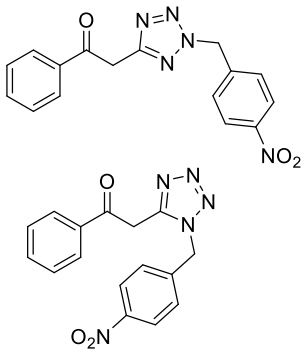
According to the general procedure: 0.699 g of nitrile **5.41e**.

Yield: 46% (0.425 g); brown solid; m.p. 181.6-183.4 ºC (lit. 181.0-183.0 ºC)[16]; IR ṽ = 2968, 2845, 1670, 1572, 1470, 1394 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 4.72 (s, 2H, $CH_2$), 6.80-6.81 (m, 1H, Ar), 7.69 (d, *J* = 3.6 Hz, 1H, Ar), 8.10 (d, *J* = 1.2 Hz, 1H, Ar) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 33.3, 112.9, 120.3, 148.8, 150.7, 181.6 ppm.

- **General procedure for the alkylation of 5-substituted-1*H*-tetrazoles 5.43 and 5.44 with benzyl bromides**.

The 5-substituted-1*H*-tetrazole were prepared following a known procedure.[16] To a suspension of the appropriate 5-substituted-1*H*-tetrazole **5.42** (10 mmol) in THF (12 mL) stirring at room temperature, triethylamine (1.48 mL, 10.5 mmol) was added. The temperature of the solution was increased to 40 °C, and benzyl bromide or *p*-nitrobenzyl bromide (10.5 mmol) in THF (6 mL) was added slowly. The solution was stirred at 40 °C for 3 h, whereupon the mixture was allowed to cool and the precipitate triethylammonium bromide was filtered and washed with cold THF. The solvent was evaporated under vacuum, giving the crude product as a variable mixture of the 1,5- and 2,5-disubstituted isomers, which were separated by flash chromatography (ethyl acetate:*n*-hexane).

**2-(1-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-phenylethanone[16] (5.43a)** and **2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone[16] (5.44a)**



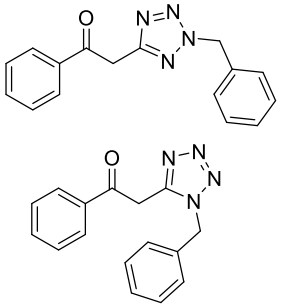
According to the general procedure: 1.88 g (10 mmol) of tetrazole **5.42a**, and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane (1:2) to (1:1)] afforded in order of elution compound **5.43a** and compound **5.44a**.

Overall yield: 70% (2.26 g); **5.43a** and **5.44a** were obtained in a (40:60) ratio.

Data for **5.43a**: yield: 42% (1.36 g); light yellow solid, m.p. 111.5-113.8 ºC (from ethyl acetate:hexane) (lit. 112.0−114.0 °C)[16]; IR ṽ = 2960, 1690, 1596, 1519, 1350 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.83 (s, 2H, $CH_2$), 6.15 (s, 2H, $CH_2$), 7.55−7.61 (m, 4H, Ar), 7.68−7.71 (m, 1H, Ar), 8.05 (d, *J* = 7.6 Hz, 2H, Ar), 8.27 (d, *J* = 8.4 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 36.6, 54.9, 124.0, 128.8, 129.5, 133.8, 135.6, 147.5, 161.3, 194.6 ppm.

Data for **5.44a**: yield: 28% (0.902 g); light brown solid; m.p. 192-194 ºC (from ethyl acetate:hexane) (lit. 192-194 ºC)[16]. IR ṽ = 2968, 1691, 1676, 1597, 1518, 1350 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ = 5.11 (s, 2H, $CH_2$), 5.82 (s, 2H, $CH_2$), 7.54−7.61 (m, 4H, Ar), 7.70−7.72 (m, 1H, Ar), 8.03 (d, *J* = 7.6 Hz, 2H, Ar), 8.22 (d, *J* = 8.2 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 33.7, 49.1, 123.7, 128.5, 128.8, 129.4, 134.1, 135.3, 141.9, 147.3, 151.2, 193.5 ppm.

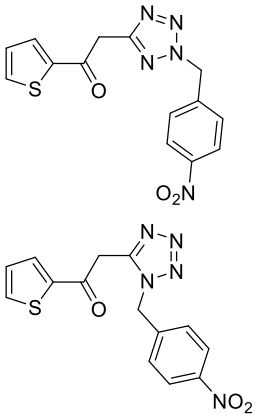
**2-(2-Benzyl-2*H*-tetrazol-5-yl)-1-phenylethanone[16] (5.43b)** and **2-(2-Benzyl-1*H*-tetrazol-5-yl)-1-phenylethanone[16] (5.44b)**



According to the general procedure with a slightly modification: 1.88 g (10 mmol) of tetrazole **5.42a**, and 1.80 g (10.5 mmol) benzyl bromide. Reaction time: 16 h. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane (1:2) to (1:1)] afforded in order of elution compound **5.43b** and compound **5.44b**. Overall yield: 67% (1.86 g); **5.43b** and **5.44b** were obtained in a (50:50) ratio.

Data for **5.43b**: yield: 34% (0.946 g); yellow solid, m.p. 62.9-64.7 ºC (from ethyl acetate:hexane) (lit. 63.0−65.0 °C)[16]; IR ṽ = 3.060, 2968, 1700, 1596, 1448, 1395, 1219, 996 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.81 (s, 2H, $CH_2$), 5.94 (s, 2H, $CH_2$), 7.34−7.42 (m, 5H, Ar), 7.55−7.58 (m, 2H, Ar), 7.67−7.71 (m, 1H, Ar), 8.05 (d, *J* = 7.6 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 35.7, 55.8, 128.2, 128.4, 128.5, 128.8, 133.7, 134.2, 135.7, 161.0, 194.7 ppm. Data for **5.44b**: yield: 33% (0.946 g); yellow solid; m.p. 119.2-121.2ºC (from ethyl acetate:hexane) (lit. 119.0-121.0 ºC)[16]. IR ṽ = 3073, 2960, 1686, 1597, 1467, 1332, 1218, 993 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$) δ = 5.07 (s, 2H, $CH_2$), 5.64 (s, 2H, $CH_2$),7.31−7.34 (m, 5H, Ar), 7.57−7.61 (m, 2H, Ar), 7.71−7.74 (m, 1H, Ar), 8.05 (d, J= 7.6 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 33.7, 50.0, 128.2, 128.5, 128.7, 128.8, 134.0, 134.5, 135.3, 150.7, 193.4 ppm.

**2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-(thiophen-2-yl)ethanone[16] (5.43c) 2-(2-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-(thiophen-2-yl)ethanone[16] (5.44c)**



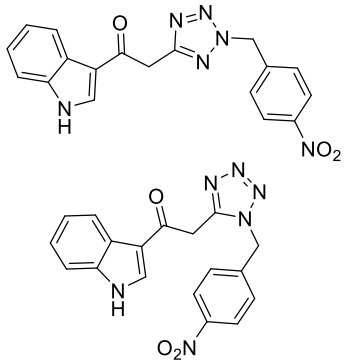
According to the general procedure: 1.94 g (10 mmol) of tetrazole **5.42b**, and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:1) to ethyl acetate] afforded in order of elution compound **5.43c** and compound **5.44c**.

Overall yield: 66% (1.86 g); **5.43c** and **5.44c** were obtained in a (58:42) ratio.

Data for **5.43c**: yield: 38% (1.25 g); brown solid, m.p. 120.1-121.8 ºC (from ethyl acetate:*n*-hexane) (lit. 120.0−122.0 °C)[16]; IR ṽ = 2981, 1658, 1416, 1342 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 5.06 (s, 2H, $CH_2$), 5.85 (s, 2H, $CH_2$), 7.32-7.35 (m, 1H, Ar), 7.55 (d, *J* = 8.8 Hz, 2H, Ar), 8.12 (d, *J* = 4.8 Hz, 1H, Ar), 8.17 (d, *J* = 3.2 Hz, 1H, Ar), 8.22 (d, *J* = 8.8 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 35.7, 54.8, 124.0, 128.9, 129.6, 134.8, 136.0, 141.3, 142.5, 147.5, 161.0, 187.4 ppm.

Data for **5.44c**: yield: 28% (0.922 g); brown solid; m.p. 173-175 ºC (from ethyl acetate:*n*-hexane) (lit. 174-176 ºC)[16]. IR ṽ = 2970, 1655, 1519, 1415, 1346 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.75 (s, 2H, $CH_2$), 5.64 (s, 2H, $CH_2$), 7.31−7.34 (m, 5H, Ar), 7.57−7.61 (m, 2H, Ar), 7.71−7.74 (m, 1H, Ar), 8.05 (d, J= 7.6 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 33.6, 49.2, 123.7, 129.0, 129.4, 135.2, 136.3, 141.9, 147.3, 150.8, 186.1 ppm.

**1-(1*H*-Indol-3-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)ethanone[16] (5.43d)** and **1-(1*H*-Indol-3-yl)-2-(2-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)ethanone[16] (5.44d)**



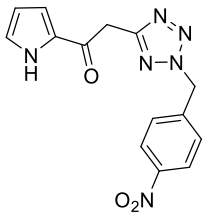
According to the general procedure: 2.27 g (10 mmol) of tetrazole **5.42c**, and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane (1:1)] afforded in order of elution compound **5.43d** and compound **5.44d**.

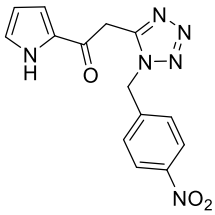
Overall yield: 62% (2.247 g); **5.43d** and **5.44d** were obtained in a (40:60) ratio.

Data for **5.43d**: yield: 25% (1.25 g); light yellow solid, m.p. 179.0-181.0 ºC (from ethyl acetate:*n*-hexane) (lit. 181.0−183.0 °C)[16]; IR ṽ = 3340, 1610, 1522, 1518, 1492, 1479,

1430, 1339, 1128 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.57 (s, 2H, $CH_2$), 6.14 (s, 2H, $CH_2$), 7.19-7.25 (m, 2H, Ar), 7.50 (d, *J* = 7.6 Hz, 1H, Ar), 7.62 (d, *J* = 8.8 Hz, 2H, Ar), 8.12 (d, *J* = 7.6 Hz, 1H, Ar), 8.27 (d, *J* = 8.8 Hz, 2H, Ar), 8.51 (d, *J* = 3.2 Hz, 1H, Ar), 12.09 (s, 1H, NH) ppm ; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 36.0, 57.7, 112.2, 115.2, 121.1, 122.0, 123.0, 124.0, 125.3, 129.5, 135.1, 136.6, 141.4, 147.5, 162.0, 188.6 ppm. Data for **5.44d**: yield: 37% (1.34 g); light yellow solid; m.p. 188.9-190.6 ºC (from ethyl acetate:*n*-hexane) (lit. 189.0-191.0 ºC)[16]; IR ṽ = 3416, 1632, 1522, 1518, 1508, 1427, 1405, 1344, 1320, 1311, 1242, 1237, 1153, 1140, 1121, 1106 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.89 (s, 2H, $CH_2$), 5.86 (s, 2H, $CH_2$), 7.19-7.24 (m, 2H, Ar), 7.51 (d, *J* = 8.0 Hz, 1H, Ar), 7.56 (d, *J* = 8.8 Hz, 2H, Ar), 8.05 (d, *J* = 7.6 Hz, 2H, Ar), 8.55 (s, 1H, Ar), 12.17 (s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, D DMSO-$d_6$) δ = 33.6, 49.3, 112.3, 115.2, 121.0, 122.1, 123.1, 123.7, 125.2, 129.3, 135.5, 136.6, 142.0, 147.2, 151.6 ppm.

**2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-(1*H*-pyrrol-2-yl)ethanone[16] (5.43e)** and 2-(2-(4-**Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-(1*H*-pyrrol-2-yl)ethanone[16] (5.44e)**





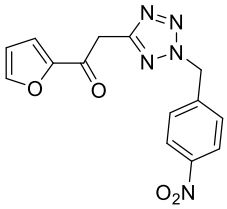
According to the general procedure: 2.27 g (10 mmol) of tetrazole **5.42d**, and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:1) to ethyl acetate] afforded in order of elution compound **5.43e** and compound **5.44e**.

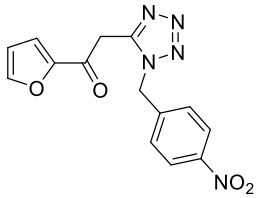
Overall yield: 72% (1.28 g); **5.43e** and **5.44e** were obtained in a (54:46) ratio.

Data for **5.43e**: yield: 39% (1.25 g); brown solid, m.p. 140.0-141.8 ºC (from ethyl acetate:*n*-hexane) (lit. 140.0−143.0 °C)[16]; IR ṽ = 3370, 1645, 1520, 1396, 1346 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.46 (s, 2H, $CH_2$), 6.14 (s, 2H, $CH_2$), 6.23-6.25 (m, 1H, Ar), 7.15 (d, *J* = 2.8 Hz, 2H, Ar), 7.62 (d, *J* = 8.4 Hz, 2H, Ar), 8.26 (d, *J* = 8.8 Hz, 2H, Ar), 11.94 (s, 1H, NH) ppm ; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 34.7, 54.8, 110.1, 118.1, 123.9, 126.5, 129.5, 130.7, 141.3, 147.5, 161.7, 183.1 ppm. Data for **5.44e**: yield: 33% (1.34 g); brown solid; m.p. 220.0-222.0 ºC (from ethyl acetate:*n*-hexane) (lit. 221.0-222.0 ºC)[16]; IR ṽ = 3412, 1641, 1516, 1395, 1350 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.80 (s, 2H, $CH_2$), 5.84 (s, 2H, $CH_2$), 6.27-6.29 (m, 1H, Ar), 7.18 (s, 1H, Ar), 7.23-7.24 (m, 1H, Ar), 7.55 (d, *J* = 8.8 Hz, 2H, Ar), 8.2 (d, *J* = 8.8

Hz, 2H, Ar), 12.00 (s, 1H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 32.4, 49.2, 110.3, 118.5, 120.0, 123.6, 126.9, 129.3, 130.2, 142.0, 147.2, 151.3, 181.7 ppm.

**1-(Furan-2-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)ethanone[16] (5.43f)** and **1-(Furan-2-yl)-2-(2-(4-nitrobenzyl)-1*H*-tetrazol-5-yl)ethanone[16] (5.44f)**





According to the general procedure: 1.79 g (10 mmol) of tetrazole **5.42e**, and 2.27 g (10.5 mmol) of *p*-nitrobenzyl bromide. Purification of the products by flash chromatography [gradient ethyl acetate:*n*-hexane from (1:1) to ethyl acetate] afforded in order of elution compound **5.43f** and compound **5.44f**.

Overall yield: 71% (1.28 g); **5.43f** and **5.44f** were obtained in a (63:37) ratio.

Data for **5.43f**: yield: 45% (1.41 g); brown solid, m.p. 105.0-107.0 ºC (from ethyl acetate:*n*-hexane) (lit. 106.0-108.0 °C)[16]; IR ṽ = 3000, 1668, 1569,1520, 1463, 1345 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 4.59 (s, 2H, $CH_2$), 6.15 (s, 2H, $CH_2$), 6.77-6.78 (m, 1H, Ar), 7.60-7.63 (m, 3H, Ar), 8.06 (s, 1H, Ar), 8.26 (d, *J* = 8.4 Hz, 2H, Ar) ppm ; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 35.0, 54.8, 112.3, 120.1, 123.9, 129.5, 141.2, 147.5, 148.6, 151.0, 160.8, 182.3 ppm.

Data for **5.44f**: yield: 26% (0.815 g); brown solid; m.p. 152.0-154.2 ºC (from ethyl acetate:*n*-hexane) (lit. 154.0-156.0 ºC)[16]. IR ṽ = 3000, 1663, 1525, 1460, 1344 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 4.89 (s, 2H, $CH_2$), 5.84 (s, 2H, $CH_2$), 6.80-6.81 (m, 1H, Ar), 7.56 (d, *J* = 8.8 Hz, 1H, Ar), 8.09 (d, *J* = 0.8 Hz, 1H, Ar), 8.23 (d, *J* = 8.8 Hz, 2H, Ar) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 39.1, 49.2, 112.9, 120.3, 123.7, 129.4, 141.9, 147.3, 148.7, 150.5, 150.6, 181.1 ppm.

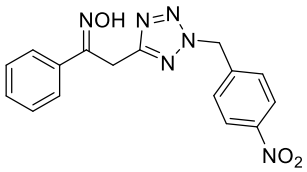
### 6.8.3 Synthesis of β-ketoxime-tetrazoles

- **General procedure for the preparation of oximes 5.45**

The oximes were prepared following a known procedure.[16] The appropriate tetrazole **5.43** or **5.44** (3.5 mmol) was dissolved in a mixture of ethanol/pyridine (1:1) (10 mL), and hydroxylamine hydrochloride (10.5 mmol, 0.73 g) was added. The reaction mixture was heated under reflux for 3 h. The solvent was evaporated under reduced pressure, and the crude was dissolved in cold water (20 mL) and extracted with ethyl acetate (3 × 25 mL).

The combined organic layers were dried over anhydrous $Na_2SO_4$ and filtered, and the solvent was evaporated, leading to the corresponding oximes, which were recrystallized in diethyl ether.

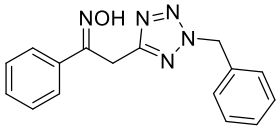
**2-(1-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-phenylethanone oxime[16] (5.45a)**



According to the general procedure: 0.928 g (3.5 mmol) of tetrazole **5.43a**.

Yield: 82% (0.971 g); white solid; m.p. 136.0-138.0 ºC (from diethyl ether) (lit. 138.0-140.0 ºC)[16]; IR $\tilde{\nu}$ = 3240, 2856, 1610, 1524, 1487,1347 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.38 (s, 2H, $CH_2$), 6.08 (s, 2H, $CH_2$), 7.36-7.37 (m, 3H, Ar), 7.66-7.67 (m, 2H, Ar), 8.21 (d, *J* = 8.4 Hz, 2H, Ar), 11.58 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 21.9, 54.6, 123.8, 126.0, 128.3, 128.8, 129.2, 134.4, 141.4, 147.4, 151.4, 163.0 ppm.

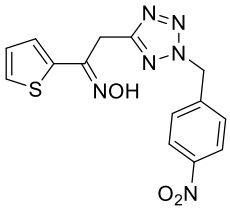
**2-(2-Benzyl-2*H*-tetrazol-5-yl)-1-phenylethanone oxime[16] (5.45b)**



According to the general procedure: 0.945 g (3.5 mmol) of tetrazole **5.43b**.

Yield: 92% (0.971 g); white solid; m.p. 100.0-100.5 ºC (from diethyl ether) (lit. 99.0-101.0 ºC)[16]; IR $\tilde{\nu}$ = 3070, 2934, 1492, 1447, 1275, 968 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.37 (s, 2H, $CH_2$), 5.85 (s, 2H, $CH_2$), 7.22-7.24 (m, 3H, Ar), 7.33-7.37 (m, 3H, Ar), 7.66-7.67 (m, 2H, Ar), 11.57 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 21.9, 55.6, 126.0, 128.0, 128.3, 128.4, 128.7, 128.9, 134.2, 135.4, 151.4, 162.7 ppm.

**2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-(thiophen-2-yl)ethanone oxime[16] (5.45c)**

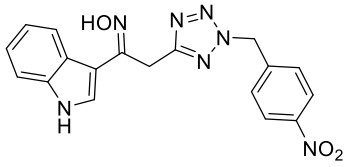


According to the general procedure: 1.15 g (3.5 mmol) of tetrazole **5.43c**. The obtained product was identified as a mixture of *E* and *Z* isomers.

Yield: 90% (1.08 g); white solid; m.p. 134.0-135.0 ºC (from diethyl ether) (lit. 133.0-138.0 ºC) [16]; IR $\tilde{\nu}$ = 3230, 3108, 2.960, 1618, 1530, 1490, 1430, 1342, 962 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.45 and 4.56 (s, 2H, $CH_2$), 5.83 and 5.91 (s, 2H, $CH_2$), 7.07 (*pseudo* t, *J* = 4.4 Hz, *J* = 4.0 Hz, 1H, Ar) and 7.16 (*pseudo* t, *J* = 4.4 Hz, 1H, Ar), 7.41 (d, *J* = 2.8 Hz, 1H, Ar), 7.56 (d, *J* = 3.2 Hz, 1H, Ar), 11.70 and 12.03 (br

s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 21.9, 55.6, 126.0, 128.0, 128.3, 128.4, 128.7, 128.9, 134.2, 135.4, 151.4, 162.7 ppm.

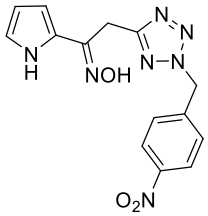
**1-(1*H*-Indol-3-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)ethanone oxime[16] (5.45d)**



According to the general procedure: 1.27 g (3.5 mmol) of tetrazole **5.43d**.

Yield: 96% (1.28 g); white solid; m.p. 194.0-195.5 ºC (from diethyl ether) (lit. 193.0-195.0 ºC)[16]; IR ṽ = 3242, 3074, 1615, 1520, 1495, 1440, 1342, 900 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 4.35 (s, 2H, $CH_2$), 6.05 (s, 2H, $CH_2$), 7.06 (*pseudo* t, *J* = 7.2 Hz, 7.6 Hz, 1H, Ar), 7.15 (*pseudo* t, *J* = 7.6 Hz, 7.2 Hz, 1H, Ar), 7.39 (d, *J* = 8.0 Hz, 1H, Ar), 7.51 (d, *J* = 8.4 Hz, 2H, Ar), 7.72 (s, 1H, Ar), 8.11 (d, *J* = 8.0 Hz, 1H, Ar), 8.21 (d, *J* = 8.4 Hz, 2H, Ar), 10.82 (br s, 1H, NH), 11.34 (br s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 22.8, 54.6, 111.9, 112.0, 119.8, 122.1, 122.4, 123.9, 124.4, 126.9, 129.2, 136.8, 141.5, 147.4, 149.3, 163.8 ppm.

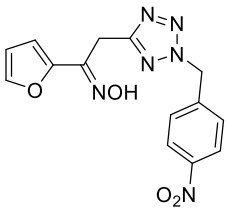
**2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-1-(1*H*-pyrrol-2-yl)ethanone oxime[16] (5.45e)**



According to the general procedure: 1.10 g (3.5 mmol) of tetrazole **5.43e**. The obtained product was identified as a mixture of *E* and *Z* isomers.

Yield: 92% (1.06 g); light brown solid; m.p. 124.2-124.9 ºC (from diethyl ether) (lit. 124.0-126.0 ºC)[16]; IR ṽ = 3438, 3151, 1603, 1512, 1343, 1195, 982 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 4.09 and 4.19 (s, 2H, $CH_2$), 6.08 and 6.09 (s, 2H, $CH_2$), 6.35 and 6.48(s, 1H, Ar), 6.77 (d, *J* = 1.2 Hz, 1H, Ar) and 6.89 (d, *J* = 1.2 Hz, 1H, Ar), 7.54 (*pseudo* t, *J* = 8.4 Hz, *J* = 4.0 Hz, 1H, Ar), 8.22-8.25 (m,4H, Ar), 8.22-8.25 (m, 4H, Ar), 7.72 (s, 1H, Ar), 10.85 and 11.00 (br s, 1H, OH), 11.30 (br s, 2H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 21.7, 28.3, 54.1, 107.2, 107.7, 109.2, 112.0, 120.1, 120.8, 123.2, 123.4, 126. 7, 128.7, 128.8, 140.9, 141.0, 142.0, 144.9, 146.6, 146.9, 146.9, 162.8, 163.6 ppm.

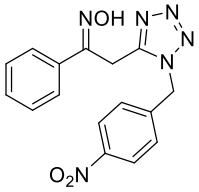
**1-(Furan-2-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)ethenone oxime[16] (5.45f)**



According to the general procedure: 1.27 g (3.5 mmol) of tetrazole **5.43f**. The obtained product was identified as mixture of *E* and *Z* isomers.

Yield: 92% (1.09 g); light yellow solid; m.p. 160.3-161.0 ºC (from diethyl ether) (lit. 160.0-162.0 ºC) [16]; IR ṽ = 3421, 3120, 1610, 1523, 1430, 1350, 1110, 985 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.31 and 4.37 (s, 2H, $CH_2$), 5.87 and 5.91 (s, 2H, $CH_2$), 6.57 (dd, *J* = 3.2 Hz, 1.6 Hz, 1H, Ar) and 6.64 (dd, *J* = 3.2 Hz, *J* = 1.6 Hz, 1H, Ar), 6.84 (d, *J* = 3.2 Hz, 1H, Ar) and 7.33 (d, *J* = 3.6 Hz, 1H, Ar), 7.50 (d, *J* = 8.8 Hz, 2H, Ar) and 7.54 (d, *J* = 8.8 Hz, 2H, Ar), 7.71 (dd, *J* = 8.0 Hz, 1.2 Hz, 2H, Ar), 8.24 (d, *J* = 8.4 Hz, 4H, Ar), 11.68 and 11.77 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 19.8, 25.1, 25.4, 48.9, 49.1, 67.0, 110.4, 111.7, 112.3, 117.2, 123.8, 123.8, 129.0, 129.3, 140.2, 141.9, 143.1, 144.2, 144.6, 147.3, 149.0, 152.4, 152.8 ppm.

**2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-1-phenylethanone oxime[16] (5.46)**



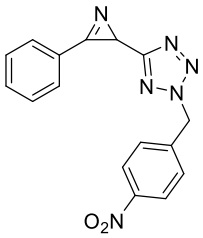

According to the general procedure: 1.13 g (3.5 mmol) of tetrazole **5.44a**. Yield: 84% (1.18 g); white solid; m.p. 172.2-173.4 ºC (from diethyl ether) (lit. 172.0-174.0 ºC)[16]; IR ṽ = 3216, 1610, 1526, 1508, 1415, 1349 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 4.43 (s, 2H, $CH_2$), 5.91 (s, 2H, $CH_2$), 7.37-7.39 (m, 3H, Ar), 7.50 (d, *J* = 8.4 Hz, 2H, Ar), 7.64-7.66 (m, 2H, Ar), 8.24 (d, *J* = 8.8 Hz, 2H, Ar), 11.8 (br s, 1H, OH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 19.9, 49.0, 123.8, 126.0, 128.4, 129.0, 129.1, 134.9, 141.8, 147.3, 150.4, 152.6 ppm.

### 6.8.4 Synthesis of tetrazolyl-2*H*-azirines

- **General procedure for the synthesis of 2*H*-Azirines 5.47 and 5.48**

The azirines were prepared following a known procedure.[16] To a solution of the appropriate oxime **5.45** or **5.46** (1.0 mmol) in dichloromethane (20 mL), at 0 ºC under a nitrogen atmosphere, was added triethylamine (1.3 equiv, 0.181 mL) and tosyl chloride (1.2 equiv, 0.229 g). The mixture was allowed to warm at room temperature, and the reaction mixture was stirred for 18 h. The solvent was evaporated under vacuum, and the crude reaction was dissolved in ethyl acetate (20 mL) and washed with water (3 × 10 mL). The organic layer was dried over anhydrous MgSO4 and filtered, and the solvent was evaporated under vacuum. The crude product was purified by flash chromatography (ethyl acetate:*n*-hexane) (1:1).

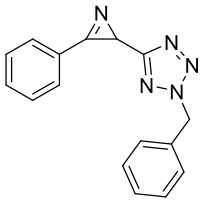
**2-(1-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-3-phenyl--2*H*-azirine[16] (5.47a)**



According to the general procedure: 0.338 g of oxime **5.45a**.

Yield: 65% (0.208 g); white solid; m.p. 129.7-131.2 ºC (from diethyl ether) (lit. 130.0-132.0 ºC)[16]; IR $\tilde{\nu}$ = 3.065, 2936, 1759, 1605, 1520, 1451, 1349 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 3.54 (s, 1H, CH), 5.80 (s, 2H, $CH_2$), 7.50 (d, $J$ = 8.4 Hz, 2H, Ar), 7.56-7.60 (m, 2H, Ar), 7.63-7.67 (m, 1H, Ar), 7.91-7.94 (m, 2H, Ar), 8.22 (d, = 8.8 Hz, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 25.8, 55.7, 122.8, 124.2, 129.3, 129.4, 130.3, 133.9, 139.7, 148.3, 160.7, 167.5 ppm.

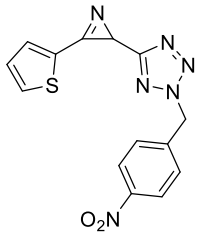
**2-(2-Benzyl-2*H*-tetrazol-5-yl)-3-phenyl-2*H*-azirine[16] (5.47b)**



According to the general procedure: 0.293 g of oxime **5.45b**.

Yield: 76% (0.209 g); light yellow solid; m.p. 82.7-84.3ºC (from diethyl ether) (lit. 83.0-85.0 ºC)[16]; IR $\tilde{\nu}$ = 3030, 1749, 1594, 1502, 140, 1274 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 3.26 (s, 1H, CH), 5.61 (d, $J$ = 15.2 Hz, 1H, CH), 5.73 (d, $J$ = 15.6 Hz, 1H, CH), 7.30-7.32 (m, 3H, Ar), 7.49-7.53 (m, 2H, Ar), 7.59-7.63 (m, 1H, Ar), 7.74-7.76 (m, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 25.0, 56.8, 123.0, 128.5, 129.0, 129.3, 130.3, 133.1, 133.7, 160.9, 167.0 ppm.

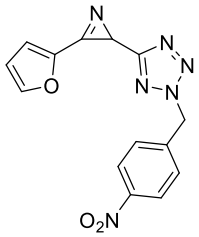
**2-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-3-(thiophen-2-yl)-2*H*-azirine[16] (5.47c)**



According to the general procedure: 0.344 g of oxime **5.45c**.

Yield: 66% (0.215 g); light yellow solid; m.p. 119.0-120.8ºC (from diethyl ether) (lit. 119-121 ºC)[16]; IR $\tilde{\nu}$ = 3109, 3070, 1740, 1605, 1562, 1452, 1330 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 3.68 (s, 1H), 6.10 (s, 2H), 7.42 (pseudo t, $J$ = 4.4 Hz, 4.0 Hz, 1H), 7.60 (d, $J$ = 8.4 Hz, 2H), 7.95 (d, $J$ = 2.8 Hz, 1H), 8.26 (d, $J$ = 8.4 Hz, 2H), 8.33 (d, $J$ = 4.8 Hz, 1H) (m, 2H, Ar), 7.59-7.63 (m, 1H, Ar), 7.74-7.76 (m, 2H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 24.9, 54.9, 124.0, 124.4, 129.3, 129.6, 136.3, 136.9, 141.1, 147.5, 153.7, 166.3 ppm.

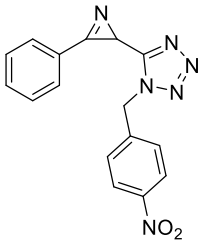
**3-(Furan-2-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2*H*-azirine[16] (5.47d)**



According to the general procedure: 0.329 g of oxime **5.45f**.

Yield: 50% (0.328 g); light brown solid; m.p. 111.0-112.9 ºC (from diethyl ether) (lit. 112.0-114.0 ºC)[16]; IR $\tilde{\nu}$ = 3.132, 3005, 1762, 1607, 1520, 1463, 1351 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 3.71 (s, 1H, CH), 6.15 (s, 2H, $CH_2$), 6.97 (d, $J$ = 1.6 Hz, 1H, Ar), 7.64− 7.68 (m, 3H, Ar), 8.31 (d, $J$ = 8.4 Hz, 2H, Ar), 8.37 (br s, 1H, Ar) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 23.8, 55.1, 113.5, 122.7, 124.0, 129.6, 139.0, 141.1, 147.5, 150.0, 150.8, 166.1 ppm.

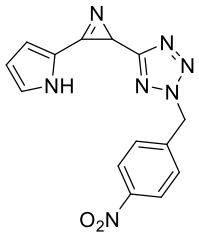
**2-(1-(4-Nitrobenzyl)-1*H*-tetrazol-5-yl)-3-Phenyl-2*H*-azirine[16] (5.48)**



According to the general procedure: 0.338 g of oxime **5.46**.

Yield: 86% (0.275 g); white solid solid; m.p. 129.5-131.5 ºC (from diethyl ether) (lit. 130.0-132.0 ºC)[16]; IR $\tilde{\nu}$ = 3.132, 3005, 1762, 1607, 1520, 1463, 1351 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) δ = 3.79 (s, 1H, CH), 6.11 (s, 2H, $CH_2$), 7.61 (d, $J$ = 8.8 Hz, 2H, Ar), 7.67 (t, $J$ = 7.6 Hz, 2H, Ar), 7.75− 7.79 (m, 1H, Ar), 7.90-7.92 ( m, 2H, Ar), 8.29 (d, $J$ = 8.8 Hz, 2H) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) δ = 21.4, 49.3, 121.6, 124.2, 129.1, 129.8, 130.4, 134.5, 142.0, 147.3, 155.4, 159.5 ppm.

**3-(1*H*-Pyrrol-2-yl)-2-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2*H*-azirine[16] (5.47e)**



According to a known procedure with slight modifications:[20]

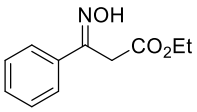
To a solutions of oxime (**5.45e**) (0.720 g, 2.2 mmol) and triethylamine (0.9 mL, 6.6 mmol, 3.0 equiv) in DCM (20 mL) at 0 ºC, was slowly added tosyl chloride (0.500 g, 2.6 mmol, 1.2 equiv), and the mixture was stirred at the same temperature for 2 h. The reaction was quenched with water, and the organic material was extracted three times with ethyl acetate (20 mL). The combined organic layers were washed with water, brine, and dried over anhydrous $Na_2SO_4$. The solvents were evaporated under vacuo, and the resulting crude materials were used immediately for the next step without further purification. To a solution of the crude ketoximetosylate in DCM (8 mL) at 0 ºC, DBU (0.4 mL, 2.6 mmol, 1.2 equiv) was slowly added, and the mixture was stirred at the same temperature for 2 h. The reaction was quenched with water, and the organic materials were extracted three times with DCM (20 mL). The combined organic layers were washed with brine and dried over anhydrous

$Na_2SO_4$. The solvents were evaporated under vacuo and the residue was purified by column chromatography (ethyl acetate:hexane) (1:1) to give the corresponding 2*H*-azirine. Recrystallization in diethyl ether.

Yield: 42% (0.286 g); light Brown oil; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 3.41 (s, 1H, CH), 6.08 (s, 2H, $CH_2$), 6.39 (dd, *J* = 2.4 Hz, 1.2 Hz, 1H, Ar), 6.95 (dd, *J* = 2.4 Hz, 1.2 Hz, 1H, Ar), 7.38 (dd, *J* = 1.2 Hz, 0.8 Hz, 1H, Ar), 7.59 (d, *J* = 8.8 Hz, 2H, Ar), 8.26 (d, *J* = 8.8 Hz, 2H), 12.49 (br s, 1H, NH) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ = 20.2, 54.8, 111.2, 114.9, 118.3, 124.0, 127.2, 129.6, 141.2, 147.5, 149.4, 167.3 ppm.

### 6.8.5 Synthesis of ethyl 3-(hydroxyimino)-3-phenylpropanoate

**Ethyl 3-(hydroxyimino)-3-phenylpropanoate[20](5.51)**

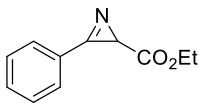



According to a known procedure with slight modifications:[20] to a mixture of ethyl 3-oxo-3-phenylpropanoate (0.423 g, 2.2 mmol, 1.0 equiv), hydroxylamine hydrochloride (0.160 g, 2.2 mmol, 1.0 equiv) and sodium acetate (0.210 g, 2.2 mmol, 1.0 equiv) was added methanol (15 mL) and water (0.7 mL). After stirring at room temperature for 4 h, the solvent was evaporated under vacuo. The reaction mixture was partitioned between $Et_2O$ and water. After separation, the organic layer was washed with saturated aqueous $NaHCO_3$, brine, dried over anhydrous $Na_2SO_4$ and solvents were evaporated under vacuo. Cold water (10 mL) and ice (10 g) were added, causing the oxime to precipitate. The oxime was filtered and recrystallized in diethyl ether.

Yield: 60% (0.273 g); white solid; m.p 125.0-126.5 ºC (from diethyl ether); IR $\tilde{\nu}$ = 3222, 3185, 2993, 1725, 1670, 1575, 1492, 1298, 1194 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ = 1.22 (t, J = 7.2 Hz, 3H, $CH_3$), 3.84 (s, 2H, $CH_2$), 4.17 (q, *J* = 7.2 Hz, 2H, $CH_2$), 7.39 (t, *J* = 3.2 Hz, 3H, Ar), 7.61-7.64 (m, 2H, Ar), 8.39 (br s, 1H, OH) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 14.1, 32.7, 61.3, 126.3, 128.6, 129.5, 135.3, 152.9, 168.8 ppm.

### 6.8.6 Synthesis of ethyl 3-phenyl-2*H*-azirine-2-carboxylate

**Ethyl 3-phenyl-2*H*-azirine-2-carboxylate[20] (5.53)**




According to a known procedure with slight modifications:[20] to a solution of oxime (0.456 g, 2.2 mmol, 1.0 equiv.) and triethylamine (0.9 mL, 6.6 mmol, 3.0 equiv) in DCM (20 mL), tosyl chloride (0.500

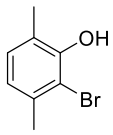
g, 2.6 mmol, 1.2 equiv) was slowly added, and the mixture was stirred at the same temperature for 2.5 h. The reaction was quenched with water, and the organic layer was extracted three times with ethyl acetate (20 mL). The combined organic layers were washed with water, brine, and dried over anhydrous $Na_2SO_4$. The solvents were evaporated under vacuo, and the resulting crude material was used immediately for the next step without further purification. To the crude of ketoximetosylate in DCM (8 mL) at 0 ºC, DBU (0.4 mL, 2.6 mmol, 1.2 equiv) was slowly added, and the mixture was stirred at the same temperature for 1h. The reaction quenched with water, and the organic layer was extracted with DCM (10 mL). The combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$. After the solvents were removed in vacuo, the residue was purified by column chromatography [ethyl:acetate:*n*-hexane (1:1)] to give the corresponding 2*H*-azirines.

Yield: 42% (0.174 g); yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) δ = 1.27 (t, *J* = 7.2 Hz, 3H, $CH_3$), 2.85 (s, 2H, $CH_2$), 4.22 (q, *J* = 7.2 Hz, 2H, $CH_2$), 7.56-7.60 (m, 2H, Ar), 7.63-7.67 (m, 1H, Ar), 7.88-7.91 (m, 2H, Ar) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 14.2, 29.7, 61.3, 122.4, 129.3, 130.5, 133.9, 158.6, 171.7 ppm.

### 6.8.7 Synthesis of 3,6-dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate

**Synthesis of 3,6-dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (5.24b)**

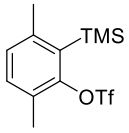
**2-bromo-3,6-dimethylphenol (5.55)**




The compound was synthesised following a known procedure with slight modifications:[21] in a flask equipped with a Soxhlet apparatus and flushed with argon, 2,5-dimethyphenol (**5.54**) (4.0 g 37,0 mmol, 1 equiv.) and diisopropylamine (0.52 ml, 0.37 g, 3.7 mmol, 0.1 equiv.) were dissolved in $CH_2Cl_2$ (100 mL). The thimble was filled with NBS (6.58 g, 37.0 mmol, 1 equiv.) and the system was heated to reflux for 16 h. During this time, the NBS was slowly consumed. After cooling to room temperature, the resulting mixture was treated with a 2M sulfuric acid solution (100 mL). The layers were separated, and the aqueous layer was extracted with *tert*-butylmethylether. The combined organic layers were washed with water and brine and dried over $Na_2SO_4$. The solvent was removed, and the crude product was purified by flash chromatography [elution with *n*-hexane]. Yield: 56% (4.17 g); colourless oil; $^1$H NMR

(400 MHz, $CDCl_3$): $\delta$ = 2.26 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 5.65 (s, 1H, OH), 6.69 (d, *J* = 7.6 Hz, 1H, Ar), 6.93 (d, *J* = 7.6 Hz, 1H, Ar) ppm.

**3,6-Dimethyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate[22] (5.24b)**



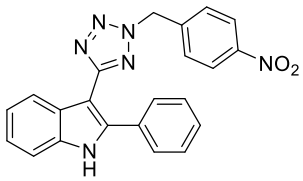

The compound was synthesised following a known procedure with slight modifications:[22] a mixture of 2-bromo-3,6-dimethylphenol (**5.55**) (4.17 g, 21.0 mmol, 1.0 equiv) and HMDS (5.22 mL, 4.02 g, 1.2 equiv) was stirred at reflux for 3 min under inert atmosphere ($N_2$). Excess of $NH_3$ and unreacted HMDS were then removed under vacuum. The formation of silyl ether was confirmed by $^1$H NMR. The crude product was dissolved in THF (0.15 M), the solution was cooled to –78 °C and *n*-BuLi ( 4.0 mL, 1.1 equiv) was added dropwise. The mixture was stirred for 3 h. Then, $Tf_2O$ (4.24 mL, 7,11g, 1.2 equiv) was added dropwise, and stirring was continued for 1 h. Cold aqueous saturated solution of $NaHCO_3$ was added, the phases were separated, and the aqueous layer was extracted with $Et_2O$. The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. Purification of the residue by column chromatography [elution with *n*-hexane] afforded the desired triflate.
Yield: 76% (5.21 g); yellow oil; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.34 (s, 3H, $CH_3$), 2.48 (s, 3H, $CH_3$), 7.09 (d, *J* = 7.6 Hz, 1H, Ar), 7.22 (d, *J* = 7.6 Hz, 1H, Ar) ppm.

### 6.8.8 Synthesis of 3-tetrazolyl-indoles

**General procedure for the synthesis of 3-Tetrazolyl-indoles**

A mixture of 18-crown-6 (0.530 g, 2.0 mmol, 4 equiv), KF (0.116 g, 2.0 mmol, 4 equiv) and the corresponding 2*H*-azirine **5.47**, **5.48 or 5.53** (0.5 mmol, 1.0 equiv) in dry THF (6 mL) under inert atmosphere was allowed to stir at 30 °C for 5 min. Then the reaction mixture was immediately transferred to a preheated oil bath at 60 °C and the stepwise addition of the appropriate aryne precursor **5.24** (1.0 mmol) dissolved in THF (6 mL) was performed. The progress of the reaction was monitored by TLC and upon completion (5 h) the mixture was filtered under celite, and the solvent was evaporated. Subsequently the crude residue was purified by flash column chromatography [eluting with gradient ethyl acetate/hexane from (1:3) to (1:1)] to afford the corresponding 3-tetrazolyl-indoles.

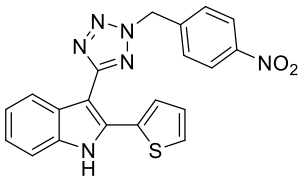
**3-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole** (**5.58a**)



According to the general procedure: 0.160 g of azirine **5.47a** and 0.298 g of triflate **5.31a**

Yield: 70% (0.112 g); yellow solid; m.p. 207.7–208.3 °C (diethyl ether/hexane); IR ṽ = 3267, 1588, 1456, 1245, 1066, 1028 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 5.82 (s, 2H, $CH_2$), 7.17 (*pseudo* t, *J* = 7.2 Hz, 2H, Ar), 7.31–7.35 (m, 1H, Ar), 7.41 (d, *J* = 6.8 Hz, 1H, Ar), 7.44-7.48 (m, 3H, Ar), 7.53 (d, *J* = 8.4 Hz, 2H, Ar), 7.59-7.61 (m, 2H, Ar), 7.69 (d, *J* = 8.0 Hz, 1H, Ar), 8.24 (d, *J* = 8.8 Hz, 2H, Ar), 9.11 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 111.4, 119.7, 120.2, 120.7, 120.9, 124.2, 124.6, 127.2, 128.1, 128.3, 129.6, 130.5, 133.3, 136.2, 139.6, 148.3, 160.4 ppm; HRMS (ESI): calcd. for $C_{22}H_{17}N_6O_2$ $[M + H]^+$ 397.1408 found 397.1407.

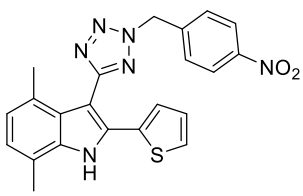
**3-(2-(4-Nitrobenzyl)-2*H*-tetrazol-5-yl)-2-(thiophen-2-yl)-1*H*-indole** (**5.58b**)



According to the general procedure: 0.163 g of azirine **5.47c** and 0.298 g of triflate **5.31a**.

Yield: 52% (0.105 g), yellow solid; m.p. 212.4–213.5 °C (diethyl ether/hexane); IR ṽ = 3239, 1585, 1525, 1232, 1087, 1028 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 5.86 (s, 2H, $CH_3$), 7.16-7.22 (m, 2H, Ar), 7.32 (*pseudo* t, *J* = 8,0 Hz, 7.2 Hz, 1H, Ar), 7.40-7.46 (m, 3H, Ar), 7.55 (d, *J* = 8.8 Hz, 2H, Ar), 7.87 (d, *J* = 8,0 Hz, 1H, Ar), 8.24 (d, *J* = 8.8 Hz, 2H, Ar), 9.25 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ = 55.8, 111.4, 112.2, 121.0, 121.1, 124.3, 124.8, 125.5, 127.1, 128.0, 129.5, 139.6, 148.3, 160.0 ppm; HRMS (ESI): calcd. for $C_{20}H_{15}N_6O_2S$ $[M + H]^+$ 403.0972 found 403.0970.

**4,7-Dimethyl-3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-(thiophen-2-yl)-1*H*-indole (5.58c)**

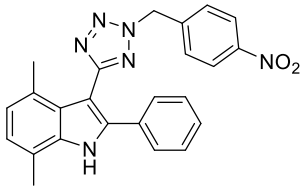


According to the general procedure: 0.163 g of azirine **5.47c** and 0.326 g of triflate **5.31b**.

Yield: 23% (0.038 g), yellow solid; m.p. 205.4–207.1 °C (diethyl ether/hexane); IR ṽ = 3229, 1595, 1514, 1341, 807, 787, 732; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 2.21 (s, 3H, $CH_3$), 2.53 (s, 3H, $CH_3$), 5.77 (s, 2H, $CH_2$), 6.82 (d, *J* = 7.2 Hz, 1H, Ar), 7.00 (d, *J* = 7.2 Hz, 1H, Ar), 7.04-7.05 (m, 1H, Ar), 7.13-7.15 (m, 1H, Ar), 7.44-7.48 (m, 3H, Ar), 8.21 (d, *J* = 8.8 Hz, Ar), 9.05 (br s, 1H, NH) ppm; $^{13}C$

NMR (100 MHz, $CDCl_3$): $\delta$ = 16.3, 19.0, 55.6, 112.2, 118.4, 122.6, 122.9, 124.1, 124.8, 126.1, 126.7, 127.1, 129.1, 129.6, 130.1, 135.5, 136.0, 139.5, 148.3, 159.9 ppm; HRMS (ESI): calcd. for $C_{21}H_{22}N_6O_2$ $[M + H]^+$ 431.1271 found 431.1281.

**4,7-Dimethyl-3-(2-(4-nitrobenzyl)-2*H*-tetrazol-5-yl)-2-phenyl-1*H*-indole (5.58d)**



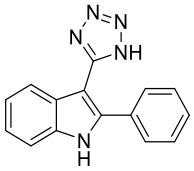
According to the general procedure: 0.160 g of azirine **5.47a** and 0.326g of triflate **5.31b**.

Yield: 37% (0.079 g), yellow solid; m.p. 209.2–211.0 °C (diethyl ether/hexane); IR $\tilde{\nu}$ = 3258, 1592, 1478, 1241, 808, 785, 734; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.08 (s, 3H, $CH_3$), 2.55 (s, 3H, $CH_3$), 5.72 (s, 2H, $CH_2$), 6.79 (d, $J$ = 7.2 Hz, 1H, Ar), 7.00 (d, $J$ = 7.2 Hz, 1H, Ar), 7.41-7.43 (m, 7H, Ar), 8.19 (d, $J$ = 8.4 Hz, 1H, Ar), 8.94 ( br s, 1H, NH) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 19.9, 29.3, 55.5, 118.3, 118.8, 120.8, 121.1, 122.2, 124.1, 124.7, 126.4, 127.2, 127.5, 129.6, 130.1, 131.3, 135.7, 139.5, 148.2, 160.3 ppm; HRMS (ESI): calcd. for $C_{24}H_{21}N_6O_2$ $[M + H]^+$ 425.1721 found 425.1718.

- **General procedure for the deprotection of compound 5.59 and synthesis of compound 5.60**

Compounds **5.59** and **5.60** were prepared following a known procedure.[23] Anhydrous ammonium formate (3.5 mmol) was added in a single portion to a stirred suspension of the appropriate indole **5.58** (0.10 mmol) and an equal weight of 10% Pd/C in methanol (10 mL) under a nitrogen atmosphere. The resulting mixture was stirred under reflux for 1 h. The mixture was cooled to room temperature, and the catalyst was removed by filtration through a Celite pad, which was further washed with methanol. The combined filtrate was evaporated under reduced pressure, and the resulting crude material was dissolved in ethyl acetate (25 mL), washed with aqueous 1 m HCl (2 x 25 mL), and dried with anhydrous $Na_2SO_4$. The solvent was evaporated, and the product was purified by crystallization from diethyl ether.

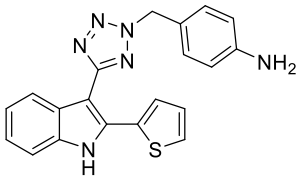
**2-Phenyl-3-(2*H*-tetrazol-5-yl)-1*H*-indole (5.59a)**



According to the general procedure: 0.040 g of indole **5.58a** and 0.220 g of ammonium formate.

Yield: 87% (0.023 g), Brown solid; m.p. > 185 °C (decomp., diethyl ether); IR $\tilde{\nu}$ = 3320, 1608, 1601, 1338, 1054, 775, 746 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 7.13-7.17 (m, 1H, Ar), 7.28-7.32 (m, 1H, Ar), 7.34-7.38 (m, 1H, Ar), 7.45-7.46 (m, 4H, Ar), 7.55 (d, *J* = 8.0 Hz, 1H, Ar), 7.64 (d, *J* = 8.4 Hz, 1H, Ar), 12.09 (br s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 112.7, 116.8 120.1, 120.9, 124.3, 127.2, 127.2, 129.0, 130.0, 130.5, 133.7, 137.2 ppm; HRMS (ESI): calcd. for $C_{15}H_{12}N_5$ $[M + H]^+$ 262.1087 found 262.1087.

**4-((5-(2-(thiophen-2-yl)-1*H*-indol-3-yl)-2*H*-tetrazol-2-yl)methyl)aniline (5.60)**



According to the general procedure: 0.040 g of indole **5.58b** and 0.220 g of ammonium formate.

Yield: 82% (0.031 g), white solid; m.p. > 185 °C (decomp., diethyl ether); IR $\tilde{\nu}$ = 3274, 1588, 1492, 1344, 1207 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ = 5.25 (s, 2H, NH), 5.75 (s, 2H, $CH_2$), 6.56 (d, *J* = 8.4 Hz, 2H, Ar), 7.12-7.18 (m, 4H, Ar), 7.26-7.30 (m, 1H, Ar), 7.37 (dd, *J* = 3.6 Hz, 2.4 Hz, 1H, Ar), 7.52 (d, *J* = 8.4 Hz, 2H, Ar), 7.58 (dd, *J* = 5.2 Hz, 4.0 Hz, 1H, Ar), 7.78 (d, *J* = 8.0 Hz, 1H, Ar), 12.15 (s, 1H, NH) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ = 56.9, 110.6, 112.7, 114.2, 120.2, 120.8, 121.0, 121.9, 124.2, 125.8, 127.5, 127.7, 130.2, 134.8, 136.9, 149.7, 159.3 ppm; HRMS (ESI): calcd. for $C_{20}H_{17}N_6S$: $[M + H]^+$ 373.1278 found 373.1281.

## 6.9 Biological assessment

### 6.9.1 Compounds 2.82a, 2.82e, 2.82d, 2.82c, 2.82f , 2.83f, 2.82g–2.82j, 2.85a–2.85d, 2.85f, 2.89 and 2.90

- **Cell culture**

For the evaluation of cytotoxic activity, six tumour cell lines were used: hepatocellular carcinoma (HepG2), human breast adenocarcinoma (MDA-MB-468), murine leukaemic monocyte macrophage (RAW 264.7), human acute monocytic leukaemia (THP1), human histiocytic leukaemia (U937) and murine T-lymphoma (thymoma; EL4); and two non-tumour cell lines: murine bone marrow (S17) and murine microglia (N9). HepG2, THP1, U937 and EL4 were cultured in RPMI 1960 culture medium supplemented with 10% heat-inactivated foetal bovine serum (FBS), 1% L-glutamine (2 mM), and 1% penicillin (50 U/ml)/streptomycin (50 mg/ml). MDA-MB-468, RAW-264.7, S17 and N9 cells were maintained in DMEM culture medium, supplemented with 10% heat-inactivated foetal bovine serum (FBS), 1% L-glutamine (2 mM), and 1% penicillin (50 U/ml)/streptomycin (50 mg/ml). All cell lines were maintained at 37 ºC in humid atmosphere with 5% $CO_2$.

- **Cytotoxicity assay**

The cytotoxic activity of the extracts was determined by the MTT colorimetric assay.[24] Briefly, exponentially growing cells were plated in 96-well tissue plates at a density of 5 x 103 cells/well (HepG2, MDA-MB-468 and S17) or 1 x 104 cells/well (THP1, U937, EL4, RAW 264.7 and N9). Adherent cell lines were previously incubated for 24 h to ensure adhesion to the wells. BIMs were applied at various concentrations (ranging between 0.078 and 200 mM) and control cells were treated with DMSO at the highest concentration used in test wells (0.5%). Cell viability was determined after 72 h of incubation with the BIMs/DMSO. Two hours prior to the end of the incubation period, 20 mL of MTT (5 mg/mL in PBS) were added to each well and further incubated at 37 ºC. 150 mL of DMSO were afterwards added to each well in order to dissolve the formazan crystals and absorbance was measured at 590 nm (Biotek Synergy 4). Results were expressed in half maximal inhibitory concentration ($IC_{50}$, mM). The selectivity of the BIMS was estimated using the following equation: Selectivity ¼ $IC_{50}$ NT/$IC_{50}$ VT, where VNT and VT indicate IC50 modulated by the compound on non-tumour and tumour cells, respectively.

### 6.9.2 Compound 2.87d

**Methods**

Briefly, HT29 human colorectal adenocarcinoma were plated in 96-well plates (100 cell/well) in undifferentiated medium in ultra-low attachment conditions for sphere formation and treated with a previously reported CSC-targeting agent (salinomycin; 1 μM - positive control), test compounds (concentrations ranging from 16 to 0.03125 μM) or vehicle control (DMSO 1 μM). Following 7 days of incubation, cell viability was assessed based on measurement of ATP, using the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega). All data analysis and $IC_{50}$ value calculations were performed using GraphPad Prism version 6.01.

After the primary screening in HT-29 cancer stem like cells (CSCs), dose-response curves were built using a 10-point concentration range of 0.03 – 16.00 μM for the hits. Compound **2.87d** showed a sigmoidal dose-response curve-fit and a half-maximal inhibitory concentration ($IC_{50}$) ≤ 2 μM and was tested on other colorectal cancer cell lines (SW-620, HCT116) enriched in CSCs. The anti-CSC effect of **2.87d** was further validated by assessing impact on tumorsphere formation. To measure tumorsphere formation, colon cancer cells were plated as single cells in 24-well ultra-low attachment plates (500 cells/well) and cultured in 1 mL serum-free DMEM/F12 supplemented with 2% B27 supplement, 1% N2 supplement, 1% non-essential amino acids, 1% sodium pyruvate, 1% penicillin-streptavidin, 4 μg/mL heparin, 40 ng/mL recombinant human EGF and 20 ng/mL recombinant human bFGF. Tumorspheres numbers were counted and spheres' area was determined using an image analysis software after spheroids images acquisition through brightfield microscopy. Salinomycin (anti-CSC agent) and 5-FU (traditional chemotherapeutic) were used as a positive control and negative control, respectively.

The $IC_{50}$ of **2.87d** was also determined on colorectal cancer cell lines plated in adherent conditions using a 10-point concentration range of 0.01 – 243.00 μM. Briefly, cell lines were seeded in 96-well plates (5,000 cells/well) in McCoy's 5A (HCT-116), RPMI 1640 (HT-29) or DMEM (SW-620), all supplemented with 10% FBS and 1% antibiotic/antimycotic solution. Seventy-two hours after plating cell viability was assessed based on measurement of MTS metabolism, using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay.

Finally, to evaluate potential compound toxicity, primary mouse hepatocytes were collected from male C57BL/6 mice and plated in 96-well plates (10,000 cells/well) in William's medium E supplemented with 10% FBS, 2% antibiotic/antimycotic and 0.1% insulin. After 4 h of plating, cells were treated with hits at concentrations ranging from 0.01 – 243.00 μM. MTS metabolism was assayed 48h after plating.

## 6.10 Minimum energy calculations

Quantum chemical calculations were carried out to explore the structure and the preferred conformations of molecules **5.47a** and **5.48**. One initial low-energy conformer of each molecule was generated using the Open Eye Omega software.[25, 26] These low-energy structures were then optimized at the DFT level of theory, using the B3LYP hybrid functional[27, 28] and the standard 6- 31G(d) basis set. All calculations were performed using the GAMESS program package.[29] Graphical representations were produced with Discovery Studio Visualizer v20.1.0.19295 software.

## 6.11 References

1. Armarego, W. L. F., *Purification of laboratory chemicals*. Eighth edition. ed.; Butterworth-Heinemann is an imprint of Elsevier: Amsterdam, **2017**, 1-1176.

2. Yoon, S. C.; Cho, J.; Kim, K., Reactions of 1-aryl-2,2-dihalogenoethanone oximes with tetrasulfur tetranitride (S4N4): a general method for the synthesis of 3-aryl-4-halogeno-1,2,5-thiadiazoles. *J Chem Soc Perk T 1* **1998**, (1), 109-116.

3. Pereira, N. A. M.; Lopes, S. M. M.; Lemos, A.; Melo, T. M. V. D. P. E., On-Water Synthesis of Dipyrromethanes via Bis-Hetero-Diels-Alder Reaction of Azo- and Nitrosoalkenes with Pyrrole. *Synlett* **2014**, *25* (3), 423-427.

4. Lemos, A.; Lourenco, J. P., Cycloaddition reactions of nitrosoalkenes, azoalkenes and nitrile oxides mediated by hydrotalcite. *Arkivoc* **2010**, 170-182.

5. Matunas, R.; Lai, A. J.; Lee, C., Iridium-catalyzed selective N-allylation of hydrazines. *Tetrahedron* **2005**, *61* (26), 6298-6308.

6. South, M. S.; Jakuboski, T. L.; Westmeyer, M. D.; Dukesherer, D. R., Synthesis and Reactions of Haloazodienes. A New and General Synthesis of Substituted Pyridazines. *J. Org. Chem.* **1996**, *61* (25), 8921-8934.

7. Sakai, K.; Hida, N.; Kondo, K., Reactions of Alpha-Polyhalo Ketone Tosylhydrazones with Sulfide Ion and Primary Amines - Cyclization to 1,2,3-Thiadiazoles and 1,2,3-Triazoles. *B. Chem. Soc. Jpn.* **1986**, *59* (1), 179-183.

8. Maynard, J. A., Use of Diethyl Phosphonate as a Solvent and Catalyst for Preparation of Arylhydrazones. *Aust. J. Chem.* **1962**, *15* (4), 867-868.

9. Bocchi, V.; Casnati, G.; Dossena, A.; Villani, F., Synthesis of N-Alkylindoles Using Tetraalkylammonium Salt Catalysis. *Synthesis-Stuttgart* **1976**, (6), 414-416.

10. Tang, Q.; Chen, X.; Tiwari, B.; Chi, Y. R., Addition of Indoles to Oxyallyl Cations for Facile Access to α-Indole Carbonyl Compounds. *Org Lett* **2012**, *14* (7), 1922-1925.

11. Wang, X.; Aldrich, C. C., Development of an imidazole salt catalytic system for the preparation of bis(indolyl)methanes and bis(naphthyl)methane. *PLoS One* **2019**, *14* (4), 1-17.

12. Pines, S. H.; Chemerda, J. M.; Kozlowsk.Ma, Cleavage of Oximes with Bisulfite . A General Procedure. *J. Org. Chem.* **1966**, *31* (10), 3446-3347.

13. Miles, D. H.; Guasch, J.; Toste, F. D., A Nucleophilic Strategy for Enantioselective Intermolecular alpha-Amination: Access to Enantioenriched alpha-Arylamino Ketones. *J. Am. Chem. Soc.* **2015**, *137* (24), 7632-7635.

14. Brintzinger, H.; Titzmann, R., Notiz über einige halogenierte aliphatische Oxime. *Ber. Dtsch. Chem. Ges.* **2006**, *85* (4), 344-345.

15. Ji, Y. H.; Trenkle, W. C.; Vowles, J. V., A high-yielding preparation of beta-ketonitriles. *Org. Lett.* **2006**, *8* (6), 1161-1163.

16. Cardoso, A. L.; Gimeno, L.; Lemos, A.; Palacios, F.; Melo, T. M. V. D. P. E., The Neber Approach to 2-(Tetrazol-5-yl)-2H-Azirines. *J. Org. Chem.* **2013**, *78* (14), 6983-6991.

17. Slatt, J.; Romero, I.; Bergman, J., Cyanoacetylation of indoles, pyrroles and aromatic amines with the combination cyanoacetic acid and acetic anhydride. *Synthesis-Stuttgart* **2004**, (16), 2760-2765.

18. Demko, Z. P.; Sharpless, K. B., Preparation of 5-Substituted 1H-Tetrazoles from Nitriles in Water. *J. Org. Chem.* **2001**, *66* (24), 7945-7950.

19. Fetter, J.; Nagy, I.; Giang, L. T.; Kajtár-Peredy, M.; Rockenbauer, A.; Korecz, L.; Czira, G., The reaction of 2-(tetrazol-5-yl)alkyl ketones and of 2-(tetrazol-5-yl)alkanoic acid derivatives with lead tetraacetate. A novel method of preparation of alk-2-ynyl ketones and alk-2-ynoic acid derivatives†. *J. Chem. Soc., Perkin trans.* **2001**, (9), 1131-1139.

20. Wang, Y.; Lei, X.; Tang, Y., Rh(ii)-catalyzed cycloadditions of 1-tosyl 1,2,3-triazoles with 2H-azirines: switchable reactivity of Rh-azavinylcarbene as [2C]- or aza-[3C]-synthon. *ChemComm* **2015**, *51* (21), 4507-4510.

21. Velder, J.; Robert, T.; Weidner, I.; Neudörfl, J.-M.; Lex, J.; Schmalz, H.-G., Modular Synthesis of Chiral Phosphine-Phosphite-Ligands from Phenolic Precursors: A New Approach to Bidentate Chelate Ligands Exploiting a P□O to P□C Migration Rearrangement. *Adv. Synth. Catal.* **2008**, *350* (9), 1309-1315.

22. Guitián, E.; Pérez, D.; Peña, D.; Cobas, A., An Efficient Procedure for the Synthesis ofortho-Trialkylsilylaryl Triflates: Easy Access to Precursors of Functionalized Arynes. *Synthesis* **2002**, (10), 1454-1458.

23. Ram, S.; Spicer, L. D., Rapid debenzylation of N-benzylamino derivatives to amino-derivatives using ammonium formate as catalytic hydrogen transfer agent. *Tetrahedron Lett.* **1987**, *28* (5), 515-516.

24. Mosmann, T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **1983**, *65* (1-2), 55-63.

25. Hawkins, P. C. D.; Nicholls, A., Conformer Generation with OMEGA: Learning from the Data Set and the Analysis of Failures. *J. Chem. Inf. Model.* **2012**, *52* (11), 2919-2936.

26. Hawkins, P. C. D.; Skillman, A. G.; Warren, G. L.; Ellingson, B. A.; Stahl, M. T., Conformer Generation with OMEGA: Algorithm and Validation Using High Quality Structures from the Protein Databank and Cambridge Structural Database. *J. Chem. Inf. Model.* **2010**, *50* (4), 572-584.

27. Becke, A. D., Density-functional exchange-energy approximation with correct asymptotic behavior. *Physical Review A* **1988**, *38* (6), 3098-3100.

28. Lee, C.; Yang, W.; Parr, R. G., Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Physical Review B* **1988**, *37* (2), 785-789.

29. Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A., General atomic and molecular electronic structure system. *Journal of Computational Chemistry* **1993**, *14* (11), 1347-1363.

# Appendices

## Appendix 1. Representative $^{1}H$, $^{13}C$ and 2D NMR spectra of the new compounds

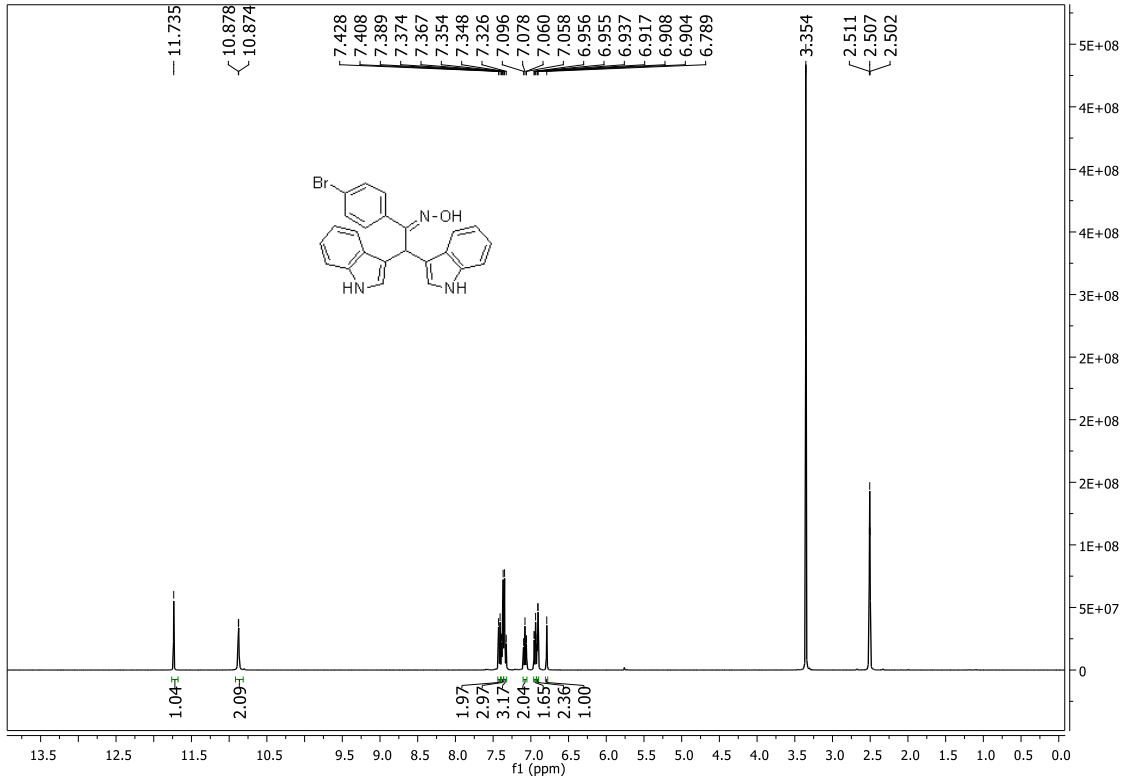



**Figure 1.** $^{1}H$ RMN spectrum of compound **2.82a** (DMSO-$d_6$).

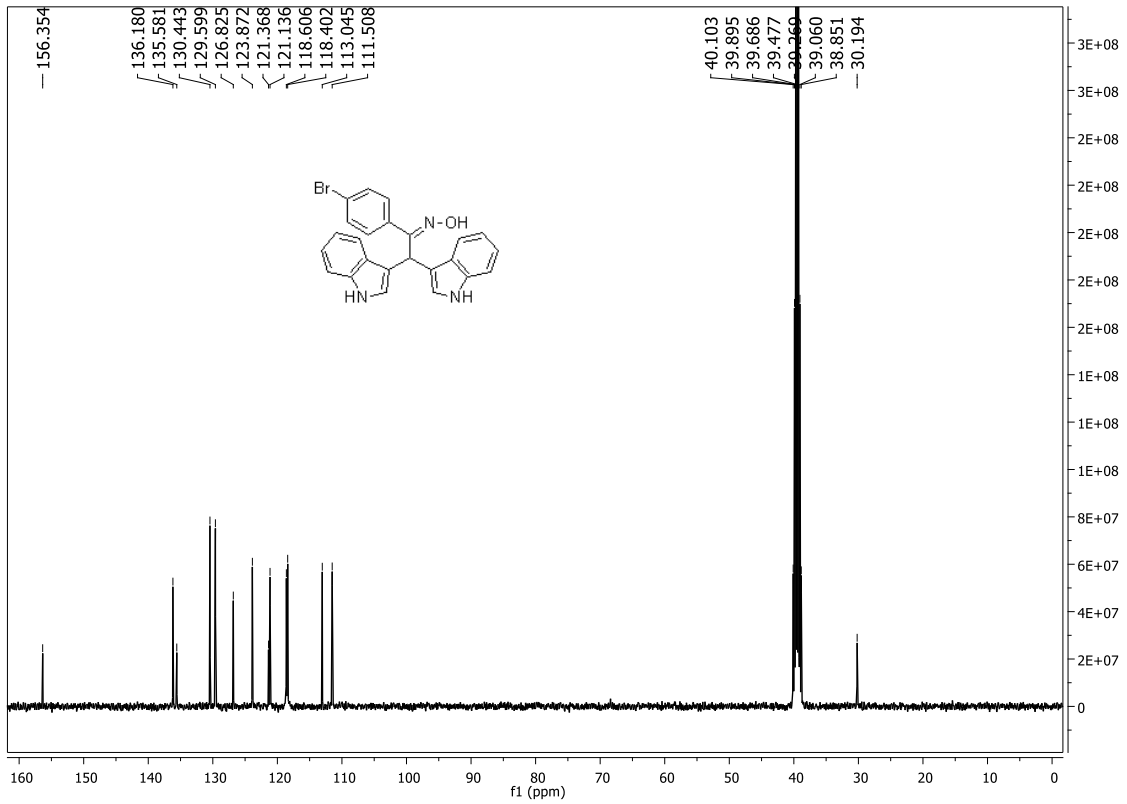



**Figure 2.** $^{13}C$ RMN spectrum of compound **2.82a** (DMSO-$d_6$).

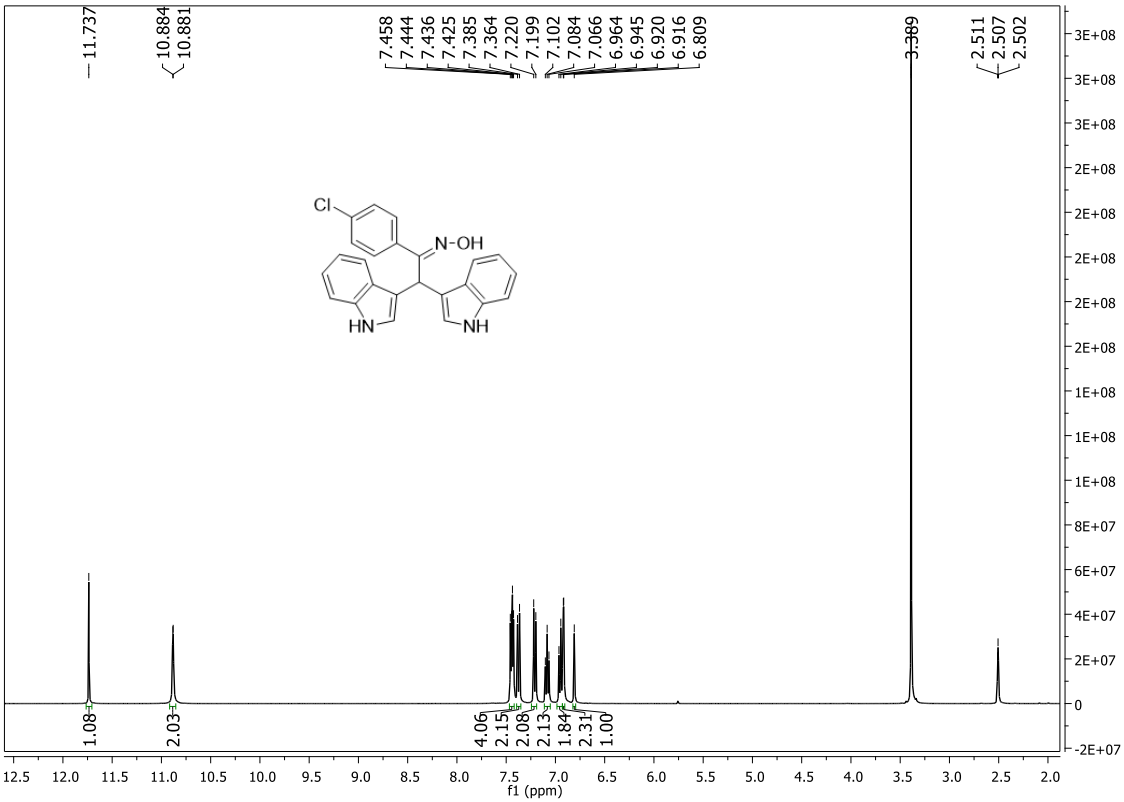



**Figure 3.** $^{1}$H RMN spectrum of compound **2.82b** (DMSO-$d_6$).

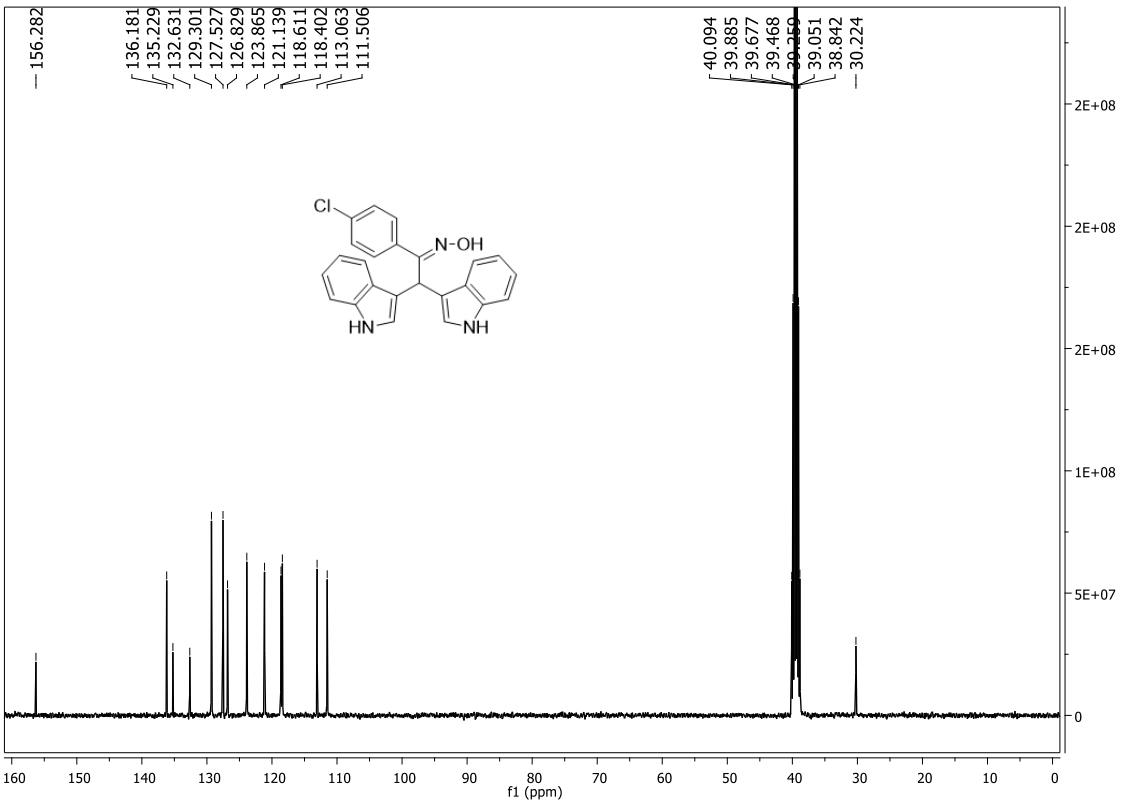



**Figure 4.** $^{13}$C RMN spectrum of compound **2.82b** (DMSO-$d_6$).

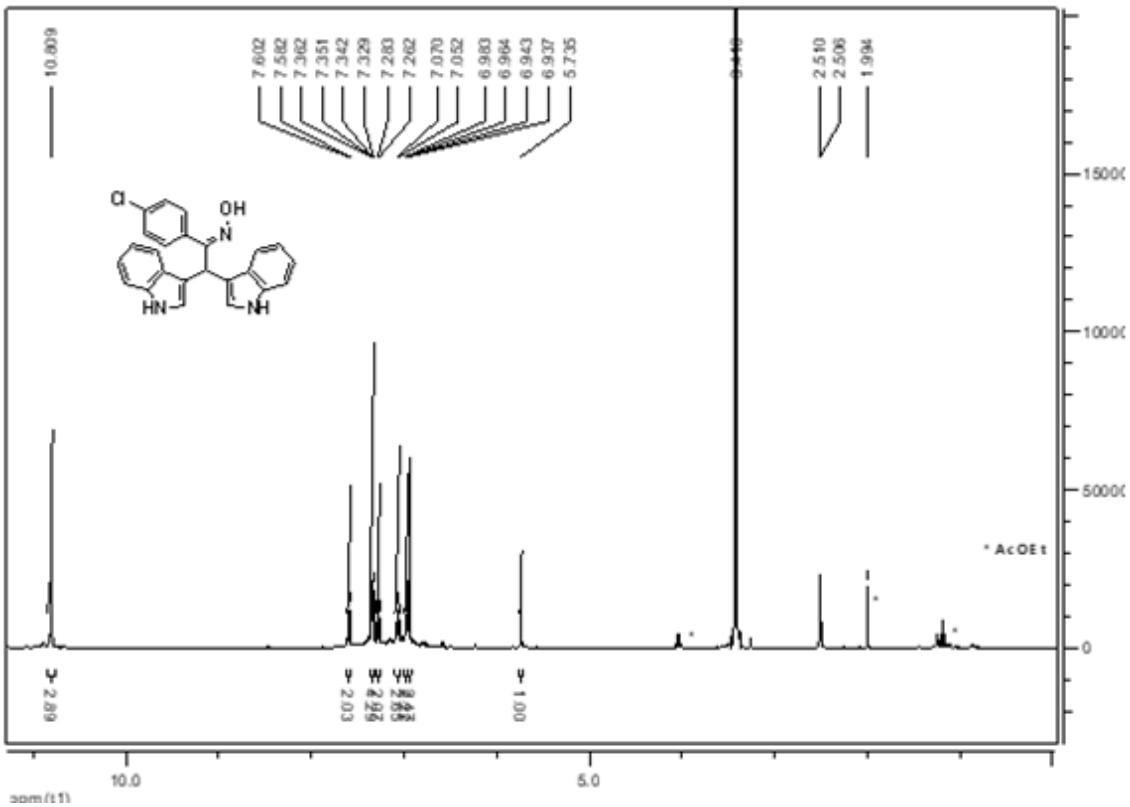



**Figure 5.** $^{1}$H RMN spectrum of compound **2.83b** (DMSO-$d_6$).

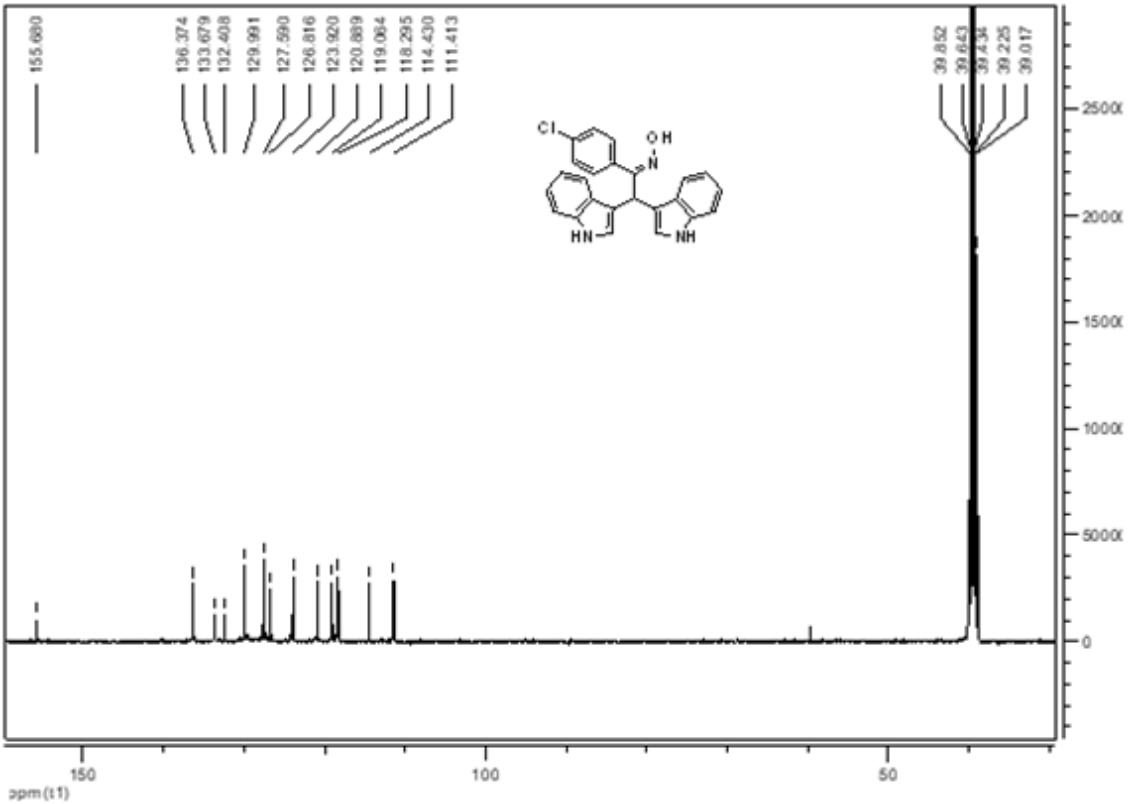



**Figure 6.** $^{13}$C RMN spectrum of compound **2.83b** (DMSO-$d_6$).

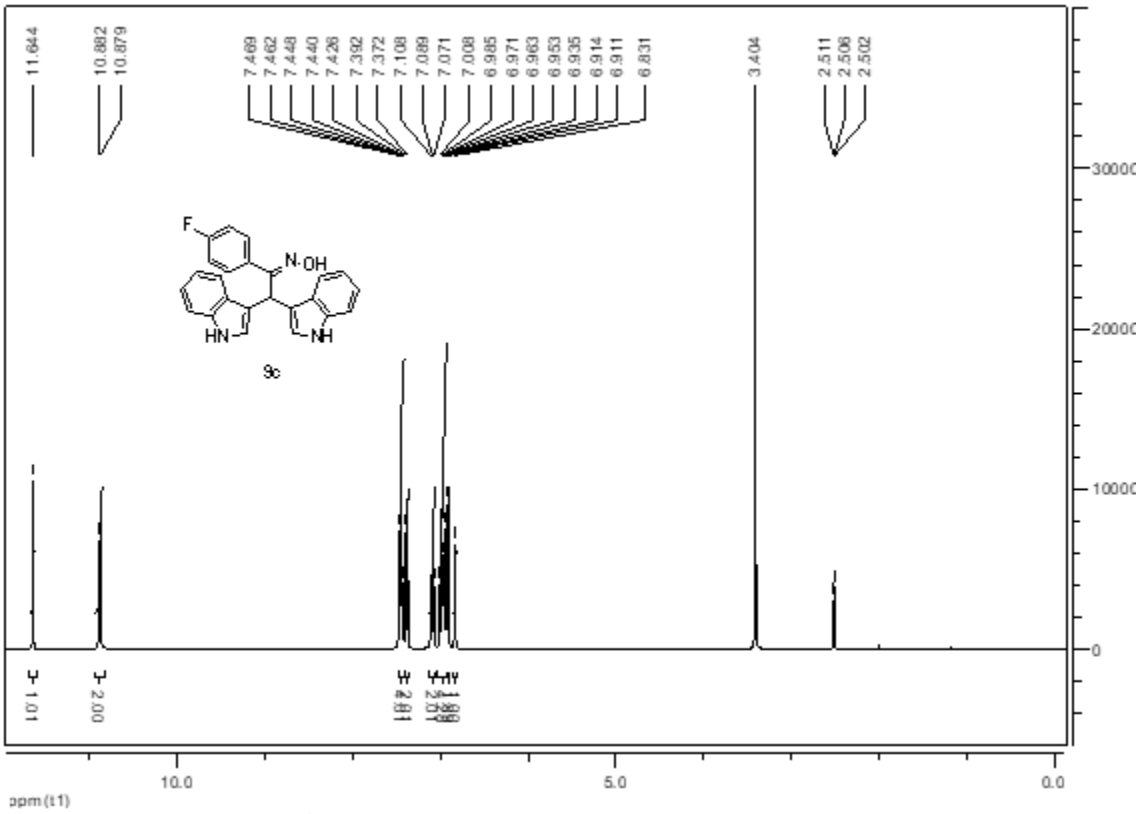



**Figure 7.** $^{1}$H RMN spectrum of compound **2.82c** (DMSO-$d_6$).

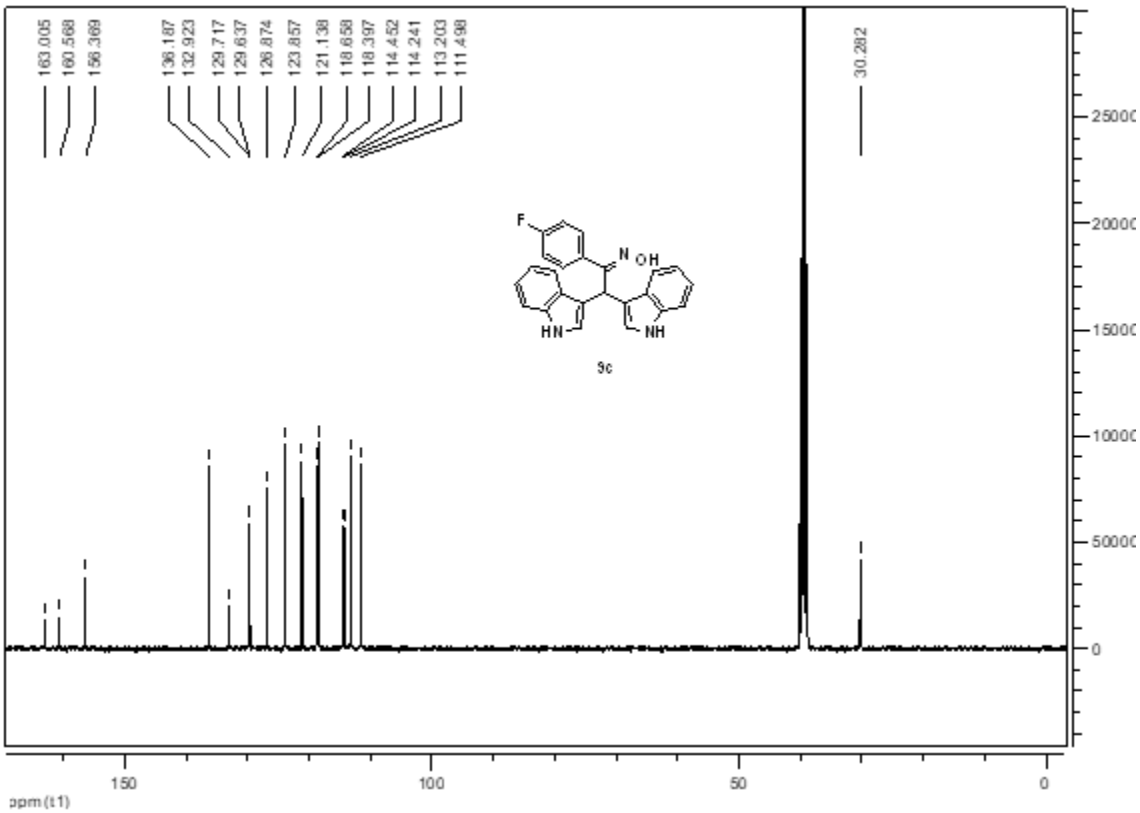



**Figure 8.** $^{13}$C RMN spectrum of compound **2.82c** (DMSO-$d_6$).

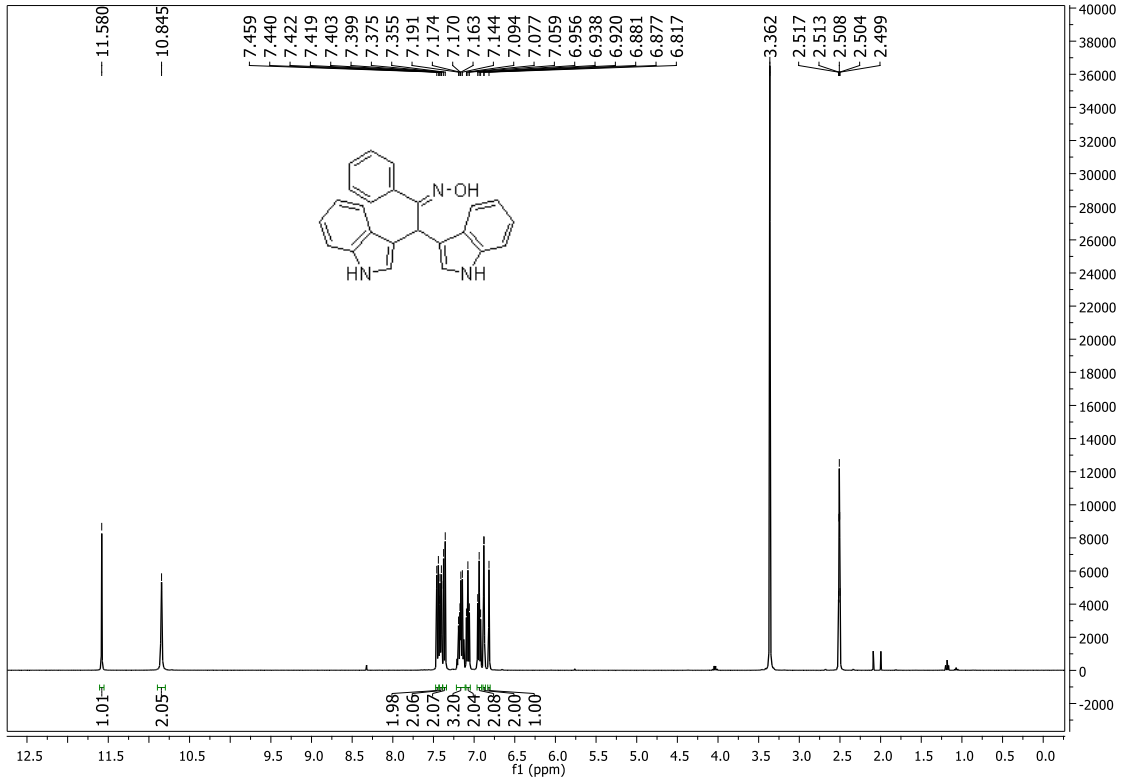



**Figure 9.** $^{1}$H RMN spectrum of compound **2.82d** (DMSO-$d_6$).

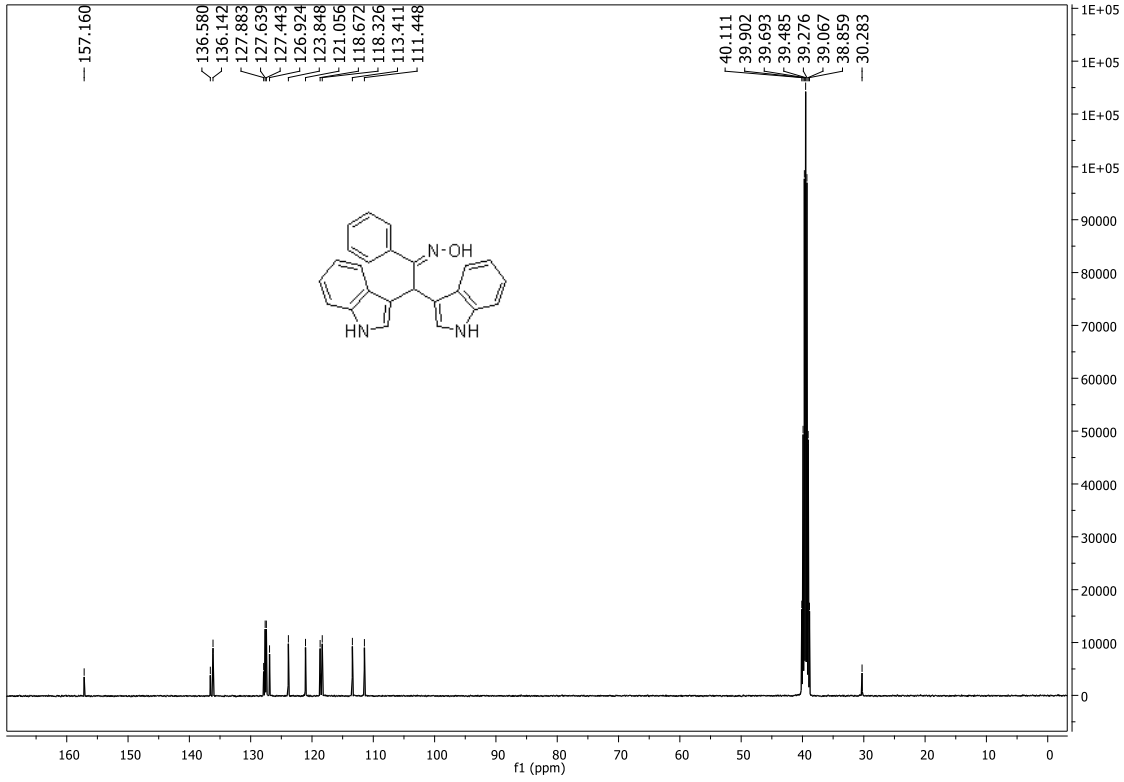



**Figure 10.** $^{13}$C RMN spectrum of compound **2.82d** (DMSO-$d_6$).

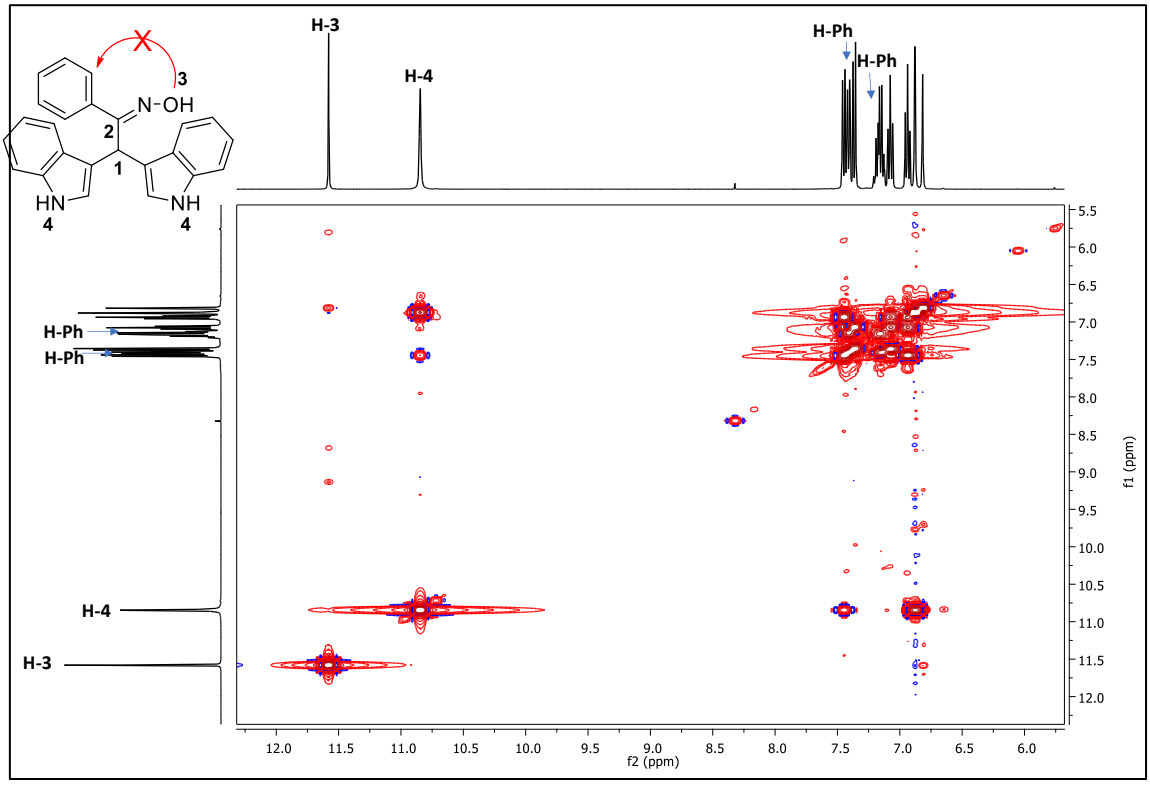



**Figure 11.** Expansion of the NOESY spectrum of compound **2.82d**.

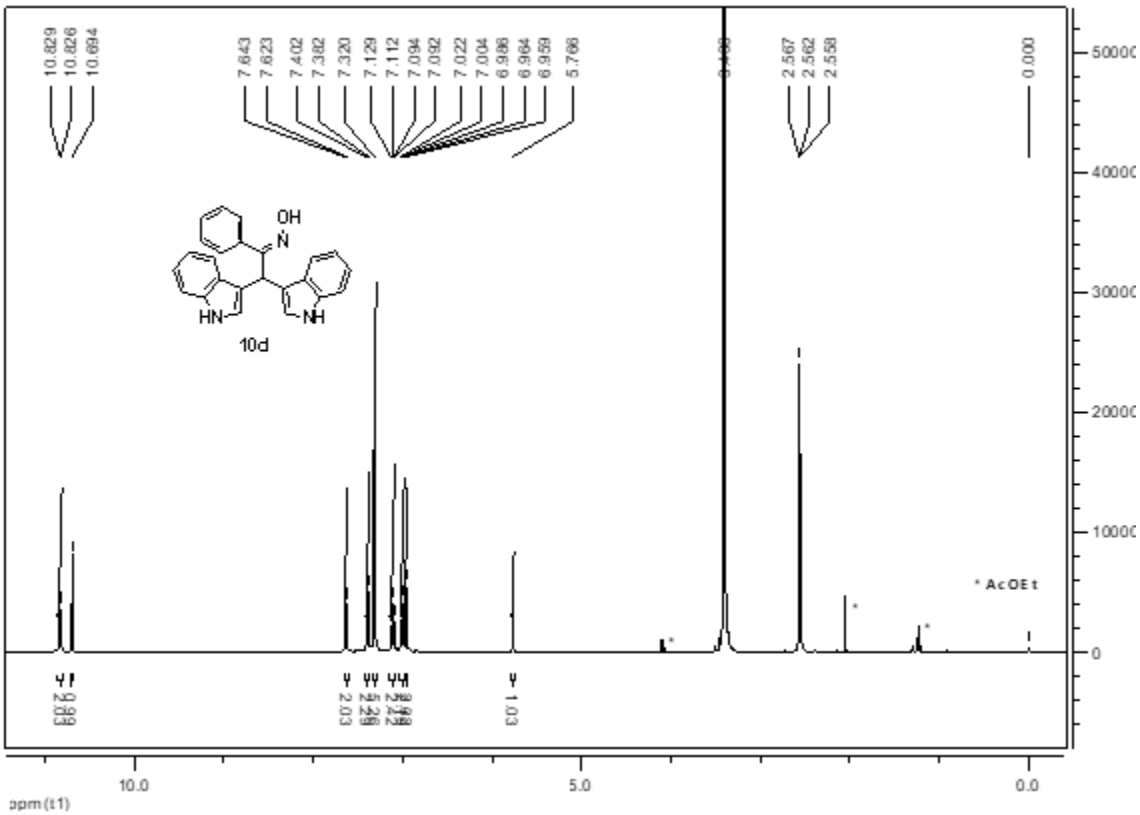



**Figure 12.** $^{1}$H RMN spectrum of compound **2.83d** (DMSO-$d_6$).

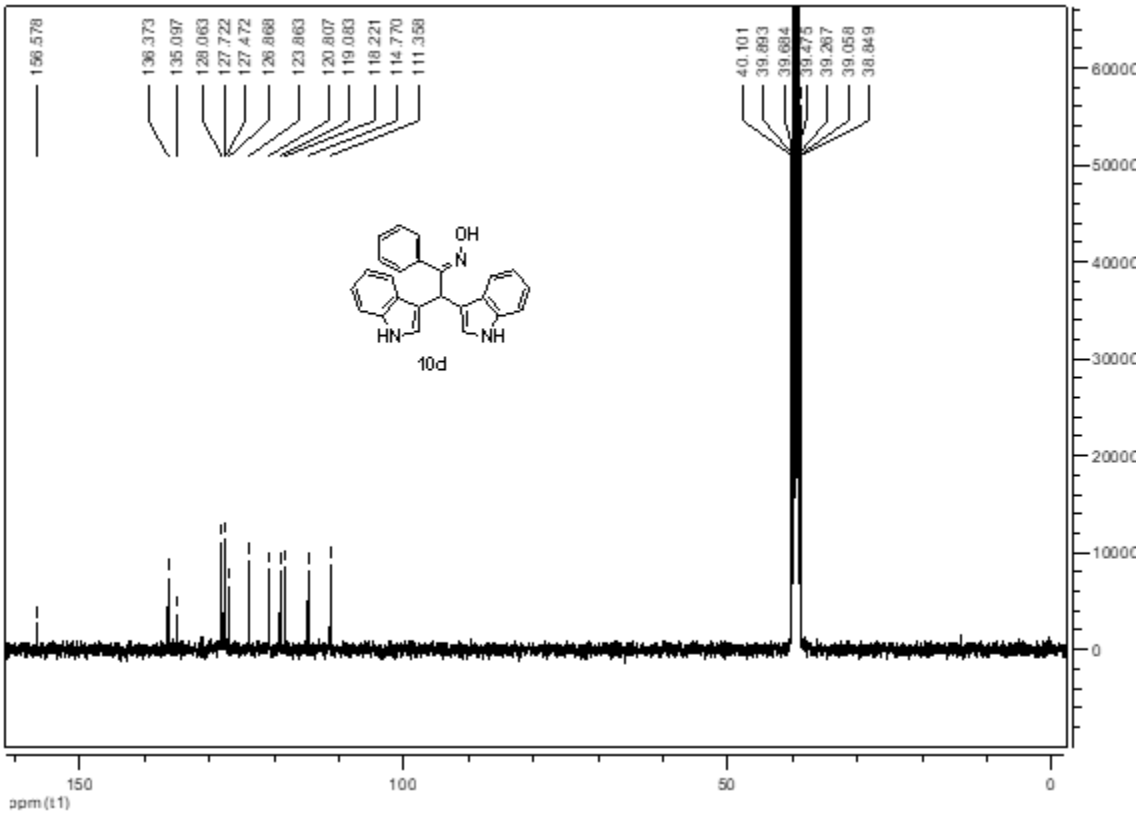



**Figure 13** $^{13}$C RMN spectrum of compound **2.83d** (DMSO-$d_6$).

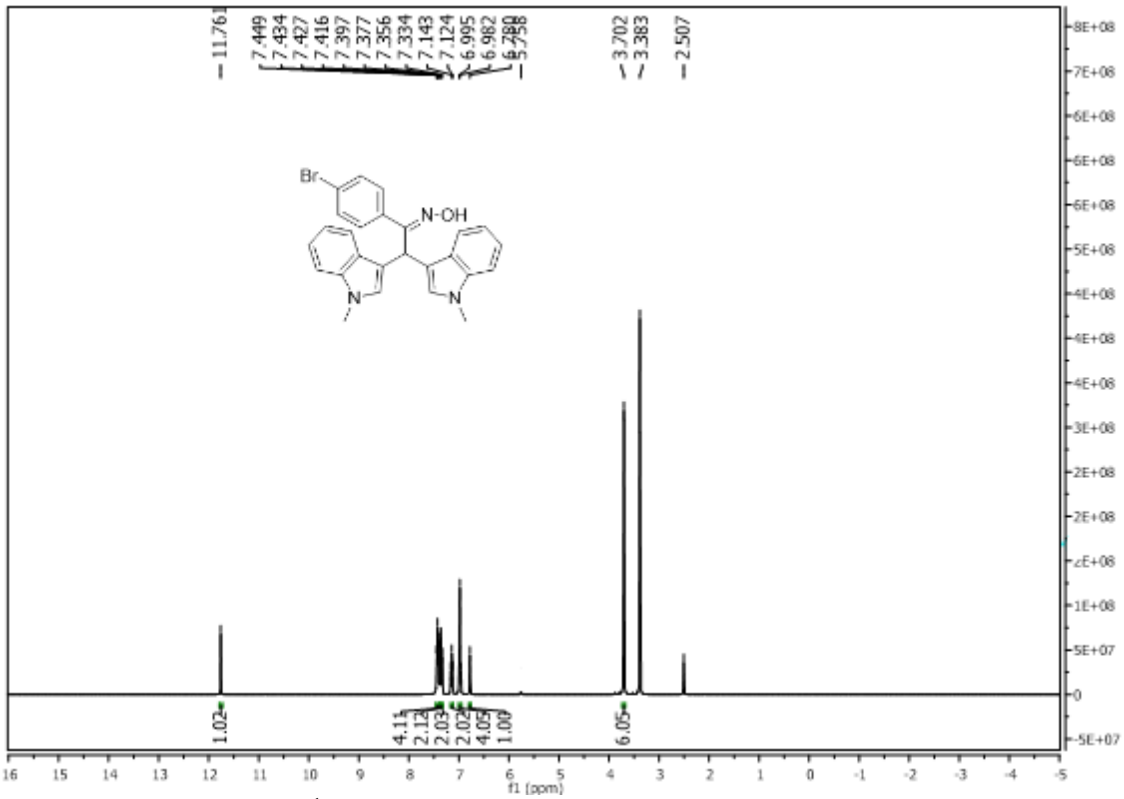



**Figure 14.** $^{1}$H RMN spectrum of compound **2.82e** (DMSO-$d_6$).

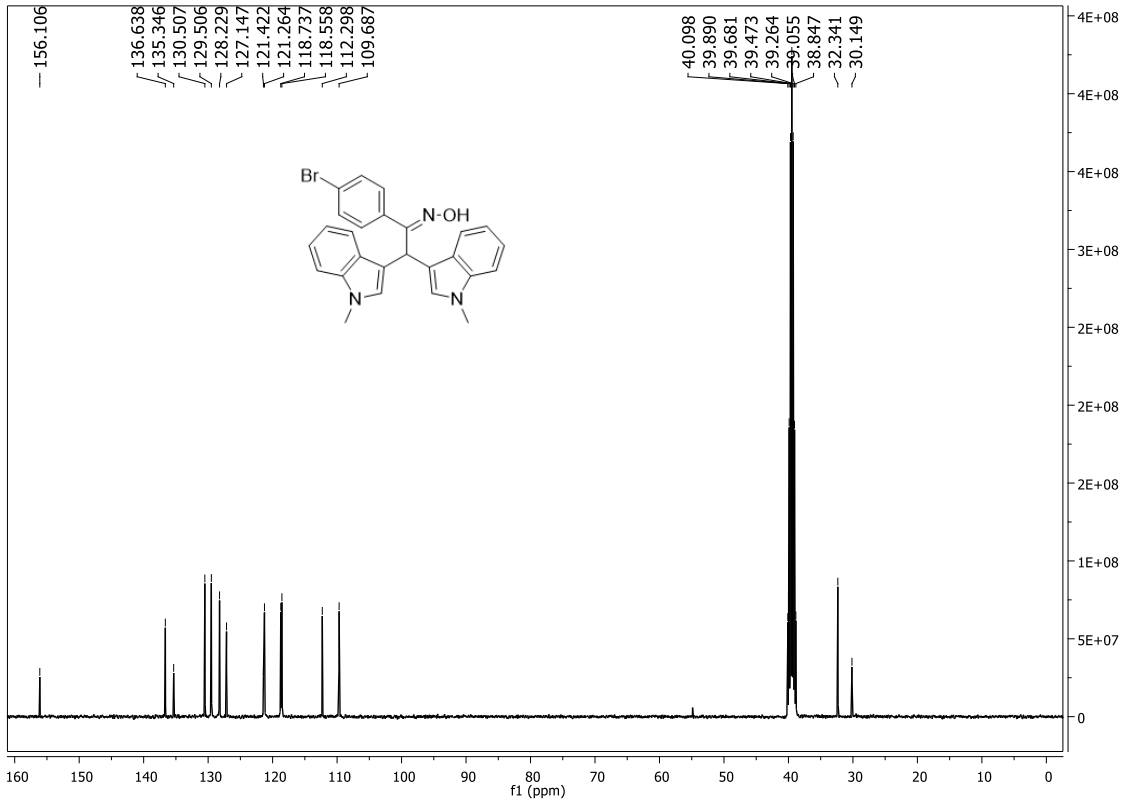



**Figure 15.** $^{13}$C RMN spectrum of compound **2.82e** (DMSO-$d_6$).

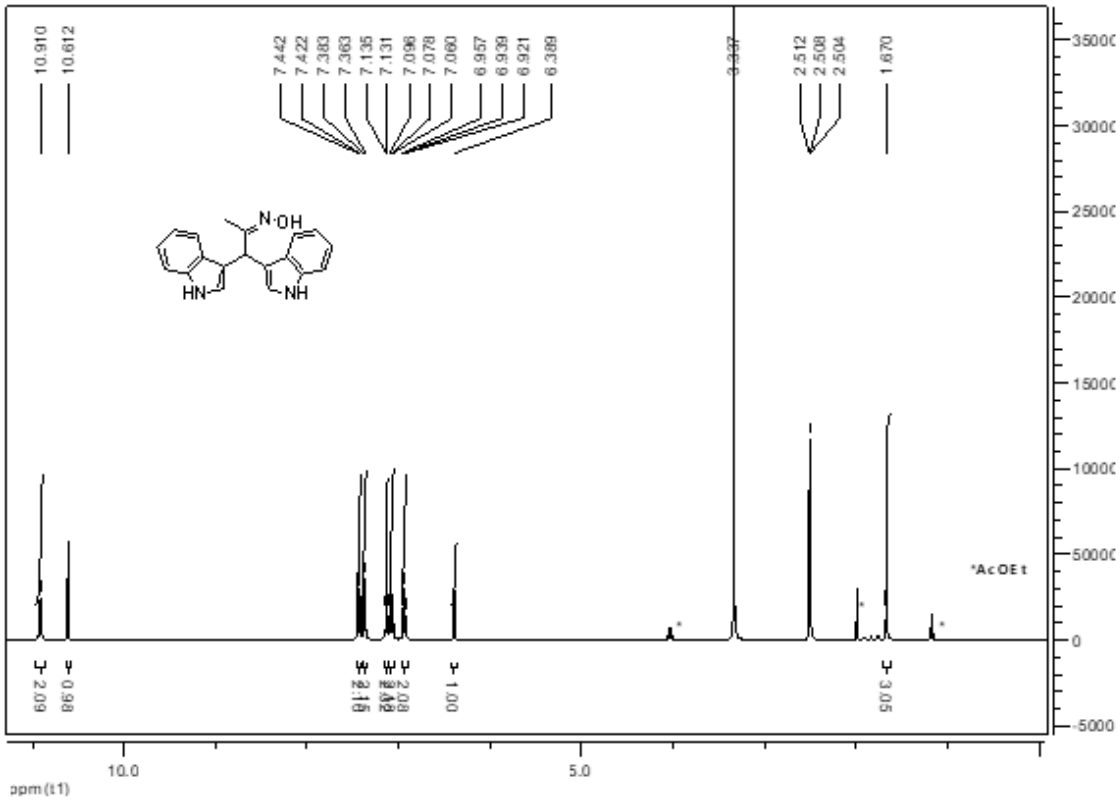



**Figure 16.** $^{1}$H RMN spectrum of compound **2.82f** (DMSO-$d_6$).

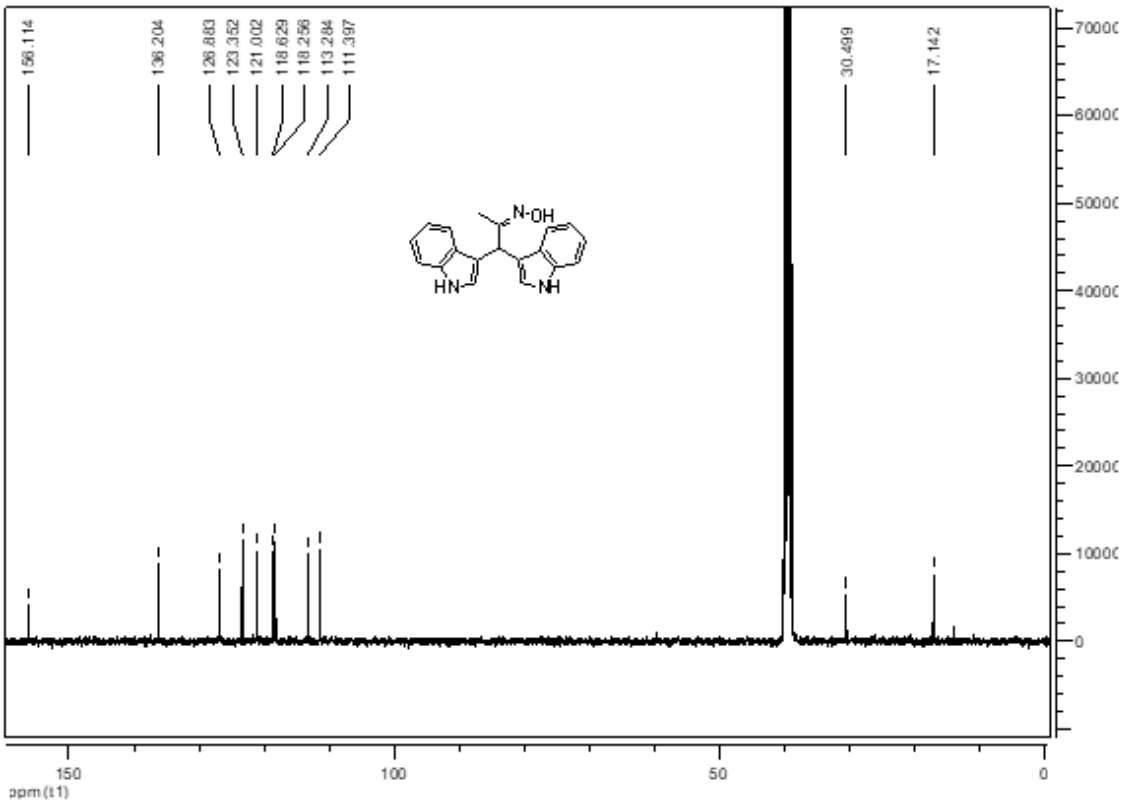



**Figure 17.** $^{13}$C RMN spectrum of compound **2.82f** (DMSO-$d_6$).

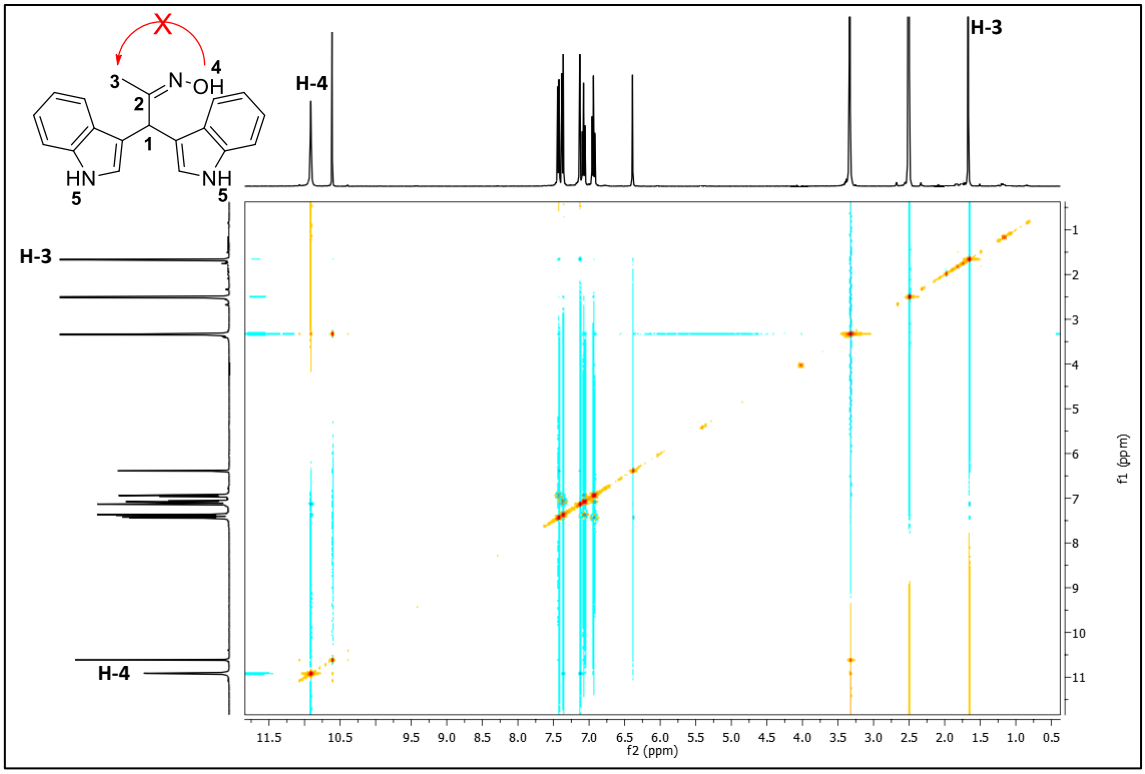



**Figure 18.** NOESY spectrum of compound **2.82f** (DMSO-$d_6$).

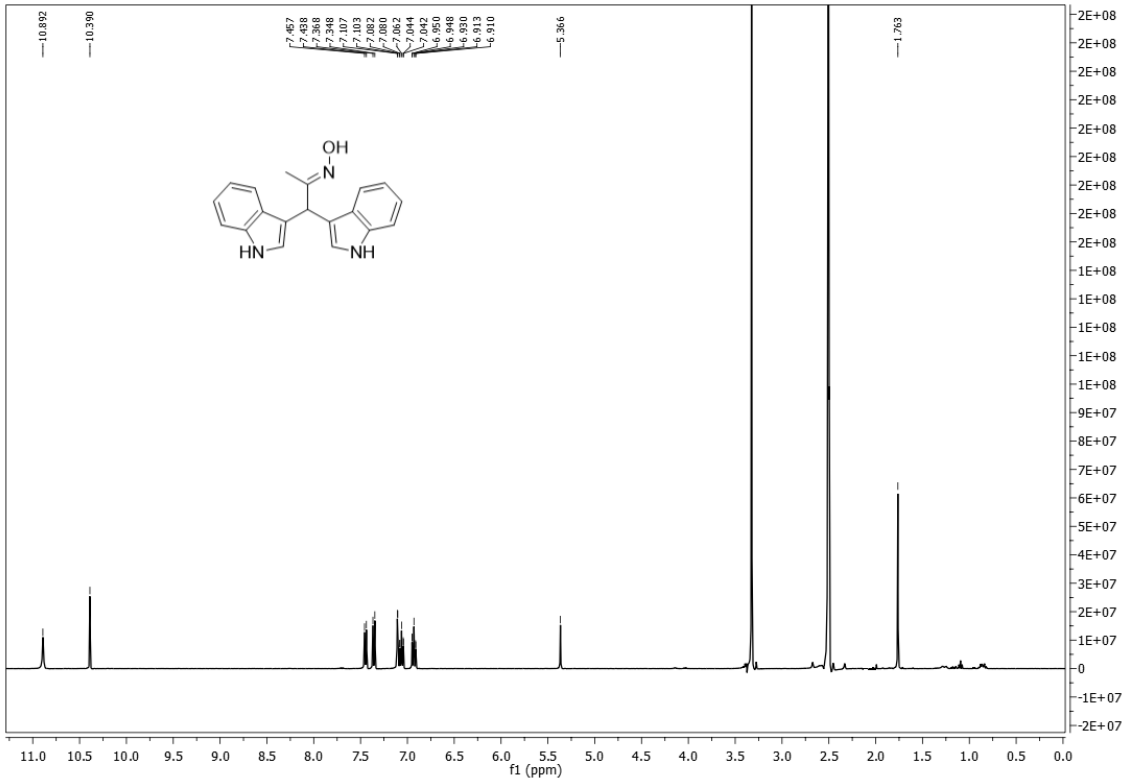



**Figure 19.** $^{1}$H RMN spectrum of compound **2.83f** (DMSO-$d_6$).

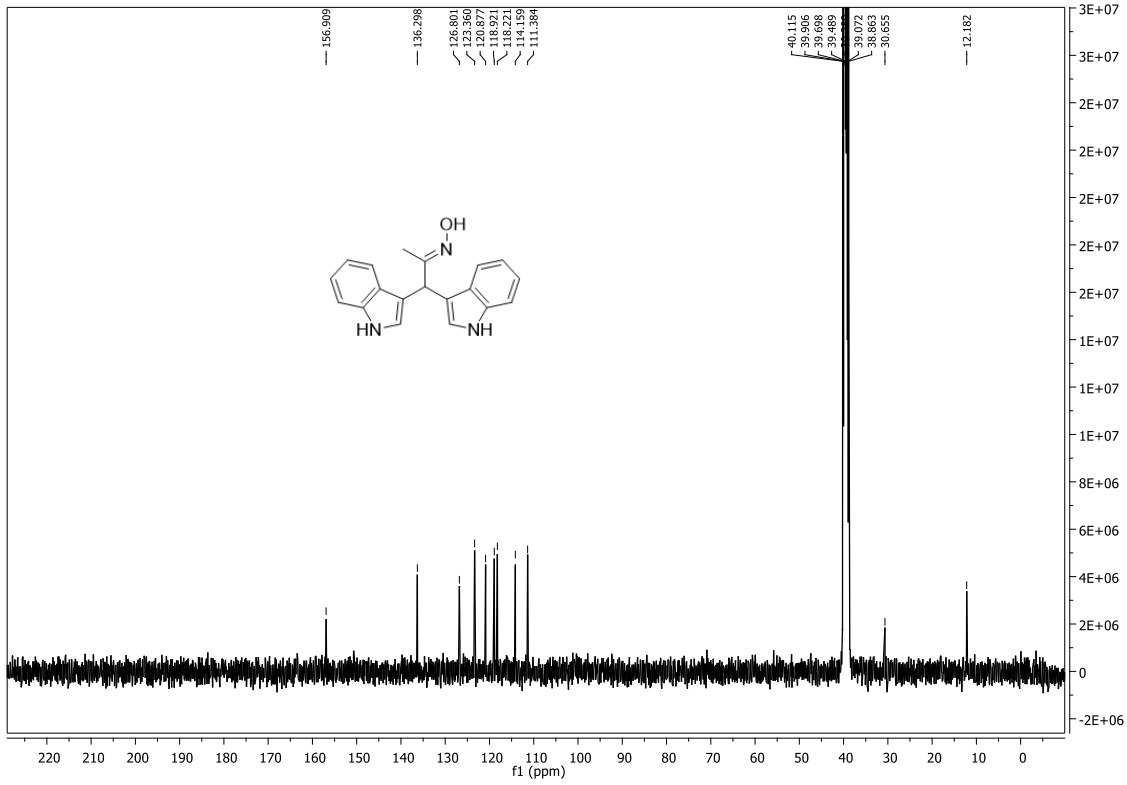



**Figure 20.** $^{13}$C RMN spectrum of compound **2.83f** (DMSO-$d_6$).

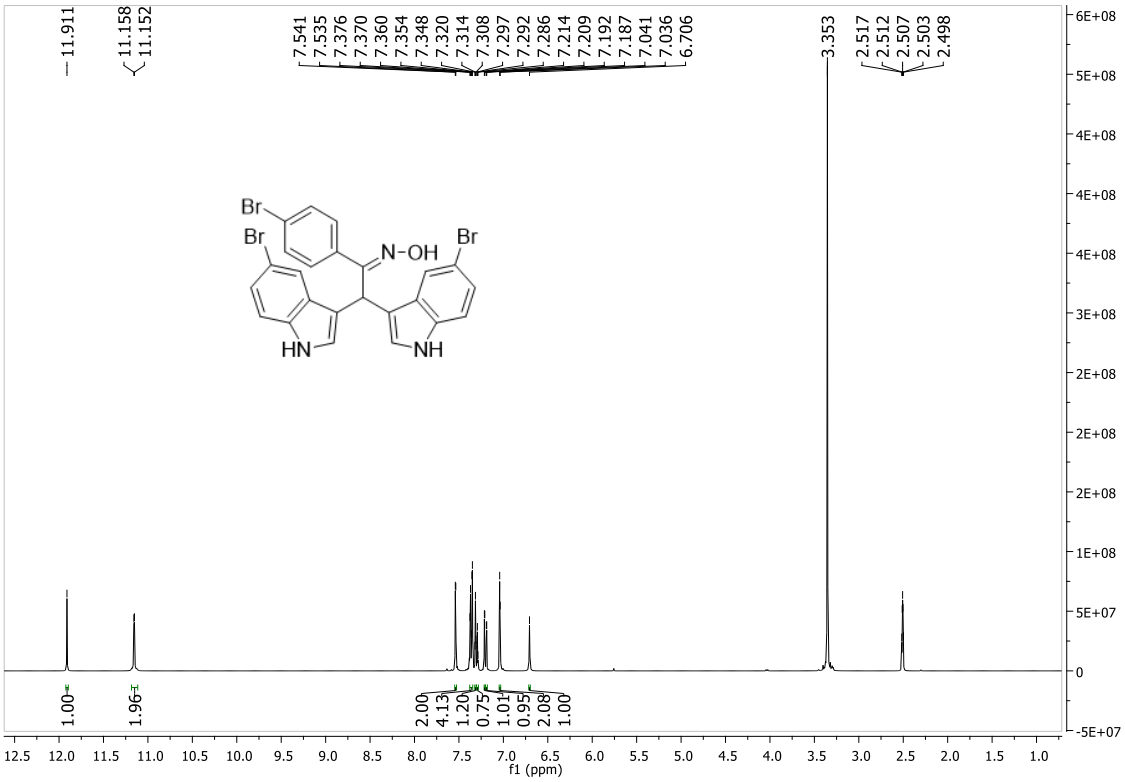



**Figure 21.** $^{1}$H RMN spectrum of compound **2.82g** (DMSO-$d_6$).

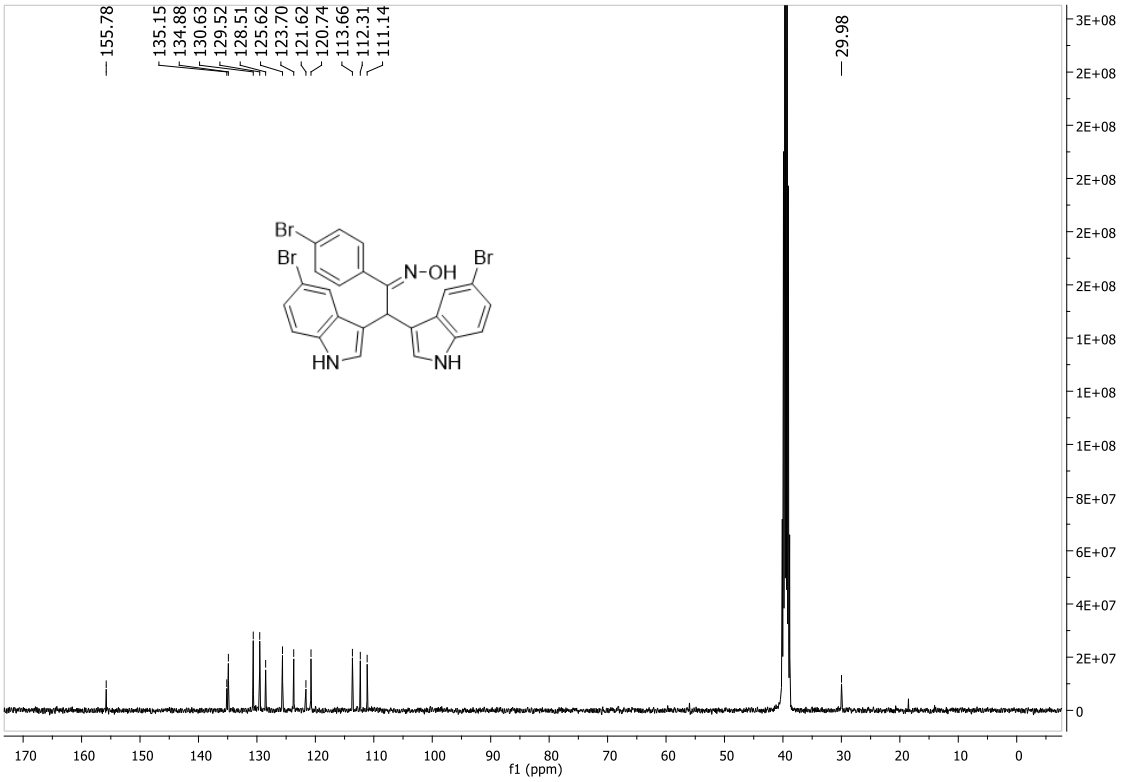



**Figure 22.** $^{13}$C RMN spectrum of compound **2.82g** (DMSO-$d_6$).

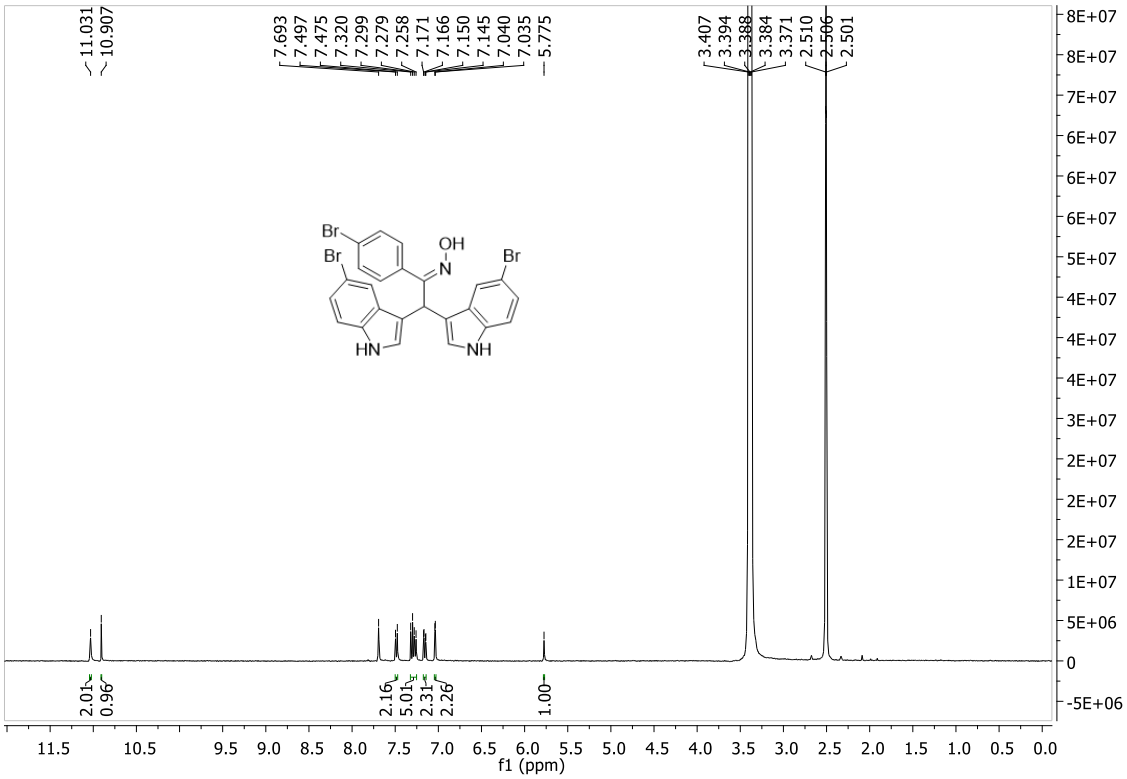



**Figure 23.** $^{1}$H RMN spectrum of compound **2.83g** (DMSO-$d_6$).

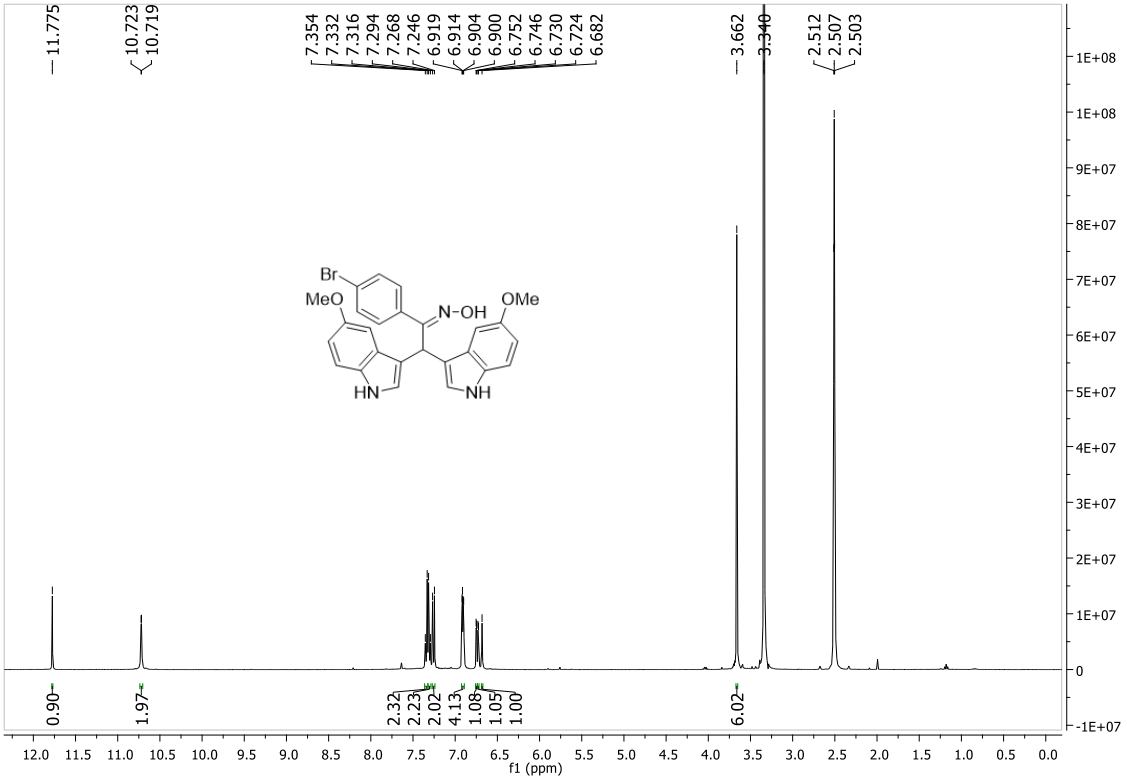



**Figure 24.** $^{1}$H RMN spectrum of compound **2.82h** (DMSO-$d_6$).

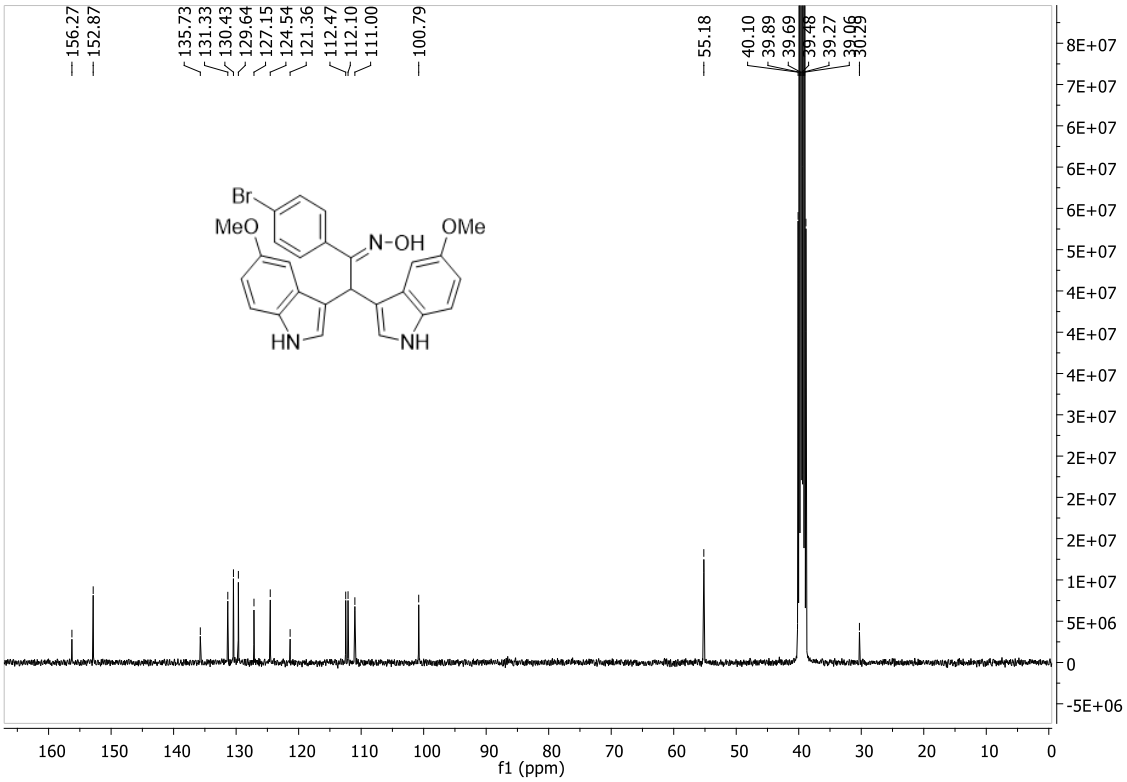



**Figure 25.** $^{13}$C RMN spectrum of compound **2.82h** (DMSO-$d_6$).

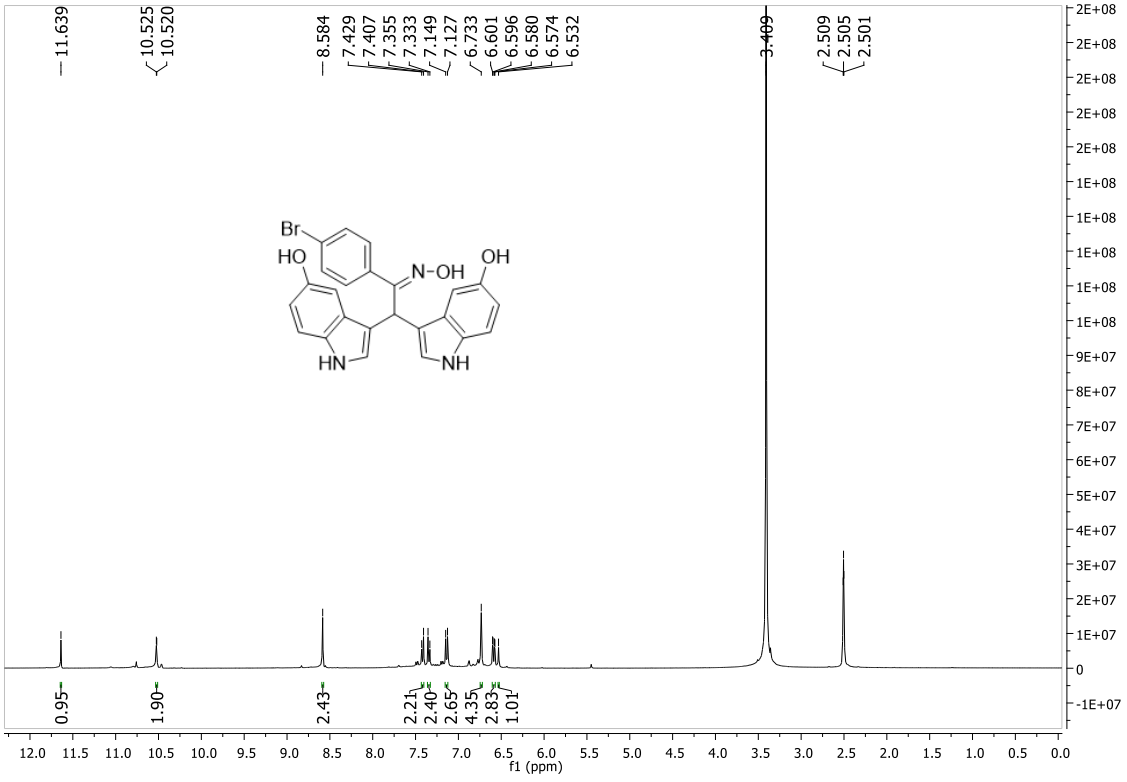



**Figure 26.** $^{1}$H RMN spectrum of compound **2.82i** (DMSO-$d_6$).

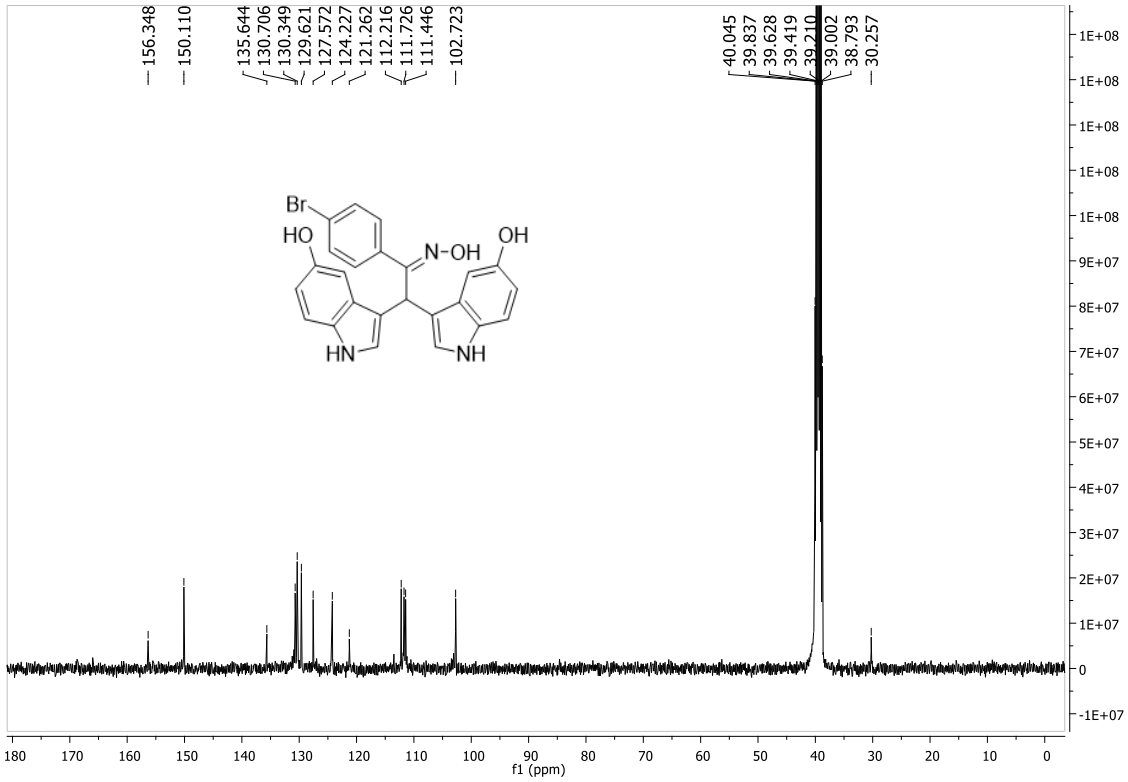



**Figure 27.** $^{13}$C RMN spectrum of compound **2.82i** (DMSO-$d_6$).

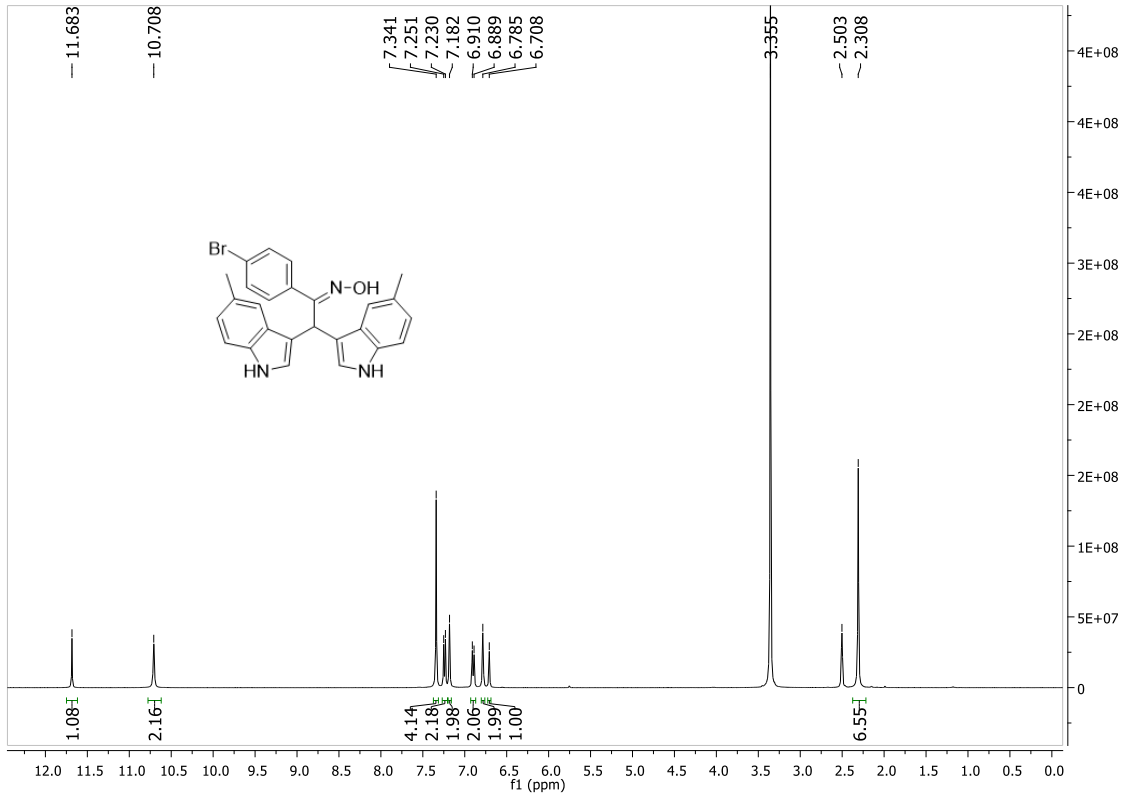



**Figure 28.** $^{1}$H RMN spectrum of compound **2.82j** (DMSO-$d_6$).

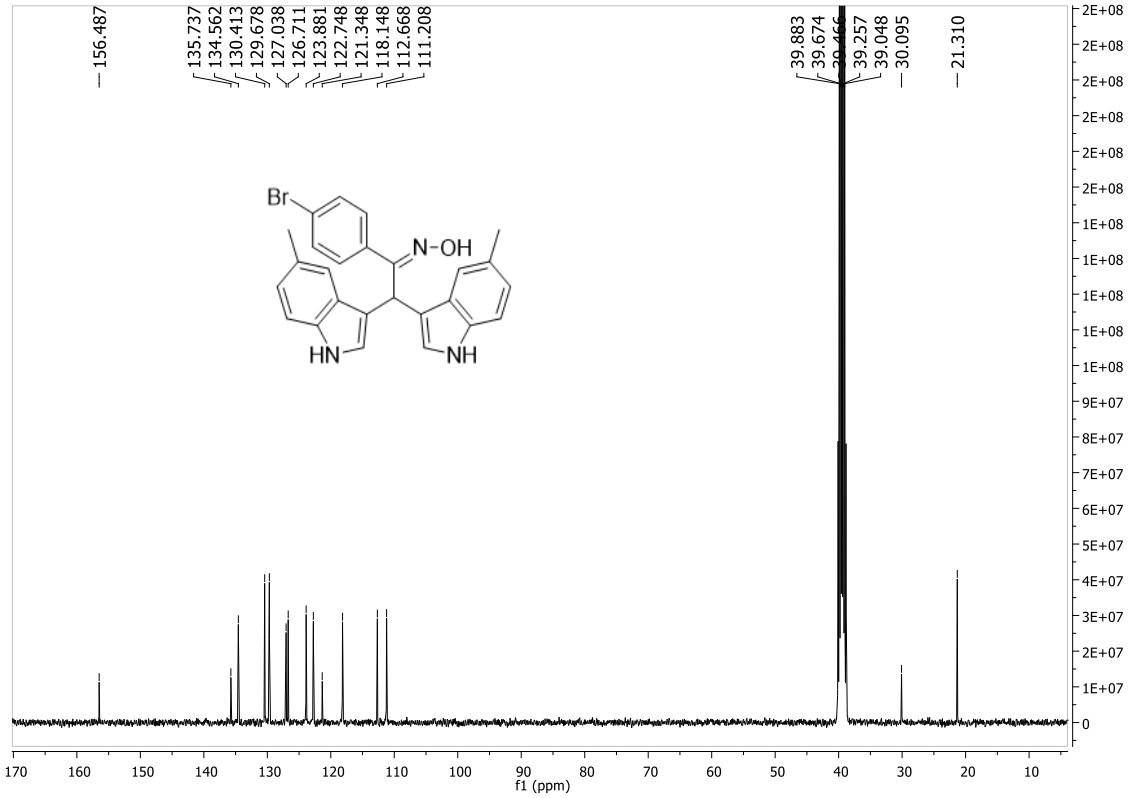



**Figure 29.** $^{13}$C RMN spectrum of compound **2.82j** (DMSO-$d_6$).

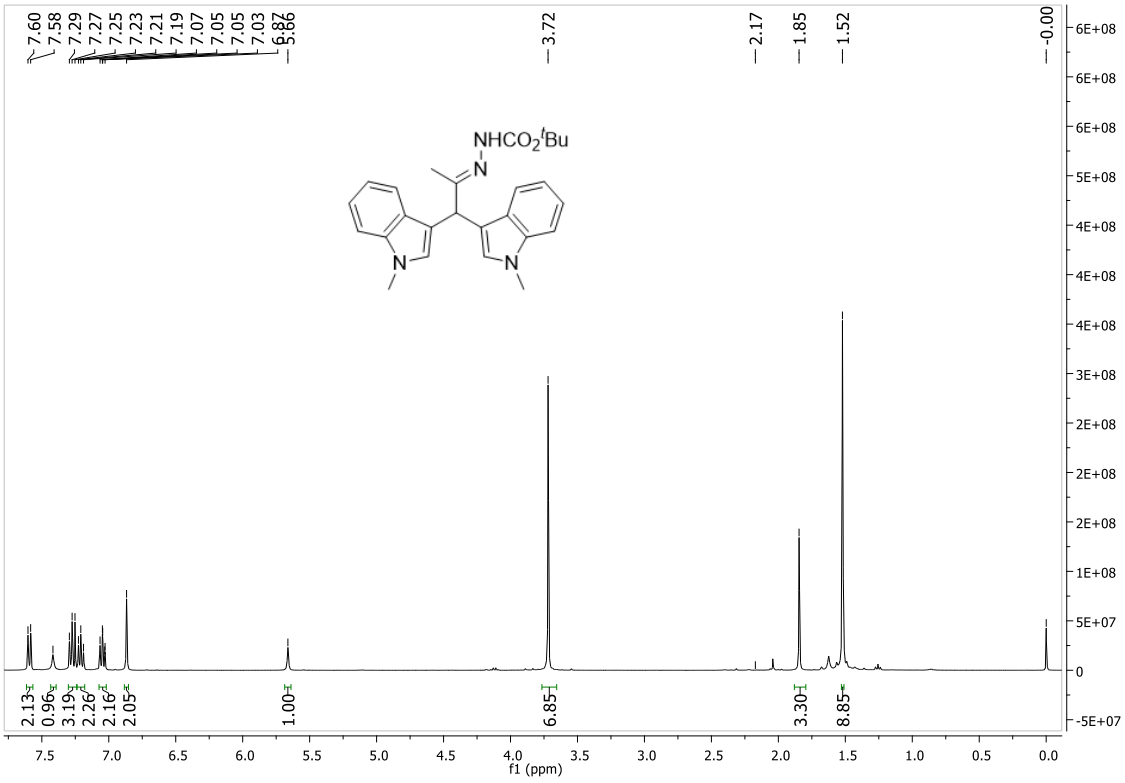



**Figure 30.** $^{1}$H RMN spectrum of compound **2.85a** ($CDCl_3$).

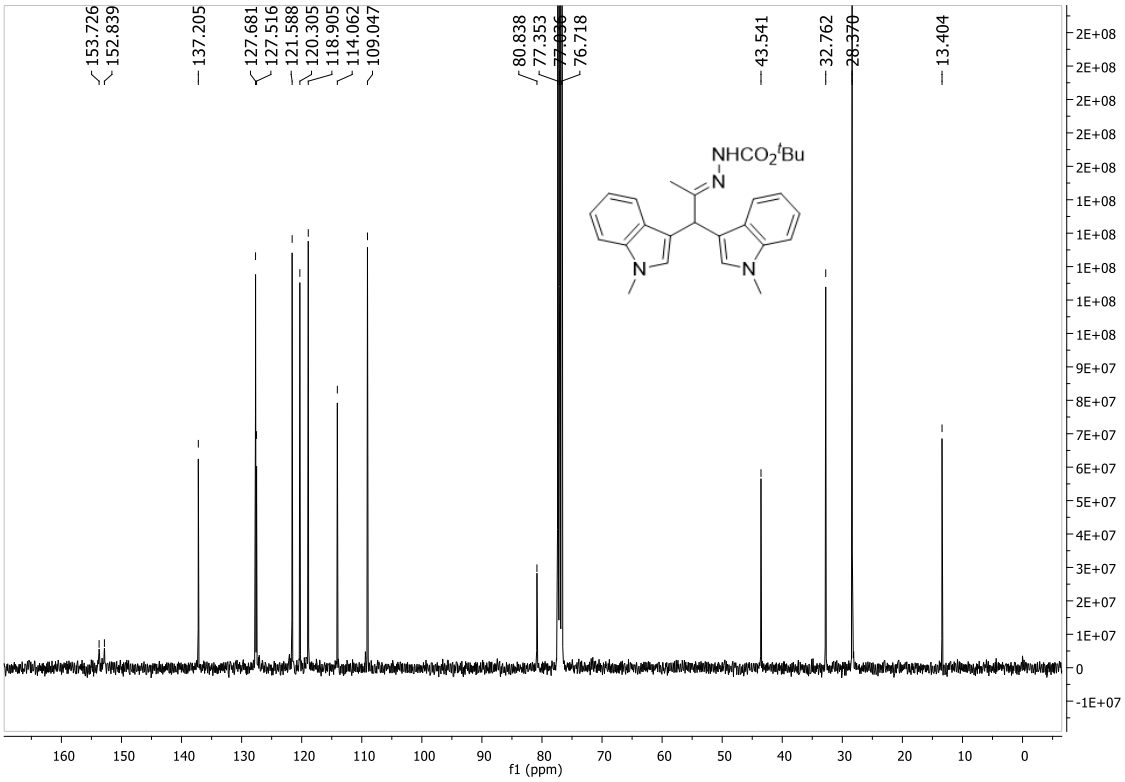



**Figure 31.** $^{13}$C RMN spectrum of compound **2.85a** ($CDCl_3$).

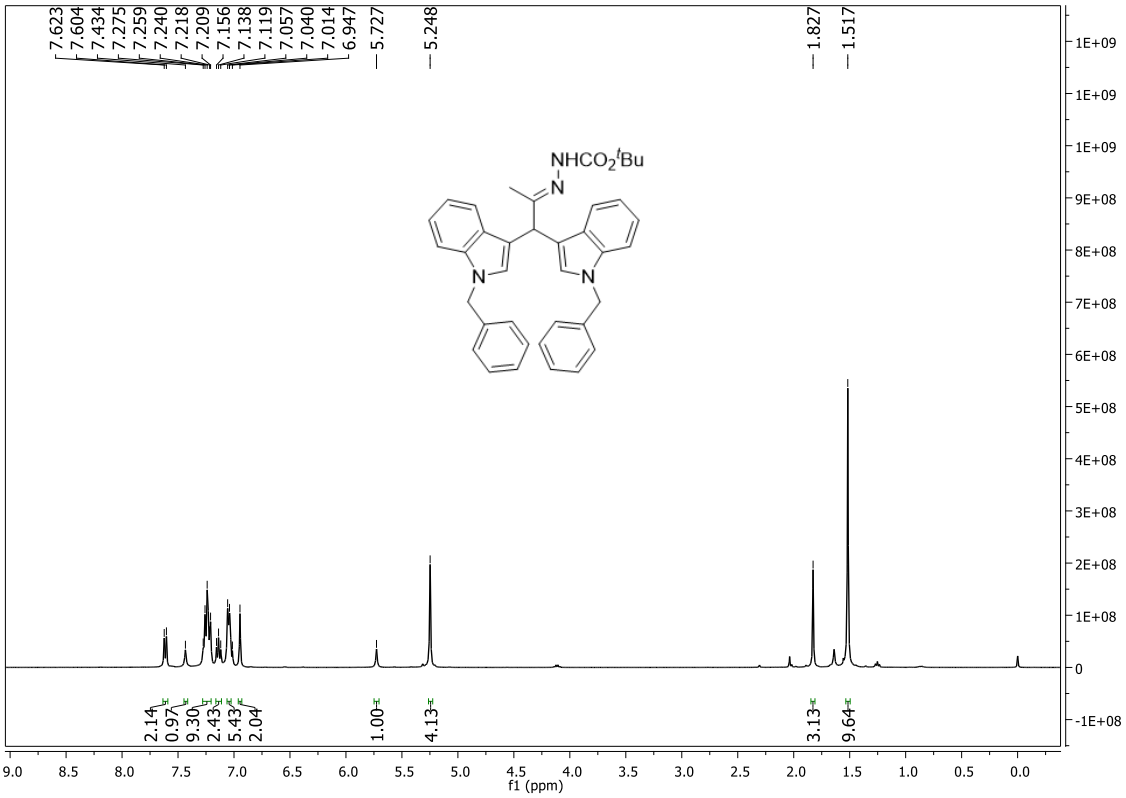



**Figure 32.** $^{1}$H RMN spectrum of compound **2.85b** ($CDCl_3$).

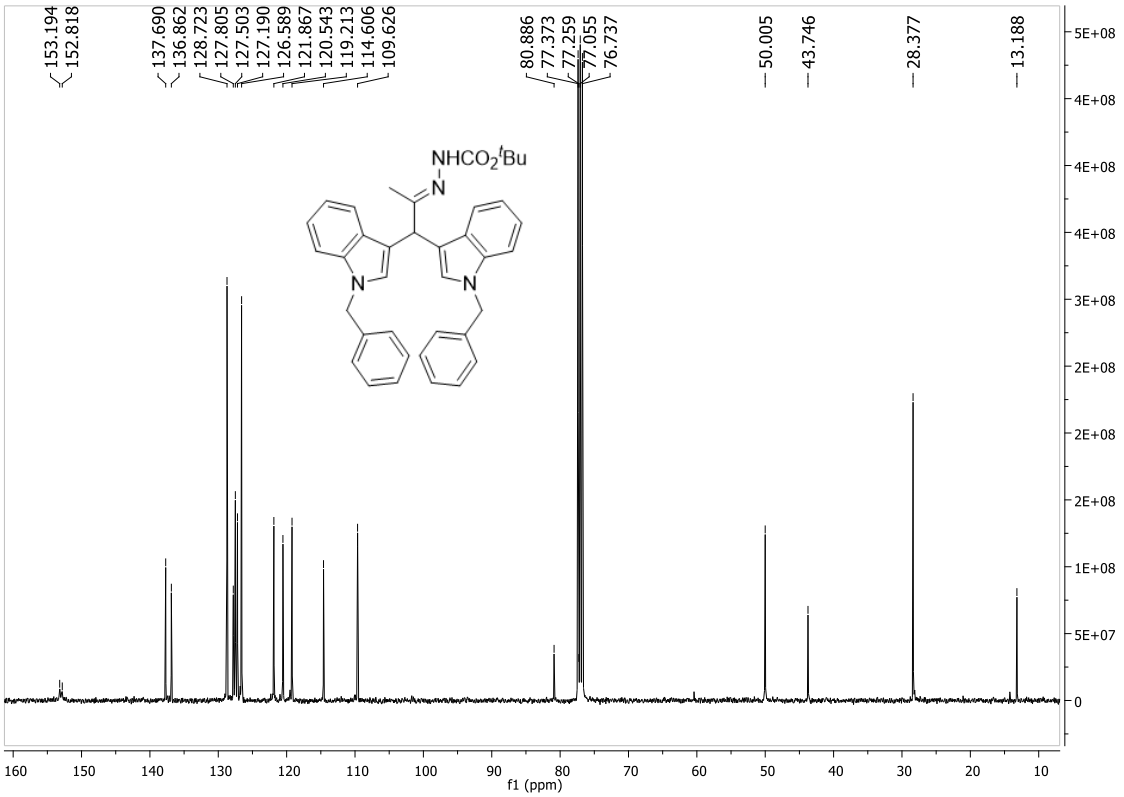



**Figure 33.** $^{13}$C RMN spectrum of compound **2.85b** ($CDCl_3$).

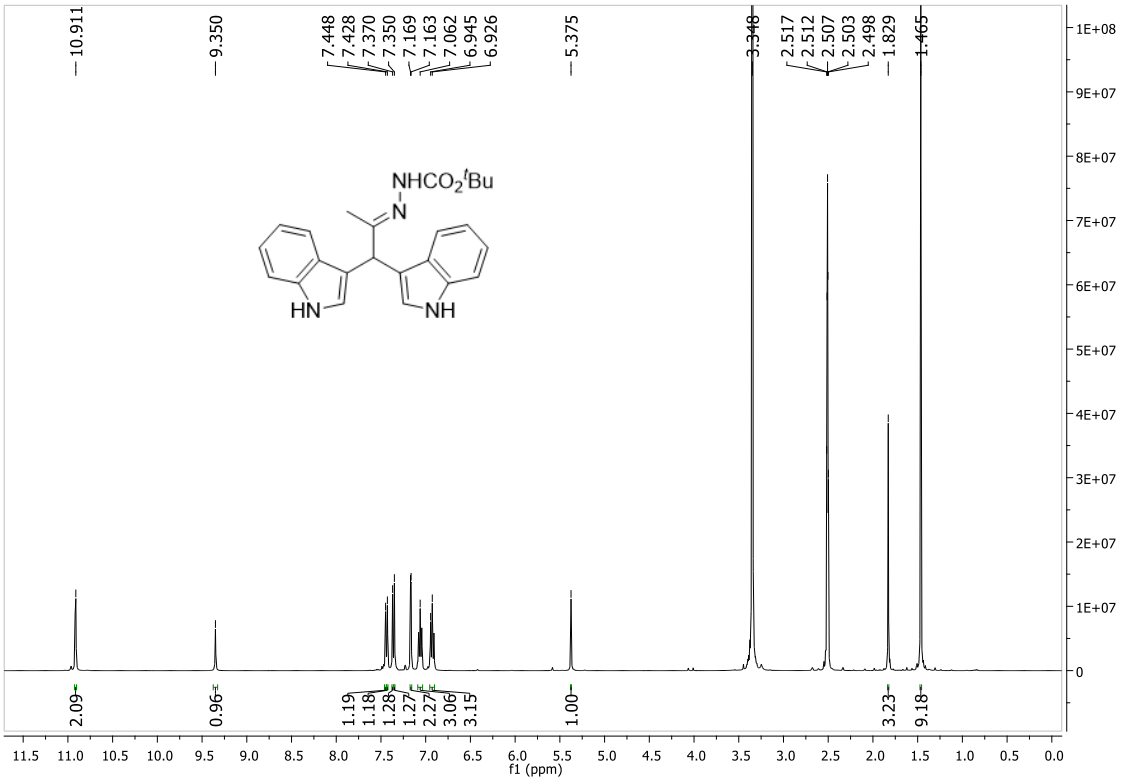



**Figure 34.** $^{1}$H RMN spectrum of compound **2.85c** (DMSO-$d_6$).

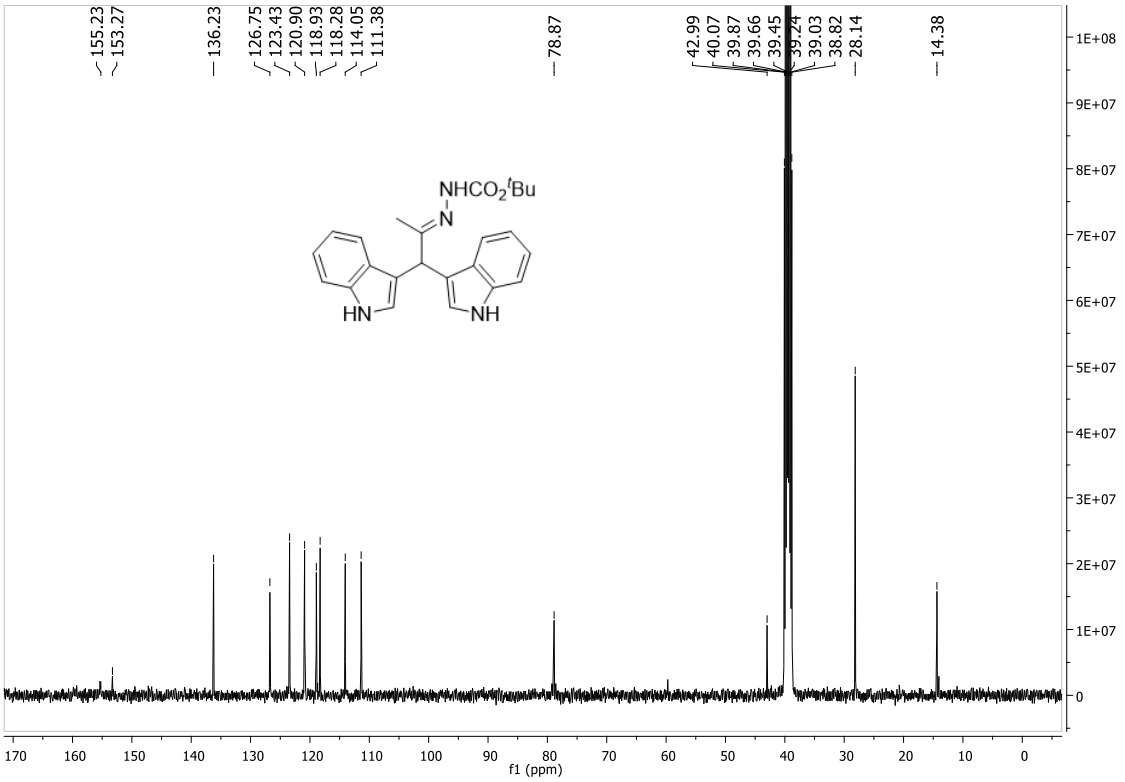



**Figure 35.** $^{13}$C RMN spectrum of compound **2.85c** (DMSO-$d_6$).

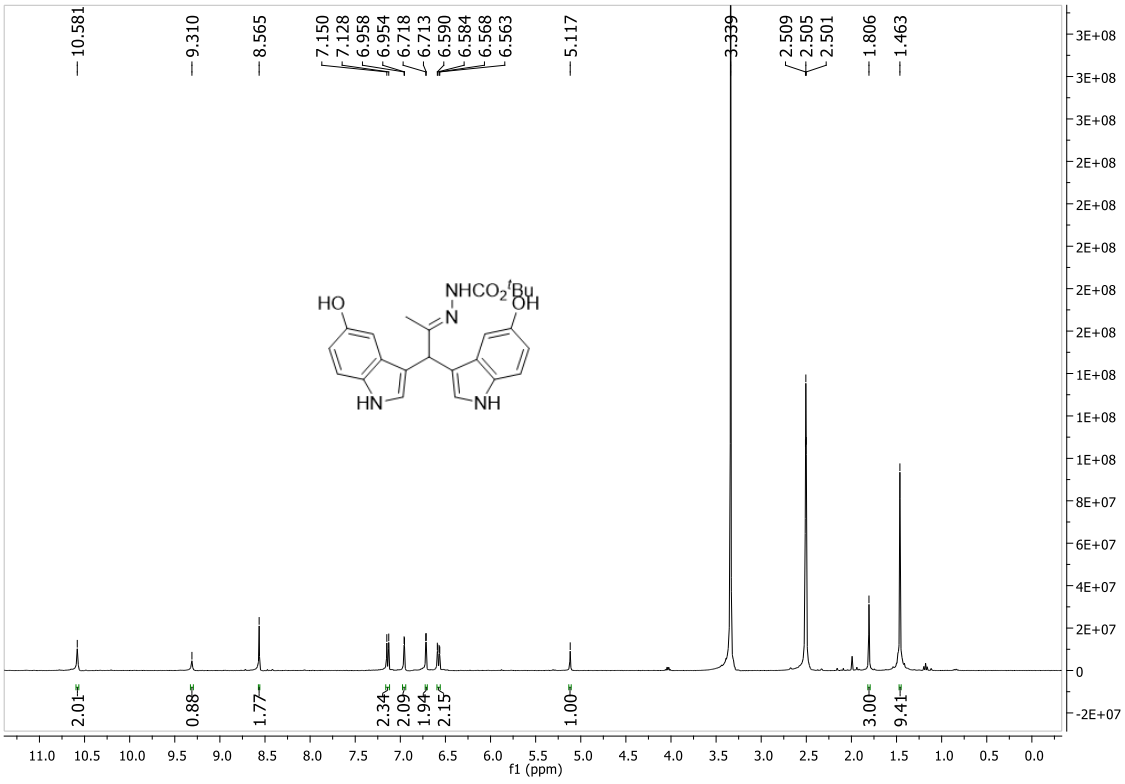



**Figure 36.** $^{1}$H RMN spectrum of compound **2.85d** (DMSO-$d_6$).

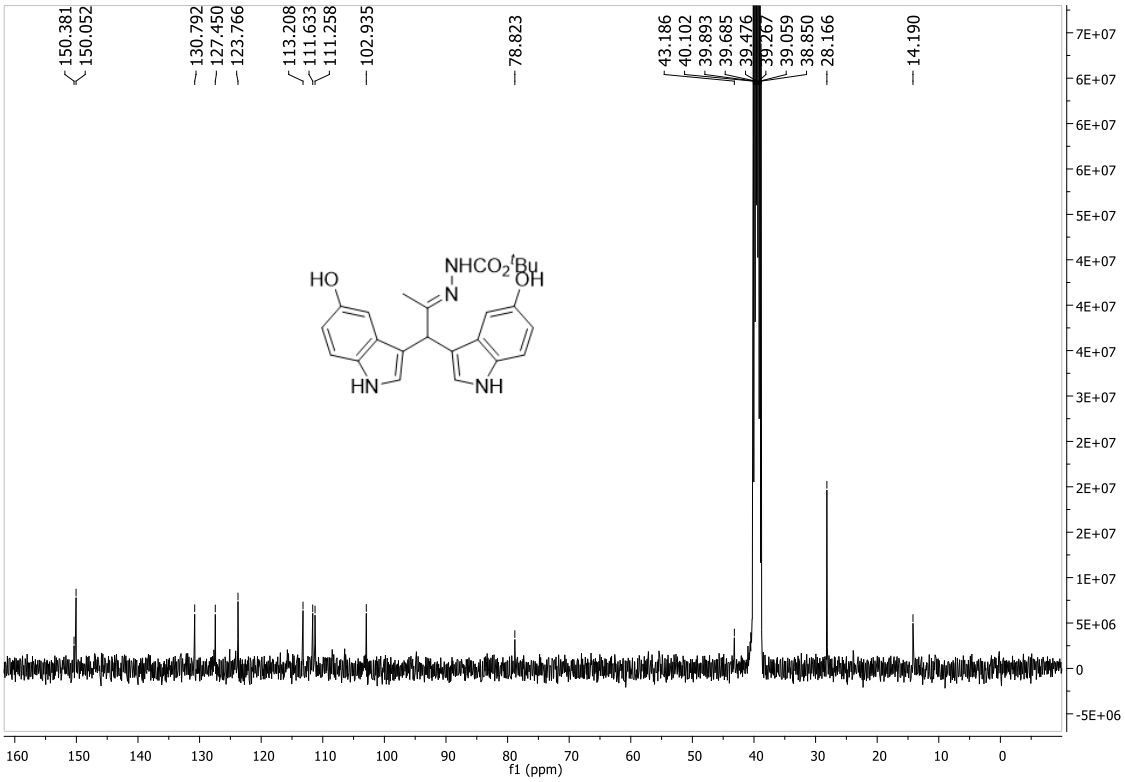



**Figure 37.** $^{13}$C RMN spectrum of compound **2.85d** (DMSO-$d_6$).

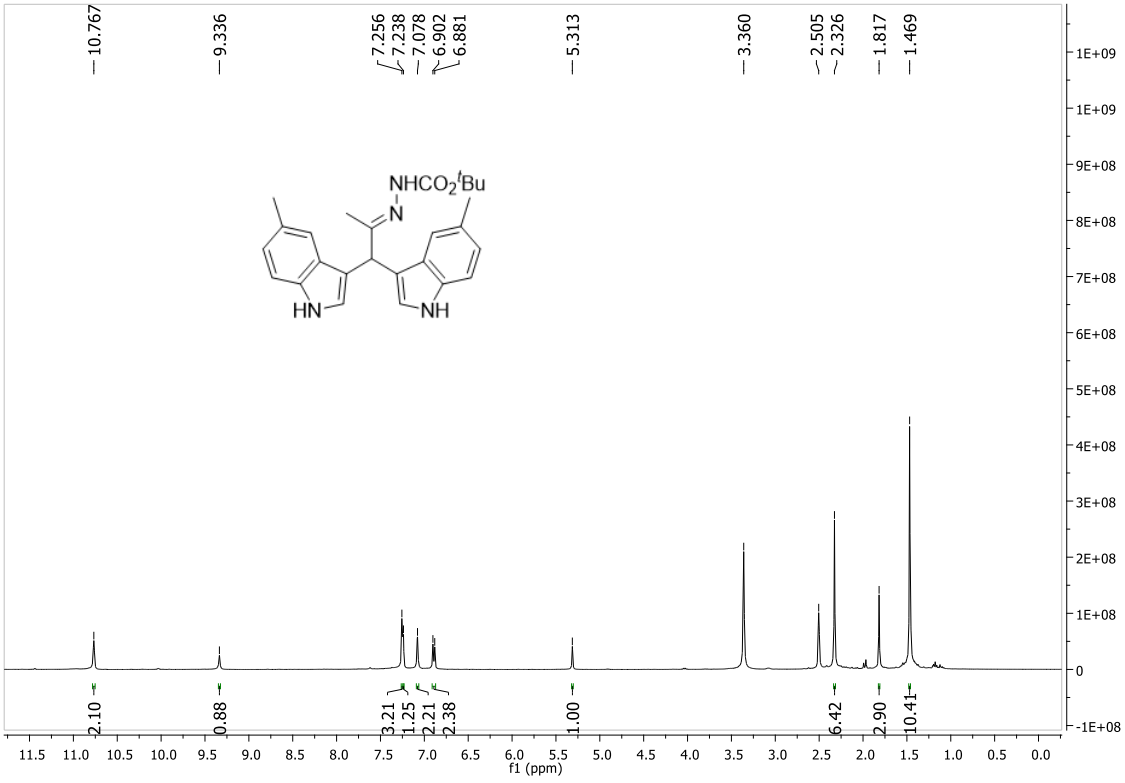



**Figure 38.** $^{1}$H RMN spectrum of compound **2.85e** (DMSO-$d_6$).

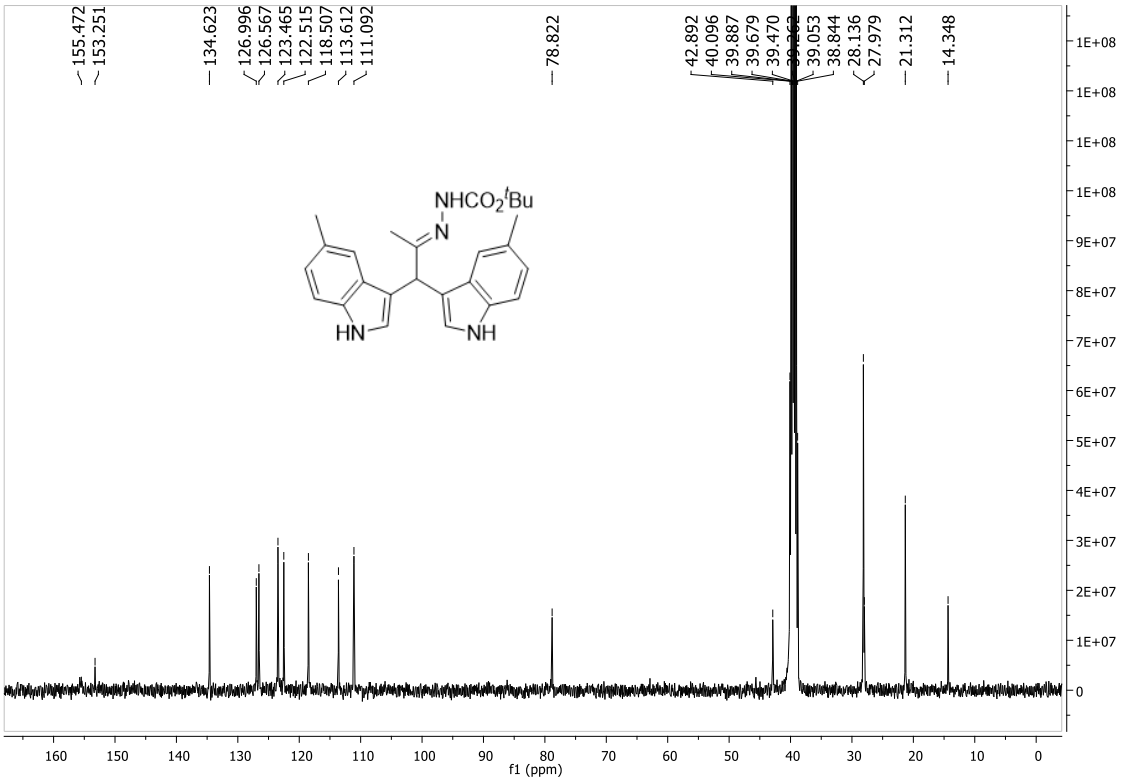



**Figure 39.** $^{13}$C RMN spectrum of compound **2.85e** (DMSO-$d_6$).

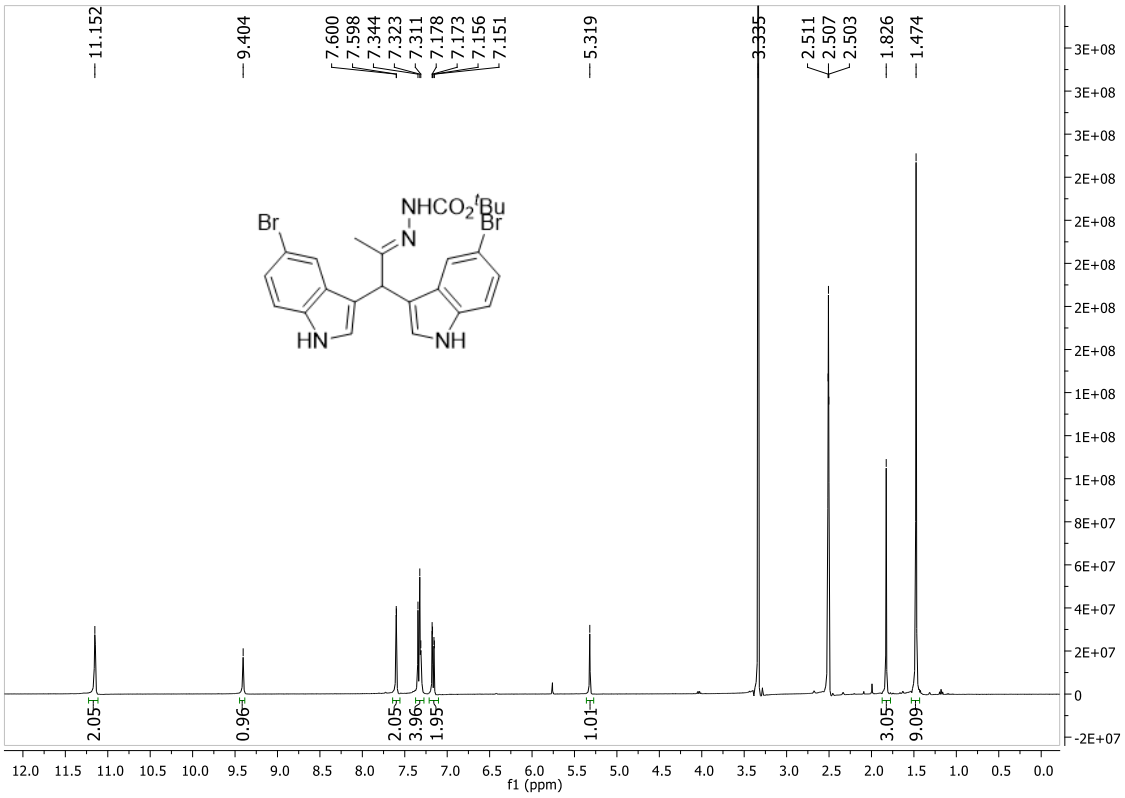



**Figure 40.** $^{1}$H RMN spectrum of compound **2.85f** (DMSO-$d_6$).

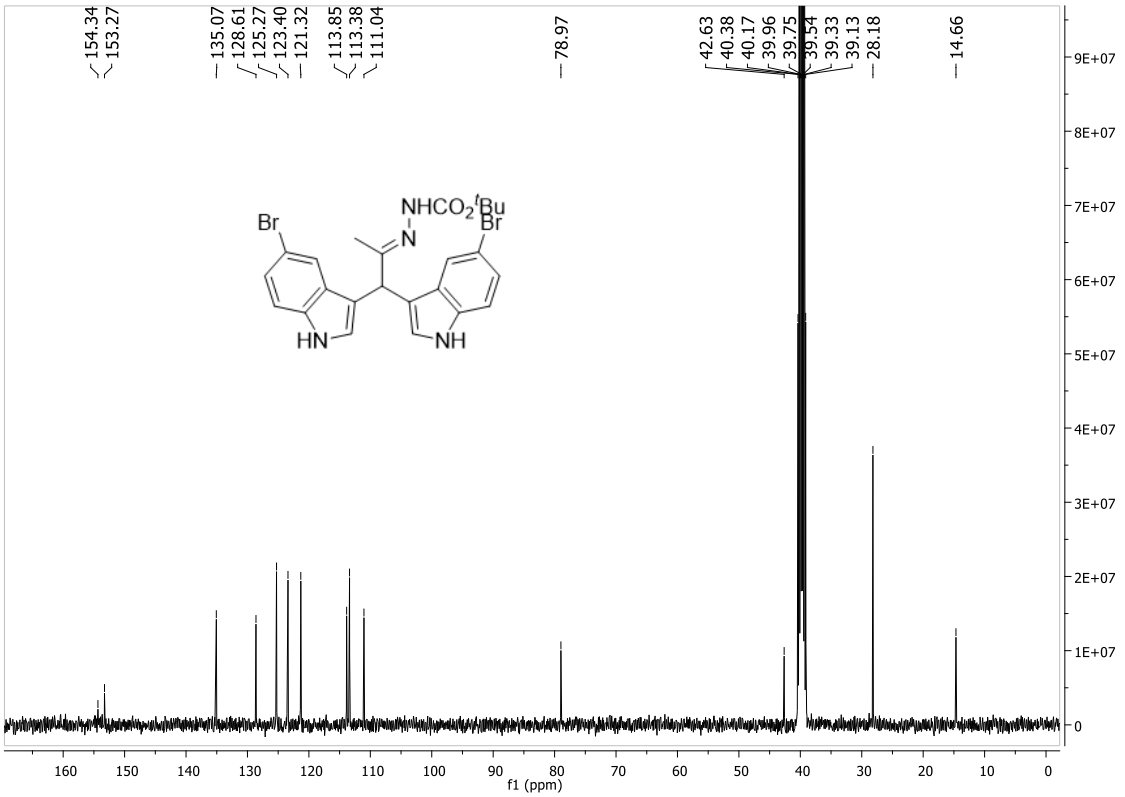



**Figure 41.** $^{13}$C RMN spectrum of compound **2.85f** (DMSO-$d_6$).

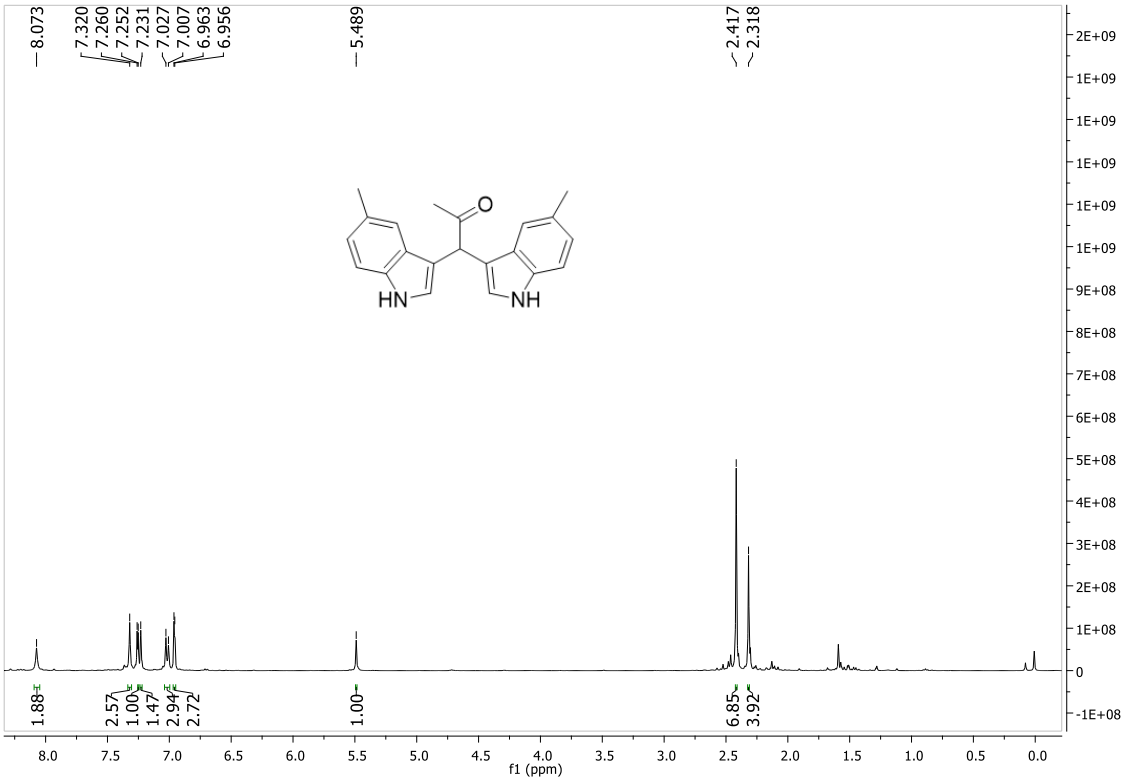



**Figure 42.** $^{1}$H RMN spectrum of compound **2.86** ($CDCl_3$).

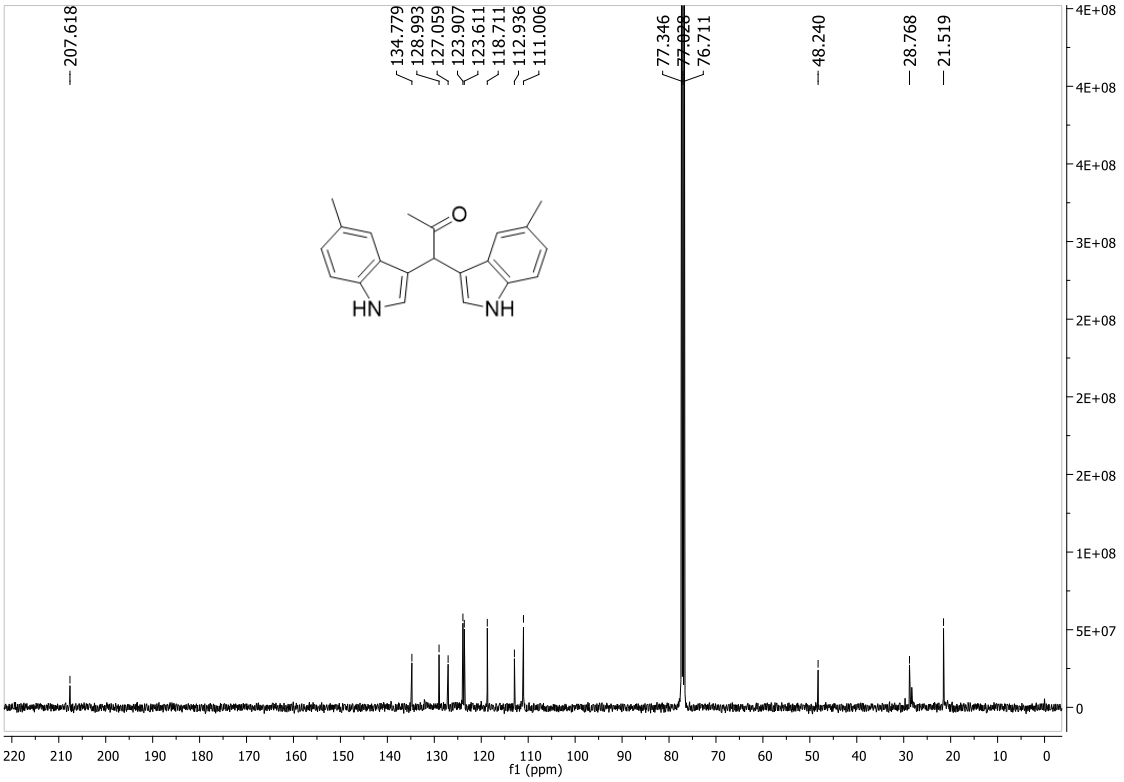



**Figure 43.** $^{13}$C RMN spectrum of compound **2.86** ($CDCl_3$).

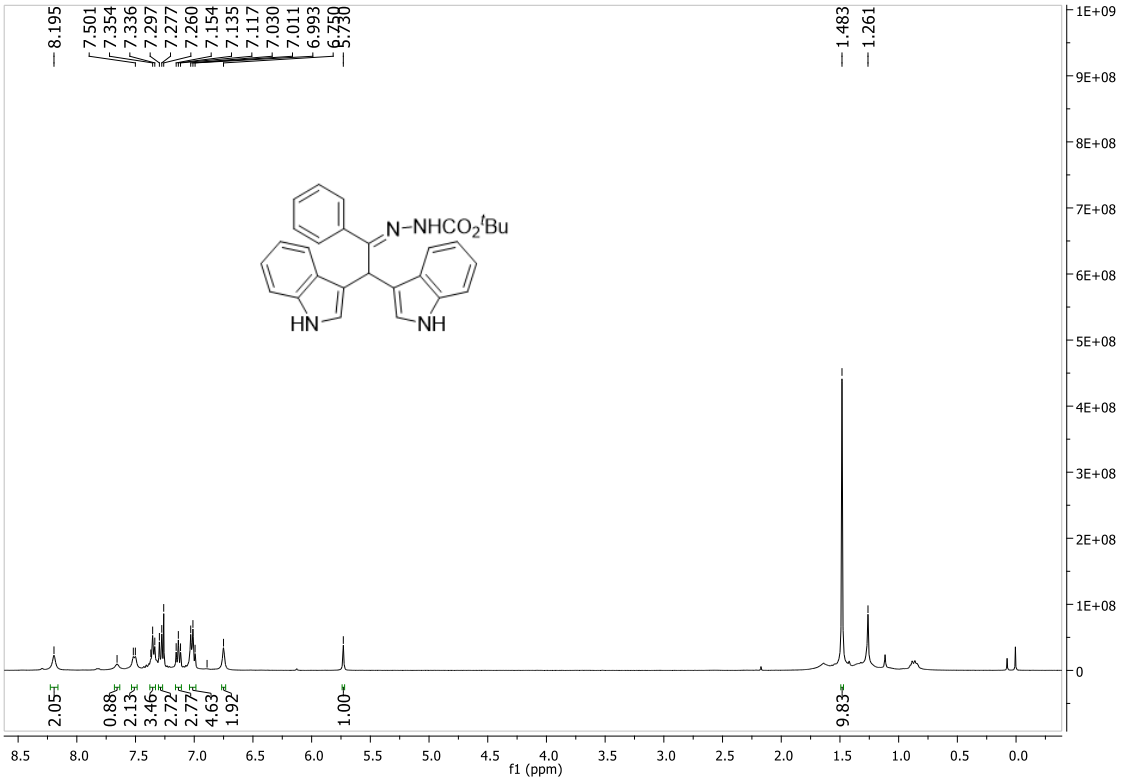



**Figure 44.** $^{1}$H RMN spectrum of compound **2.87a** ($CDCl_3$).

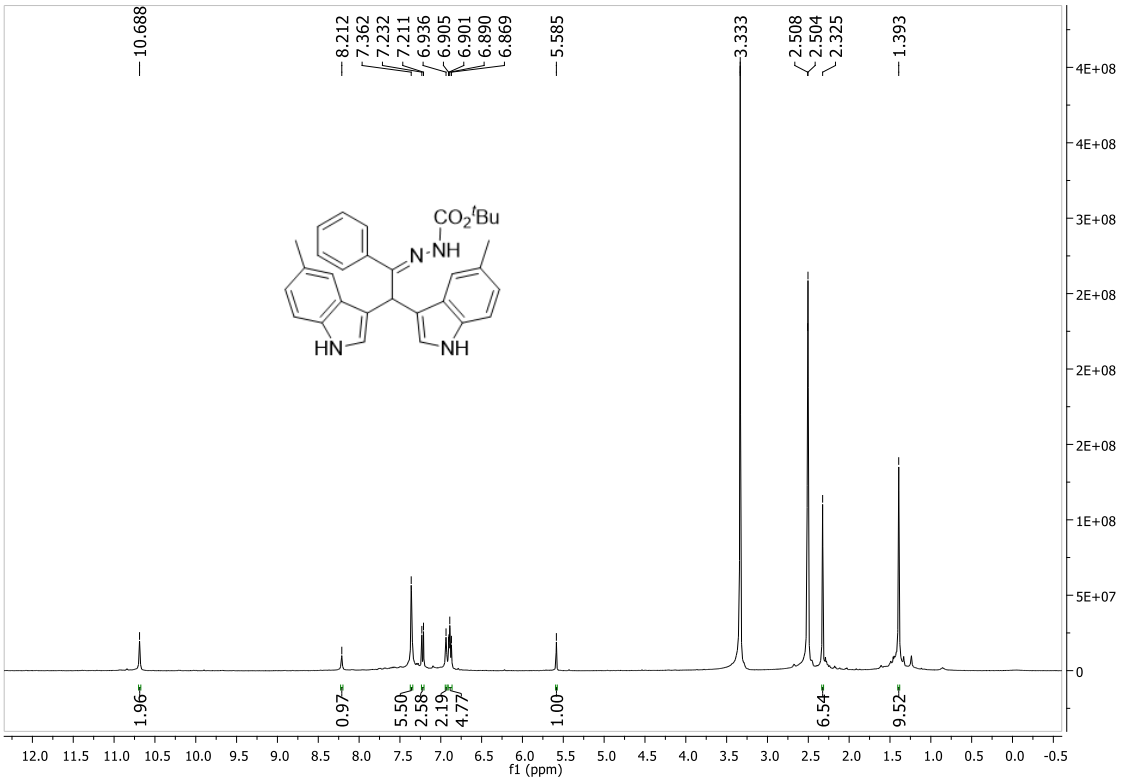



**Figure 45.** $^{1}$H RMN spectrum of compound **2.87b** ($CDCl_3$).

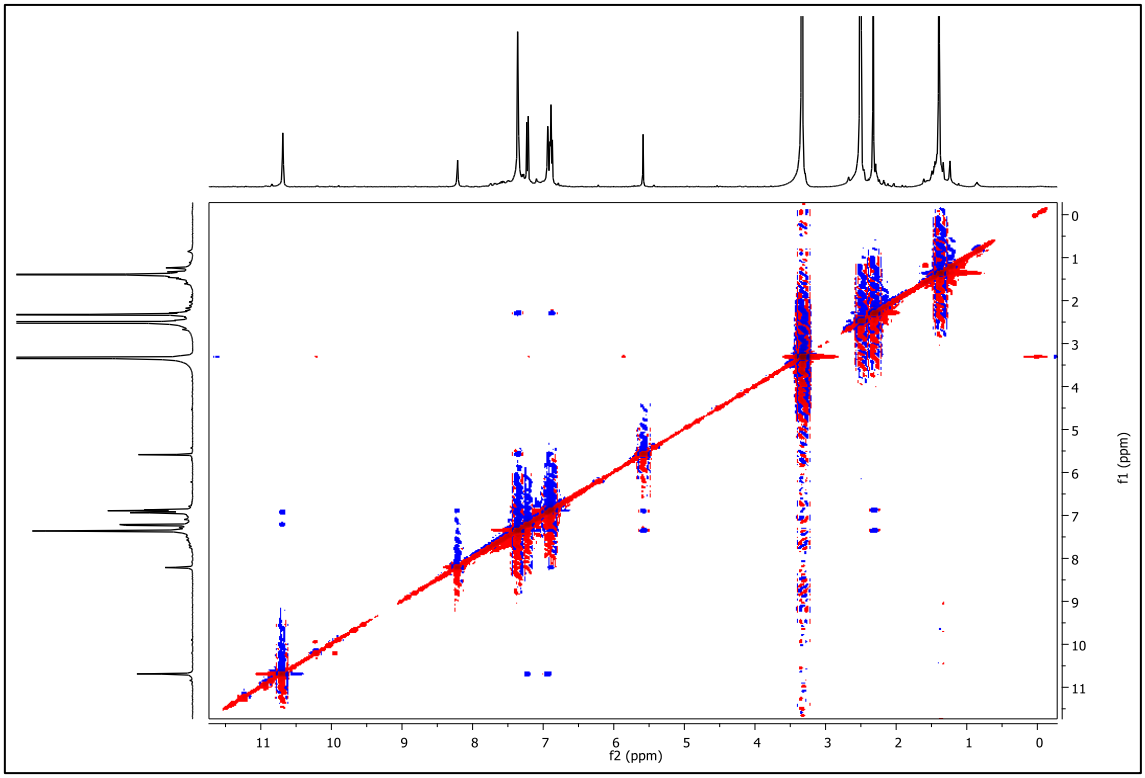



**Figure 46.** NOESY spectrum of compound **2.87b** ($CDCl_3$).

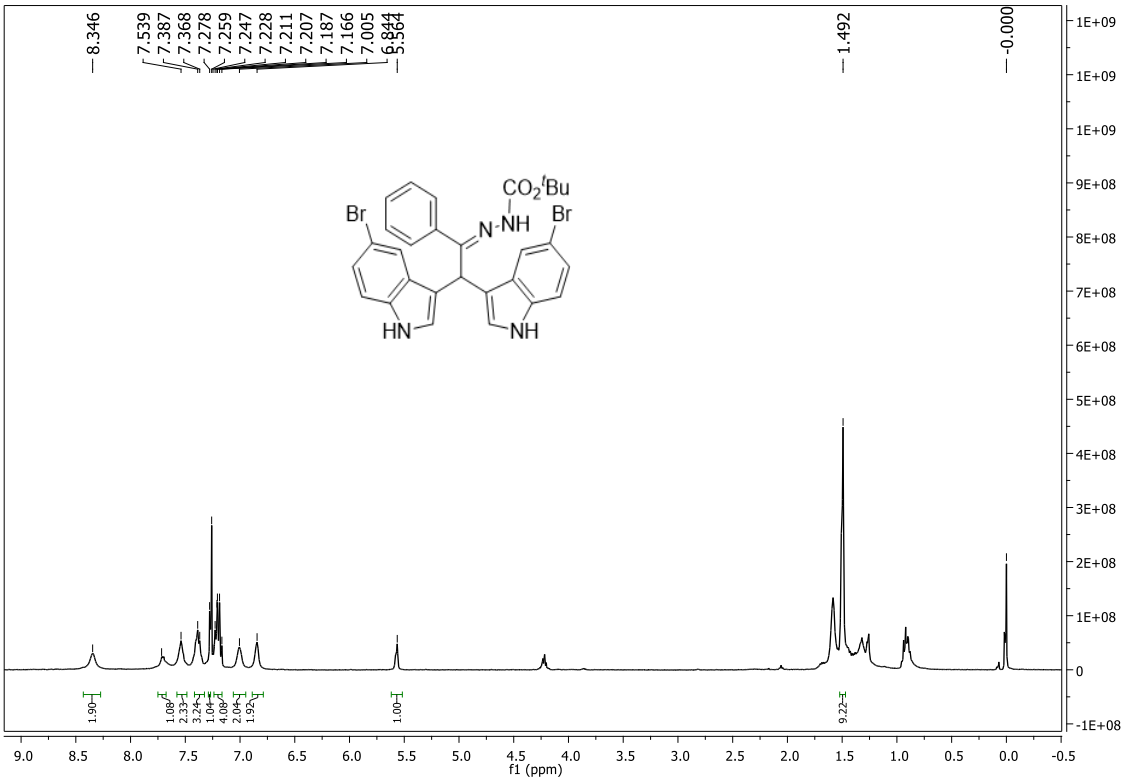



**Figure 47.** $^{1}$H RMN spectrum of compound **2.87c** ($CDCl_3$).

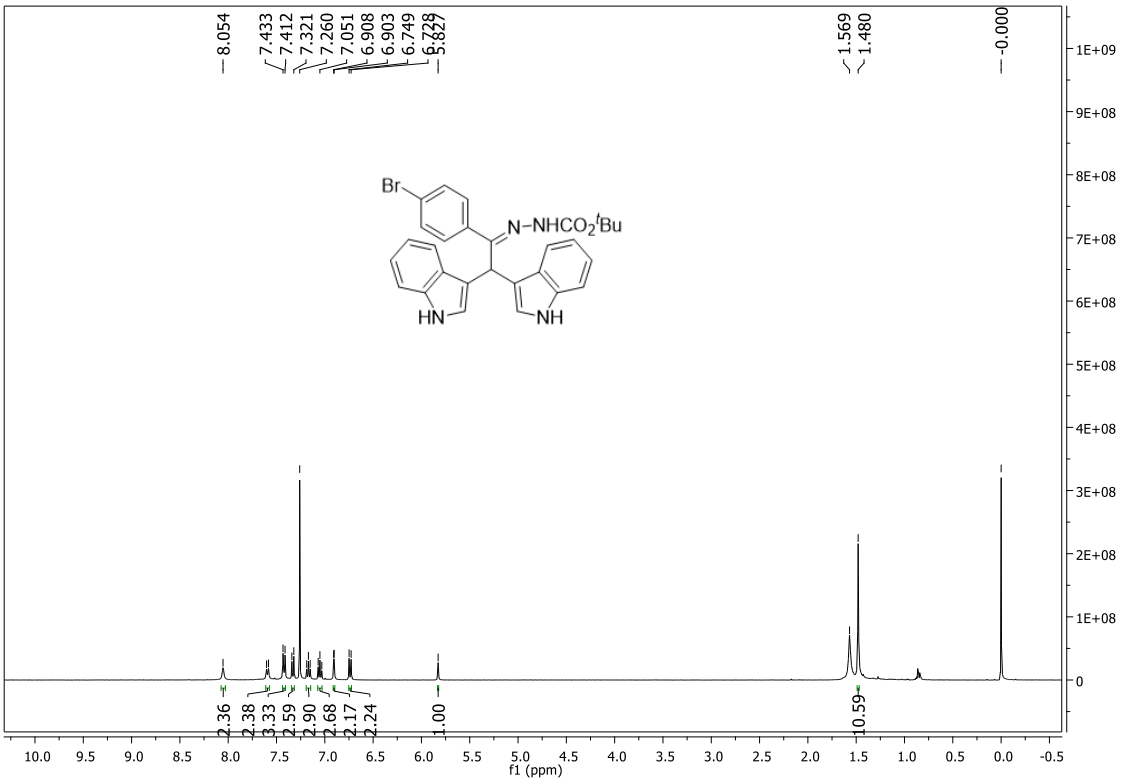



**Figure 48.** $^{1}$H RMN spectrum of compound **2.87d** ($CDCl_3$).

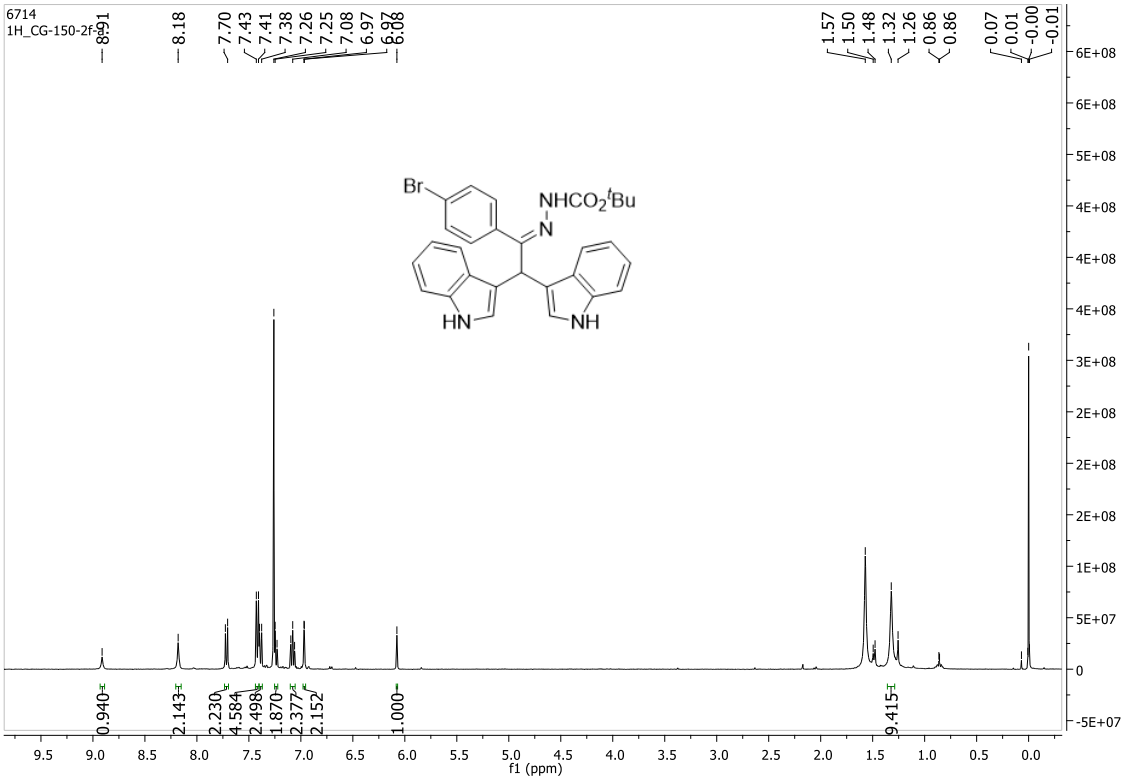



**Figure 49.** $^{1}$H RMN spectrum of compound **2.88d** ($CDCl_3$).

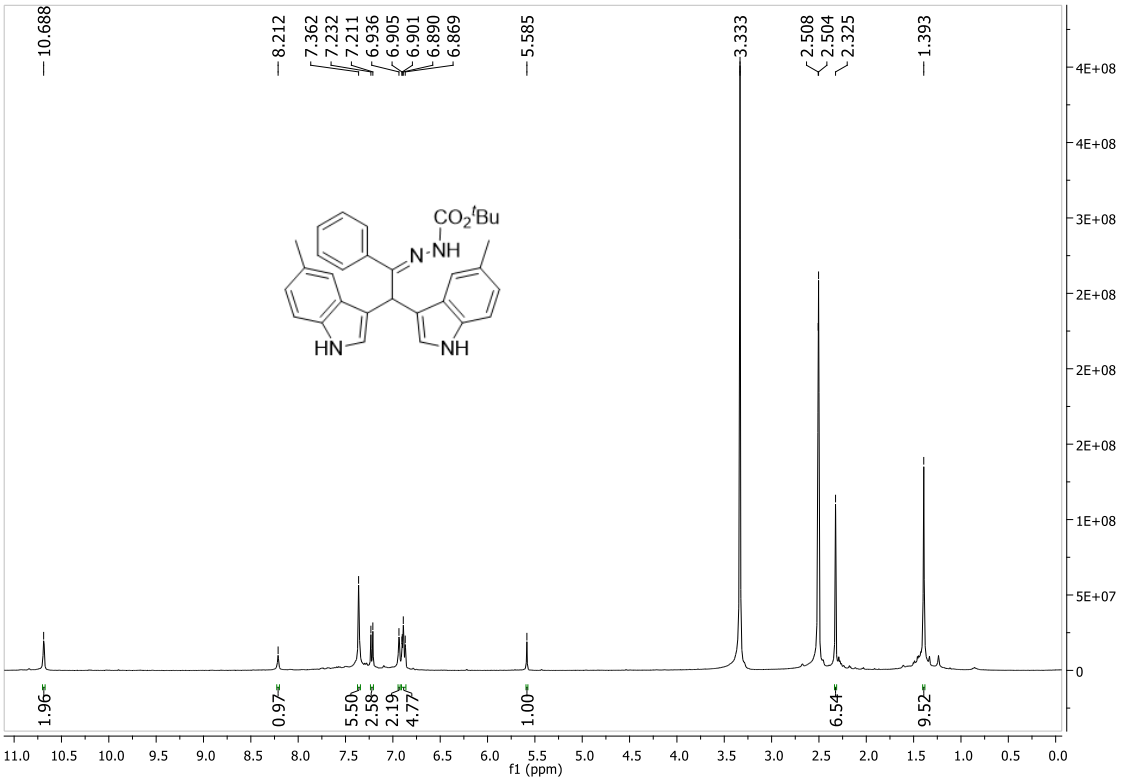



**Figure 50.** $^{1}$H RMN spectrum of compound **2.87e** (DMSO-$d_6$).

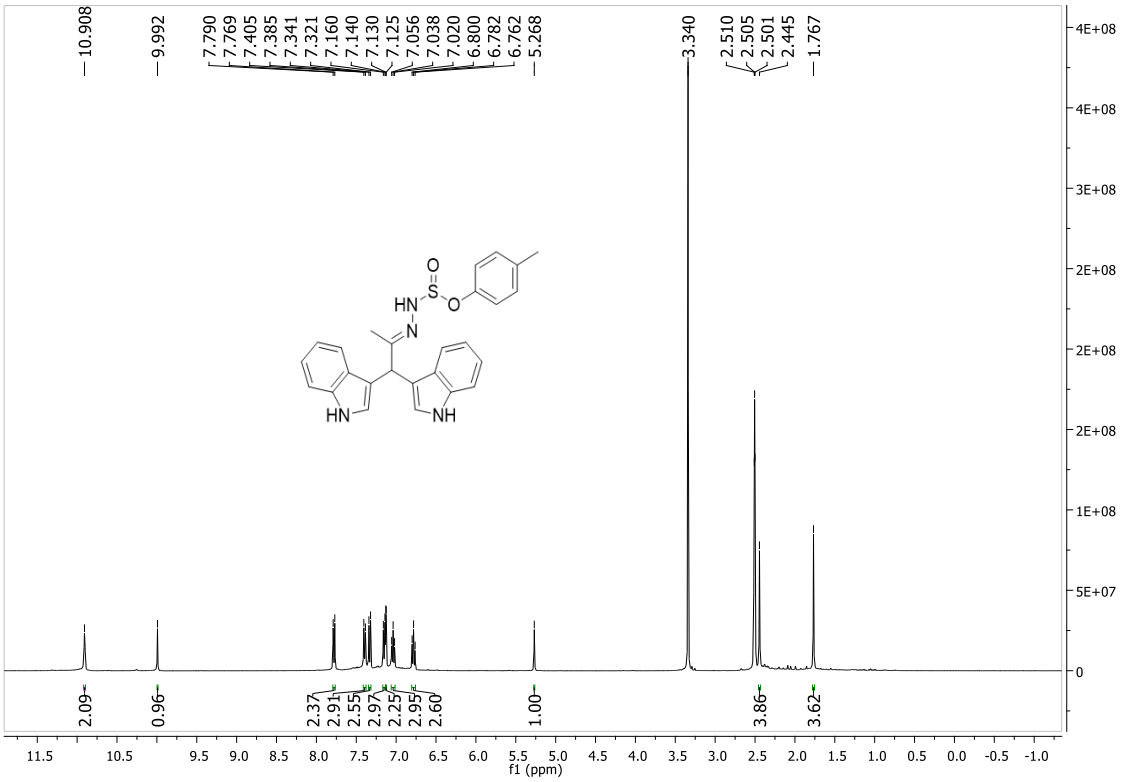



**Figure 51.** $^{1}$H RMN spectrum of compound **2.89a** (DMSO-$d_6$).

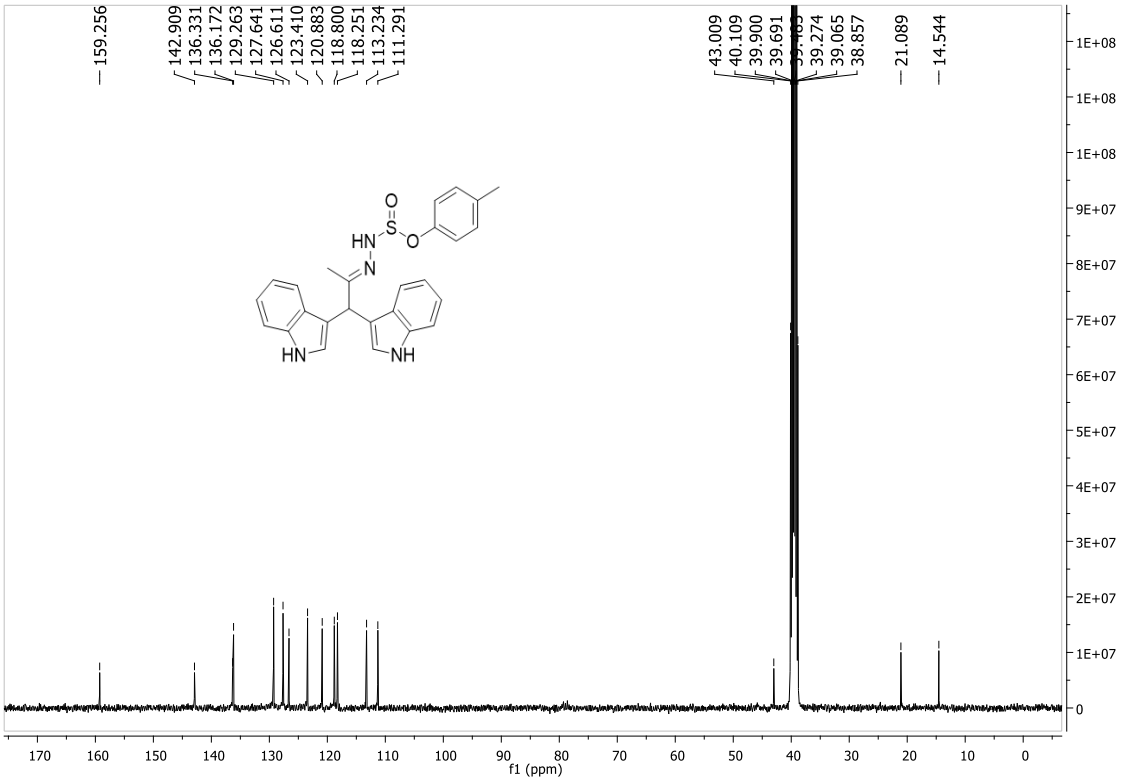



**Figure 52.** $^{13}$C RMN spectrum of compound **2.89a** (DMSO-$d_6$).

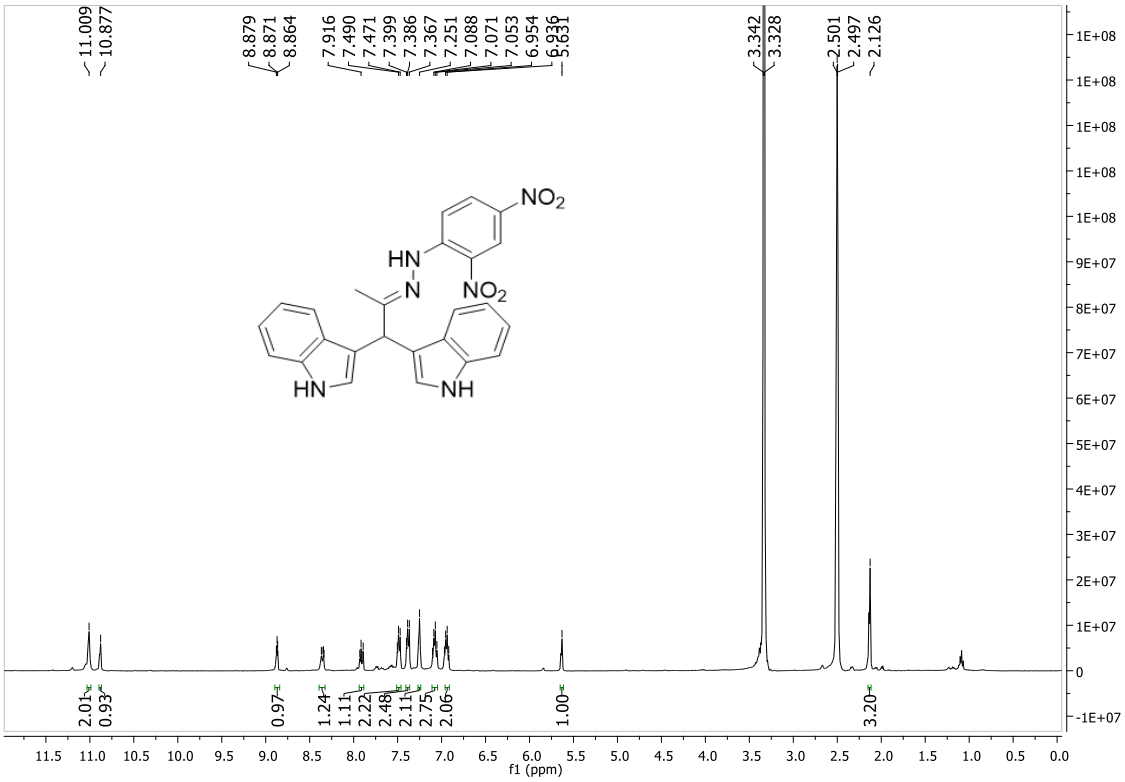



**Figure 53.** $^{1}$H RMN spectrum of compound **2.89b** (DMSO-$d_6$).

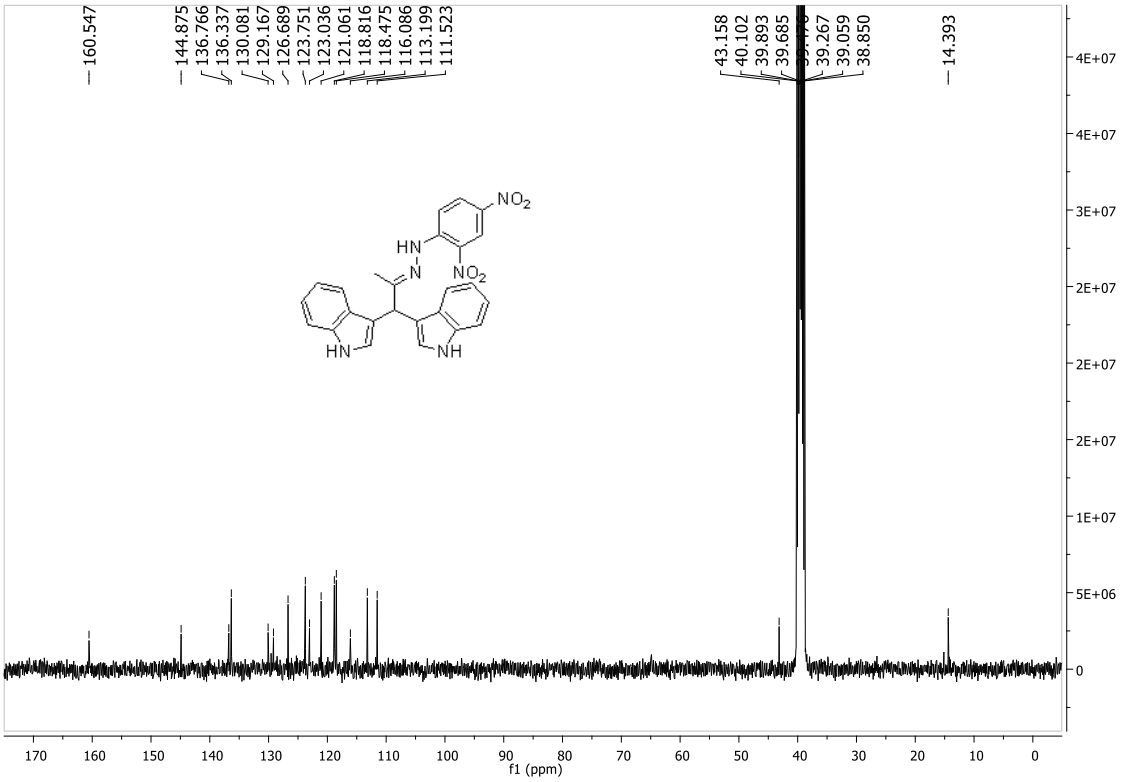



**Figure 54.** $^{13}$C RMN spectrum of compound **2.89b** (DMSO-$d_6$).

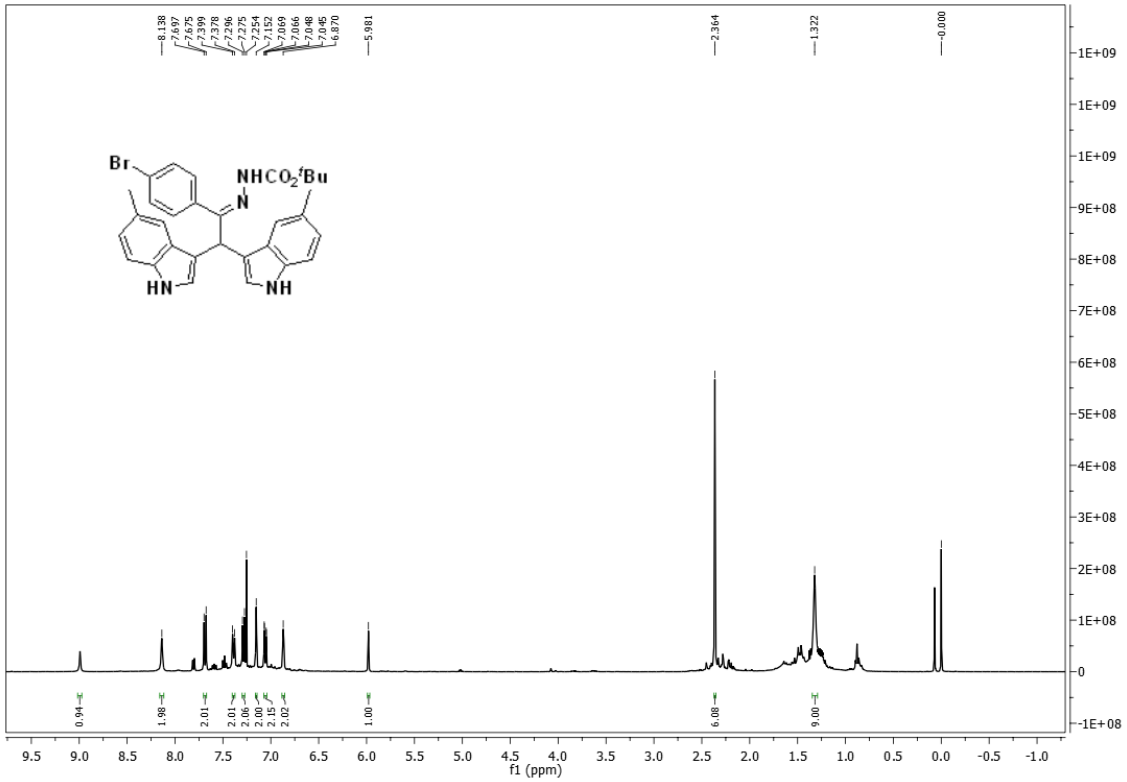



**Figure 55.** $^{1}$H RMN spectrum of compound **3.43b** ($CDCl_3$).

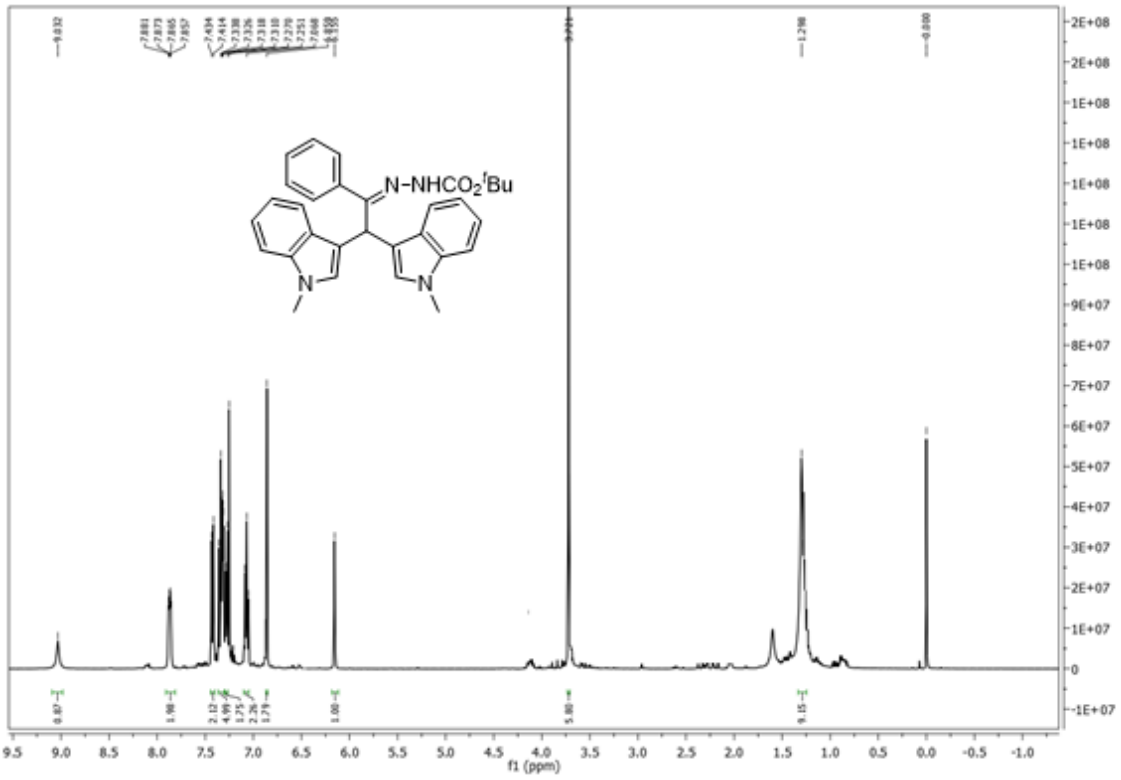



**Figure 56.** $^{1}$H RMN spectrum of compound **3.42d** ($CDCl_3$).

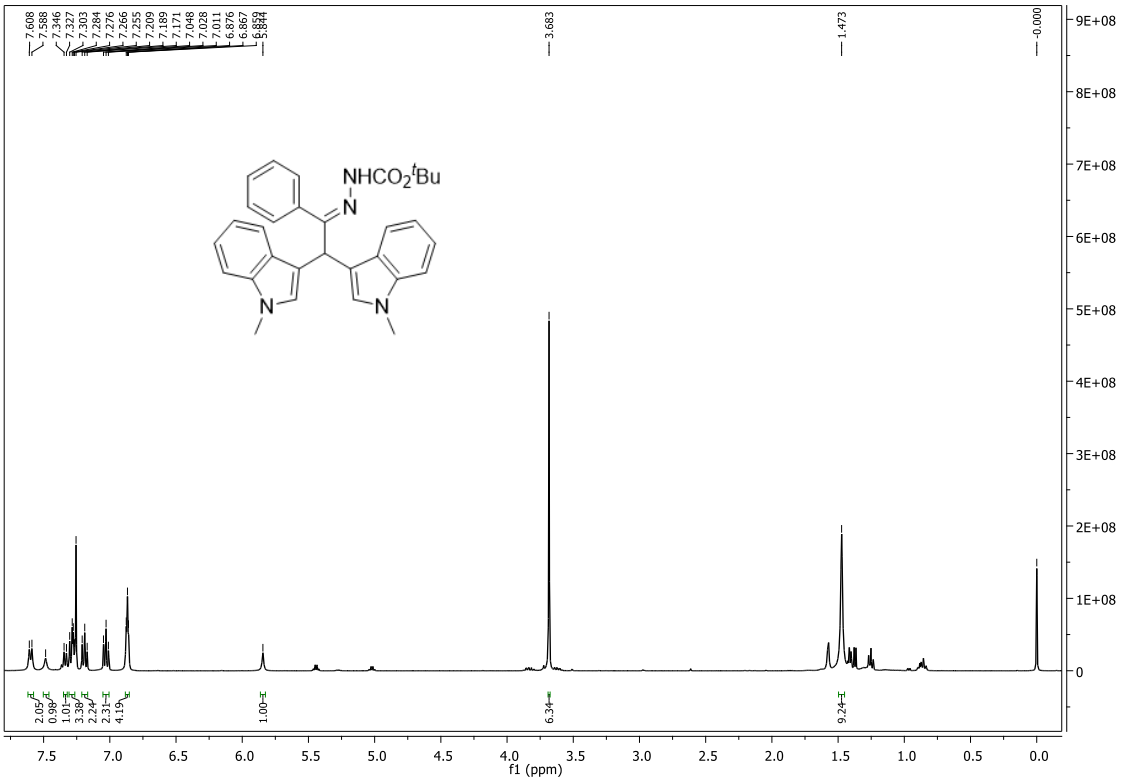



**Figure 57.** $^{1}$H RMN spectrum of compound **3.43c** ($CDCl_3$).

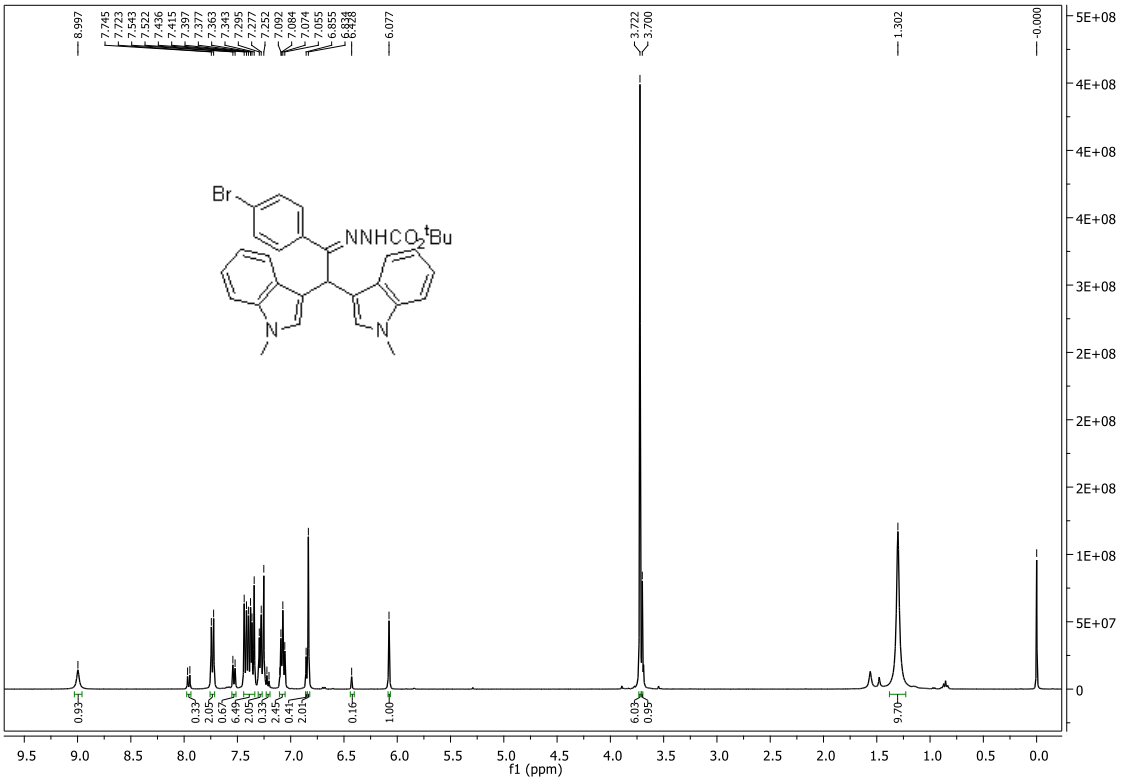



**Figure 58.** $^{1}$H RMN spectrum of compound **3.42e/3.43d** ($CDCl_3$).

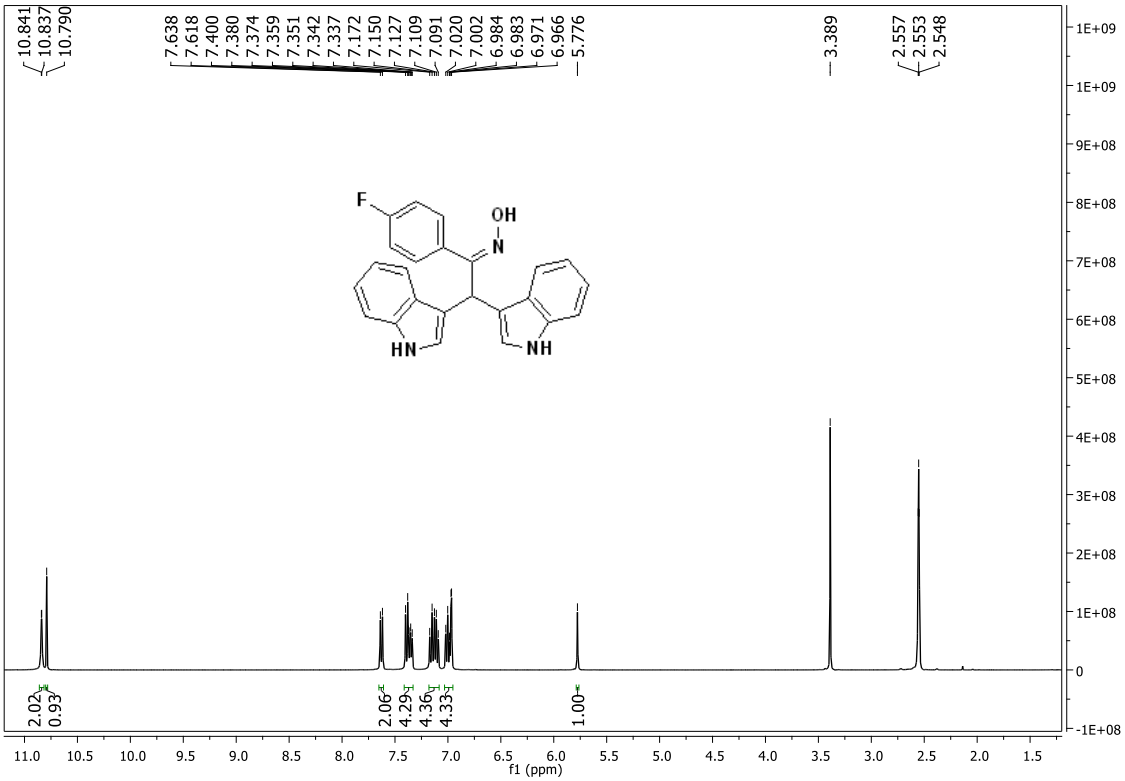



**Figure 59.** $^{1}$H RMN spectrum of compound **3.44a** (DMSO-$d_6$).

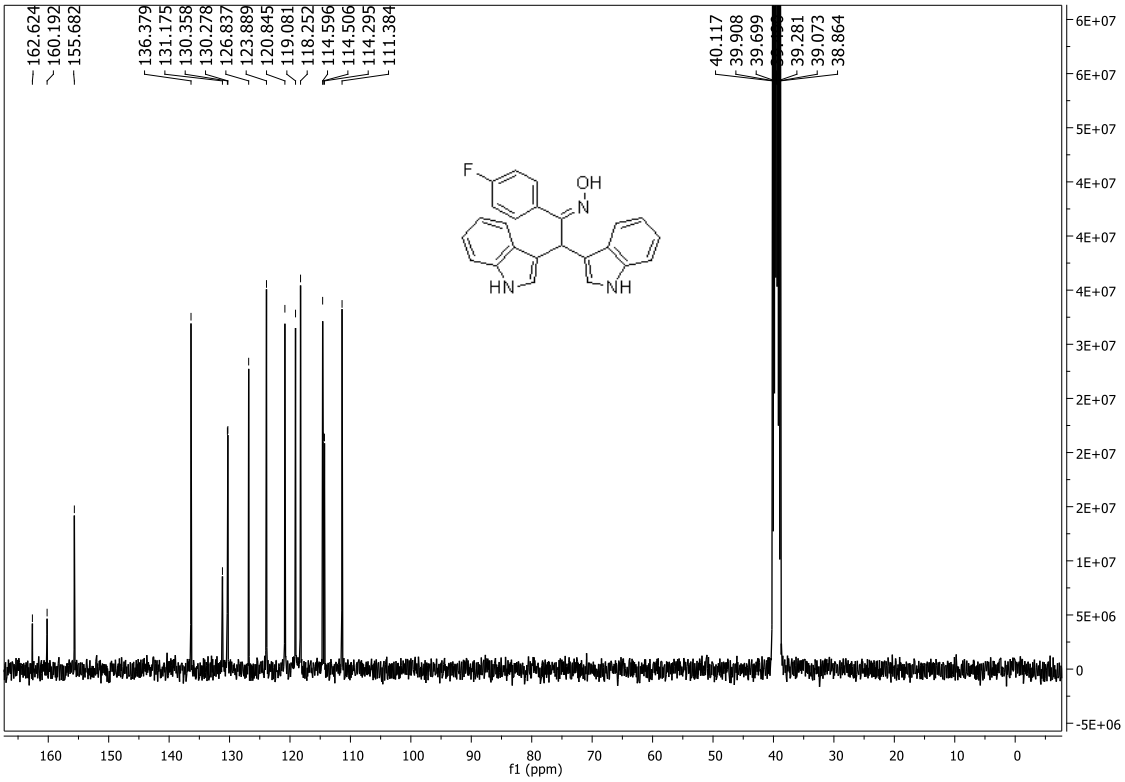



**Figure 60.** $^{13}$C RMN spectrum of compound **3.44a** (DMSO-$d_6$).

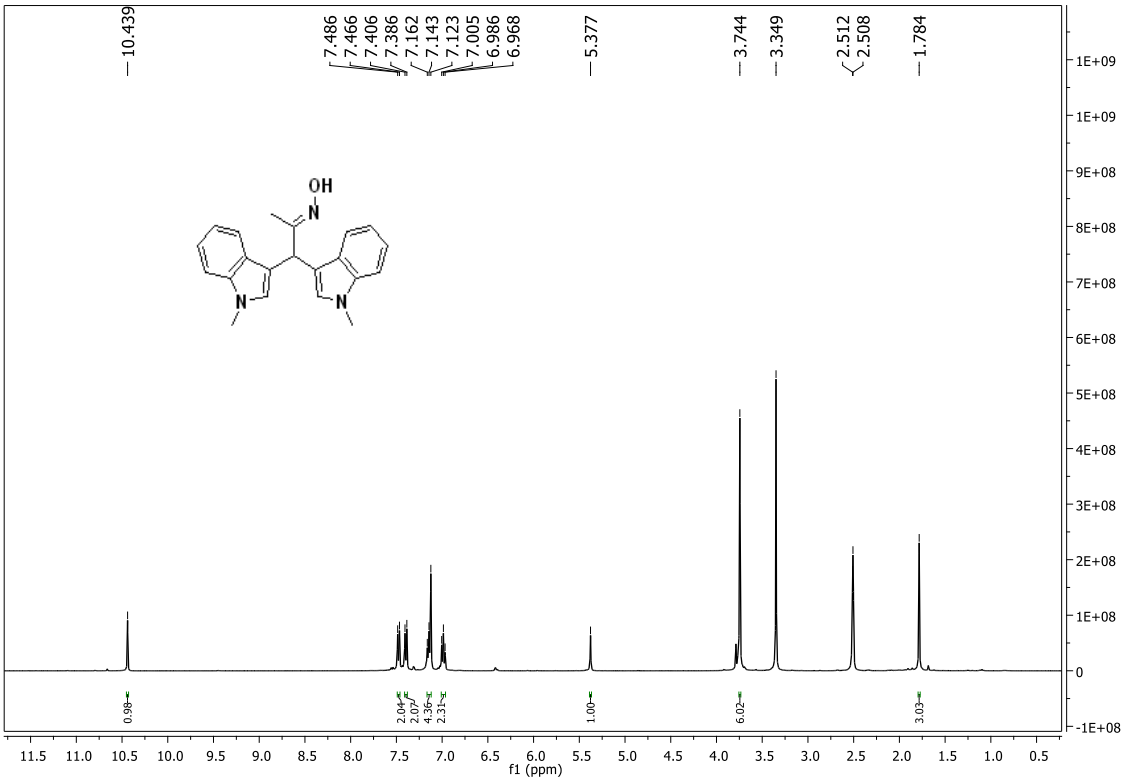



**Figure 61.** $^{1}$H RMN spectrum of compound **3.41c** (DMSO-$d_6$).

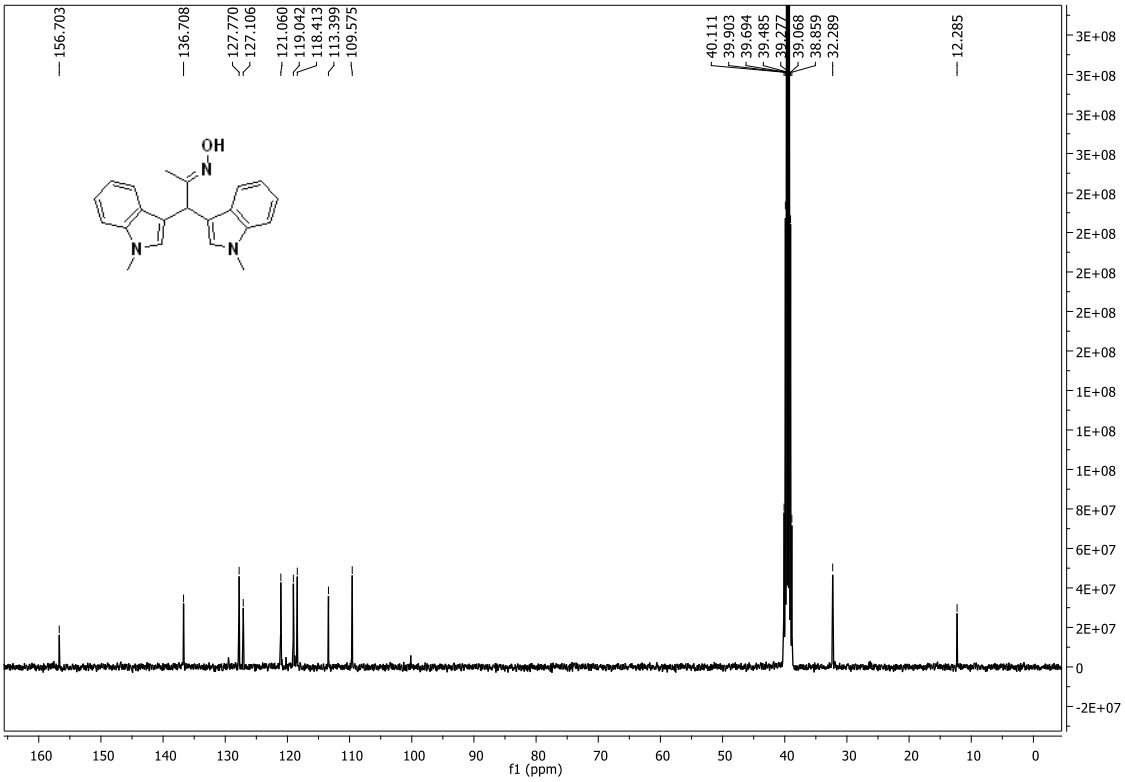



**Figure 62.** $^{13}$C RMN spectrum of compound **3.41c** (DMSO-$d_6$).

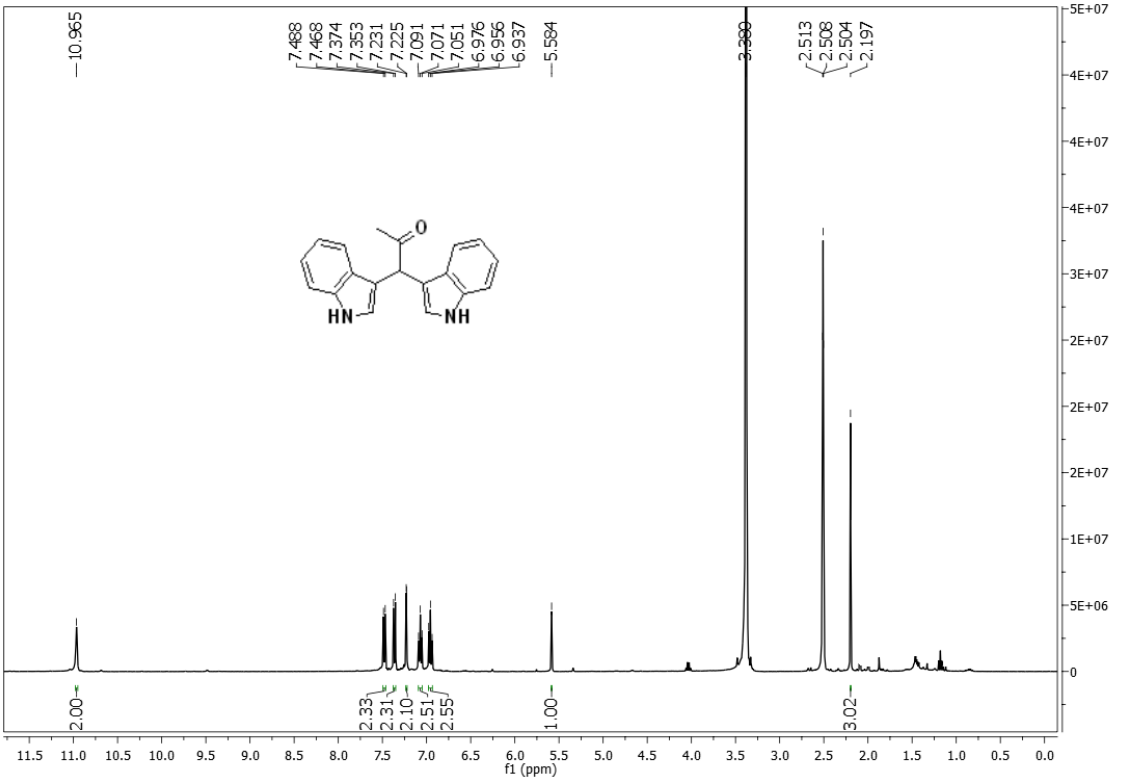



**Figure 63.** $^{1}$H RMN spectrum of compound **3.45a** (DMSO-$d_6$).

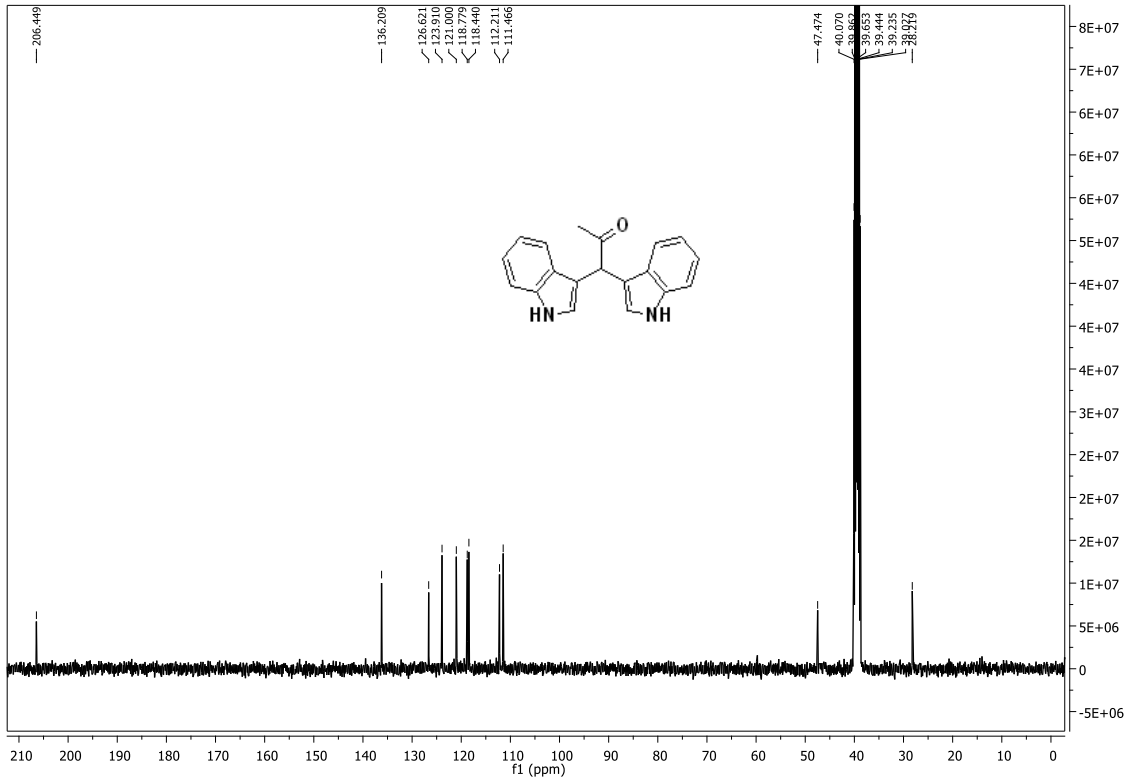



**Figure 64.** $^{13}$C RMN spectrum of compound **3.45a** (DMSO-$d_6$).

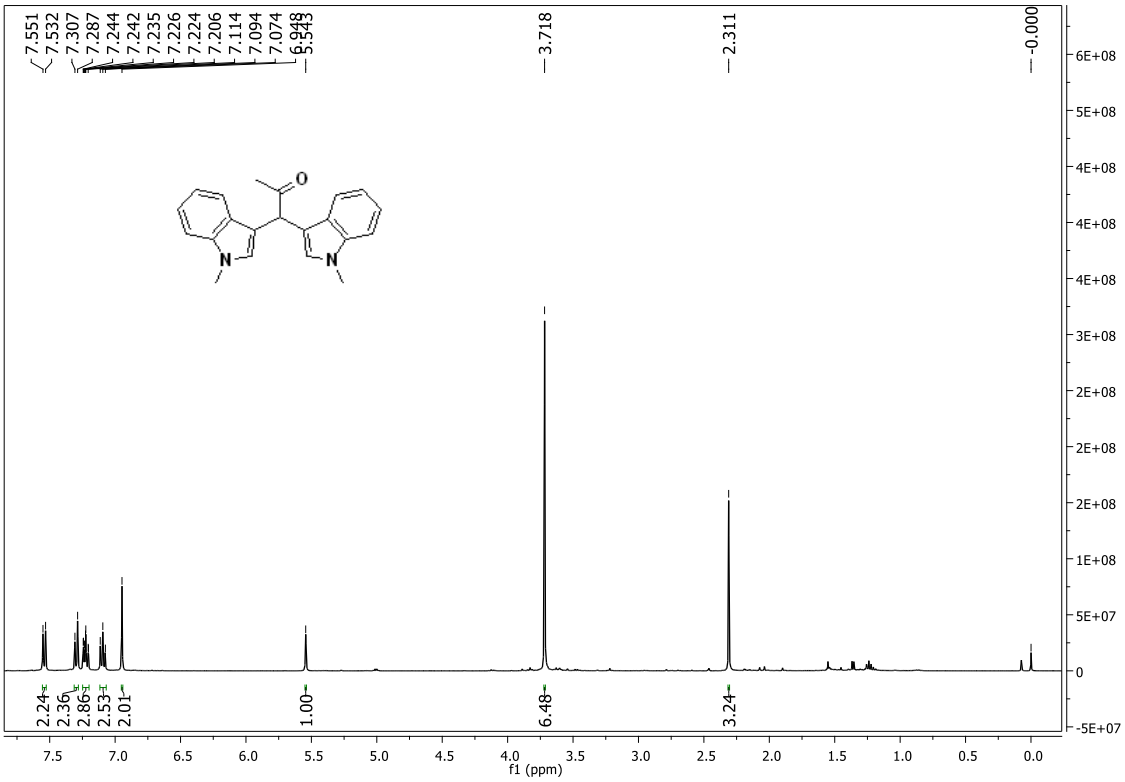



**Figure 65.** $^{1}$H RMN spectrum of compound **3.45b** ($CDCl_3$).

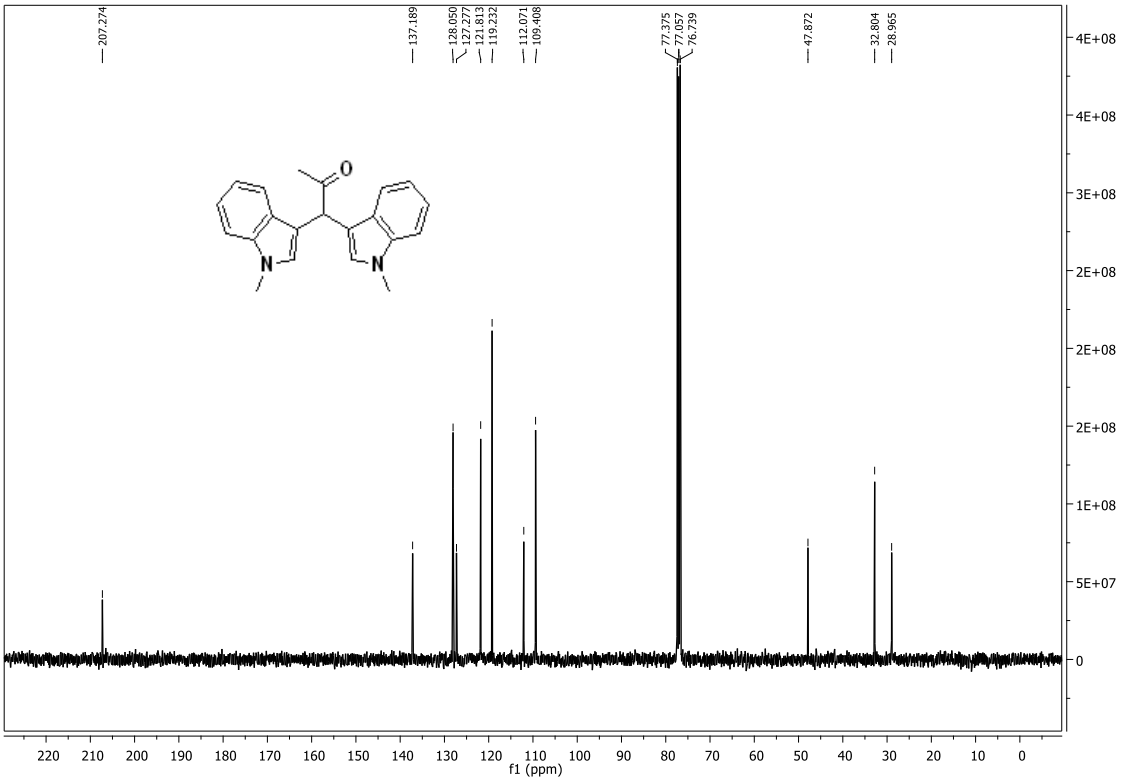



**Figure 66.** $^{13}$C RMN spectrum of compound **3.45b** ($CDCl_3$).

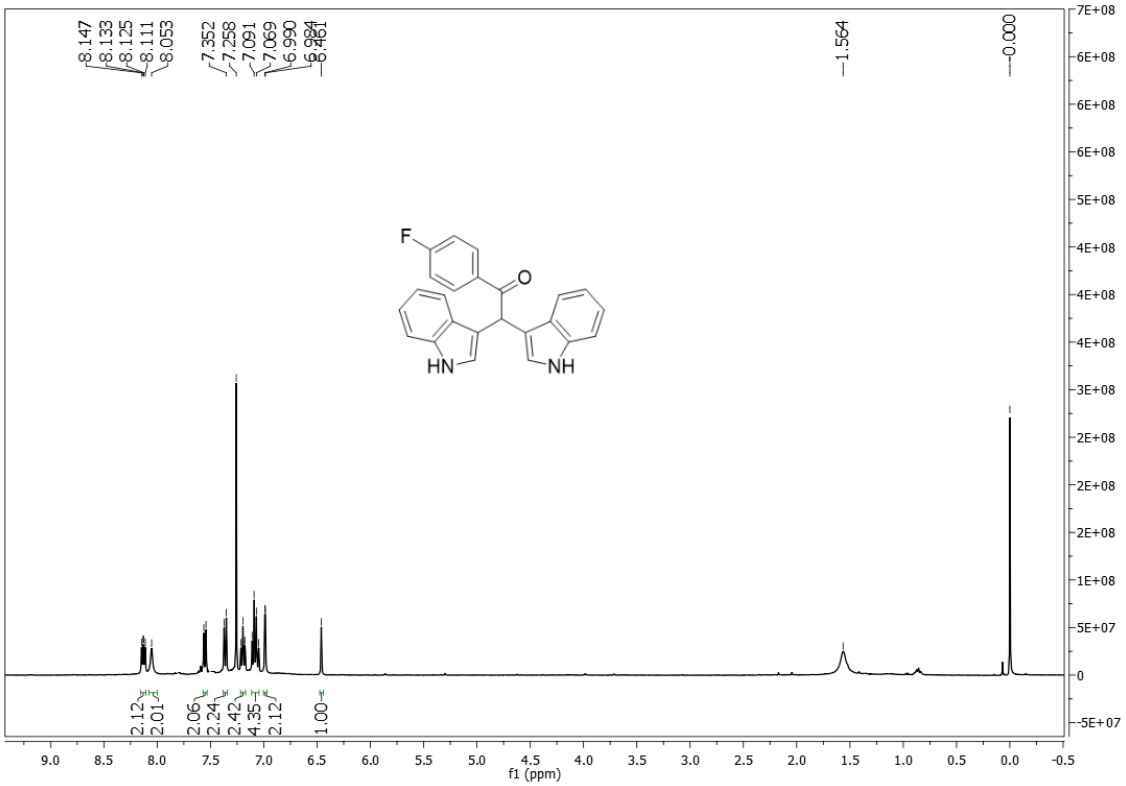



**Figure 67.** $^{1}$H RMN spectrum of compound **3.45d** ($CDCl_3$).

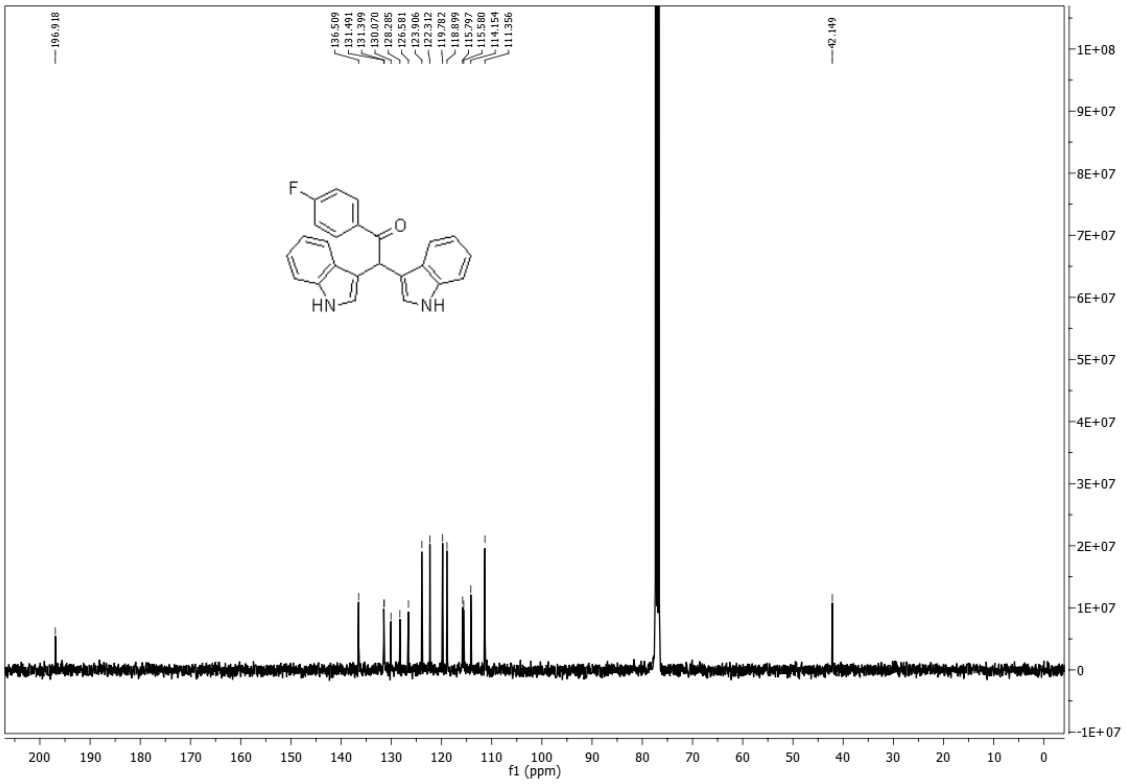



**Figure 68.** $^{13}$C RMN spectrum of compound **3.45d** ($CDCl_3$).

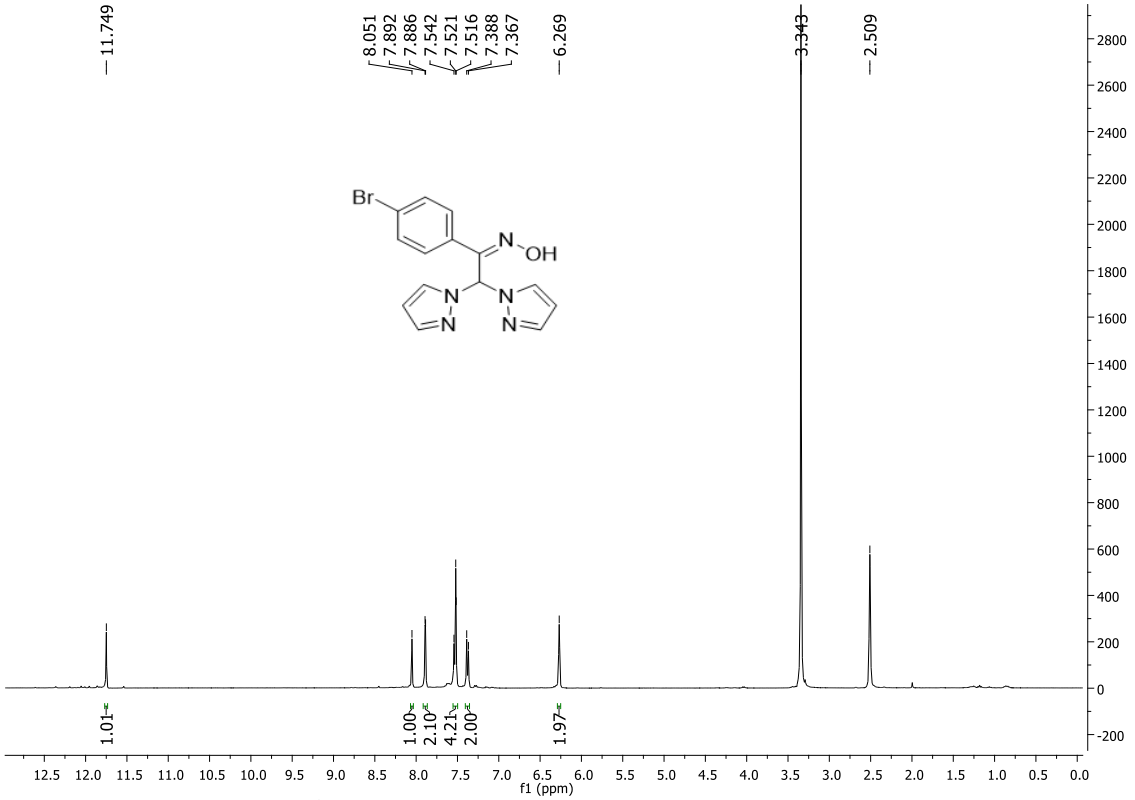



**Figure 69.** $^{1}$H RMN spectrum of compound **4.27a** (DMSO-$d_6$).

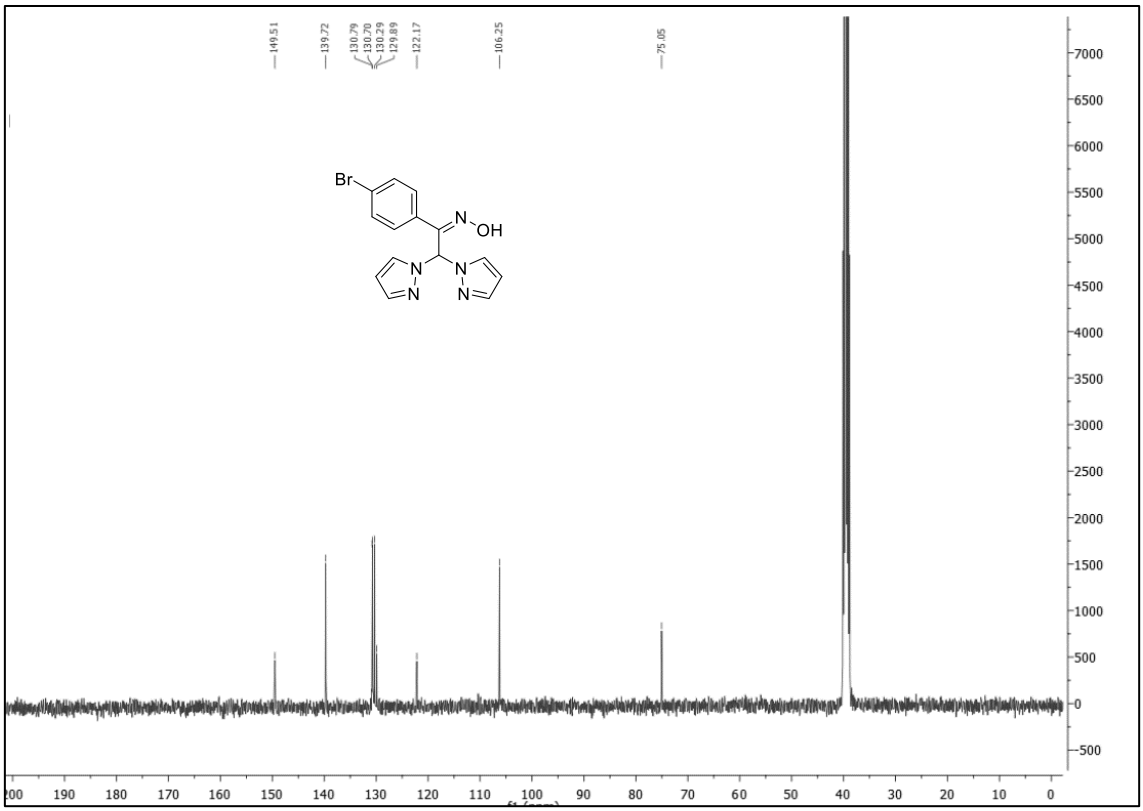



**Figure 70.** $^{13}$C RMN spectrum of compound **4.27a** (DMSO-$d_6$).

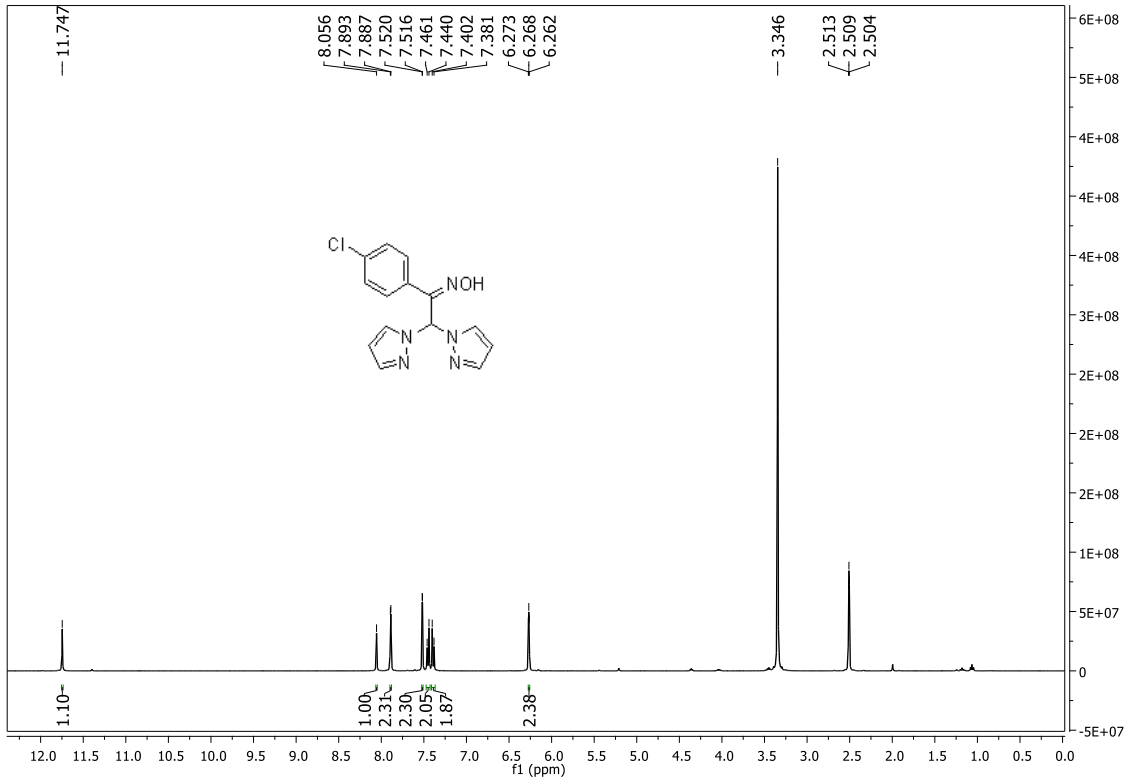



**Figure 71.** $^{1}$H RMN spectrum of compound **4.27b** (DMSO-$d_6$).

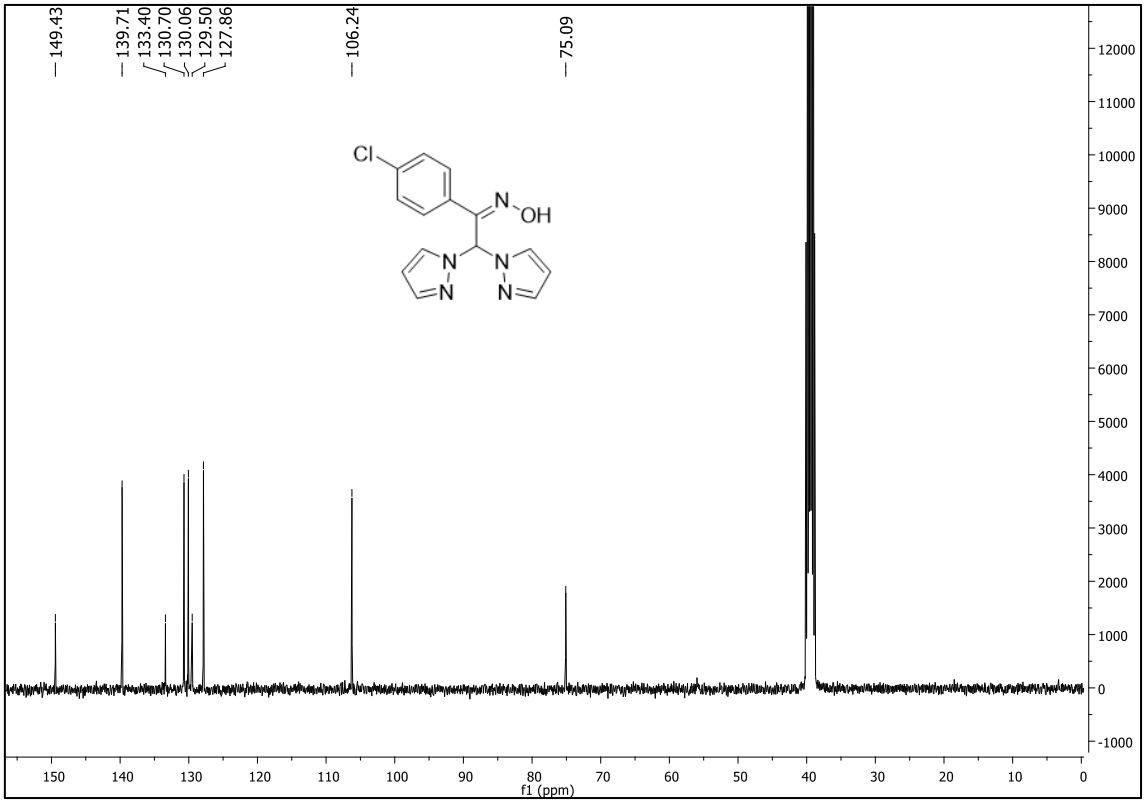



**Figure 72.** $^{13}$C RMN spectrum of compound **4.27b** (DMSO-$d_6$).

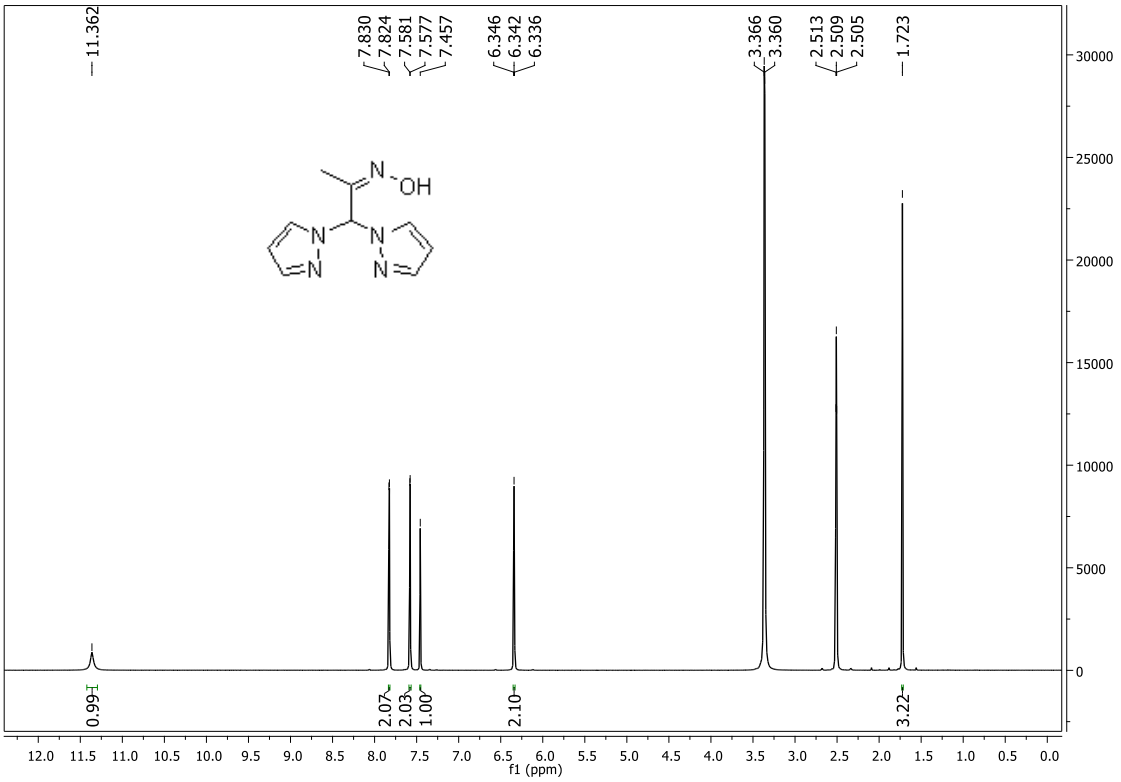



**Figure 73.** $^{1}$H RMN spectrum of compound **4.27c** (DMSO-$d_6$).

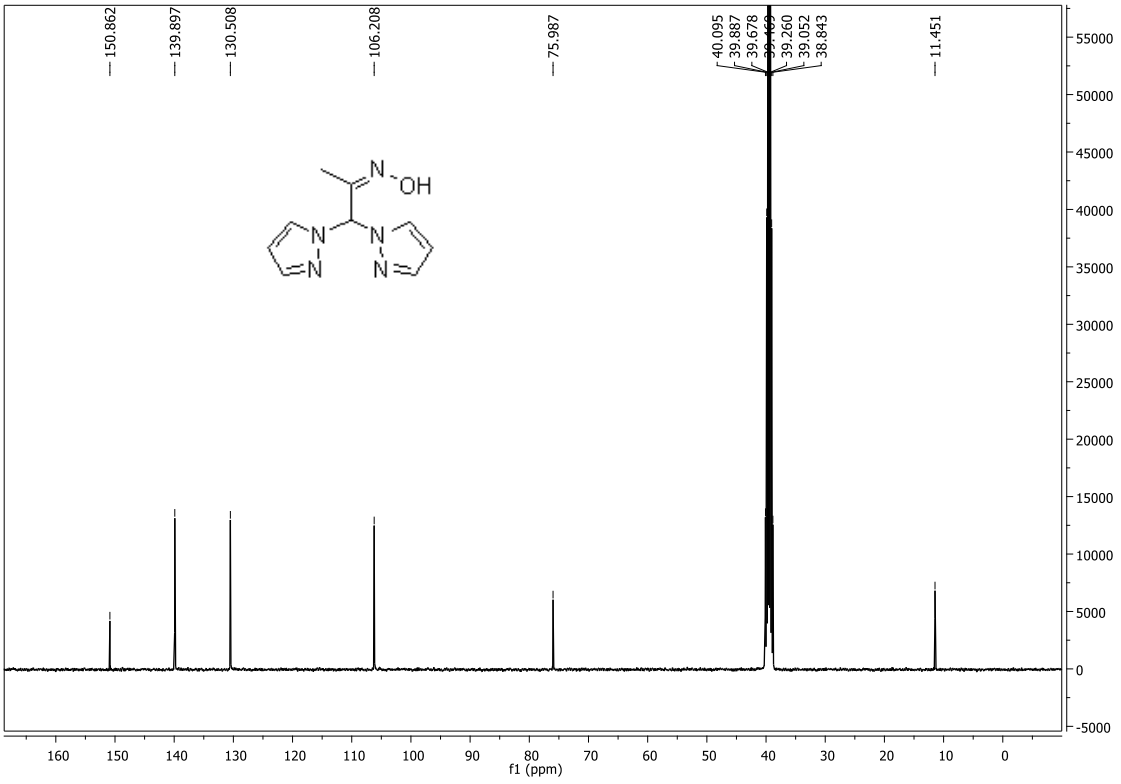



**Figure 74.** $^{13}$C RMN spectrum of compound **4.27c** (DMSO-$d_6$).

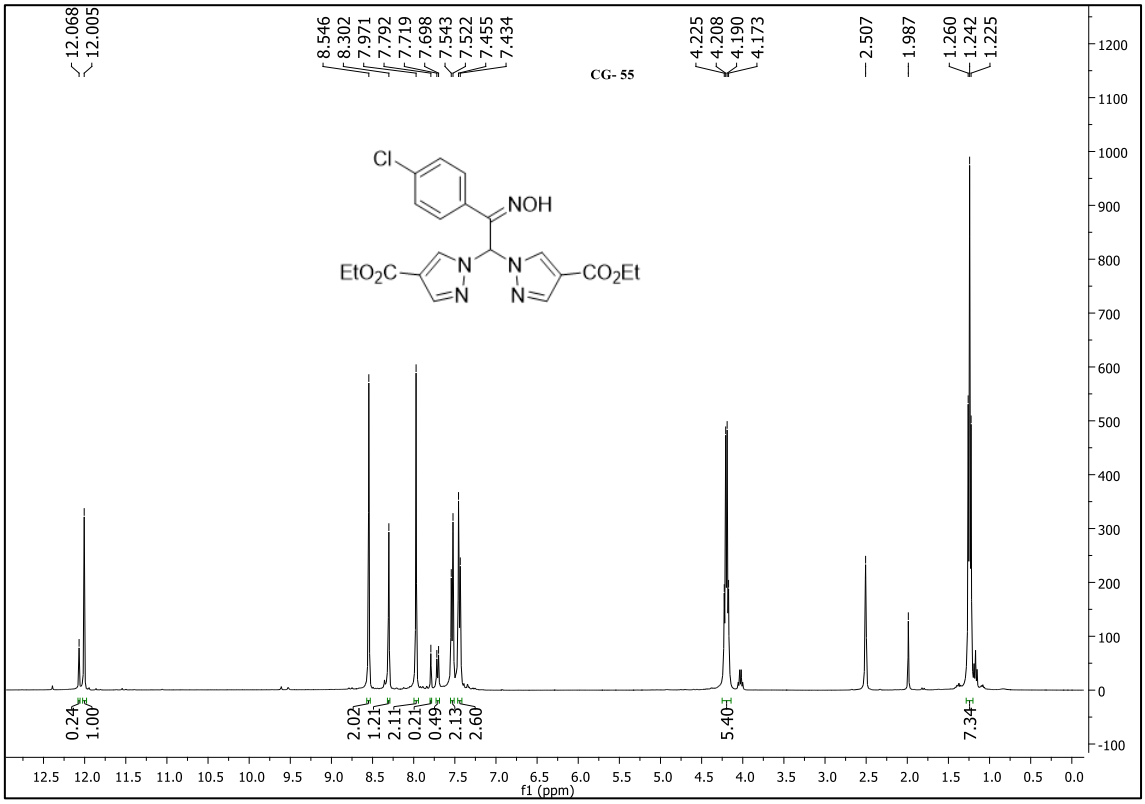



**Figure 75.** $^{1}$H RMN spectrum of compound **4.27d** (DMSO-$d_6$).

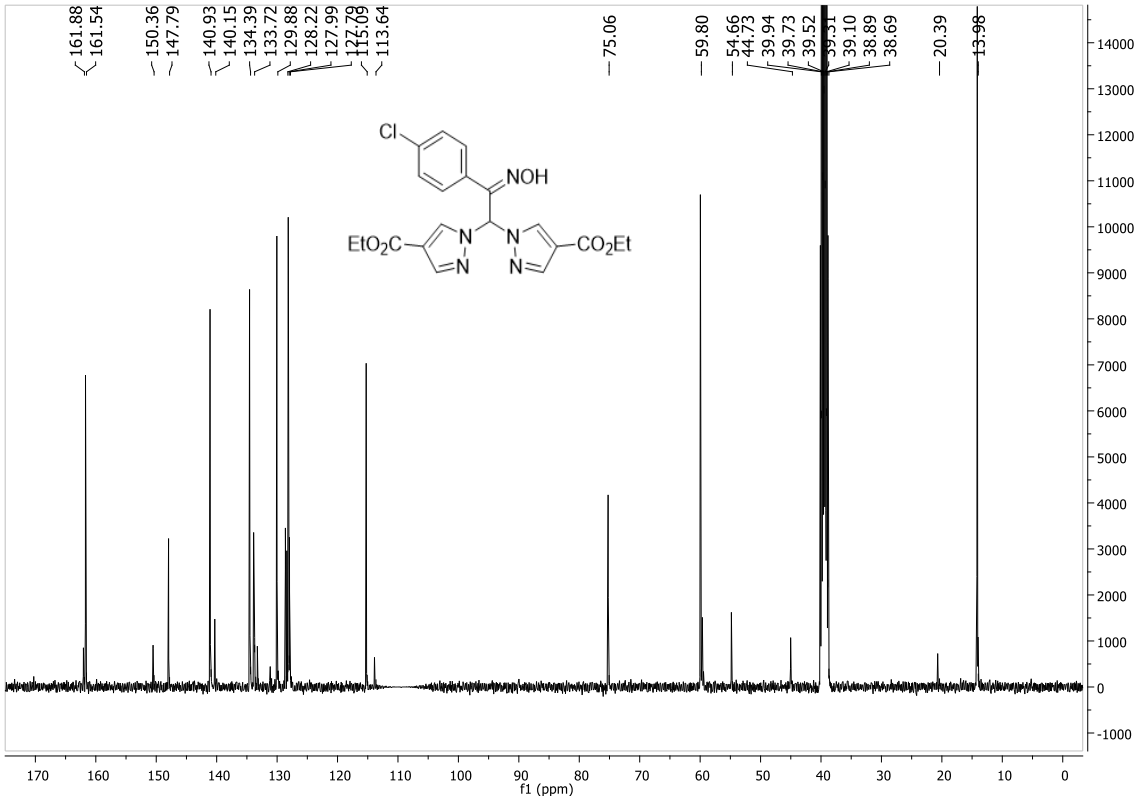



**Figure 76.** $^{13}$C RMN spectrum of compound **4.27d** (DMSO-$d_6$).

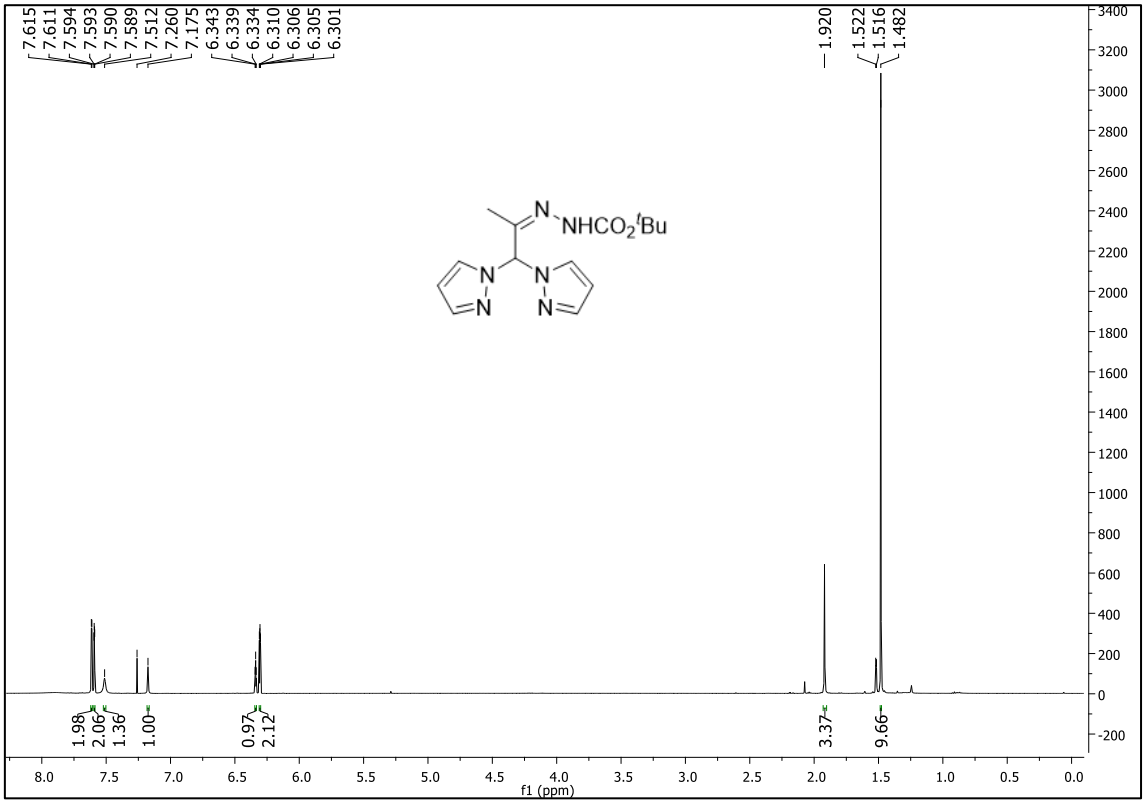



**Figure 77.** $^{1}$H RMN spectrum of compound **4.28a** ($CDCl_3$).

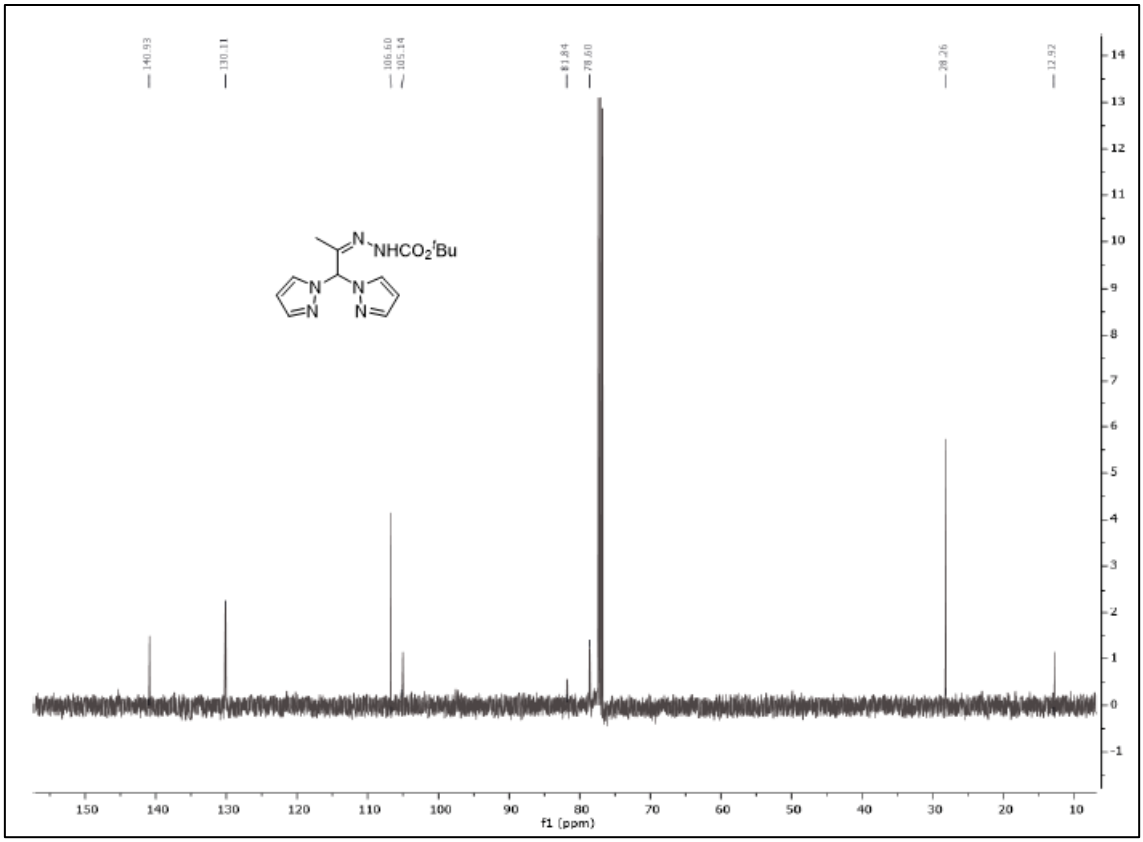



**Figure 78.** $^{13}$C RMN spectrum of compound **4.28a** ($CDCl_3$).

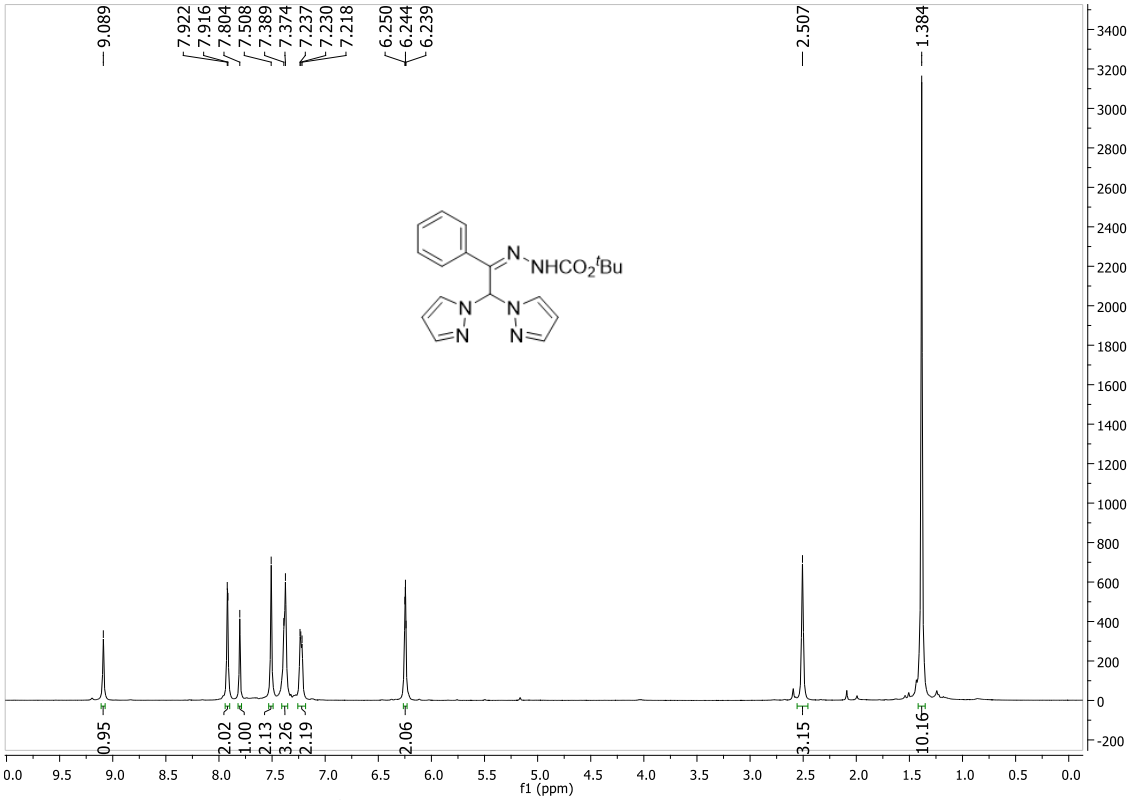



**Figure 79.** $^{1}$H RMN spectrum of compound **4.28b** ($CDCl_3$).

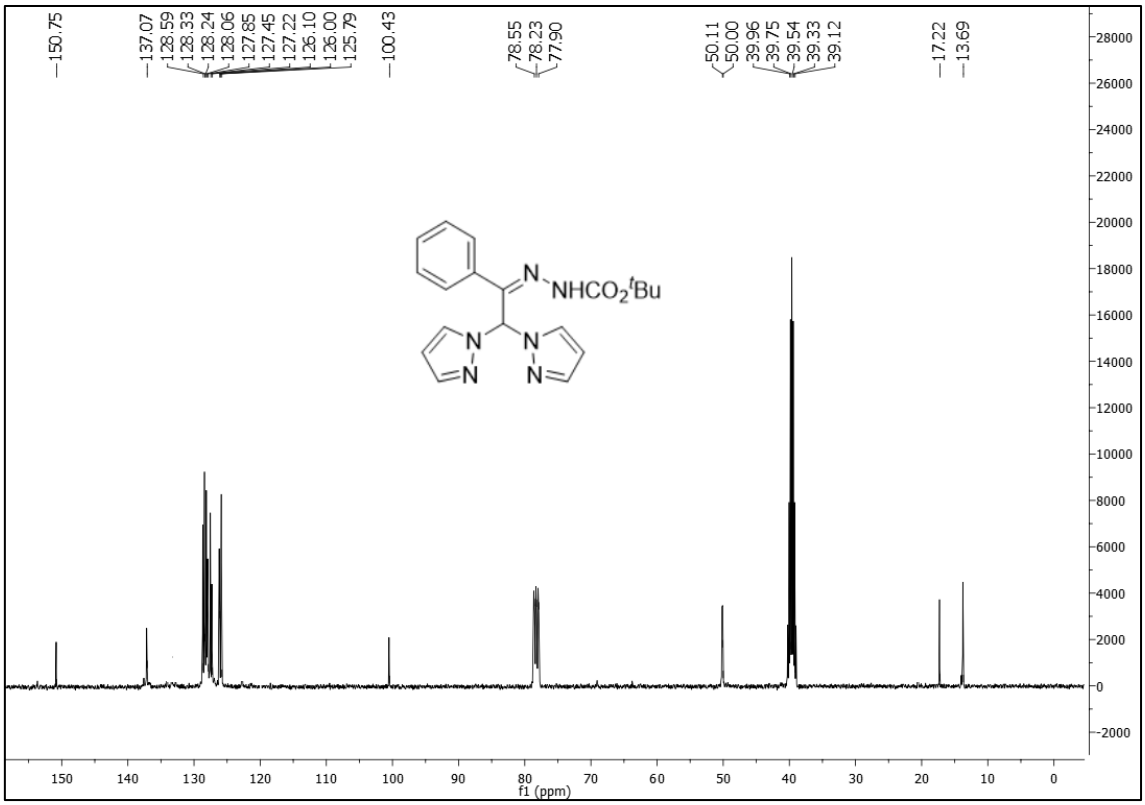



**Figure 80.** $^{13}$C RMN spectrum of compound **4.28b** ($CDCl_3$).

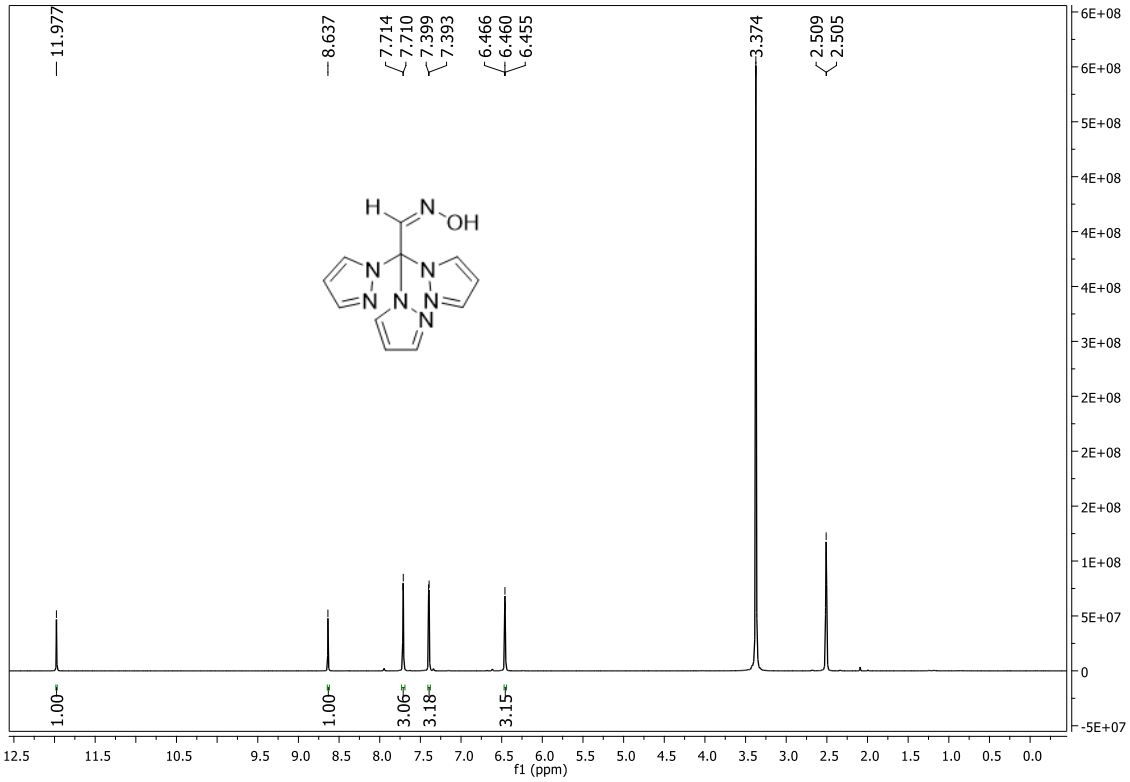



**Figure 81.** $^{1}$H RMN spectrum of compound **4.31** (DMSO-$d_6$).

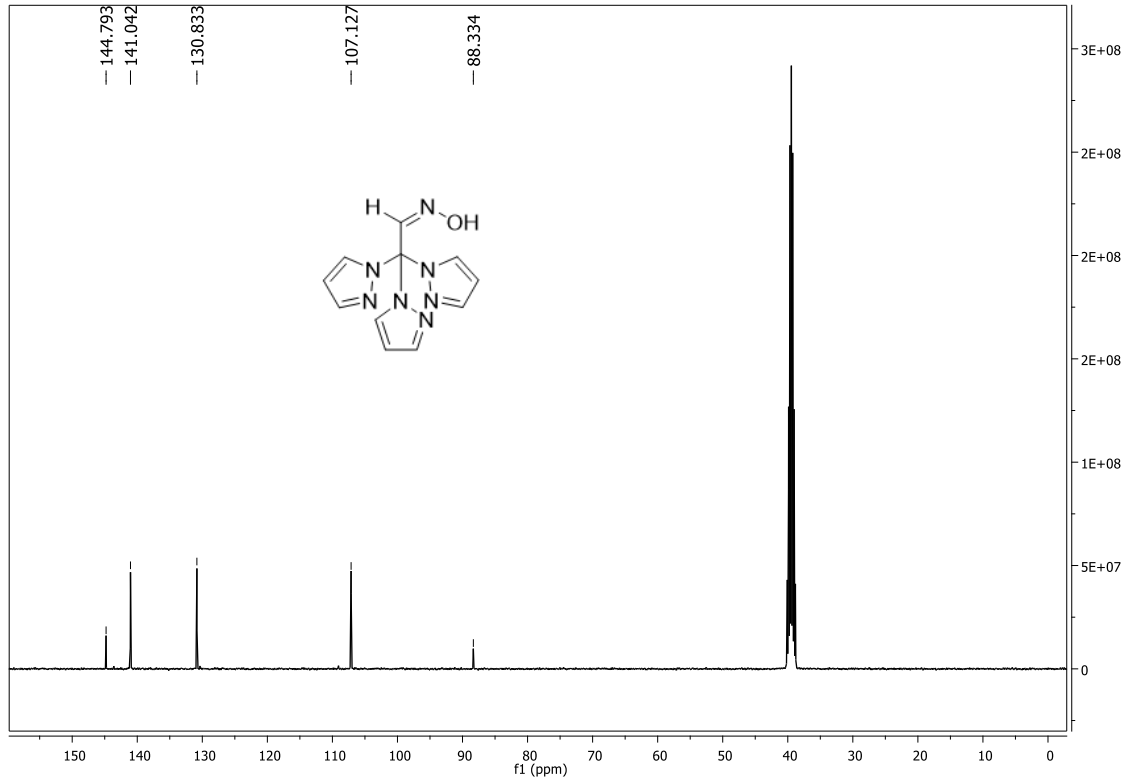



**Figure 82.** $^{13}$C RMN spectrum of compound **4.31** (DMSO-$d_6$).

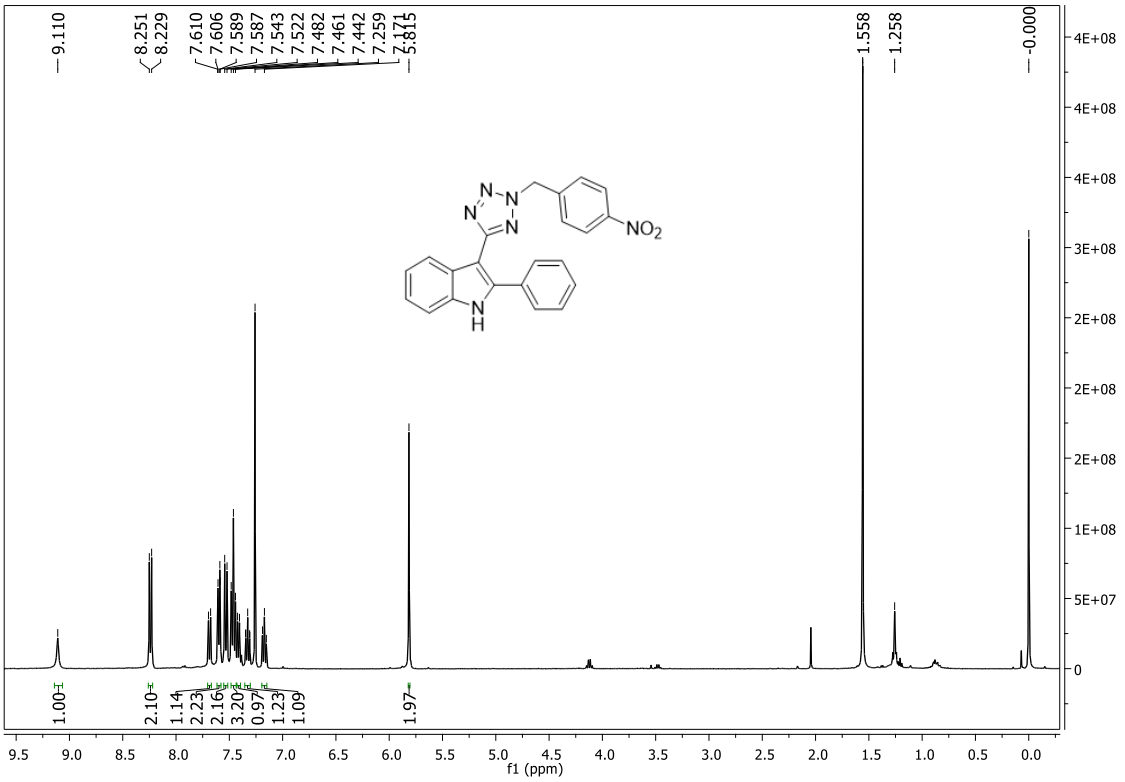



**Figure 83.** $^{1}$H RMN spectrum of compound **5.58a** ($CDCl_3$).

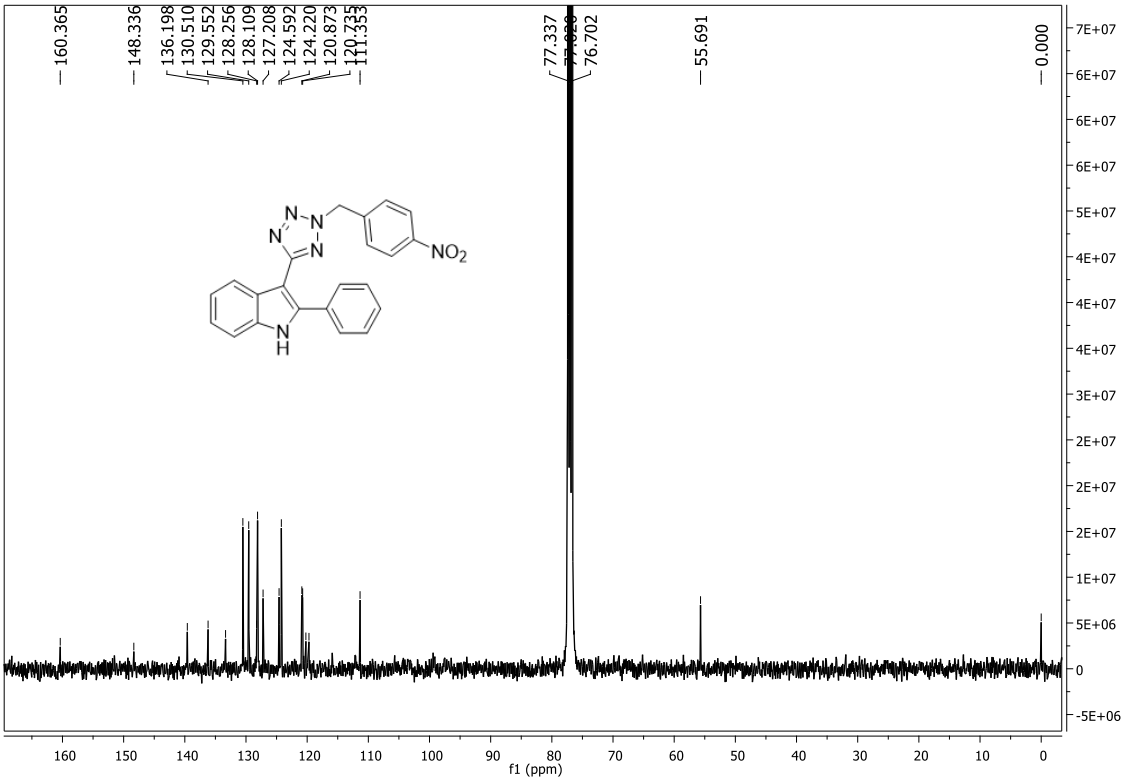



**Figure 84.** $^{13}$C RMN spectrum of compound **5.58a** ($CDCl_3$).

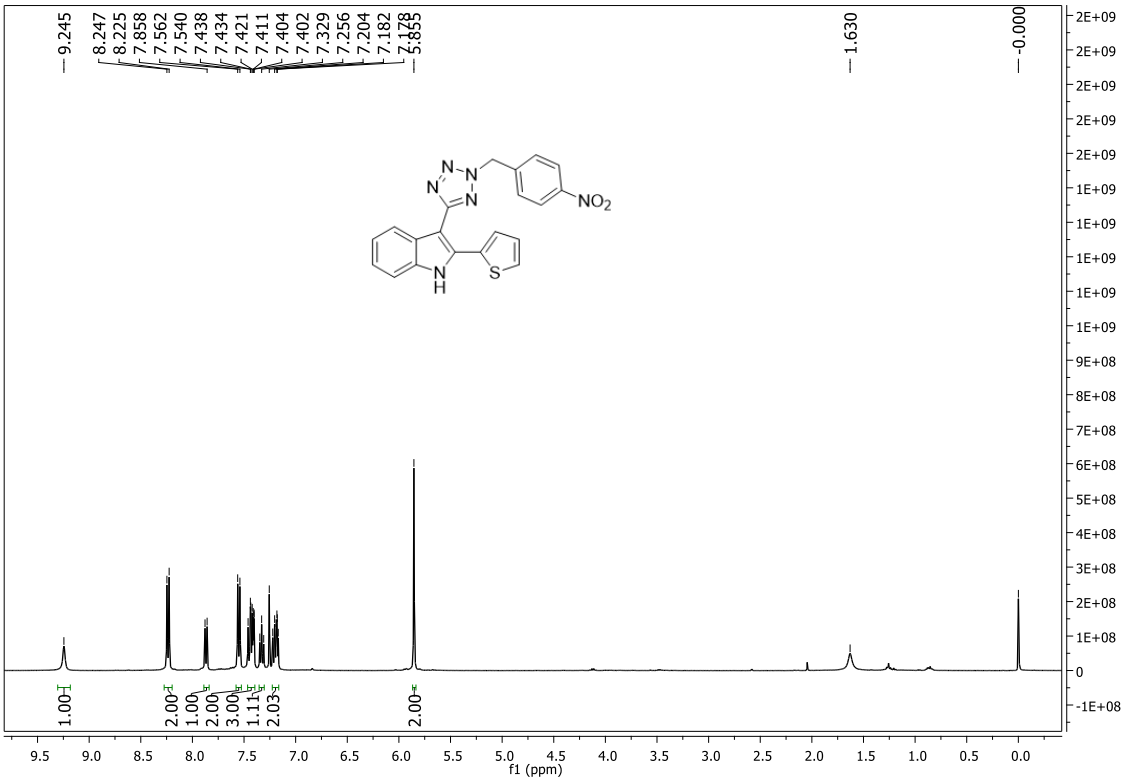



**Figure 85.** $^{1}$H RMN spectrum of compound **5.58b** ($CDCl_3$).

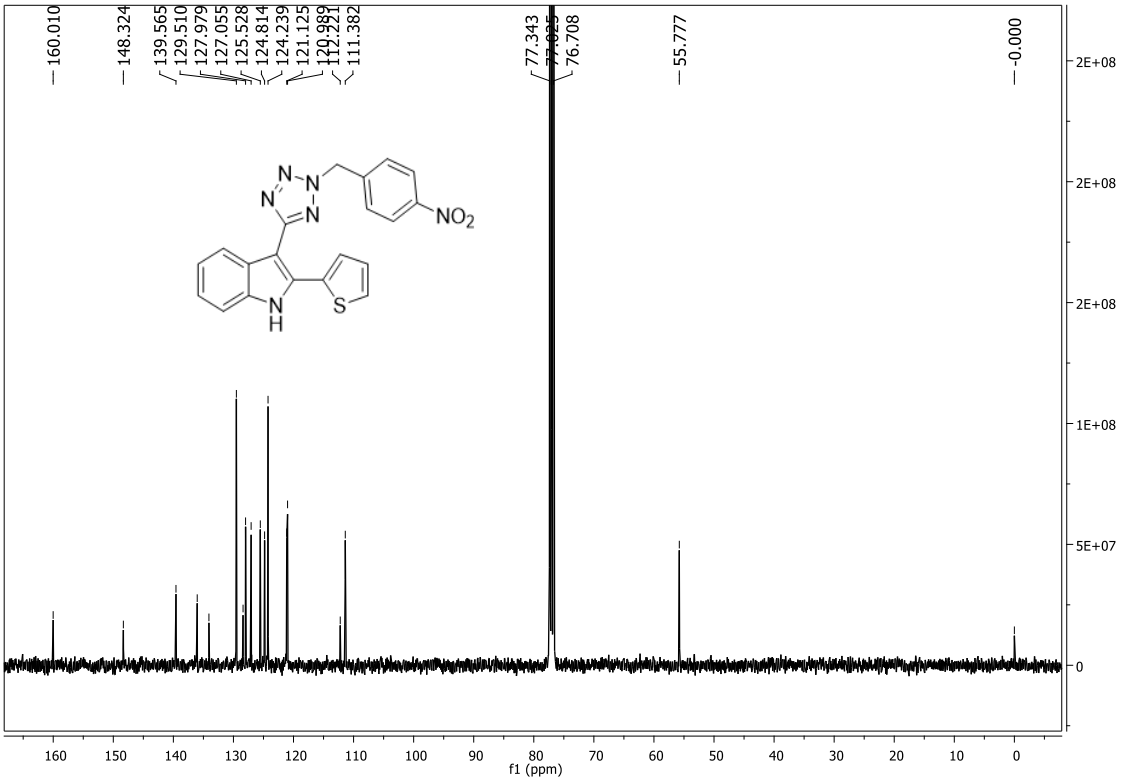



**Figure 86.** $^{13}$C RMN spectrum of compound **5.58b** ($CDCl_3$).

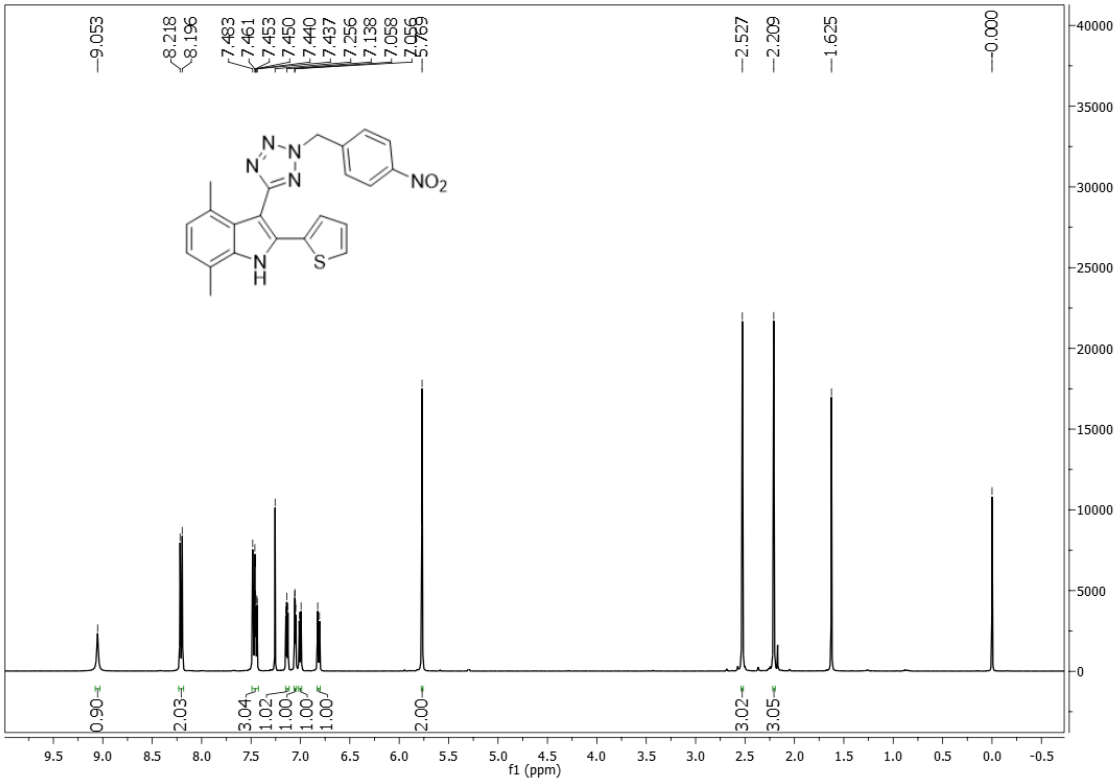



**Figure 87**. $^{1}$H RMN spectrum of compound **5.58c** ($CDCl_3$).

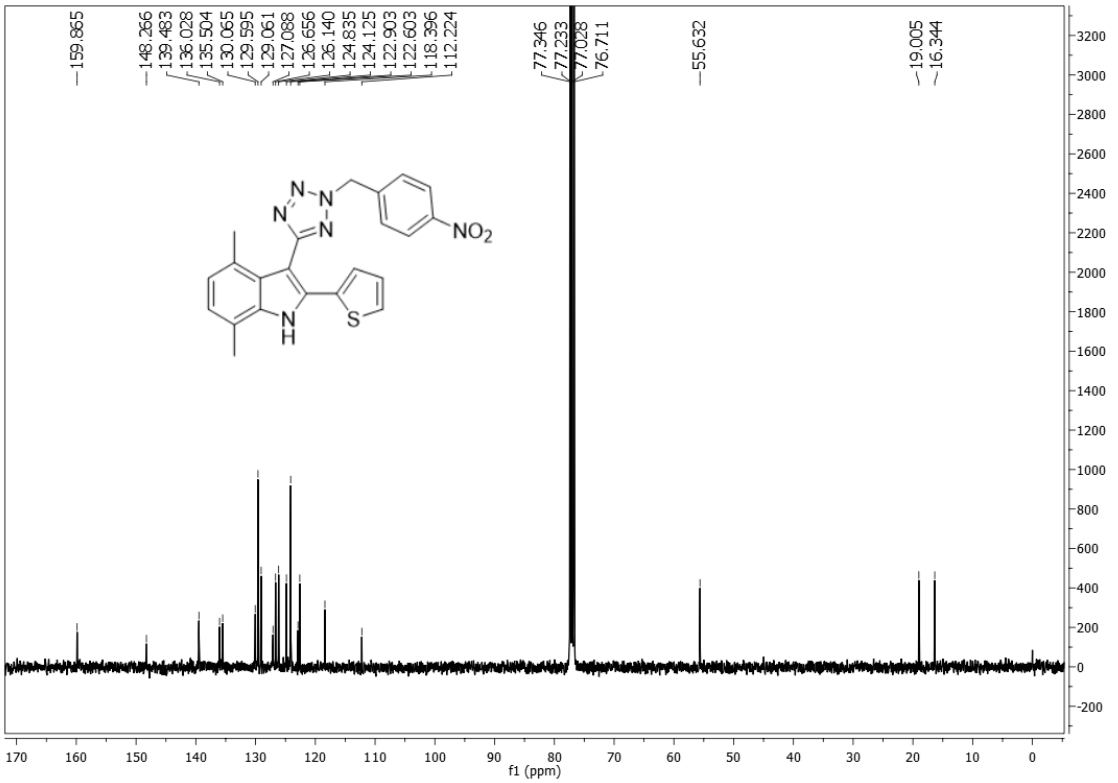



**Figure 88.** $^{13}$C RMN spectrum of compound **5.58c** ($CDCl_3$).

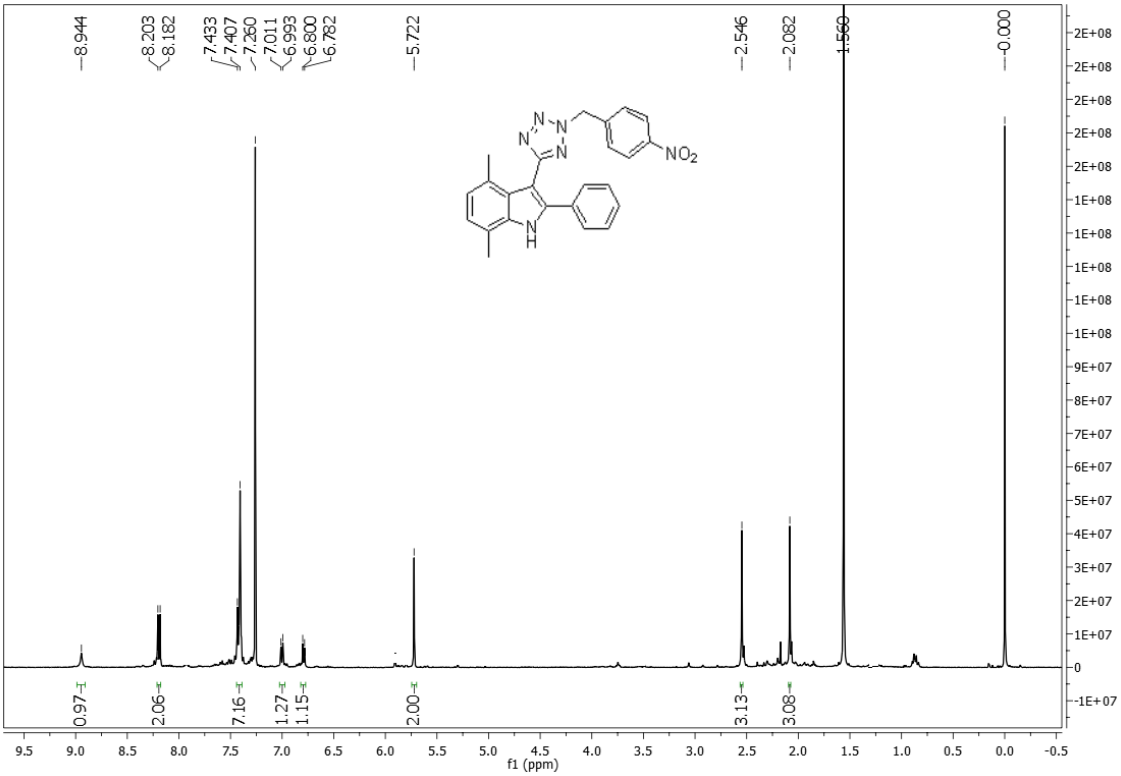



**Figure 89.** $^{1}$H RMN spectrum of compound **5.58d** ($CDCl_3$).

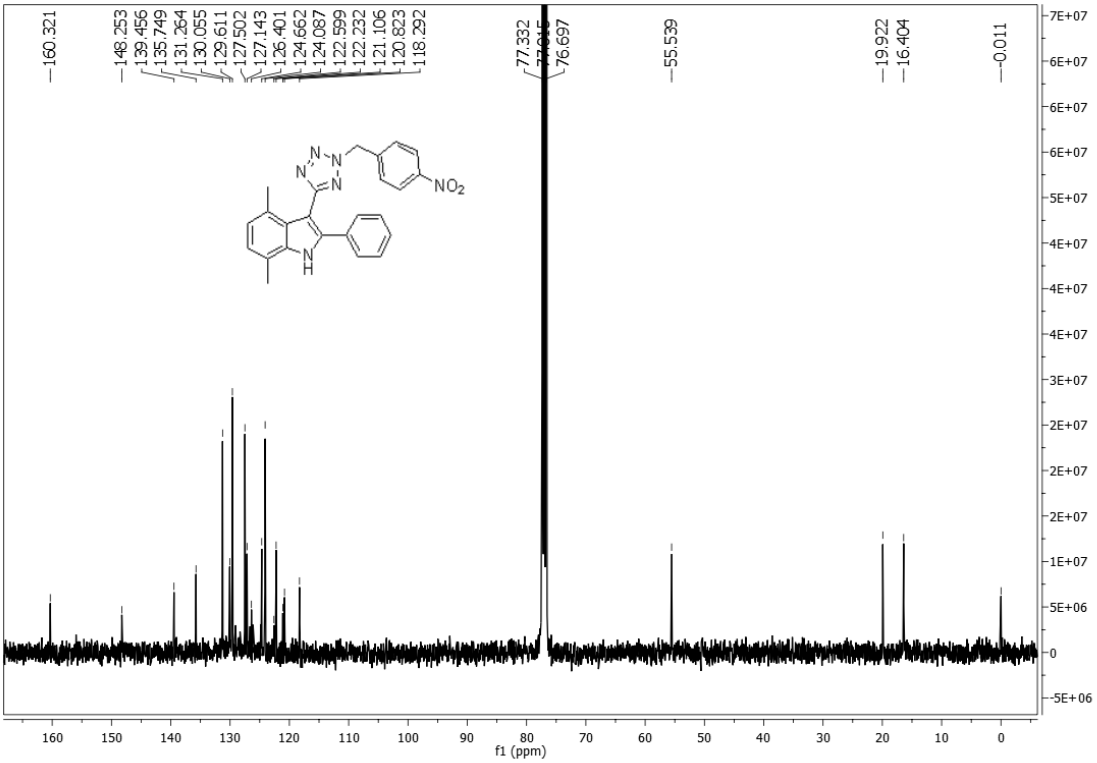



**Figure 90.** $^{13}$C RMN spectrum of compound **5.58d** ($CDCl_3$).

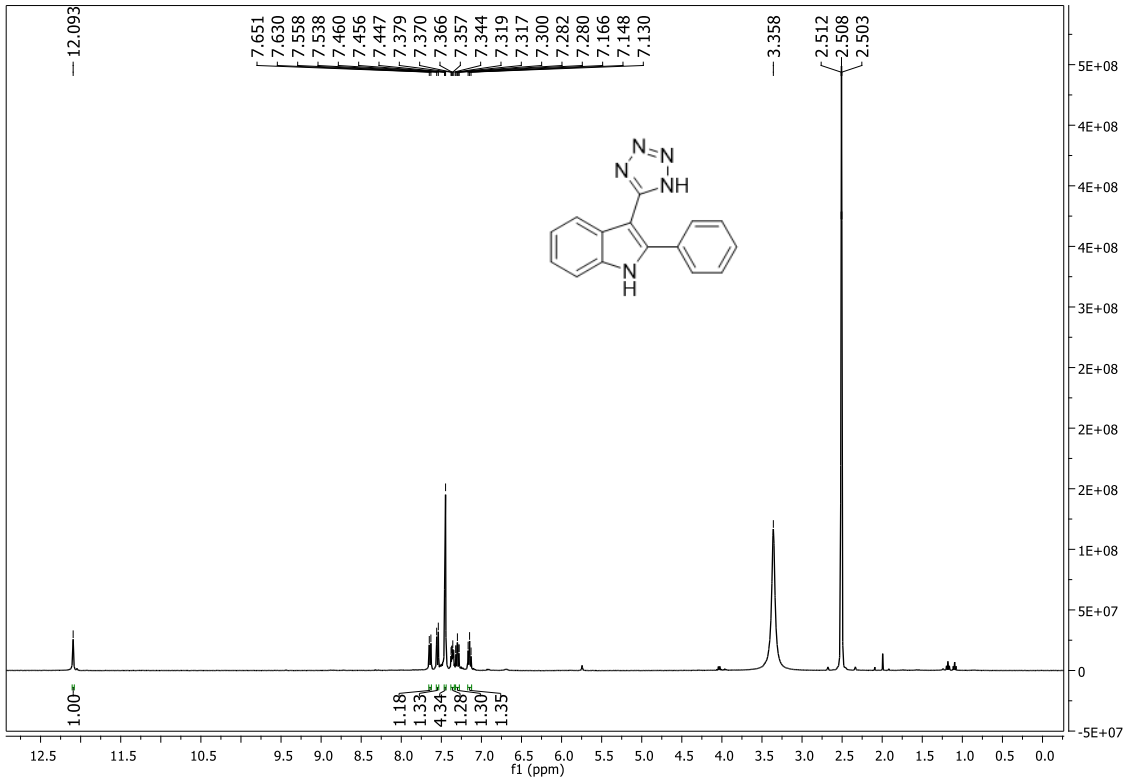



**Figure 91.** $^{1}$H RMN spectrum of compound **5.59a** (DMSO-$d_6$).

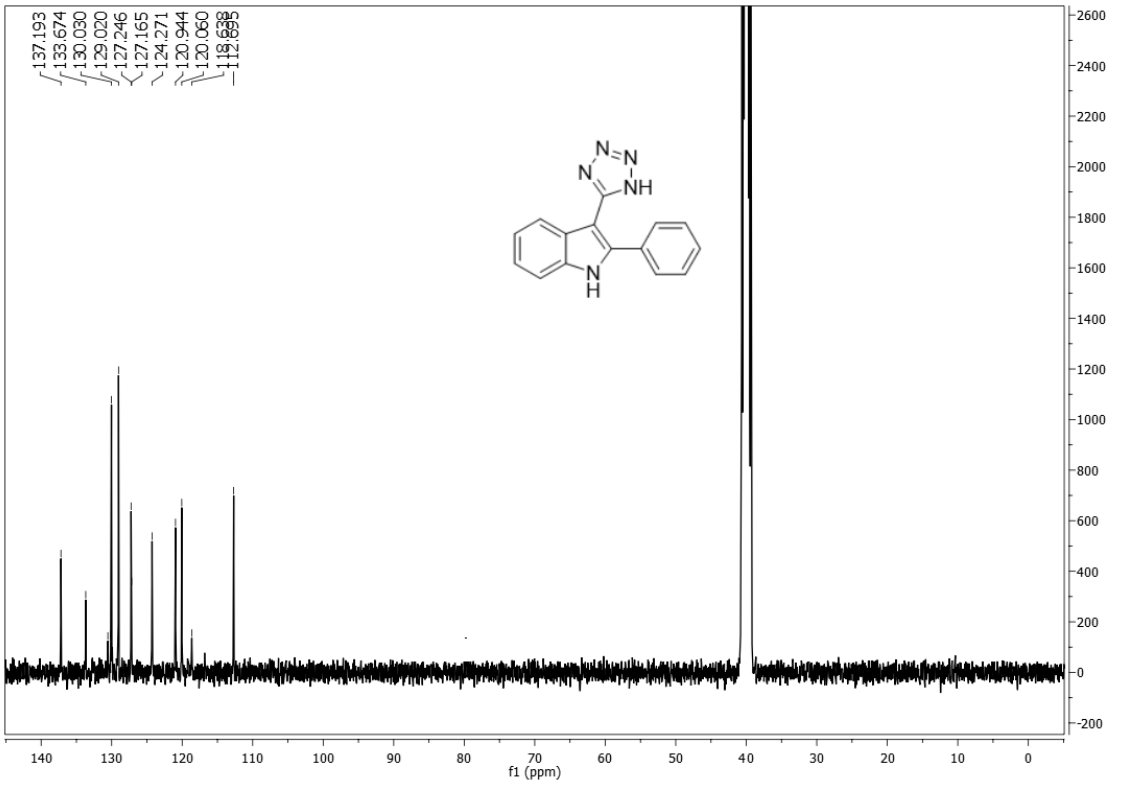



**Figure 92.** $^{13}$C RMN spectrum of compound **5.59a** (DMSO-$d_6$).

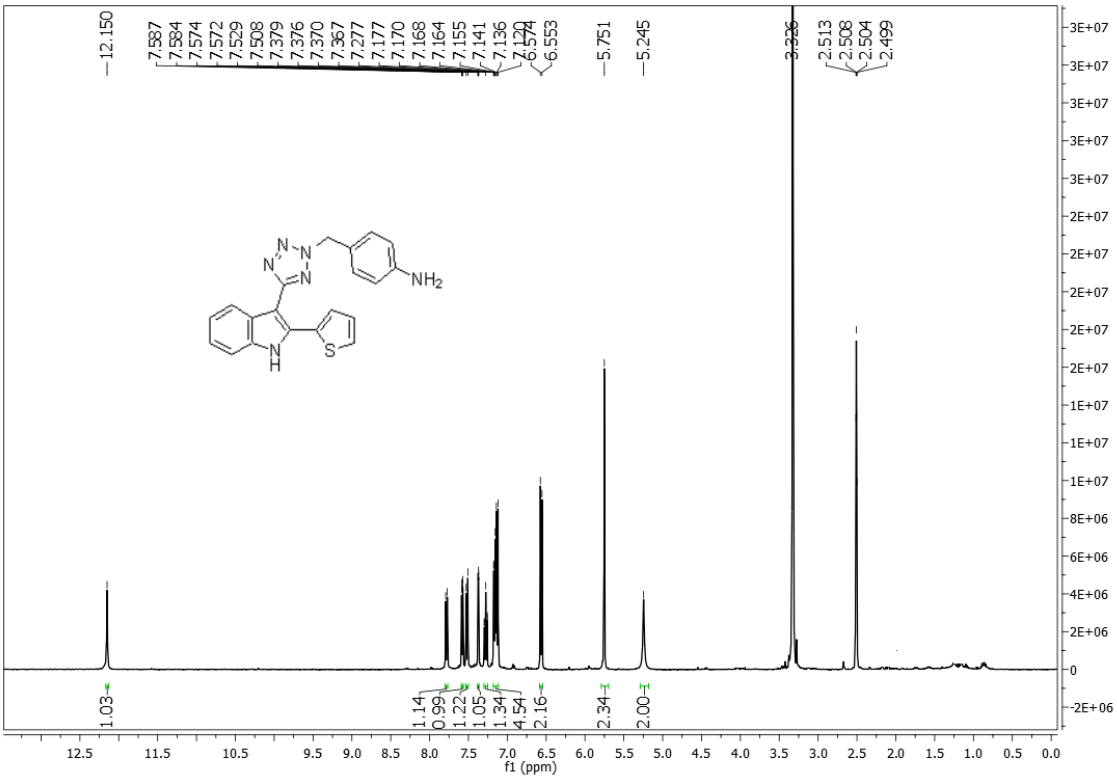



**Figure 93.** $^{1}$H RMN spectrum of compound **5.60a** (DMSO-$d_6$).

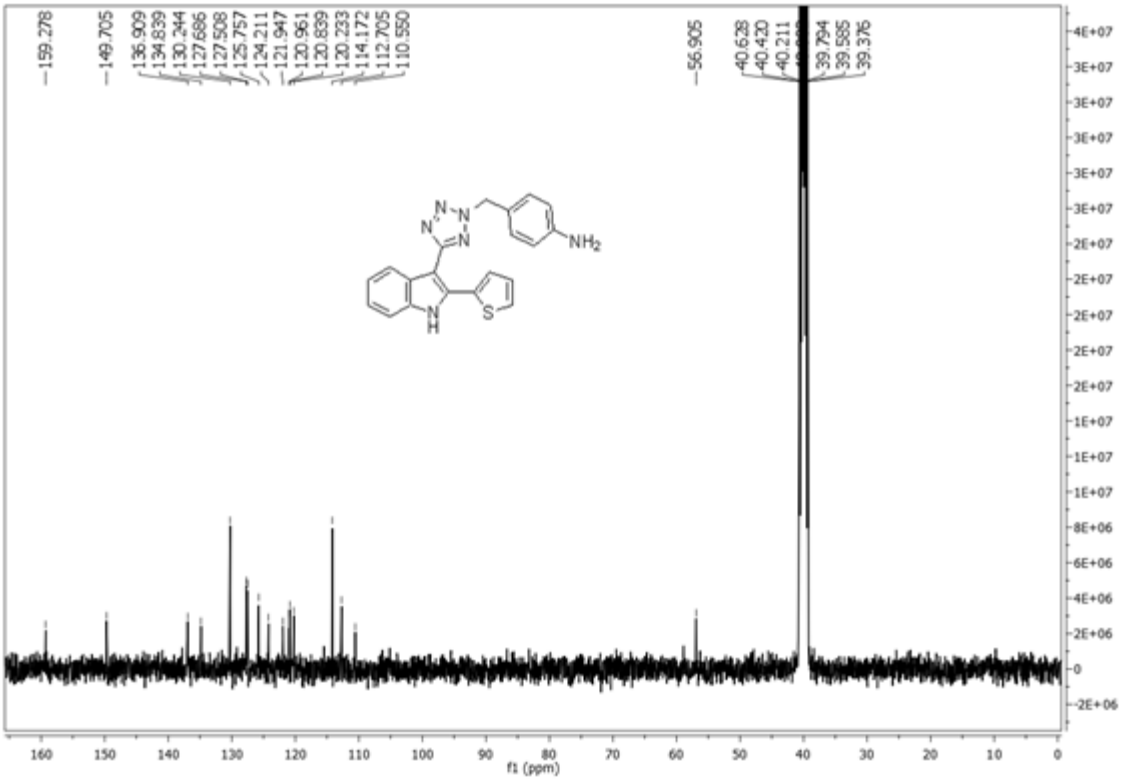



**Figure 94.** $^{13}$C RMN spectrum of compound **5.60** (DMSO-$d_6$).

## Appendix 2. Theoretical computational calculations at the DFT level - Optimised geometries, minimum energy and Cartesian coordinates

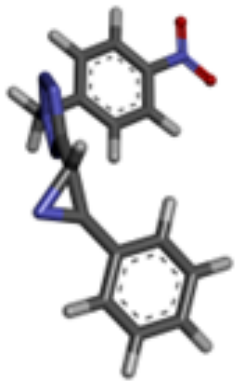


**Figure 1.** Optimized geometry (B3LYP/6-31G(d) level) of 2-(tetrazol-5-yl)-2*H*-azirines **5.47a**. Colour coding: Grey for carbon; red for oxygen; blue for nitrogen and white for hydrogen.

**Table 1.** Minimum energy and respective Cartesian coordinates (Å) calculated at the B3LYP/6-31G(d) level of theory for compound **5.47a**.

$E_h$ = -1095.0668990974

| Atom | Charge | X | Y | Z |
|---|---|---|---|---|
| N | 7.0 | -1.3278061940 | -1.8560082050 | -0.4724330115 |
| C | 6.0 | -0.1000719327 | -1.5937638935 | -0.3037405767 |
| C | 6.0 | -0.3512954372 | -3.0241429327 | -0.2145172705 |
| C | 6.0 | 0.8725958726 | -0.5291048946 | -0.2421908155 |
| C | 6.0 | -0.3765907556 | -3.7242337079 | 1.0931791673 |
| C | 6.0 | 2.1878967612 | -0.8303103730 | 0.1478517322 |
| C | 6.0 | 3.1359579760 | 0.1883151127 | 0.2152708618 |
| C | 6.0 | 2.7762646586 | 1.4989294626 | -0.1098746689 |
| C | 6.0 | 1.4652984491 | 1.8002125794 | -0.4982498262 |
| C | 6.0 | 0.5102112264 | 0.7912703174 | -0.5630794626 |
| N | 7.0 | -1.4750389116 | -4.0867400199 | 1.7976708101 |

| | | | | |
|---|---|---|---|---|
| N | 7.0 | -1.0646443360 | -4.7448375265 | 2.9083221516 |
| N | 7.0 | 0.2279537988 | -4.7796454264 | 2.8611794710 |
| N | 7.0 | 0.6916174448 | -4.1547526808 | 1.7473619250 |
| C | 6.0 | -2.9018950517 | -3.8484761759 | 1.5550190023 |
| C | 6.0 | -3.4745968279 | -2.7252570087 | 2.4018441092 |
| C | 6.0 | -3.8842636968 | -1.5291714967 | 1.7988090321 |
| C | 6.0 | -4.4359613200 | -0.5033123851 | 2.5639616201 |
| C | 6.0 | -4.5661790007 | -0.6913715028 | 3.9374347614 |
| C | 6.0 | -4.1590298615 | -1.8677757823 | 4.5660823437 |
| C | 6.0 | -3.6139616334 | -2.8850711865 | 3.7889161062 |
| N | 7.0 | -5.1509786325 | 0.3882691872 | 4.7520005239 |
| O | 8.0 | -5.4840649673 | 1.4231510722 | 4.1747350651 |
| O | 8.0 | -5.2717446252 | 0.1906635235 | 5.9605446821 |
| H | 1.0 | -0.1316240491 | -3.6629964580 | -1.0712387093 |
| H | 1.0 | 2.4433747517 | -1.8535225222 | 0.4100322065 |
| H | 1.0 | 4.1530516708 | -0.0382030541 | 0.5217306979 |
| H | 1.0 | 3.5187464412 | 2.2910411244 | -0.0593692332 |
| H | 1.0 | 1.1928165678 | 2.8217190896 | -0.7481099239 |
| H | 1.0 | -0.5110681100 | 1.0076121951 | -0.8632698147 |
| H | 1.0 | -3.0117257530 | -3.6245962709 | 0.4935561333 |
| H | 1.0 | -3.4126095399 | -4.7919838779 | 1.7681459329 |
| H | 1.0 | -3.7538960999 | -1.3951663621 | 0.7292783594 |
| H | 1.0 | -4.7608274516 | 0.4289249628 | 2.1180813066 |
| H | 1.0 | -4.2751792061 | -1.9695752902 | 5.6381147258 |
| H | 1.0 | -3.2793322254 | -3.8044895934 | 4.2603505854 |

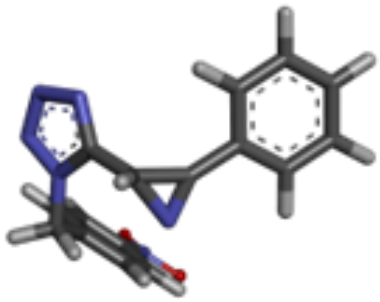


**Figure 2.** Optimized geometry (B3LYP/6-31G(d) level) of 2-(tetrazol-5-yl)-2*H*-azirines and **5.48**. Colour coding: Grey for carbon; red for oxygen; blue for nitrogen and white for hydrogen.

**Table 2.** Minimum energy and respective Cartesian coordinates (Å) calculated at the B3LYP/6-31G(d) level of theory for compound **5.48**.

$E_h$ = -1095.0710273077

| Atom | Charge | X | Y | Z |
|---|---|---|---|---|
| N | 7.0 | -4.4968136265 | -3.7953954837 | -2.8817059811 |
| C | 6.0 | -5.3621492304 | -3.0633471930 | -2.3229607607 |
| C | 6.0 | -4.0421354814 | -2.8224781553 | -1.7572466491 |
| C | 6.0 | -6.7790198373 | -2.7581717226 | -2.3496858698 |
| C | 6.0 | -3.6008942672 | -3.4032880452 | -0.4771377064 |
| C | 6.0 | -7.3222387012 | -1.8443290699 | -1.4347446525 |
| C | 6.0 | -8.6847564182 | -1.5530202747 | -1.4706124402 |
| C | 6.0 | -9.5050959565 | -2.1672493225 | -2.4202659325 |
| C | 6.0 | -8.9653873558 | -3.0774924430 | -3.3363196804 |
| C | 6.0 | -7.6068693634 | -3.3761095898 | -3.3030988889 |
| N | 7.0 | -4.2835384615 | -4.3002904325 | 0.2384761568 |
| N | 7.0 | -3.4433798087 | -4.5237855353 | 1.2488071965 |
| N | 7.0 | -2.3222786490 | -3.8094395247 | 1.1915826377 |
| N | 7.0 | -2.4054919331 | -3.0932151918 | 0.0977674015 |
| C | 6.0 | -3.7437123332 | -5.4266300823 | 2.3610033696 |
| C | 6.0 | -4.2270357472 | -4.6927104285 | 3.5970596333 |
| C | 6.0 | -5.5382702876 | -4.2006940458 | 3.6624345340 |

| | | | | |
|---|---|---|---|---|
| C | 6.0 | -5.9840747030 | -3.5232479921 | 4.7933916640 |
| C | 6.0 | -5.1008020415 | -3.3466221857 | 5.8580124392 |
| C | 6.0 | -3.7923836488 | -3.8215817345 | 5.8207828065 |
| C | 6.0 | -3.3619085787 | -4.4952997146 | 4.6799470446 |
| N | 7.0 | -5.5685067500 | -2.6366356143 | 7.0618477259 |
| O | 8.0 | -6.7264209450 | -2.2199532598 | 7.0639529409 |
| O | 8.0 | -4.7730285886 | -2.5042842625 | 7.9909864604 |
| H | 1.0 | -3.4389226139 | -1.9765812272 | -2.0824619243 |
| H | 1.0 | -6.6723678851 | -1.3718640352 | -0.7033336875 |
| H | 1.0 | -9.1064582730 | -0.8463278528 | -0.7610740929 |
| H | 1.0 | -10.5669026972 | -1.9368768606 | -2.4490183207 |
| H | 1.0 | -9.6064154431 | -3.5518074727 | -4.0745352115 |
| H | 1.0 | -7.1723277719 | -4.0804500228 | -4.0071111947 |
| H | 1.0 | -2.8286663974 | -5.9840366425 | 2.5740526501 |
| H | 1.0 | -4.4993531152 | -6.1187557798 | 1.9837943874 |
| H | 1.0 | -6.2101656314 | -4.3441670183 | 2.8203163007 |
| H | 1.0 | -6.9935460142 | -3.1376591809 | 4.8671625668 |
| H | 1.0 | -3.1383692134 | -3.6607533960 | 6.6691497859 |
| H | 1.0 | -2.3419122305 | -4.8661492070 | 4.6296852913 |