**$N,N'$-Ethylenebis(pyridoxylideneiminito) and $N,N'$-Ethylenebis(pyridoxylaminato): Synthesis, Characterization, Potentiometric, Spectroscopic, and DFT Studies of Their Vanadium(IV) and Vanadium(V) Complexes**


**Abstract:** The Schiff base $N,N'$-ethylenbis(pyridoxylideneiminito) (H$_2$pyr$_2$en, 1) was synthesized by reaction of pyridoxal with ethylenediamine; reduction of H$_2$pyr$_2$en with NaBH$_4$ yielded the reduced Schiff base $N,N'$-ethylenbis(pyridoxylaminato) (H$_2$Rpyr$_2$en, 2); their crystal structures were determined by X-ray diffraction. The totally protonated forms of 1 and 2 correspond to H$_3$L$_4$$^+$, and all protonation constants were determined by pH-potentiometric and $^1$H NMR titrations. Several vanadium(IV) and vanadium(V) complexes of these and other related ligands were prepared and characterized in solution and in the solid state. The X-ray crystal structure of $[V^{V}O_2(HRpyr_2en)]$ shows the metal in a distorted octahedral geometry, with the ligand coordinated through the N-ammine and O-phenolato moieties, with one of the pyridine-N atoms protonated. Crystals of $[(V^{V}O_2)(pyren)_2]$H$_2$O were obtained from solutions containing H$_2$pyr$_2$en and oxovanadium(IV), where Hpyren is the “half” Schiff base of pyridoxal and ethylenediamine. The complexation of V$^{IV}O_2^+$ and V$^{V}O_2^+$ with H$_2$pyr$_2$en, H$_2$Rpyr$_2$en and pyridoxalamine in aqueous solution were studied by pH-potentiometry, UV/Vis absorption spectroscopy, as well as by EPR spectroscopy for the $V^{IV}O$ systems and $^1$H and $^{35}V$ NMR spectroscopy for the $V^{V}O_2$ systems. Very significant differences in the metal-binding abilities of the ligands were found. Both 1 and 2 act as tetradentate ligands. H$_2$Rpyr$_2$en is stable to hydrolysis and several isomers form in solution, namely cis-trans type complexes with V$^{IV}O_2$ and $\alpha$-cis- and $\beta$-cis-type complexes with V$^{V}O_2$. The pyridinium-N atoms of the pyridoxal rings do not take part in the coordination but are involved in acid–base reactions that affect the number, type, and relative amount of the isomers of the $V^{IV}O_2$-H$_2$Rpyr$_2$en and $V^{V}O_2$-H$_2$Rpyr$_2$en complexes present in solution. DFT calculations were carried out and support the formation and identification of the isomers detected by EPR or NMR spectroscopy, and the strong equatorial and axial binding of the O-phenolato in $V^{IV}O_2$ and $V^{V}O_2$ complexes. Moreover, the DFT calculations done for the $[V^{IV}O_2(H_2Rpyr_2en)]$ system indicate that for almost all complexes the presence of a sixth equatorial or axial H$_2$O ligand leads to much more stable compounds.

**Keywords:** coordination modes · geometric isomers · N, O ligands · speciation · vanadium

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author. Some additional X-ray data for 1, 2, 4, and 9 (SI-1); further discussion of IR spectra (SI-2); UV/Vis absorption data for the ligands and V$^{IV}O_2$ complexes prepared in this work (SI-3); determination of the dissociation constants of [H$_2$Rpyr$_2$en]$^+$ in aqueous solution by $^1$H NMR spectroscopy (SI-4); EPR spectra of solutions containing V$^{IV}O_2^+$ and H$_2$pyr$_2$en (SI-5); 2D NOESY, ROESY and TOCSY spectra (SI-6); information on experimental and calculated $^1$H and $^{35}V$ NMR data (SI-7); determination of the pK$_a$ values of the pyridine protons of complexes [V$^{IV}O_2$(Rpyr$_2$en)H$_2$O] from $^1$H and $^{35}V$ NMR (SI-8); some general information about the structures of [M(Rpyr$_2$en)H$_2$] complexes (M = V$^{IV}O_2$, V$^{V}O_2$, and n = 0–2) calculated by DFT (SI-9); determination of the vanadium content in samples (SI-10); hydrolytic species of V$^{IV}O_2^+$ and V$^{V}O_2^+$ and corresponding formation constants (SI-11).
Introduction

Vanadium is a bioessential element that is found in remarkably high concentrations in marine ascidians,[1] certain mushrooms,[2] and polychaete worms.[3] Three types of vanadium-containing enzymes are known: vanadium nitrogenases,[4] vanadium-dependent haloperoxidases,[5] and vanadium-containing nitrate reductases.[6-7] Among other biological effects, vanadium’s insulin-like action[8] and anticancer activity[9] have stimulated a considerable amount of research. However, our understanding of the role of vanadium in living organisms is far from complete. For instance, in the insulin-like action, it is known that the originally supplied vanadium(IV) or vanadium(V) complexes undergo considerable transformations in the organism, including ligand-exchange processes and redox reactions, before participating in various phosphorylation/dephosphorylation reactions involved in the metabolism of glucose.[10] Most of vanadium’s biologically important reactions occur in water-based environments such as blood plasma and intracellular fluids. Therefore the knowledge of the distribution and chemical speciation of the vanadium compounds in aqueous solution is of the utmost importance.

Several vanadium complexes of the Schiff base sal2en \([N,N\text{-}ethylenediaminebis(salicylideneiminato)}\) and related ligands have been proposed as insulin-enhancing agents, and for the treatment of obesity and hypertension.[11,12] To date, only \([V^{IV}\text{O(sal2en)}]\) has been tested in vivo for insulin-mimetic activity. Pyridoxal and pyridoxamine are forms of vitamin B₆, known cofactors required by many enzymes. They are nontoxic metabolites and fairly soluble in aqueous solution. Pyridoxal-containing vanadium complexes are therefore of potential therapeutic interest.

In this work we study the Schiff base derived from the condensation of pyridoxal with ethylenediamine: H₂pyr2en (1; see Scheme 1). One disadvantage of Schiff bases (hereafter designated by SB) is their tendency to hydrolyze. This may yield the half SB (and one aldehyde), or proceed further to the ethylenediamine and a second molecule of pyridoxal. The complex \([[\text{VO}_2]\text{Hpyr2en}]=2\text{H}_2\text{O}\) was obtained from solutions containing H₂pyr2en and VOSO₄, where Hpyren is the “half” SB 3 (see Scheme 1). However, this instability was overcome by reduction of the SB to give an amine: H₂Rpyr2en (2). Both compounds 1 and 2 were isolated, characterized by X-ray diffraction, and their acid–base properties studied. H₂Rpyr2en was found to be quite stable.
to hydrolysis throughout the whole pH range. The vanadium(iv) complexes of the two ligands, of pyridoxamine (HpyrN) and of the SB derived from the reaction of methylamine with pyridoxal (HMepryR) were synthesized and characterized in the solid state.

EPR, $^{1}H$, and $^{51}V$ NMR spectroscopies were used to provide detailed information for the rather complicated solution equilibria of these ligands with $^{51}VO^{2+}$ and $^{51}VO_{2}^{+}$. Ab initio $^{[13]}$ and DFT $^{[14]}$ calculations were carried out and support the formation and identification of the isomers present in both systems, and help to explain the experimental data. Overall the present systems are remarkable examples of the complexity of the types of isomers that may form in solutions of $^{51}VO$ and $^{51}VO_{2}$ complexes (as indeed of many other metal–ligand systems), and how pH-potentiometry, spectroscopic techniques, DFT calculations, and computer handling of the experimental data may be used to characterize the systems in solution, and evaluate the various subtle effects that determine the isomers that form.

Results and Discussion

X-ray diffraction studies

$H_{2}pyr_{2}en$ (1) and $H_{2}Rpyr_{2}en$ (2): Figure 1 shows ORTEP representations of ligands $H_{2}pyr_{2}en$ (1) and $H_{2}Rpyr_{2}en$ (2). Only half of 1 and 2 were found in the asymmetric unit, the inversion centers being located at the midpoint of the CH$_{2}$–CH$_{2}$ bonds. The overall geometrical parameters of $H_{2}pyr_{2}en$ compare well with other ethylenebis(salicylidenedimine) derivatives $^{[15–17]}$, namely those of sal$_{2}en$ $^{[15]}$ (see Table 1) $^{[a]}$ (see Table 1)

![Table 1](image)

for selected data). The N–CH and O–C lengths of 1 (1.277(5) and 1.349(5) Å, respectively) are consistent with the double-bond N–C and single-bond O–C character of these bonds, while the NH–CH$_{2}$ and O–C lengths of 2

Figure 1. Structures of A) $H_{2}pyr_{2}en$ (1) and B) $H_{2}Rpyr_{2}en$ (2) (ORTEP diagrams; the thermal ellipsoids of the non-hydrogen atoms are drawn at the 30% probability level). There are short intramolecular hydrogen bonds: in 1 for O3–H3A–N1 (O–N 2.534(5) Å, H–N 1.698(1) Å, O–H–N 135.6(6)°), and in 2 for O3–H3–N1 (O–N 2.597(2) Å, H–N 1.71(3) Å, O–H–N 153.9(5)°). See also the Supporting Information (SI-1).
(1.471(2) and 1.357(2) Å, respectively) are consistent with their single-bond character. The configurations of 1 and 2 are determined by the strong intramolecular H bonds between O\textsubscript{phenolic}–N\textsubscript{amine}. Their main differences are in the planarity of each half-molecule of H\textsubscript{2}pyr\textsubscript{2}en (planar within 0.012(3) Å), partly due to the conjugation of the C=N bond with the heteroaromatic ring. The two halves of 2 are non-planar with respect to each other. For example the torsion angles C5-C4-C3-N1 and O3-C5-C4-C3 are $\pm$1.8(7)° and $\pm$0.3(7)° in the H\textsubscript{2}pyr\textsubscript{2}en, and 35.6(2)° and $\pm$2.3(3)° in H\textsubscript{2}Rpyr\textsubscript{2}en.

[V\textsuperscript{V}O\textsubscript{2}(HRpyr\textsubscript{2}en)]\textsubscript{3}H\textsubscript{2}O (4): Upon coordination, the N\textsubscript{amine} atoms become dissymmetric centers. The unit cell of the crystal contains four molecules: in two of them the configurations of the N\textsubscript{amine} are S,S, while in the other two they are R,R (enantiomers of the S,S molecules). Figure 2A shows an ORTEP diagram with the atom-labeling scheme, and selected bond lengths and angles are given in Table 1. The molecule is neutral with one of the pyridinium nitrogens protonated, and corresponds to the symmetrical isomer, sometimes designated by 'α-cis' (see below).

In complex 4 the HRpyr\textsubscript{2}en\textsuperscript{−} ligand coordinates the VO\textsubscript{2}\textsuperscript{+} moiety by means of two phenolate-O\textsuperscript{−} and two amine-N atoms forming a quite distorted octahedral coordination polyhedron. This is due both to the specific constraints of the VO\textsubscript{2} fragment and to the tetradeinate coordination, which imposes stericchemical strain, but overall the coordination geometry of this compound is rather similar to other monomeric V\textsuperscript{V}O\textsubscript{2} complexes. The distortion is reflected in the coordination distances and angles and there are two short bonds (V=O bonds of 1.628(5) and 1.683(4) Å) that are trans to the two V–N long bonds of 2.308(6) and 2.247(5) Å. The O–V–O angle in the VO\textsubscript{2} moiety is similar to those previously reported for related complexes with nitrogen trans to the oxo groups, for example, the α-cis isomer of the EDTA complex and β-cis isomer of the EDDA complex. Other authors also found a rather similar type of isomerism and used the notation cis- and trans-phenolates. Hereafter we will use the α-cis and β-cis notation; however, as will be clear below, in solution the V\textsuperscript{V}–Rpyr\textsubscript{2}en system is much more complex, because several α-cis and β-cis complexes form.

The differences observed in the V=O distances of [V\textsuperscript{V}O\textsubscript{2}(HRpyr\textsubscript{2}en)]\textsubscript{3}H\textsubscript{2}O (4) are partly due to the different involvement of the two oxygen atoms in H bonding in the 3D structure. Atom O2 is involved in two short intermolecular H bonds (with N4 and O100 of two neighboring molecules), while atom O1 is only involved in a weaker one with O6 of a symmetry-related molecule.

General structures of the α-cis and β-cis-type complexes here: The V–O\textsubscript{phenolate} and V–N\textsubscript{amine} bonds are within the normal range found for this type of compound. The ligand coordinates vanadium forming a (6+5+6)-membered fused chelate system. The rings are not planar partly due to the greater flexibility of the reduced SB ligand, which is not constrained to remain planar when coordinated to the metal ion. Due to the fact that atom N4 is protonated and N3 is not, the two heteroaromatic rings have some differences namely: the N3–C8 and N4–C15 bonds, which differ by 0.014 Å, and some angles (e.g., C6-N3-C8 and C13-N4-C15 differ by 7.6°).

Comparing the bond lengths of ligand 2 and its complex, there are some small differences upon coordination; for example, the C–O\textsubscript{phenolic} bond decreases by 0.04 Å. However, there are no significant differences between the C3–N1 and C10–N2 internuclear separations.
[(V\textsubscript{II}O\textsubscript{3})(pyren\textsubscript{3-})\textsubscript{2}]\textsubscript{2}H\textsubscript{2}O \ (9): Although the initial mixture contained V\textsuperscript{IV} and H\textsubscript{3}pyren\textsubscript{en}, a V\textsuperscript{V} complex with the half-Schiff-base monomionic ligand Hpyren \textsubscript{3} \ (3) was obtained, where one of the imine bonds hydrolyzed. The oxidation of V\textsuperscript{IV} was probably due to diffusion of air into the solution. An ORTEP diagram of \textsubscript{9} is shown in Figure 2B, and selected bond lengths and angles are in Table 1.

Compound \textsubscript{9} is a dinuclear V\textsuperscript{V} Schiff base with a bis(\(\mu\)-oxo) bridge.\textsuperscript{25-29,31-33} Each V\textsuperscript{V} ion is six-coordinate, and the \(\mu\)-oxo atoms are trans to the V=O bonds. The coordination polyhedra obtained can be best described as two edge-shared octahedra that are significantly distorted. This distortion is mainly due to the O1-V1-O2 angle of 107.3\textdegree\textsuperscript{9\textsuperscript{p}}, a value comparable to that obtained in compound \textsubscript{4} as well as in other VO\textsubscript{2} units found in the literature.\textsuperscript{37,21-23,30-33} The V1–O1 bond has a typical V=O length of 1.602(16) Å, while O2 is involved in the bridge between V1 and the symmetry-generated V1A atom (\(-x+2, y, -z\)). This coordination gives rise to a rather distorted V\textsubscript{2}O\textsubscript{4} core with two strong V=O\textsubscript{m} bonds, and two weak V–O interactions (see Table 1). The remaining three coordination sites are occupied by the tridentate pyren\textsubscript{3-} units, comprising the pheno- late-O3, the imine-N1, and the amine-N2 atoms. The metal is above the plane defined by N1, N2, O2, and O3 toward O1 by 0.354(1) Å. The V–V separation is 3.166(1) Å, comparable to the values found in related complexes that range from 3.103 Å to 3.372 Å.\textsuperscript{30,31,33,34} Owing to the presence of strong V=O interactions, in agreement with the magnetic susceptibility measurements (see below). The binding of an O=O\textsubscript{m} to an adjacent vanadium atom, trans to its vanadyl-O atom, lengthens and weakens the bond, thereby lowering the V=O\textsubscript{m} stretching frequency. For complexes 7 and 8 strong bands appear at 910 and 915 cm\textsuperscript{-1} (medium-strong) are assigned to the v(C12–O4)\textsubscript{phenolate} and v(C5–O3)\textsubscript{phenolate}, respectively. For the V\textsuperscript{IV} compounds these bands appear in the range 1273–1321 cm\textsuperscript{-1} (see Table SI-3 in the Supporting Information) and, in some cases, two bands may also be distinguished. The v(V–O1) and v(V–O2) bonds were calculated to appear at 1001 and 974 cm\textsuperscript{-1}, while the experimental values are 924 and 903 cm\textsuperscript{-1}, respectively. The lower experimental values arise from the hydrogen bonding involving both O1 and O2.

**Electronic absorption spectra:** The electronic absorption spectra of bis(salicylaldimines) and of their transition-metal complexes have been extensively studied.\textsuperscript{40-43} The electronic absorption spectra of the V\textsubscript{IV}O\textsubscript{2} complexes (see Table SI-4 in the Supporting Information) were measured in DMSO. The V\textsubscript{IV}O–Schiff base complexes 5 and 6 show a strong absorption with \(\lambda_{\text{max}}\) at 370–380 nm that is absent in the reduced Schiff base complexes. This band can be assigned to azomethine \(\pi\textrightarrow\pi^*\), but also has a contribution from a LMCT (phenolate-O to d orbitals on the vanadyl) transition. Although three (or four) d–d bands are expected to appear in the absorption spectra of V\textsubscript{IV}O\textsubscript{2} complexes, they are often overlapped or under strong CT bands. The spectrum of [V\textsubscript{IV}O(pyr\textsubscript{2}en)] shows band I (d\textsubscript{xy},–d\textsubscript{xz},–d\textsubscript{yz}) at approximately 730 nm, and band II (d\textsubscript{xy},–d\textsubscript{xz},–d\textsubscript{yz}) at 580 nm. Band III (d\textsubscript{xy},–d\textsubscript{yz}) occurs below 500 nm and is under the much stronger band at \(~380\) nm. For complex 8, band I is red-shifted but has about the same intensity as in 5, and band II is blue shifted but less intense. In the 470–860 nm range the spectrum of [V\textsubscript{IV}O(pyr\textsubscript{2}en)] is more intense than that of [V\textsubscript{IV}O(pyr\textsubscript{2}en)]. As expected the spectra of [V\textsubscript{IV}O(pyr\textsubscript{2}en)] and [V\textsubscript{IV}O(pyr\textsubscript{2}en)] are similar and the d–d bands I and II occur at about the same wavelength.

**EPR spectra:** The Hamiltonian parameters obtained by computer simulation of the experimental X-band EPR spectra of frozen solutions of the vanadium complexes in DMSO, using the computer program from Rockenbauer,\textsuperscript{44} are listed in Table 2. The spectra of complexes 5, 6, and 8 show slight rhombic distortions, which can be seen in the perpendicular lines \(M_1=7/2, 5/2, 3/2\), indicating a distorted geometry around the metal center. The \(g_g, g_v\) values are in the range 0.005 to 0.009 while the \(|A_x–A_y|\) are \(~6\) to \(8\times10^{-4}\) cm\textsuperscript{-1}. The degree of rhombic distortion increases in the order of complex 6<8<5. In complex 6 the ligand is less rigid than in 5, allowing the molecule to assume a more symmetric geometry around the metal center. In complexes 5 and 8 the ligands are Schiff bases, but in 6 instead of an ethylene bridge there are two methyl groups, which possibly explains the higher distortion in complex 5. The additivity rule\textsuperscript{45-48} was developed to allow the determination of the identity of the equatorial donor groups in complexes of square-pyramidal geometry (or octahedral with a weak sixth ligand), but has also been successfully applied to structurally distorted molecules.\textsuperscript{49-53} The spectrum of 7 shows axial symmetry, and if we consider that the two ligand molecules bind vanadium in equatorial positions through the four donor atoms (2×N\textsubscript{amine}×2×O\textsubscript{phenolate})\textsubscript{eq}, the estimated \(|A_x,–A_y|\) is 158×10\textsuperscript{-4} cm\textsuperscript{-1}. If the binding mode involves (2×O\textsubscript{phenolate}×N\textsubscript{amine},
The pH-range 2–7 was followed by 1H NMR spectroscopy, but at pH > 7 a very weak signal corresponding to one of the aldehyde protons of pyridoxal could be detected. This slight extent of hydrolysis may explain the significantly higher uncertainties in the protonation constants of pyridoxal (see Table 3). No hydrolysis was detected in the pH range 1–13, as also confirmed by 1H NMR spectroscopy. Table 3 also includes the protonation constants of pyridoxine (obtained in this work), and those of pyridoxal. For 2 and pyridoxine the deprotonation processes overlap each other and the $pK_a$ values were calculated from both the pH-metric and 1H NMR data.

For pyridoxal the $pK_{a}$ of 12.9 corresponds to the deprotonation of its hemiacetal form,[62] and in the case of pyridoxamine the $pK_{a}$ of ~10.3 corresponds mainly to the amino and phenolic groups. In neutral aqueous solution the zwiterionic form predominates (the phenol deprotonated and the pyridine-N protonated), but a significant amount of the uncharged forms are also present; microequilibria must be considered. The $pK_a$ of ~8 of pyridoxal and of pyridoxamine are largely associated with the pyridinium protons, and the $pK_a$ of ~3–4 to both the hydroxyl and the pyridinium protons.[44] However, in the case of our ligands the existence of H bonds between the O phenolate and the N amine/imine may change further the protonation constants are included in Table 3.

Schiff bases of the sal-enzyme type may hydrolyze in solution forming the half SB or eventually totally decompose at low pH,[57,58] and their VIVO complexes may be involved in disproportionation reactions.[59–61] All these processes depend on the solvent used. The $H_3[Rpyr]_2$ ligand is soluble in water, and much more stable in this solvent than sal-enzyme, and pH-potentiometry could be used to determine the protonation constants (see Table 3). No hydrolysis was detected in the pH range 2–7 by 1H NMR spectroscopy, but at pH > 7 a very weak signal corresponding to one of the aldehyde protons of pyridoxal could be detected. This slight extent of hydrolysis may explain the significantly higher uncertainties in the protonation constants of I, about twice those of $H_3[Rpyr]_2$ (2), which is also reasonably soluble in water but fairly stable throughout the pH range 1–13, as also confirmed by 1H NMR spectroscopy. Table 3 also includes the protonation constants of pyridoxine (obtained in this work), and those of pyridoxal. For 2 and pyridoxine the deprotonation processes overlap each other and the $pK_a$ values were calculated from both the pH-metric and 1H NMR data.

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DMSO$_{eq}$ (N$_{amino}$)$_{eq}$ taking $A_0$(DMSO) = 42 × 10$^{-4}$ cm$^{-1}$ (see Table 2), then the estimated parameters fit better with the simulated ones. This is probably the coordination mode of 7 in DMSO. For complexes 5, 6, and 8 the EPR parameters (Table 2) fit well with the tetradentate binding mode in involving either (2 × N$_{amino}$, 2 × O phenolate)$_{eq}$ or (2 × N$_{amino}$, 2 × O phenolate)$_{eq}$.

**Magnetic properties of the vanadium(vi) complexes**: The magnetic susceptibilities, $\chi$, of the vanadium(vi) complexes 5 and 6 were measured by the Faraday method in the temperature range of 3–287 K. Data were corrected for the diamagnetic contribution using the Pascal constants.[52] The magnetic susceptibility of [V$^{IV}$O(pyr$^2$en)$_2$]$(\mu_0)$ fits well the Curie–Weiss law (with $\chi_0$(287 K) = 1.35 × 10$^{-3}$ emu mol$^{-1}$, $C = 0.381$ emu K mol$^{-1}$, $\theta = -0.32$ K). Yamada et al.[53] reported a $\mu_0$ of 1.53 $\mu_B$ for this complex at room temperature (RT). However, we obtained a $\mu_0$(RT) of 1.76 $\mu_B$ (roughly constant until approximately 25 K), typical of monomeric V$^{IV}$O compounds. This is in agreement with its green color and with the $\chi$(V=O) value obtained. In contrast, for complex [V$^{IV}$O(Rpyr$^2$en)$_2$], smaller $\mu_0$(RT) values were obtained: 1.50 $\mu_B$ and these remain approximately constant until approximately 25 K. At low temperatures, an increase was observed, which suggests that ferromagnetic interactions become important. Low magnetic moments were found for several vanadium complexes[53,54] with sal-enzyme type ligands, and these low magnetic moments have been explained by the existence of V=O−V=O interactions. Possibly this is also the case for [V$^{IV}$O(Rpyr$^2$en)$_2$], which is in agreement with the low $\chi$(V=O) value for this complex (see above).

**Ligand protonation by pH-potentiometry and 1H NMR spectroscopy**

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Table 2. Spin Hamiltonian parameters obtained by using a computer program from Rockenbauer.[60]

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$[\text{V}^{IV}\text{O(pyr}^2\text{en}$_2$]$(\mu_0)$ taking $A_0$(DMSO) = 42 × 10$^{-4}$ cm$^{-1}$ (see Table 2), then the estimated parameters fit better with the simulated ones. This is probably the coordination mode of 7 in DMSO. For complexes 5, 6, and 8 the EPR parameters (Table 2) fit well with the tetradentate binding mode involving either (2 × N$_{amino}$, 2 × O phenolate)$_{eq}$ or (2 × N$_{amino}$, 2 × O phenolate)$_{eq}$.

**Magnetic properties of the vanadium(vi) complexes**: The magnetic susceptibilities, $\chi$, of the vanadium(vi) complexes 5 and 6 were measured by the Faraday method in the temperature range of 3–287 K. Data were corrected for the diamagnetic contribution using the Pascal constants.[52] The magnetic susceptibility of [V$^{IV}$O(pyr$^2$en)$_2$]$(\mu_0)$ fits well the Curie–Weiss law (with $\chi_0$(287 K) = 1.35 × 10$^{-3}$ emu mol$^{-1}$, $C = 0.381$ emu K mol$^{-1}$, $\theta = -0.32$ K). Yamada et al.[53] reported a $\mu_0$ of 1.53 $\mu_B$ for this complex at room temperature (RT). However, we obtained a $\mu_0$(RT) of 1.76 $\mu_B$ (roughly constant until approximately 25 K), typical of monomeric V$^{IV}$O compounds. This is in agreement with its green color and with the $\chi$(V=O) value obtained. In contrast, for complex [V$^{IV}$O(Rpyr$^2$en)$_2$], smaller $\mu_0$(RT) values were obtained: 1.50 $\mu_B$ and these remain approximately constant until approximately 25 K. At low temperatures, an increase was observed, which suggests that ferromagnetic interactions become important. Low magnetic moments were found for several vanadium complexes[53,54] with sal-enzyme type ligands, and these low magnetic moments have been explained by the existence of V=O−V=O interactions. Possibly this is also the case for [V$^{IV}$O(Rpyr$^2$en)$_2$], which is in agreement with the low $\chi$(V=O) value for this complex (see above).

**Ligand protonation by pH-potentiometry and 1H NMR spectroscopy**

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Table 2. Spin Hamiltonian parameters obtained by using a computer program from Rockenbauer.[60]
Vanadium(IV) and Vanadium(V) Complexes

 acidity of the phenolic-OH, the acid–base microequilibria becoming more complicated.

The pH-potentiometry technique allows the determination of the protonation constants of the ligands (Table 3), but cannot provide information on the sequence of protonation of their basic sites. The protonation of the N and O atoms generally result in a deshielding of the nonlabile H atoms associated to adjacent carbon atoms. Therefore, a 1H atoms generally result in a deshielding of the nonlabile H atoms attached to adjacent carbon atoms. [65] Therefore, a 1H atoms generally result in a deshielding of the nonlabile H atoms.

Table 3. Protonation and formation constants[b] of species M,L,H formed in the V^{4+}-H,Rpyr \text{en} and V^{5+}-H,Rpyr \text{en}. H,Rpyr \text{en} and pyridoxamine systems calculated from the pH-potentiometric data with the PSEQUAD computer program,[63] and from 1H NMR data (some pK_a values).

<table>
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<tr>
<th>Species</th>
<th>logK_a</th>
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<th>logK_c</th>
<th>logK_d</th>
<th>LogK_e</th>
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<tr>
<td>H,L</td>
<td>10.07(2)</td>
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<td>H,L,L</td>
<td>18.86(3)</td>
<td>8.79(5)</td>
<td>19.67(1)</td>
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<td>H,L</td>
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<td>8.08(6)</td>
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<tr>
<td>H,L,L</td>
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<td>6.89(7)</td>
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<td>5.98(2)</td>
<td>3.36(2)</td>
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<tr>
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<td>4.43(9)</td>
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<td>3.04(3)</td>
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<tr>
<td>H,L,L</td>
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<td>36.68(5)</td>
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<td>H,L</td>
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<td>H,L</td>
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<td>H,L,L</td>
<td>36.86(2)</td>
<td>2.23(4)</td>
<td>38.60(2)</td>
<td>2.23(4)</td>
<td>2.23(4)</td>
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</tbody>
</table>

[a] For each logK_h value listed obtained by pH-potentiometry, the standard deviation obtained in the particular calculation corresponding to the values shown are included in (parentheses). To account for the range of logK_h values obtained in the several equilibrium models tested, each standard deviation may be multiplied by about three times the values presented. [b] For the similar compound pyridoxal, the protonation constants (at 25°C and 1M KCl) are: 12.93, 8.28, 4.00. [c] From the 1H NMR titration study only an average value for pK_a and pK_b was obtained for pyridoxamine (7.99), which has a protonated positively charged side chain and the O phenolate deprotonated. [d] Difference between the experimental and the calculated titration curves expressed as the volume (mL) of titrant.
[\text{V}^{IV}\text{OLH}] (pK_a = 4.9) and [\text{V}^{IV}\text{OL}] (neutral, pK_a ~ 6.1, corresponding to structure II), which is less soluble than [\text{VOL}] in the case of the \text{V}^{IV}\text{O}-\text{H}_2\text{Rpyr}_2\text{en} system.

In agreement with the speciation curves (Figure 3A), the spectra of solutions containing \text{V}^{IV}\text{O}^{2+} and \text{H}_2\text{pyr}_2\text{en} start to deviate from that of [\text{V}^{IV}\text{O}(\text{H}_2\text{O})_3]^{3+} at pH > 3. As the pH is increased, the blue solutions become green and the bands at 370–470 nm (\pi \rightarrow \pi^* \text{imine} and \text{LMCT} bands) develop further, indicating the coordination of both \text{N}_{\text{imine}} and \text{O}_\text{phenolate} atoms. For the \text{V}^{IV}\text{O}-\text{H}_2\text{Rpyr}_2\text{en} system the changes in the visible spectra are also in agreement with the speciation model. At pH 1.8 the spectrum consists of a broad d–d transition band at 775 nm, a shoulder at about 680 nm (due to the aqua vanadyl ion), and a band at about 555 nm, showing that a significant amount of [\text{V}^{IV}\text{OLH}] is present at this pH. Between pH 3 and 4.5 all the oxovanadium(iv) is in the form of [\text{V}^{IV}\text{OLH}] and there are no spectral changes.

Figures 4 and SI-6 in the Supporting Information depict the high-field region of the X-band EPR spectra of frozen aqueous solution samples of the \text{V}^{IV}\text{O}-\text{H}_2\text{Rpyr}_2\text{en} and
the EPR spectra are observed. The rhombic distortion of the spectra, (e.g., Figure SI-6B in the Supporting Information), is an indication of the distorted environment around the vanadyl center, due to the rigid structure of the ligand.}

Figure 3B shows that for the V\textsuperscript{IV}O-H\textsubscript{2}Rpyr\textsubscript{2}en system the complexation starts at pH 2, with the formation of [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+}. Further deprotonation yields [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+} and [V\textsuperscript{IV}OL\textsubscript{2}], which then precipitates. The EPR spectra, for example, Figure 4, show that for each stoichiometry two well separated signals can be ascribed. Evaluated from the intensities of the \( M_1=5/2 \) and 7/2 components, the relative amount of the complexes is approximately the same in the pH range 2–5, suggesting that they are structural isomers. An H\textsubscript{2}O ligand may coordinate in an equatorial position (cis to the \( O_{vanadyl} \) atom) or axial (trans to the \( O_{vanadyl} \) atom), as is common for [V\textsuperscript{IV}OL\textsubscript{2}] complexes, \( L \) being a bidentate ligand\textsuperscript{[69,71]} However, the type and number of isomers that may form in the present systems is complex, and a more systematic and comprehensive discussion about the stoichiometries and isomers that may form in solution is given below in connection with the DFT calculations.

In the V\textsuperscript{IV}O-Hpyr\textsubscript{2}en system (note that pyr\textsubscript{N} is assigned as a cis to the O\textsubscript{vanadyl} atom), complex formation starts at pH \( \sim 2.5 \) with the formation of [V\textsuperscript{IV}OL\textsubscript{2}]\textsuperscript{+} (see Figure 3C), with a binding mode similar to that shown in structure I (with \( N_{amine} \) instead of \( N_{amine} \)). Deprotonation of the noncoordinated NH\textsuperscript{+}pyridine of this complex yields [V\textsuperscript{IV}OL\textsubscript{2}]\textsuperscript{+}, while coordination of a second pyr\textsubscript{N} leads to the formation of [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+}. Further deprotonation of the NH\textsuperscript{+}pyridine of the bis complex yields [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+} and [V\textsuperscript{IV}OL\textsubscript{2}], the latter is neutral and precipitates. The EPR spectra for this system (Table 2) indicate the presence of three distinct types of species in the pH range 2–6: the aqua vanadyl ion, [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}] and [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]. The simulated \( A_1 \) value for [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}] fit well with the estimated value for the binding mode (\( N_{amine} \text{phenolate}, 2 \times H_2O \)). Deprotonation of this species corresponds to only a slight decrease in the \( A_1 \) values. In agreement with the proposed (2 \( \times N_{amine} \), 2 \( \times O_{phenolate} \)) binding mode the spectra of [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}] correspond to lower \( A_1 \) values than those of [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]. The linewidths and shape of the \( M_1=7/2 \) components of the spectra indicate the existence of structural isomers also in this system. The visible spectra of the V\textsuperscript{IV}O-pyrN system show a pattern somewhat similar to those of the previous systems: as the pH increases the d–d transition at \( \lambda_{max} \sim 760 \text{ nm} \) shifts to lower energy, and the one at \( \lambda_{max} \sim 570 \text{ nm} \) shifts to higher energy. The intensity of both bands progressively increases.

### Vanadium(iv) complexes studied by pH-potentiometry, and \textsuperscript{1}H and \textsuperscript{51}V NMR spectroscopy:

The ligand H\textsubscript{2}pyr\textsubscript{2}en does not form complexes with V\textsuperscript{V} stable enough to allow the use of pH-potentiometry. However, in the \textsuperscript{51}V NMR spectra of solutions containing 3 mm of V\textsuperscript{V} and 6 mm of I, a new signal at \( \delta \sim 577 \text{ ppm} \) was detected in the pH range 5–9, indicating complex formation. With pyridoxamine, the \textsuperscript{51}V NMR measurements with 3 mm V\textsuperscript{V} and 12 mm of ligand did not show any complex formation in the same pH range. In the V\textsuperscript{V}–H\textsubscript{2}Rpyr\textsubscript{2}en system pH-potentiometry clearly indicated complex formation, and the stability constants given in Table 3 were obtained by the evaluation of the pH-metric titration data. The corresponding speciation diagram is shown in Figure 5\textsuperscript{[70]} At pH > 3.5, complexes [\textsuperscript{51}V\textsuperscript{II}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+} → [\textsuperscript{51}V\textsuperscript{II}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+} are the predominant species with increasing pH, and at pH > 9.5 the ligand molecule is displaced from the coordination sphere and the oxoanions H\textsuperscript{2}O\textsubscript{V}/\textsuperscript{O} are formed. As in the V\textsuperscript{IV}O–H\textsubscript{2}pyr\textsubscript{2}en and V\textsuperscript{IV}O–H\textsubscript{2}Rpyr\textsubscript{2}en systems, these deprotonation processes involve the NH\textsuperscript{+}pyridine protons, and 2 acts as a tetradeutate ligand in [V\textsuperscript{IV}OL\textsubscript{2}H\textsubscript{2}]\textsuperscript{+}, [V\textsuperscript{IV}OL\textsubscript{2}], and [V\textsuperscript{IV}O\textsubscript{2}]\textsuperscript{3–} (see below).

Between pH 3.5 and 6 the \textsuperscript{51}V NMR spectra show only two relatively broad signals (Figure 6A and SI-7 in the Supporting Information). In the pH range 6–9, as the NH\textsuperscript{+}pyridine groups successively deprotonate, the two peaks gradually shift, but the ratios of the peak areas are almost independent on both the total V concentration or the L:M ratio. This indicates the formation of two mononuclear complexes for each stoichiometry, that is, structural isomers. There are two types of structural isomers (structures III and IV in Scheme 2), and for structure IV (\( \beta\text{-cis} \) isomer) the protons of the two half-molecules are not equivalent. Separate \textsuperscript{1}H NMR resonances are observed for the two isomers, due to slow exchange conditions, this being especially clear for the aromatic pyridoxal protons (e.g., signals assigned as \( \alpha\text{-cis} \) and \( \beta\text{-cis} \) in Figure 6B). The assignments of all the other \textsuperscript{1}H signals were confirmed by using 2D COSY spectra. Comparing the \textsuperscript{51}V NMR spectra with the aromatic region of the \textsuperscript{1}H NMR spectra (at 1:1 and other metal-to-ligand ratios, for example, Figure 6A, Figure 6B and SI-4 in the Supporting Information), and taking into account the relative areas of the NMR peaks, we conclude that while in the \( \alpha\text{-cis} \) complex the two aromatic protons are equivalent, in the \( \beta\text{-cis} \) they correspond to two separate signals. This also occurs with the other pyridoxal protons (of CH\textsubscript{3}, CH\textsubscript{2}OH, and CH\textsubscript{2}N), as well as with the en bridge -CH\textsubscript{2}–CH\textsubscript{2}– protons. This data supports structures III and IV.
The pH below 2.5. The UV/Vis measurements indicated an nonequilibrium species.

While for the cis isomer was confirmed by using 2D NOESY and ROESY spectra (see SI-6 in the Supporting Information). The two stepwise protonation steps of the NH\textsuperscript{+} pyridine in the \( \alpha \)-cis and \( \beta \)-cis isomers, there is a slight change in the ratio of the two types of isomers between pH 6 and 9, with a minimum of \( \alpha \)-cis/\( \beta \)-cis at pH 7.8, where the population of the monoprotonated forms of [VO\textsubscript{2}LH\textsubscript{2}]\textsuperscript{2+} shows its maximum (Figure 5 and SI-8A in the Supporting Information).

The species involved in the microscopic deprotonation scheme of the NH\textsuperscript{+} pyridine groups of the \( \alpha \)-cis and \( \beta \)-cis isomers together with the corresponding microconstants are shown in Scheme 3. Starting with [VO\textsubscript{2}LH\textsubscript{2}]\textsuperscript{2+} and increasing the pH, each isomer has two deprotonation steps. The first step for the \( \alpha \)-cis\textsubscript{H\textsubscript{2}} and \( \beta \)-cis\textsubscript{H\textsubscript{2}} isomers can proceed by two different paths (deprotonation either on the ring A or ring B of pyridoxal), yielding either \( \alpha \)-cisH(B\textsuperscript{3+}) or \( \alpha \)-cisH(A\textsuperscript{3+}), and \( \beta \)-cisH(B\textsuperscript{3+}) or \( \beta \)-cisH(A\textsuperscript{3+}), respectively. As the \( \alpha \)-cis isomers are symmetrical, the \( \alpha \)-cisH(B\textsuperscript{3+}) and \( \alpha \)-cisH(A\textsuperscript{3+}) are identical and simply represented by \( \alpha \)-cisH. Therefore, theoretically there are seven different species, these being schematically depicted in Scheme 3.

Based on the pH dependence of the chemical shifts of the 1H and 31V NMR spectra and on the equilibria involved, the microconstants shown in Scheme 3 could be calculated (see SI-8 in the Supporting Information). The two stepwise \( K_{a} \) values of \( \alpha \)-cisH\textsubscript{2} (pK\textsubscript{a}H\textsuperscript{2} = 7.19) differ more (0.89) than the expected value based on statistical consideration (0.61). This is due to the charge of the molecule, which becomes zero after the first deprotonation. The first deprotonation of \( \beta \)-cisH\textsubscript{2} (pK\textsubscript{a}H\textsuperscript{2} = 7.19) is lower than the pK\textsubscript{a}H\textsuperscript{2} (7.44), and this changes the ratio of the \( \alpha \)-cis: \( \beta \)-cis isomers from \(~3:1\) (for the stoichiometry [VO\textsubscript{2}LH\textsubscript{2}]) to \(~1:7:1\) (for the [VO\textsubscript{2}LH\textsubscript{2}] stoichiometry). The difference in the second range 4–10. However, due to the different acidity of the NH\textsuperscript{+} pyridine in the \( \alpha \)-cis and \( \beta \)-cis isomers, there is a slight change in the ratio of the two types of isomers between pH 6 and 9, with a minimum of \( \alpha \)-cis/\( \beta \)-cis at pH 7.8, where the population of the monoprotonated forms of [VO\textsubscript{2}LH\textsubscript{2}]\textsuperscript{2+} shows its maximum (Figure 5 and SI-8A in the Supporting Information).

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stepwise deprotonation constants between the two isomers (pK_a^H = 8.33 and pK_b^H = 8.67) is almost the same (0.35) as for the first (0.25), but now the pK_a of β-cis is higher, therefore the isomer ratio is settled back to ~3:1.

The acidity of one of the NH^+_pyridine of β-cis is lower by ~0.42 units than the other. The assignment of the pK_a^H and pK_b^H is based on the DFT calculations. The gas-phase structure of β-cisH(B^H) is less stable than that of β-cisH(A^H) by 3.5 kcal mol^-1, and we assume the same trend occurs in aqueous solution. Thus the first deprotonation occurs mainly at the NH^+_pyridine(B) which is more acidic, and the second deprotonation involves the NH^+_pyridine(A) which is more basic. The complete resolution of the microscopic dissociation scheme, which is a good example of how the joint evaluation of parallel pH/H NMR and pH/β^H NMR data can be used to describe such a system revealing all fine details, is given in the Supporting Information (SI-8).

**DFT calculations:** Gas-phase structures are obtained by DFT calculations, but these normally give good approximations of the solution and solid-state molecular structures. The stability of the isomers is dependent on solvation and ionic interactions occurring in solution, and these environmental effects may affect the predominance of the isomers in solution. However, DFT calculations certainly may give good clues in understanding the ratio of the various binding isomers present in solution, the type of bonding present and to explain the spectroscopic properties.
DFT calculations were carried out for most of the possible isomers of diprotonated (MLH₂), monoprotonated (MLH) and deprotonated (ML) VIVO and VVO₂ complexes of 2, in order to evaluate their relative energies, to understand several aspects related to their structural preferences and electronic structure, and to determine some properties. The structure of the complexes was simplified by replacing the -CH₃ and -CH₂OH groups by -H in the pyridoxal ring. For the main objective of our calculations their effect is expected to be small, although they are relevant for intermolecular association and H-bonding interaction with solvent molecules. The Supporting Information (e.g., SI-9) summarizes the structural data, relative energies, and some properties obtained in the DFT calculations, namely the experimental and calculated ¹H NMR data for the isomers corresponding to III and IV.

For the VVO₂ complexes, the DFT calculations were carried out both including and excluding a coordinated H₂O ligand, revealing interesting aspects on the energetics of H₂O coordination in VVO₂ complexes. In fact, for all stoichiometries and isomers (except for one isomer and stoichiometry of the VVO₂-Rpyr₂en system, see below), the coordination of one H₂O molecule corresponds to an exothermic reaction with calculated ΔE between −100 and −75 kJ mol⁻¹ (−24 and −18 kcal mol⁻¹). For the [VIVO] stoichiometry corresponding to structure IX (Scheme 2), no six-coordinate complex with a coordinated water molecule in the axial position trans to V=O could be obtained, all optimization attempts leading to an isolated H₂O molecule and the five-coordinate complex. This emphasizes the subtle electronic/charge effects that may be operating in this type of complex.

DFT calculations for the VVO₂ isomers with two trans-Ooxo atoms yielded structures corresponding to energies higher (by approximately 146 kJ mol⁻¹ (~35 kcal mol⁻¹)) than those with two cis-Ooxo ligands. This is because while in the cis-VVO₂ compounds the strongly π-donating Ooxo ligands have exclusive use of one dₓz, dᵧz orbitals each, and share a third one (dₓᵧ), in the trans-configuration, the Ooxo ligands would have to share two dₓᵧ orbitals and leave one unused.

The fully optimized DFT structure (the bonding parameters are given in Table 1) corresponding to compound 4 may be used for comparison with the molecular structure determined by X-ray diffraction. As may be seen in Table 1, the calculated bond lengths and angles compare well with those for 4. Some of the differences arise from the fact that an iso-
lated molecule was considered in the theoretical calculations, while as was revealed by X-ray diffraction, several intramolecular hydrogen bonds occur in the crystal structure of complex 4. As for the X-ray structure, the calculated V–O(phenolate, Namine) bond lengths of the half-molecule containing the protonated NH$_{\text{pyridine}}$ moiety are about 0.05–0.15 Å longer than in the deprotonated half-molecule.

The DFT structural data reveal that for all isomers of MLH$_2$, MLH and ML complexes (M = V$^{\text{IV}}$O and V$^{\text{V}}$O$_2$) of 2, in the DFT structures the (O = V–O) bond lengths of the cis bonded molecule are about 2.2–2.4 Å. In the cases where the group is trans to a Ophenolate donor (as O4 in compound 4). For the [V$^{\text{V}}$O(OH)$_2$], [V$^{\text{V}}$O(OH) and [V$^{\text{V}}$O] complexes, five, eight, and five isomers were considered, respectively (see Scheme 2), the average V–O$_{\text{aco}}$ bond lengths being 1.599, 1.611, and 1.619 Å, respectively. A similar trend was observed for the V$^{\text{V}}$O complexes. The longer V–O$_{\text{aco}}$ bond lengths (approximately 1.61–1.63 Å) correspond to the complexes where the O$_{\text{aco}}$ atom is trans to a Ophenolate atom.

The (O = V–O) bond lengths are within the range 1.94–2.20, and 2.25–2.42 Å, respectively. For several of the more stable isomers of the V$^{\text{V}}$O and V$^{\text{V}}$O$_2$ complexes the (O = V–O) bond lengths are shorter than expected. The lower values (1.94 Å) being found for some of the V$^{\text{V}}$O complexes. There are only a few complexes with (O = V–O) bond lengths (trans) intermediate between those of the cis form, when the Ophenolate is in the equatorial plane (approximately 1.9 Å), and those of the compounds containing neutral oxygen donors, including the HOphenolate group (approximately 2.2–2.4 Å). Thus, the trans influence of the O$_{\text{aco}}$ ligand is still expressed when one compares the lengths ~1.9 and ~2.1 Å.

In agreement with the spectroscopic (EPR and NMR) results, the DFT calculations indicate that, for each stoichiometry [ML], [MLH], and [MLH]$_2$ (M = V$^{\text{IV}}$O and V$^{\text{V}}$O$_2$) there are several isomeric structures corresponding to similar energies. For the V$^{\text{V}}$O complexes the binding modes correspond to structures V–IX included in Scheme 2, and the only one that appears to be energetically disfavored corresponds to structure VII. Nevertheless, for each stoichiometry at least three types of isomers may exist in solution with A$_i$ values of either 158 or 164–5×10$^{-8}$ cm$^{-1}$. This is in good agreement with the experimental values (Figure 4 and Table 2), which showed the existence of two distinct signals with A$_i$ values of 157–8 and 164–6×10$^{-8}$ cm$^{-1}$ in the pH range 2–8, which is also, for all stoichiometries.

For the V$^{\text{V}}$O$_2$ complexes all types of isomers considered in the DFT calculations (binding modes III–IV in Scheme 2) correspond to similar energies. The maximum energy difference found was 14.6 kJ mol$^{-1}$ (3.5 kcal mol$^{-1}$), and this for two isomers of the [V$^{\text{V}}$O$_2$LH] stoichiometry with equal binding mode (structure IV: β-cisH(A$^{\text{IV}}$) and β-cisH(B$^{\text{II}}$) in Scheme 3), and differing in the type of pyridine-N atom that is protonated.

**Conclusion**

The Schiff-base ligand of pyridoxal and ethylenediamine, 1, and its hydrolytically more stable reduced derivative 2 proved to be efficient binders of both vanadium(iv) and vanadium(v). X-ray diffraction studies show the Schiff base pyr$_{\text{en}}$ and the reduced Schiff base Rp,yr$_{\text{en}}$ with strong intramolecular hydrogen bonds. Several V$^{\text{V}}$O and V$^{\text{V}}$O$_2$ complexes of these and related ligands were prepared and their properties studied. One of these, namely [V$^{\text{V}}$O$_2$(HRpyr$_{\text{en}}$)], was isolated in crystalline form and structurally characterized by X-ray diffraction. Both pyr$_{\text{en}}$ and Rp,yr$_{\text{en}}$ were found to form basically similar complexes, the tetradentate coordination of the ligands through 2×Ophenolate and 2×Namin/iminic, being the predominant binding mode. [V$^{\text{V}}$O$_2$–(pyridoxaminato)$_2$] was also characterized and involves one of the vitamin B$_6$ forms as the ligand, the binding mode being similar.

IR and magnetic susceptibility measurements with the V$^{\text{V}}$O complex of the reduced SB revealed weak V–O...O interactions between the metal ion centers, which are not present in the corresponding complex formed with the Schiff base.

The acid–base properties of the ligands, and complexation with V$^{\text{V}}$O$^{\text{IV}}$+ and V$^{\text{V}}$O$_2$+ in aqueous solution were studied by pH-potentiometry, visible absorption, EPR, $^1$H and $^{51}$V NMR spectroscopy. We highlight the differences in the binding abilities of the SB and its reduced derivative towards both V$^{\text{V}}$O$^{\text{IV}}$+ and V$^{\text{V}}$O$_2$+ that is clearly demonstrated by the values of the proton displacement constants ($K^+$) characteristic to the formation equilibrium: $K^+$ = H$_2$L$^+$$^+$ + V$^{\text{V}}$O$_2$+ $\rightarrow$ [V$^{\text{V}}$O$_2$L]$^+$ + H$_2$L$^+$$^+$; the log $K^+$ values are ~14.1 and ~14.6 for pyr$_{\text{en}}$ and Rp,yr$_{\text{en}}$, respectively. This difference in the stability may probably be explained by the much higher flexibility of the reduced Schiff base ligand, lacking the –C=N double bonds, thus resulting in significantly less strain and/or better ligand–metal orbital overlap in the complexes formed. The Schiff base may also hydrolyze, and this was confirmed by both solution studies and X-ray characterization of [(V$^{\text{V}}$O$_2$)$_2$(pyr)$_2$]2H$_2$O, containing the half Schiff base pyr$^-$ as ligand.

EPR (V$^{\text{IV}}$–Rp,yr$_{\text{en}}$ system) and $^1$H and $^{51}$V NMR (V$^{\text{V}}$O$_2$–Rp,yr$_{\text{en}}$ system) studies indicated the presence of various isomeric species in solution. The combined quantitative treatment of the pH-metric and spectral data provided the complete description of the equilibrium system. It was shown that distinct species result from the protonation/deprotonation of the NH$_{\text{pyridine}}$ of the ligand. For the V$^{\text{IV}}$O$_2$–Rp,yr$_{\text{en}}$ system microequilibrium constants between seven types of complexes were calculated from the measured $^1$H and $^{51}$V NMR chemical shifts. DFT calculations provided sensible molecular structures, and energy values for the various isomers, confirming the assumptions made on the EPR and NMR data.

Overall the present systems are remarkable examples of the complexity of the types of isomers that may form in solutions of V$^{\text{IV}}$O and V$^{\text{V}}$O$_2$ complexes (and indeed in many other metal–ligand systems), and how pH-potentiometry, spectroscopy, DFT calculations, and handling of the experi-
mental data may be used to characterize the systems in solution.

The complexes prepared are slightly soluble in water and the order of stability of the complexes formed is pyridoxamine < H_{2}pyr-< H_{2}pyr-en. Pyridoxal and pyridoxamine are forms of vitamin B_{6} and are nontoxic metabolites. Their vanadium complexes may therefore be good candidates for therapeutic use. Toxicity, insulin-mimetic, and other biologi-

Experimental Section

Materials: All chemicals used for the synthetic work were obtained from Merck, Sigma-Aldrich or Calbiochem were of reagent grade. They were used without further purification.

Synthesis of the ligands

Preparation of H_{2}pyr- (1): Pyridoxal-HCl (2.24 g, 11 mmol) was dissolved in H_{2}O (40 mL) and the pH was set to 6.5 by addition of concen-

The mixture was stirred under reflux for 1 h. The yellow precipitate formed was separated (5 mL) was added dropwise to the pyridoxal suspension. The mixture was used without further purification.

Preparation of H_{2}pyr-en (2): NaBH_{4} (0.113 g, 3.00 mmol) dissolved in methanol containing KOH was added to a suspension of H_{2}pyr-en (1.00 g, 2.79 mmol) in methanol/chloroform (3:2, 50 mL). The mixture was stirred at about 5°C overnight, and the yellow solution turned colorless. HCl (1.0 M) was added until pH 4.5 was reached, and the solution was stirred for 2 h. The pH was then increased to 10 by addition of KOH (3x). The white precipitate was filtered, washed with water, ethanol, and diethyl ether, and dried under vacuum. Yield: 97%; elemental analysis calcd (%) for C_{60}H_{62}O_{32}: H 6.19, N 15.63; found: C 60.66, H 6.3, N 15.7; MS: m/z: 358; NMR (D_{2}O) (pH 4.3): 2.49 (s, 6H; CH_{2}), 3.31 (s, 4H; CH=CH-OH), 5.1 (dd, 4H; -CH=CH-), 6.58 (s, 2H; CH=CH-N), 7.77 (s, 2H; CH_{nernst}). The solution of the filtrate was kept at room temperature; after several weeks yellow crystals were obtained and characterized by X-ray diffraction.

Preparation of H_{2}pyr-en (3): NaN_{3} (0.54 g, 8.3 mmol) was dissolved in water was slowly added and the pH was set to 7.5±8 by addition of 3x KOH. After precipitation of the complex, the solution was filtered, washed, and dried under vacuum.

Preparation of H_{2}pyr-en (4): The pH of a solution containing H_{2}pyr-en (3 mm) and KOCl (3 mm) was adjusted to about 7 by addition of 0.2 mL KOH. After about 24 h at room temperature yellow crystals suitable for X-ray diffraction were collected.

Preparation of H_{2}Rpyr2en (2): Three-dimensional, room-
temperature X-ray data were collected on a Siemens Smart1000 CCD instru-

Preparation of H_{2}Rpyr2en (3): The 2D COSY, NOESY, ROESY, and TOCSY spectra were also obtained at 25±1°C, on the same NMR spectrometer using the same 3-mm broad band probe.

X-ray crystal structure determination of 1, 4, and 9: For the three com-

Preparation of V^{4+}O(Me-pyrN)_{2} (8): The Schiff base Me-pyrN was formed in situ by reaction of pyridoxal-HCl (1 equiv) with methyleneimine (1 equiv). In the end an orange compound precipitated. Yield: 65%; elemental analysis calcd (%) for C_{37}H_{49}NO_{6}V: 2.5H_{2}O [formulation: V^{4+}O(Me-pyrN)_{2}·2.5H_{2}O]: C 45.96, H 5.79, N 11.91; found: C 46.2, H 5.9, N 11.6.

Crystals of (V^{4+}O)_{2}((pyren))·2H_{2}O (9): After about two weeks, some crystals were formed from the filtrate obtained in the preparation of 5, which were left in a flask and in contact with air at about 4°C. These were removed and characterized by X-ray diffraction.

Physical and spectroscopic studies: IR spectra were recorded with a BioRad FTS 3000 MX FTIR spectrometer. Visible spectra were recorded either with a Hitachi U-2000 or a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrophotometer. The EPR spectra were recorded at 77 K (on glasses made by freezing solutions in liquid nitrogen) with a Bruker ESP 300E X-band spectrometer. The magnetic susceptibilities were measured in the range 5–296 K using a 7-Tesla Faraday Oxford Instruments system cou-

X-ray crystal structure determination of 1: Three-dimensional, room-
temperature X-ray data were collected on a MACCHI Enraf-Nomius diffractometer with two graphite-monochromated radiation. The crystal structures were solved by direct methods (program SIR97) and refined by SHEXL97, all in the package WinGX-Version 1.6403B. All non-

hydrogen atoms were refined anisotropically and hydrogen atoms for 1 and 9 that were located in the Fourier maps were refined isotropically. In compound 4 the hydrogen atoms were included in calculated positions and allowed to be refined riding on the parent atom, except for the nitrogen and the water hydrogen atoms, which were refined and isotropically with some constraints. Further details of the crystal structure determinations are given in Table 4 and in the Supporting Infor-
mation (SI-1). Graphical representations were prepared using ORTE-
PH[77] and SCHAKAL99.[80]

X-ray crystal structure determination of 2: Three-dimensional, room-
temperature X-ray data were collected on a Siemens Smart 1000 CCD instru-
mctures were solved by direct methods and refined by full-

matrix least-squares on the same NMR spectrometer using the same 5-mm broad band probe.

Determination of the vanadium content in samples: A procedure was de-
voped for this purpose (see Supporting Information SI-10).

pH-potentiometric titrations: All measurements were made in water. The purity of the ligands was checked pH-potentiometrically and the exact concentration of solutions were determined by the Gran method.[85] The stock solution of V^{4+}O was prepared and standardized as reported earli-

er[84] and also as mentioned above. The H_{2}O^{+} concentration in the stock solutions was determined by pH-potentiometry. The V^{4+} stock solution was prepared by dissolving KVO_{3} in KOH solution of known molarity and its H_{2}O^{+} concentration was calculated. The vanadium content in the commercial KVO_{3} was determined as described above.

All solutions were manipulated in an inert atmosphere (high purity N_{2} or purified argon). The ionic strength was adjusted to 0.20 M KCl and the temperature was 25.0±0.1°C. The pH was measured with an Orion 710 A precision digital pH meter equipped with an Orion Ross 8103B/2 type combined glass electrode, calibrated for hydrogen ion concentration as described earlier.[84] The ionic product of water was pK_{w}=13.76.

Stability constants were determined by pH-metric titration of 10.0 or 25.0 mL samples. The ligand concentrations were in the range 0.0005–0.005 M (0.0005–0.01 M for pyridoxamine), and the L:M ratio from 1 to 4 (2 to 12 for pyridoxamine). Titration results were normally done from pH 2.0

Preparation of [V^{4+}O(Me-pyrN)]_{2} (8): The Schiff base Me-pyrN was formed in situ by reaction of pyridoxal-HCl (1 equiv) with methyleneimine (1 equiv). In the end an orange compound precipitated. Yield: 65%; ele-

mental analysis calcd (%) for C_{37}H_{49}NO_{6}V: 2.5H_{2}O [formulation: V^{4+}O(Me-pyrN)_{2}·2.5H_{2}O]: C 45.96, H 5.79, N 11.91; found: C 46.2, H 5.9, N 11.6.

Crystals of (V^{4+}O)_{2}((pyren))·2H_{2}O (9): After about two weeks, some crystals were formed from the filtrate obtained in the preparation of 5, which was left in a flask and in contact with air at about 4°C. These were removed and characterized by X-ray diffraction.

Physical and spectroscopic studies: IR spectra were recorded with a BioRad FTS 3000 MX FTIR spectrometer. Visible spectra were recorded either with a Hitachi U-2000 or a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrophotometer. The EPR spectra were recorded at 77 K (on glasses made by freezing solutions in liquid nitrogen) with a Bruker ESP 300E X-band spectrometer. The magnetic susceptibilities were measured in the range 5–296 K using a 7-Tesla Faraday Oxford Instruments system cou-

X-ray crystal structure determination of 1, 4, and 9: For the three com-

Preparation of H_{2}Rpyr2en (3): NaN_{3} (0.54 g, 8.3 mmol) was dissolved in water was slowly added and the pH was set to 7.5±8 by addition of 3x KOH. After precipitation of the complex, the solution was filtered, washed, and dried under vacuum.

Preparation of H_{2}Rpyr2en (4): The pH of a solution containing H_{2}pyr-en (3 mm) and KOCl (3 mm) was adjusted to about 7 by addition of 0.2 mL KOH. After about 24 h at room temperature yellow crystals suitable for X-ray diffraction were collected.

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Preparation of [V^{4+}O(Me-pyrN)]_{2} (8): The Schiff base Me-pyrN was formed in situ by reaction of pyridoxal-HCl (1 equiv) with methyleneimine (1 equiv). In the end an orange compound precipitated. Yield: 65%; elemental analysis calcd (%) for C_{37}H_{49}NO_{6}V: 2.5H_{2}O [formulation: V^{4+}O(Me-pyrN)_{2}·2.5H_{2}O]: C 45.96, H 5.79, N 11.91; found: C 46.2, H 5.9, N 11.6.
Vanadium(v) and Vanadium(v) Complexes

Table 4. Crystal and structure refinement data.

<table>
<thead>
<tr>
<th></th>
<th>H2pyr, en (1)</th>
<th>H2Rpyr, en (2)</th>
<th>[VO2(HRpyr2en)]·3H2O</th>
<th>[VO2(pyren)3]·2H2O</th>
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<td>C2H7N2O</td>
<td>C2H10N2O</td>
<td>C2H12N2O3V</td>
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<td>298(2)</td>
<td>293(2)</td>
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<td>P21/a</td>
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<td>8.791(3)</td>
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<td>–</td>
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<tr>
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</table>

until 11.5, unless very extensive hydrolysis, precipitation or very slow equilibration was detected, with KOH solution of known concentration (approximately 0.2 mol dm⁻³). The reproducibility of titration points included in the evaluation was within 0.005 pH units in the whole pH range.

The concentration stability constants βpyr = [M⋯H2pyr][M][H]²⁺ were calculated by using the PSEQUAD computer program.[60] The formation indices (all data) were of the NMR instrument.[90] The full-matrix least-squares on F² were prepared in D₂O (99.995% D₂O) to obtain ⁵¹V NMR spectra, or in a D₂O solution of the V⁺⁺ salt when the samples were analyzed by one-(1D) and two-dimensional (2D) ¹H NMR spectroscopy, to have the desired L:M ratios. Several sets of experiments as a function of pH, with different L:M ratios and total concentrations were carried out.

The ¹H and ⁵¹V NMR chemical shifts were referenced relative to TSS at 0 ppm and to a VOCI external solution at 0 ppm, respectively. A presaturation pulse sequence was used for ¹H NMR spectra to eliminate the residual water signal. The aqueous V⁺⁺ ·H₂pyr, en system was also studied by 2D ¹H NMR techniques, including COSY, NOESY, TOCSY, and ROESY, using the respective pulse sequences installed in the software of the NMR instrument. ⁵¹V NMR acquisition parameters were: 33 kHz spectral width, 30 μs pulse width, 1 s acquisition time, and 10 Hz line broadening. The signal intensities of the NMR resonances were obtained using the program NUTS.[91]

Molecular orbital calculations: The calculations were performed with the B3LYP HF/DFT hybrid functional as implemented in the Gaussian 98 set of programs.[92] The functional includes a mixture of Hartree-Fock[13] exchange and DFT[14] exchange-correlation, given by Becke’s three parameter functional[93] with the Lee, Yang, and Parr correlation functional, which includes both local and nonlocal terms.[94,95] All the optimized geometries are the result of full optimizations without any symmetry constraints, done with model complexes with the CH₃ and CH₂OH substituents of the pyridyl rings replaced by hydrogen atoms. Spin unrestricted calculations were done to optimize all the possible isomers of the V⁺⁺ complexes in the three protonation states (di-, mono-, and deprotonated), with and without water coordinated, in a total of 32 different species. A standard LanL2DZ basis set[96-98] was used for the optimizations. In the case of the isomers presenting two equatorial N atoms, two equatorial O atoms, and one axial H₂O trans to the V=O bond (structure V in Scheme 2), the B3LYP optimized structures described the H₂O coordination poorly, with long V-O(water) lengths (>2.4 Å) and enhanced H(water)-O(axial) hydrogen interactions. Thus, the Barone and Adamo one parameter functional[99] with modified Perdew-Wang exchange and Perdew-Wang 91 correlation[100,101] (MPW1PW91) was used to re-optimize those complexes, since this functional is known to describe weak interactions better than B3LYP.[102] Indeed, the structures obtained

in this way, and discussed in the text, present shorter V–O(water) and longer H(water)–O_{\text{bridge}} separations than the ones resulting from the B3LYP optimizations. Single-point energy calculations with the B3LYP functional and a standard 6–31G(d,p) basis set \cite{28} were done for all the optimized structures. Spin contamination was carefully monitored for all the unrestricted calculations performed, and the values of \langle S^2 \rangle (0.7500±0.7501) indicate minor spin contamination.

All possible isomers in the three protonation states of the VVO_2 complexes (in a total of 10 different species) were optimized using the B3LYP functional and a 6–31G(d,p) basis set. \cite{31} The Hartree–Fock level using a 6–311G(2d,p) basis set. The calculations were done for the several V–Rpyr2en complexes, VOCl_3, VO_4^{3–}, and their relative energies were calculated. Single-point energy calculations with the B3LYP functional and a standard 6–31G(d,p) basis set. \cite{106,117} NMR shielding tensors were calculated using the Gauge-Independent Atomic Orbital method (GIAO) \cite{118} at the Hartree–Fock level using a 6–311G(d,p) basis set. The calculations were done for all the unrestricted calculations performed, and the values of \langle S^2 \rangle (0.7500±0.7501) indicate minor spin contamination.

Acknowledgement

This work was carried out in the frame of a COST D21 project. The authors are grateful to A. R. Tomé for help with the use of the program Origin 6.3, and the financial support of the Hungarian National Research Fund (OTKA T31986/2000). Hungarian Academy of Sciences, Fundo Europeu para o Desenvolvimento Regional, Fundação para a Ciência e Tecnologia, POCTI Programme (project POCTI/35368/QUI/2000), and the Hungarian–Portuguese Intergovernmental S & T Cooperation Programme for 2000–2001.


