Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 2309–2313
Carbohydrate RESEARCH

Note

The revised NMR chemical shift data of carrageenans

Fred van de Velde,[a,b,*] Leonel Pereira[c] and Harry S. Rollema[b]

[a]*Wageningen Centre for Food Sciences, PO Box 557, 6700 AN Wageningen, The Netherlands*
[b]*NIZO food research, Product Technology Department, Kernhemseweg 2, PO Box 20, 6710 BA Ede, The Netherlands*
[c]*Department of Botany, University of Coimbra, Arcos do Jardim-3000 Coimbra, Portugal*

Received 5 March 2004; accepted 14 July 2004
Available online 11 August 2004

Abstract—A new set of ^{13}C and ^{1}H NMR chemical shifts of most common carrageenan types is given relative to DSS as the internal standard according to the IUPAC recommendations. Moreover, the chemical shifts of characteristic signals for pyruvate acetal and floridean starch are reported. Additionally, chemical shifts of common internal standards, such as methanol, DMSO and acetone, were measured at different temperatures and pH values.

Keywords: Carrageenan; Hybrids; NMR spectroscopy; Structure analysis; Chemical shift data

Carrageenans represent one of the major texturising ingredients in the food industry.[1] Carrageenan is a generic name for a family of linear, sulfated galactans, obtained by extraction from certain species of marine red algae (Rhodophyta). They are composed of alternating 3-linked β-D-galactopyranose (G-units) and 4-linked α-D-galactopyranose (D-units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA-units), forming the disaccharide repeating unit of carrageenans (see Fig. 1). The letter codes in Figure 1 refer to the nomenclature developed by Knutsen et al.[2] Since natural carrageenan is a mixture of nonhomologous polysaccharides, the term disaccharide repeating unit refers to the idealised structure.

The sulfated galactans are classified according to the presence of the 3,6-anhydro bridge on the 4-linked galactose residue and the position and number of sulfate groups. The most common types of carrageenan are traditionally identified by a Greek prefix. The three commercially most important carrageenans are called ι-, κ- and λ-carrageenan, and the corresponding IUPAC-inspired names and letter codes are carrageenose 2,4′-disulfate (G4S-DA2S), carrageenose 4′-sulfate (G4S-DA) and carrageenan 2,6,2′-trisulfate (G2S-D2S,6S). In addition to these three major carrageenan types, two other types, called μ- and ν-carrageenan (letter code G4S-D6S and G4S-D2S,6S, respectively), are often encountered in commercial carrageenan samples and are the biological precursors of, respectively, κ- and ι-carrageenan.

Since natural carrageenans are mixtures of different sulfated polysaccharides, their composition differs from batch to batch. Therefore, the quantitative analysis of carrageenan batches is of greatest importance for both ingredient suppliers and food industries to ensure ingredient quality. From the pioneering work of Usov and co-workers,[3,4] NMR spectroscopy is nowadays the preferred technique to determine and quantify the composition of carrageenan batches.[5] Starting from the early work of Usov and co-workers, chemical shifts of carrageenan resonances are generally converted to values relative to tetramethylsilane (TMS) via an internal dimethyl sulfoxide (DMSO) or methanol (MeOH) standard.[3,6–8] The use of DMSO or MeOH as internal standards resulted in a generally accepted set of chemical shifts for different types of carrageenans as summarised by van de Velde et al.[5] However, to convert chemical shifts from aqueous internal DMSO or MeOH to values relative to TMS is not obvious, as TMS is only sparingly soluble in highly polar solvents such as water

* Corresponding author. Tel.: +31-318-659-582; fax: +31-318-650-400; e-mail: fred.van.de.velde@nizo.nl

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.07.015

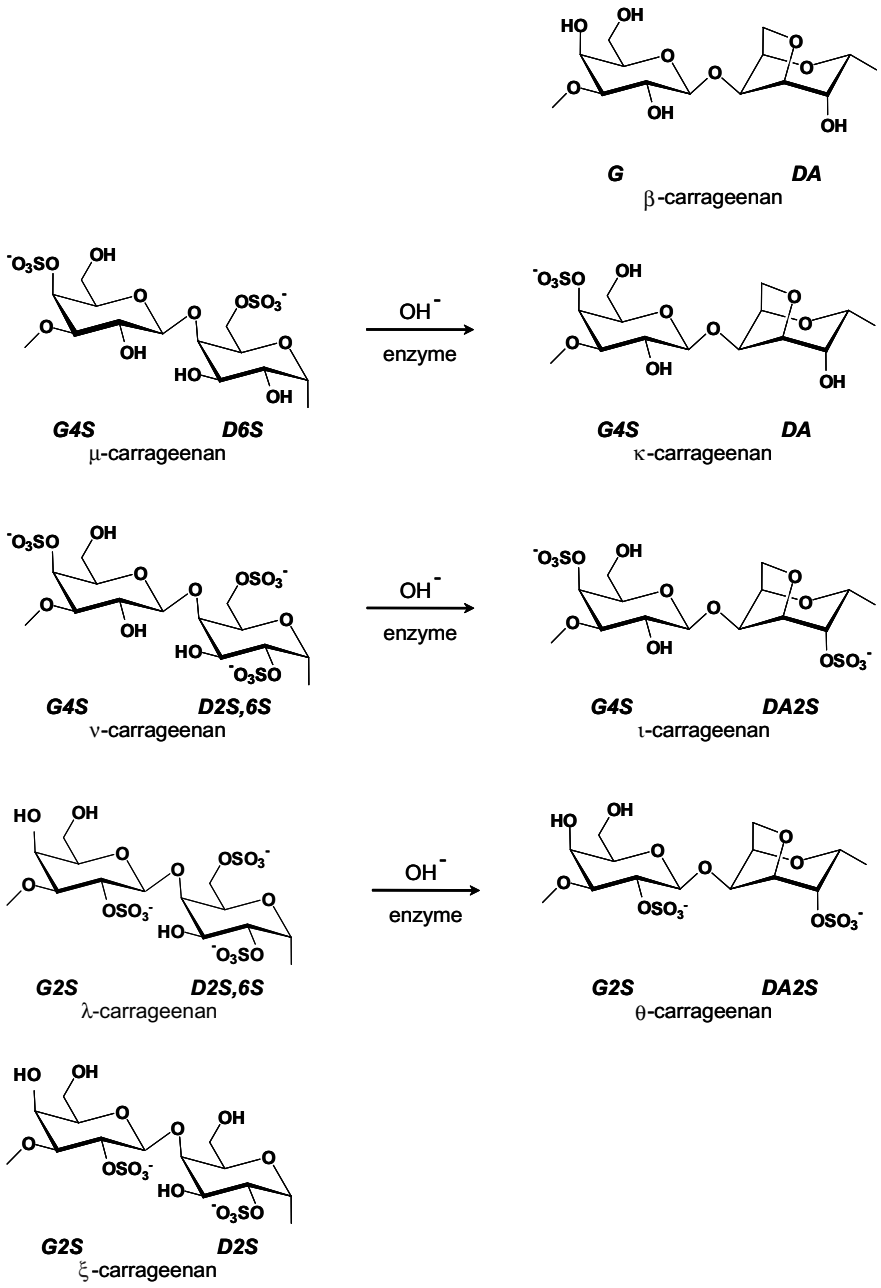

Figure 1. Schematic representation of the different structures of the repeating units of carrageenans. The letter codes refer to the nomenclature of Knutsen et al.[2]

or D_2O. Therefore, the IUPAC commission for molecular structure and spectroscopy recently recommended the use of 2,2-dimethyl-2-silapentane-3,3,4,4,5,5-d_6-5-sulfonate sodium salt (DSS) as the primary reference for both ^1H and ^{13}C NMR spectroscopy in highly polar solvents. For most purposes the difference between DSS and TMS when dissolved in the same solvent are negligible, and, therefore, the data from DSS and TMS scales may be validly compared without correction.[9]

However, application of the IUPAC recommendations in NMR spectroscopy of carrageenans results in changed chemical shifts for all common carrageenan types. Pereira et al.[10] reported chemical shift values relative to DSS for κ- and ι-carrageenan that are 2.5 ppm larger than those reported by Usov.[3] Therefore, we studied the application of DSS as an internal standard for NMR spectroscopy of carrageenans in more detail.

In this work, both ^{13}C and ^1H NMR spectra of representative carrageenan samples containing the most common repeating units, for example, κ (kappa)-, ι (iota)-, λ (lambda)-, μ (mu)-, ν (nu)-, θ (theta)-, β (beta)- and ξ (ksi)-carrageenan were recorded. The chemical shifts of all carbon atoms as well as the anomeric protons are reported relative to DSS internal standard ($\delta = 0.000$ ppm)

according to IUPAC recommendations,[9] providing the scientific community with a new set of chemical shift data for different carrageenan types.

1. Discussion

First of all ^{13}C and ^{1}H NMR spectra of mixtures of different internal standards, for example, DSS, DMSO, MeOH, acetone and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP, as an alternative for DSS[9]), were recorded at three temperatures (24, 45 and 65 °C) in D_2O containing 20 mM phosphate adjusted to the following pH values: pH 5.5, 7.2, 9.7 and 11.8. Chemical shifts of the different internal standards are given in Table 1. According to the IUPAC recommendations, DSS is set at a chemical shift of 0 ppm for all temperatures and pH values.[9]

Measured within the pH range from pH 5.5 to 11.7, the chemical shifts of the internal standards, TSP, DMSO and MeOH were pH insensitive. Although acetone showed good signal at pH 5.5 and 7.2, the intensity of this signal dropped dramatically at higher pH values. This is probably due to ketone–enol rearrangements. Therefore, acetone is not the internal reference of choice at higher pH values. Temperature dependency of the chemical shift of the internal standards was observed in the ^{13}C spectra for DMSO. The chemical shift of the DMSO resonance in the ^{13}C spectra shifted from 41.29 ppm at 24 °C to 41.53 ppm at 65 °C.

TSP has a chemical shift of $\delta = -0.18$ (^{13}C) and $\delta = -0.017$ (^{1}H) that allows the use of TSP as an alternative for DSS. The measured ^{13}C NMR chemical shifts of DMSO ($\delta = 41.53$) and MeOH ($\delta = 51.43$) are significantly different from those reported and used by Usov et al.,[6] respectively, $\delta = 39.45$ and $\delta = 50.12$ for DMSO and MeOH. This difference explains for the greater part the chemical shift differences observed by Pereira et al.[10] Usov et al.[6] already mentioned in their comments on chemical shift calculation a systematic discrepancy (up to 1 ppm) in chemical shifts reported by different authors. However, in our opinion the chemical shifts of DMSO and MeOH reported are measured in apolar solvent to allow the use of TMS as internal standard and, thereby, are significantly different from those measured in aqueous solution.

Furthermore, both ^{13}C and ^{1}H NMR spectra were recorded for relevant carrageenan samples containing the most common carrageenan repeating units, for example, κ-, ι-, λ-, μ-, ν-, θ-, β- and ξ-carrageenan. Assignment of the resonances in the NMR spectra of the common carrageenan types was based on spectra and data summarised in literature.[5] For the ξ-carrageenan ^{13}C NMR spectrum, only a few resonances are assigned in literature.[11] A detailed study on the interpretation of both the ^{13}C and ^{1}H NMR spectra of a ξ/θ-hybrid carrageenan is currently carried out and the results will be published separately (Usov, A. I.; Shashkov, A. S.; Rollema, H. S.; Pereira, L.; Van de Velde, F. *Carbohydr. Res.*, in preparation). The chemical shift data obtained in the current study are summarised in, respectively, Tables 2 and 3. The ^{13}C NMR chemical shifts of κ- and ι-carrageenan relative to DSS are in good agreement with those reported in literature,[10] although they were measured under different conditions. Generally, the chemical shifts given in Table 2 are larger (on average 2.1 ppm) than those summarised by Van de Velde et al.[5] This difference is equal to the difference between the measured chemical shift of DMSO relative to DSS and the chemical shift of DMSO reported by Usov et al.[6] The ^{1}H NMR chemical shifts of the α-anomeric protons of carrageenans (Table 3) are only slightly different from those reported in literature.[5,12]

In addition to the common carrageenan repeating units, the analysed carrageenan samples contained some minor constituents and contaminants that are frequently encountered in carrageenan batches. Pyruvic acid is a common component of many complex carrageenans. It forms a cyclic acetal at positions 4 and 6 of 3-linked galactose residues. This substituent can be identified by characteristic signals of its carbons together with specific substitution effects on the corresponding carbon atoms of 3-linked D-galactose.[5,11,13] Relative to DSS the ^{13}C NMR chemical shifts of these characteristic

Table 1. Chemical shifts for common internal standards for NMR spectroscopy in aqueous systems

Compound abbr.	Chemical shift (ppm)[a]		Compound chemical name
	^{13}C	^{1}H	
DSS	0.000	0.000	2,2-Dimethyl-2-silapentane-3,3,4,4,5,5-d_6-5-sulfonate sodium salt
TSP	−0.18	−0.017	3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt
MeOH	51.43	3.337	Methanol
DMSO	41.53[b]	2.696	Dimethyl sulfoxide
Acetone[c]	32.69	2.208	Acetone

[a] Recorded in D_2O containing NaH_2PO_4 and Na_2HPO_4 (20 mM phosphate; pH 7.2) at 65 °C. Constant chemical shifts measured between 24 and 65 °C and between pH 5.5 and pH 11.8, with the exception of DMSO where a slight temperature dependence was observed, and acetone, which is not applicable at high pH (see text).

[b] Slight temperature dependency observed ranging from a chemical shift of 41.29 ppm at 24 °C to 41.53 ppm at 65 °C.

[c] Only valid at pH 5.5 and 7.2.

Table 2. ^{13}C NMR chemical shifts for the most common carrageenan structural units[a]

Carrageenan	Unit[b]	Chemical shift (ppm) relative to DSS as internal standard					
		C-1	C-2	C-3	C-4	C-5	C-6
β (beta)	G	104.81	71.72	82.58	68.56	77.55	63.49
	DA	96.81	72.40	81.64	80.33	79.26	71.72
ι (iota)	G4S	104.43	71.53	79.06	74.34	77.04	63.52
	DA2S	94.29	77.15	80.04	80.55	79.29	72.02
κ (kappa)	G4S	104.70	71.72	80.98	76.25	77.00	63.49
	DA	97.34	72.11	81.41	80.54	79.07	71.72
λ (lambda)	G2S	105.61	79.61	77.99	66.35	76.51	63.45
	D2S,6S	93.85	77.04	71.76	82.61	70.89	70.25
μ (mu)[c]	G4S	107.00	72.69	80.54	76.25	77.1	63.48
	D6S	100.26	70.7	72.8	81.40	70.5	69.89
ν (nu)	G4S	106.96	72.40	82.42	73.36[d]	77.15	63.58
	D2S,6S	100.53	78.58	70.37	82.13	70.37	70.02
θ (theta)[e]	G2S	102.57	79.8	79.4	69.97	77.05	63.38
	DA2S	97.81	77.05	79.6	81.75	79.2	72.35
ξ (ksi)[f]	G2S	105.44			66.92		
	D2S	94.94					

[a] Carrageenan (30 mg mL^{-1}), DSS (10 mM) and Na_2HPO_4 (20 mM) in D_2O recorded at 65 °C.
[b] Codes refer to the nomenclature developed by Knutsen et al.[2]
[c] Chemical shifts given with one decimal place are obtained from literature and corrected with the offset calculated for the chemical shifts of the anomeric carbon atoms.[12]
[d] Chemical shift differs from the value given in the literature.[12]
[e] Chemical shifts given with one decimal place are recalculated from literature;[18] moreover, they may be interchanged. TSP is used as the internal standard; however, chemical shifts are given relative to DSS.
[f] Only a few resonances of the ξ-carrageenan spectrum are assigned in literature.[11] A detailed study on the interpretation of the NMR spectra of a ξ/θ-hybrid carrageenan is currently in progress and the results will be published separately (Usov, A. I.; Shashkov, A. S.; Rollema, H. S.; Pereira, L.; Van de Velde, F. *Carbohydr. Res.*, in preparation). TSP is used as the internal standard; however, chemical shifts are given relative to DSS.

Table 3. Chemical shifts (ppm) of the α-anomeric protons of carrageenans referred to DSS as internal standard at 0 ppm[a]

Carrageenan	Monosaccharide[b]	Chemical shift (ppm)
β (beta)	DA	5.074
ι (iota)	DA2S	5.292
κ (kappa)	DA	5.093
λ (lambda)	D2S,6S	5.548
ν (nu)	D2S,6S	5.501
μ (mu)	D6S	5.238

[a] Carrageenan (30 mg mL^{-1}), DSS (10 mM) and Na_2HPO_4 (20 mM) in D_2O recorded at 65 °C.
[b] Codes refer to the nomenclature developed by Knutsen et al.[2]

signals are 27.61, 103.55 and 177.96 ppm for, respectively, the methyl, acetal and carboxyl carbon atoms. The pyruvic acid acetals are also detected in the ^1H NMR spectra by the methyl proton resonances with a chemical shift of 1.44 ppm relative to DSS. In addition to the methyl signal, characteristic signals in the anomeric proton region are observed at 5.30 and 5.49 ppm. Small amounts of 3-linked 6-*O*-methyl-D-galactose residues were found in κ-carrageenan from *Kappaphycus alvarezii*[14] and in several other polysaccharides.[8] The specific signal for OMe is found at a chemical shift relative to DSS of 61.14 ppm in the ^{13}C NMR spectrum.

Floridean starch, a branched (1→4, 1→6)-α-D-glucan structurally related to plant amylopectins and animal glycogens, is a storage polysaccharide of red algae. It is soluble in water and can accompany carrageenans in the extraction and precipitation steps. The presence of floridean starch can be confirmed by the well-known set of signals of 4-linked α-D-glucopyranose residues in the ^{13}C NMR spectrum.[5,15,16] In carrageenan ^{13}C NMR spectra, floridean starch signals are detected at chemical shifts relative to DSS at 102.55, 74.34 and 74.04 ppm for, respectively, the C-1, C-2 and C-5 carbon atoms. In the ^1H NMR spectra, floridean starch is detected by the signal of the anomeric proton of the α-(1→4)-linked D-glucopyranosyl moiety at 5.35 ppm. The resonance of the anomeric proton of the α-(1→6)-linked D-glucopyranosyl appears at a chemical shift of 0.39 ppm upfield.[16] 2-Propanol is generally applied to precipitate carrageenans from the extraction

liquid.[1] Therefore, 2-propanol can be observed in the NMR spectra of carrageenan batches. In the ^{1}H NMR spectra 2-propanol gives a characteristic triplet at 1.169 ppm. In the ^{13}C NMR spectra the methyl carbon of 2-propanol is detected at 26.38 ppm.

In conclusion, we report here a new set of chemical shifts for most common types of carrageenan repeating units relative to DSS as the internal standard as recommended by the IUPAC.[9] In addition to the chemical shifts of carrageenan repeating units, the chemical shifts of minor substituent and contaminants of carrageenan batches are reported.

2. Experimental

2.1. Carrageenan samples

Alkaline- and water-extracted carrageenan samples from the following species were used to record the NMR spectra: *Betaphycus gelatinum*, *Chondracanthus teedei*, *Eucheuma denticulatum*, *Gigartina skottsbergii*, *Kappaphycus alvarezii*, *Sarconema scinaioides*. To reduce the viscosity of the NMR samples, carrageenan samples were first sonicated according the following procedure. Carrageenan (250 mg; 5 mgmL^{-1}) was dissolved in phosphate buffer (50 mL; 20 mM Na_2HPO_4) at 80 °C for 30 min and allowed to cool to room temperature. This solution was sonicated for three times 30 min in melting ice (Heat Systems XL 2020 sonicator, 12 mm tip, power 475 W, frequency 20 kHz), the solution was centrifuged at elevated temperature (above the gelation temperature) to remove insoluble material. The sonicated solutions were dialysed against phosphate buffer (20 mM Na_2HPO_4; 3×2 L), water (1×2 L) and lyophilised.

2.2. NMR spectroscopy of carrageenans

NMR samples were prepared by dissolving the sonicated carrageenans (30 mgmL^{-1}) in D_2O containing 20 mM Na_2HPO_4 and 10 mM internal standard (DSS). ^{1}H NMR spectra were taken at 65 °C on a Bruker DRX500 spectrometer operating at 500.13 MHz. Typically 64 scans were taken with an interpulse delay of 5 s (T_1 values for the resonances of the anomeric protons of κ- and ι-carrageenan are smaller than 1.5 s). ^{13}C NMR spectra were recorded at 65 °C on a Bruker DRX500 spectrometer operating at 125.76 MHz essentially as described elsewhere.[17] Chemical shifts (δ) are relative to internal DSS standard ($\delta = 0.000$ ppm for both ^{1}H and ^{13}C according to the IUPAC recommendations[9]). Assignments of the NMR spectra were based on spectra and data summarised by Van de Velde et al.[5]

2.3. Internal standards

Phosphate·buffered D_2O solutions of different pH values were prepared by dissolving either NaH_2PO_4 (20 mM; pH 5.5), a mixture of NaH_2PO_4 and Na_2HPO_4 (20 mM phosphate; pH 7.2), Na_2HPO_4 (20 mM; pH 9.7), or a mixture of Na_2HPO_4 and NaOH (20 mM phosphate; pH 11.8) in D_2O. pH values given throughout this paper are the pH readings of our electrode. Mixtures of the different internal standards were prepared by dissolving DSS (0.15 M), TSP (0.28 M), MeOH (0.85 M), DMSO (0.50 M) and acetone (0.45 M) in the D_2O/phosphate solutions. NMR spectra were taken at 24, 45 and 65 °C on a Bruker DRX500 spectrometer.

References

1. Van de Velde, F.; De Ruiter, G. A. In *Biopolymers Vol. 6: Polysaccharides II: Polysaccharides from Eukaryotes*; Steinbüchel, A., DeBaets, S., VanDamme, E. J., Eds.; Wiley-VCH: Weinheim, 2002; Vol. 6, pp 245–274.
2. Knutsen, S. H.; Myslabodski, D. E.; Larsen, B.; Usov, A. I. *Bot. Mar.* **1994**, *37*, 163–169.
3. Usov, A. I. *Bot. Mar.* **1984**, *27*, 189–202.
4. Yarotsky, S. V.; Shashkov, A. S.; Usov, A. I. *Bioorg. Khim.* **1977**, *4*, 1135–1137.
5. Van de Velde, F.; Knutsen, S. H.; Usov, A. I.; Rollema, H. S.; Cerezo, A. S. *Trends Food Sci. Technol.* **2002**, *13*, 73–92.
6. Usov, A. I.; Yarotsky, S. V.; Shashkov, A. S. *Biopolymers* **1980**, *19*, 977–990.
7. Miller, I. J.; Blunt, J. W. *Bot. Mar.* **2000**, *43*, 263–271.
8. Chiovitti, A.; Bacic, A.; Craik, D. J.; Munro, S. L. A.; Kraft, G. T.; Liao, M.-L. *Carbohydr. Res.* **1998**, *305*, 243–252.
9. Harris, R. K.; Becker, E. D.; CabralDeMenezes, S. M.; Goodfellow, R.; Granger, P. *Pure Appl. Chem.* **2001**, *73*, 1795–1818.
10. Pereira, L.; Sousa, A.; Coelho, H.; Amado, A. M.; Ribeiro-Claro, P. J. A. *Biomol. Eng.* **2003**, *20*, 223–228.
11. Falshaw, R.; Furneaux, R. H. *Carbohydr. Res.* **1995**, *276*, 155–165.
12. Ciancia, M.; Matulewicz, M. C.; Finch, P.; Cerezo, A. S. *Carbohydr. Res.* **1993**, *238*, 241–248.
13. Chiovitti, A.; Bacic, A.; Craik, D. J.; Kraft, G. T.; Liao, M.-L.; Falshaw, R.; Furneaux, R. H. *Carbohydr. Res.* **1998**, *310*, 77–83.
14. Bellion, C.; Brigand, G.; Prome, J.-C.; Bociek, D. W. S. *Carbohdr. Res.* **1983**, *119*, 31–48.
15. Colson, P.; Jennings, H. J.; Smith, J. C. P. *J. Am. Chem. Soc.* **1974**, *96*, 8081–8087.
16. Knutsen, S. H.; Grasdalen, H. *Bot. Mar.* **1987**, *30*, 497–505.
17. Van de Velde, F.; Peppelman, H. A.; Rollema, H. S.; Tromp, R. H. *Carbohydr. Res.* **2001**, *331*, 271–283.
18. Falshaw, R.; Furneaux, R. *Carbohydr. Res.* **1994**, *252*, 171–182.