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A ^{13}C-N M R STUDY OF A DI- AND A TRI-SACCHARIDE CONTAINING THE α-D-GALACTOPYRANOSYL GROUP

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ABSTRACT

The ^{13}C-n m r spectra of methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**1**) and methyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**) in D_2O were recorded Comparison of these spectra with the spectra of methyl α-D-galactopyranoside (**4**) and methyl β-lactoside (**5**) provided substantial confirmation of the structures of **1** and **2**

INTRODUCTION

The use of ^{13}C-nuclear-magnetic resonance spectroscopy for structural elucidation in carbohydrate chemistry becomes increasingly valuable as the data base broadens In our program[1,2] to synthesize the ceramide trisaccharide related to Fabry's disease, we prepared methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside[1] (**1**) and methyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside[2] (**2**) by halide ion-catalyzed condensation of 2,3,4,6-tetra *O*-benzyl-D-galactopyranosyl bromide[3] with methyl 2,3,6-tri-*O*-benzoyl-α D-galactopyranoside[4,5] and with methyl 2,3,6,2′,3′,6′-hexa-*O*-benzoyl-β-lactoside[2], respectively In each case it was necessary to establish the structural identity of the major product Halide ion-catalyzed conditions were expected to induce *cis*-glycosylation at the free hydroxyl group[6,7] However it was possible for benzoyl migration to occur prior to condensation and for glycosylation to proceed subsequently at a position other than at the desired 4-hydroxyl group Acyl migration prior to glycosylation was observed by Hall *et al*[8] when 1,2,3,6-tetra-*O*-acetyl-α-D-glucopyranose (**3**) was condensed with hepta-*O*-acetyl-α-cellobiosyl bromide The product, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(hepta-*O*-acetyl-β-cellobiosyl)-D-glucopyranose resulted from O-6 to O-4 acetyl migration in **3**, followed by glycosylation at the newly freed 6-hydroxyl group Natural-abundance ^{13}C-n m r spectroscopy was used to prove the structures of the unknown products of our glycosylation reactions The spectra offered convincing evidence that benzoyl migration did not occur, and that glycosylation indeed proceeded in the *cis* manner, at the desired 4-hydroxyl group

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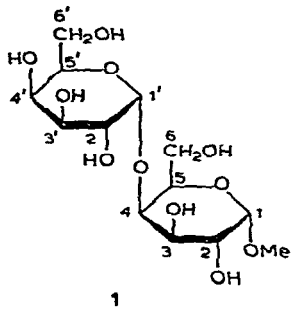

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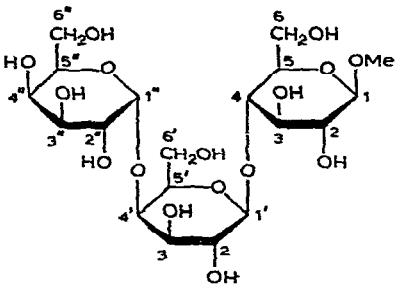

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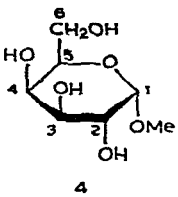

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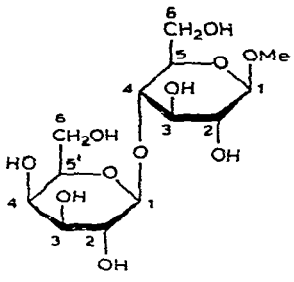

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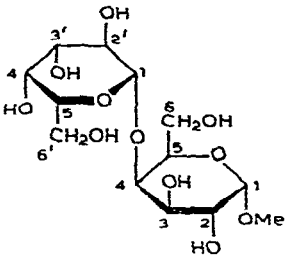

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EXPERIMENTAL

Proton-decoupled ^{13}C-n m r spectra were recorded with a Varian XL100-15 Fourier-transform spectrometer modified for multinuclear operation[9] and equipped with a Varian 620L computer for data handling The probe temperature was maintained at 35° For each carbon-13 spectrum (25 2 MHz), 15–20 mg of sample was dissolved in 0 25 ml of D_2O contained in a 5-mm tube A drop of 1,4-dioxane was added as the internal reference The spectra are reported with chemical shifts downfield from tetramethylsilane assuming the 1,4-dioxane peak to be located at δ 67 45 in each spectrum

Methyl 4-O-α-D-*galactopyranosyl*-α-D-*galactopyranoside* (**1**) was prepared as described in ref 1 *Methyl 4'*-O-α-D-*galactopyranosyl*-β-*lactoside* (**2**) was prepared as described in ref 2 *Methyl* α-D-*galactopyranoside* (**4**) was purchased as the hydrate from Pfanstiehl Laboratories, Inc . Waukegan, Illinois *Methyl* β-*lactoside* (**5**) was prepared according to Smith and Van Cleve[10], except that mercuric cyanide was used in lieu of silver carbonate

RESULTS AND DISCUSSION

The halide ion-catalyzed condensations of tetra-*O*-benzyl-D-galactopyranosyl bromide[3] with methyl 2 3,6-tri-*O*-benzoyl-α-D-galactopyranoside[4,5] and methyl 2,3,6,2′,3′,6′-hexa-*O*-benzoyl-β-lactoside[2], followed by chromatographic purification

and deprotection of the major condensation product, gave, in the first case[1], the pure disaccharide methyl glycoside **1** and, in the second cases[2], the pure trisaccharide methyl glycoside **2**

Table I lists the ^{13}C-n m r chemical shifts of methyl α-D-galactopyranoside (**4**), methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**1**), methyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**), and methyl β-lactoside (**5**) The spectral lines have been assigned, although few assignments are absolute An exception is the entry for methyl α-D-galactopyranoside (**4**), the complete spectrum of which was unambiguously assigned with the aid of deuterium labelling by Gorin and Mazurek[11] Walker and coworkers[12] have recently demonstrated the advantages of isotopic labelling for assignment of the ^{13}C-n m r spectra of even the simplest sugars As our spectra were of unlabelled compounds, the assignments were based largely on a comparison of the four sets of spectral data listed in Table I This assignment method revealed large differences and strong similarities between the spectra of related compounds Comparison of the spectrum of a compound of unproven structure with the spectra of known compounds provided substantial proof of the structure of the unknown*

The proton-decoupled, ^{13}C-n m r spectrum of the unknown disaccharide methyl glycoside (Fig 1, **1** in Table I) showed eleven of the thirteen lines theoretically possible Two of these peaks (δ 61 48 and 71 89) were of nearly double intensity and thus constituted coincident resonances The two anomeric carbon resonances (δ 100 35 and 101 35) were obvious, as were the two C 6 atoms (δ 61 48) and the glycosidic methyl group signal (δ 56 07) The presence of the two overlapping peaks at δ 61 48 ruled out *O*-galactosylation at C-6, as the chemical shift was characteristic of a C-6 atom bearing a free hydroxyl group[13] Instead only the line at δ 79 78 (C-2, C-3, or C-4) reflected the strong downfield shift expected from *O*-galactosylation One of the two coincident resonances at δ 71 89 was unambiguously assigned to C-5′ by comparison with C-5 in **4** The six resonances (δ 69 32, 69 50, 69 91, 70 03, 70 11, and 71 89) remaining unassigned spanned a range of only 2 6 p p m As the chemical shifts of C-4 and C-5 in methyl β-D-galactopyranoside differ by more than 6 p p m [11], our disaccharide thus contained of two 1 2-*cis*(α)-D-galactopyranosyl groups

The condensation had not resulted in *O*-glycosylation at C-3 Substitution at O-3 would have induced a 3–4-p p m upfield shift in the C-4 resonance, relative to that of C-4 in **4** Lemieux and Driguez[14] reported such shifts for C-4 in 3-*O*-α-D-galactopyranosyl-α-D-galactopyranose (3 7 p p m upfield) and in 3-*O*-α-D-galactopyranosyl-β-D-galactopyranose (3 9 p p m upfield), relative to C-4 in α-D-galactopyranose and in β-D-galactopyranose, respectively That methylation of a hexo-

*The spectral lines of α-D-galactose derivatives were assigned by Voelter *et al* [13] and by Lemieux and Driguez[14] by comparison with incorrect assignments of the spectra of α-D-galactose and of **4** Our discussion refers to shift assignments from refs 13 and 14 that we have corrected by comparison with the absolute assignments of the spectral lines of α-D-galactose and of **4**, published by Gorin and Mazurek[11]

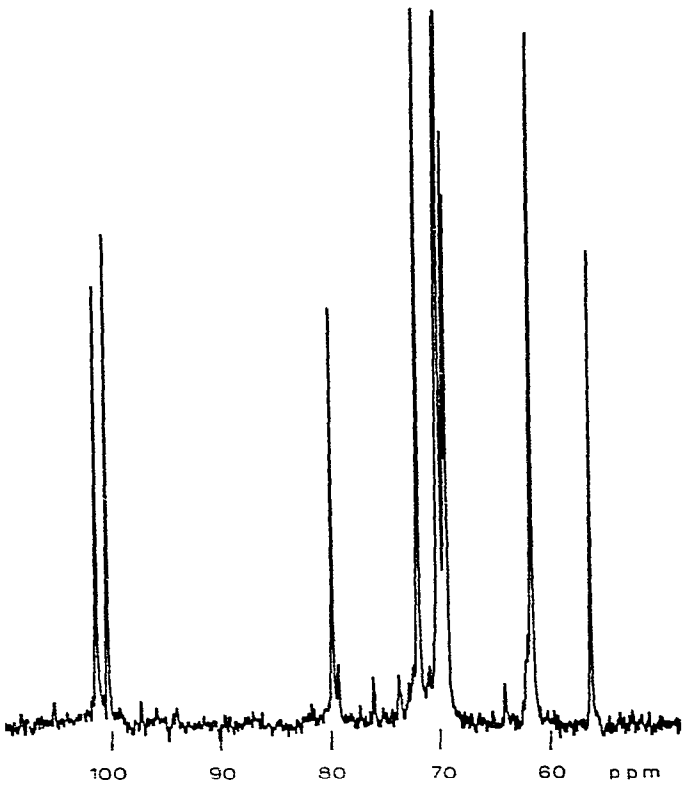

Fig 1 The ^{13}C-n m r spectrum of **1** in D_2O

pyranose hydroxyl group causes a significant (~4 5 p p m) upfield shift on β-carbon atoms bearing axial hydroxyl groups has been stated as a general tule by Voelter *et al* [13], based on their work on the methylation of galactopyranose and on the studies of methylated inositols reported by Dorman *et al* [15] This upfield shift has also been reported[16 17] for the C-2 resonances of mannose derivatives *O*-substituted at C-3 As none of the six unassigned lines in the disaccharide spectrum revealed an upfield shift of more than 0 8 p p m from the C-4 line of **4** (δ 70 14), the possibility of *O*-galactosylation at C-3 was eliminated

It was expected that *O*-substitution at C-2 would have caused an upfield shift at the C-1 resonance Voelter *et al* [13] reported an upfield shift of 2 7 p p m for C-1 of methyl 2,6-di-*O*-methyl-α-D-galactopyranoside, relative to the shift of C-1 in **4** Lemieux *et al* [18] found that the C-1 resonances of 8-(methoxycarbonyl)octyl 2-*O*-α-D-glucopyranosyl-β-D-galactopyranoside and 8-(methoxycarbonyl)octyl 2-*O*-β-D-glucopyranosyl-β-D-galactopyranoside were shifted upfield 0 5 p p m and 1 8 p p m, respectively, relative to the C-1 line of 8-(methoxycarbonyl)octyl β-D-galactopyranoside A small upfield shift for C-1 was also reported by Lemieux and Driguez in the case of a 2-*O*-substituted α-D-galactopyranose[14] the C-1 resonance of 2 *O*-α-

L-fucopyranosyl-α-D-galactopyranose was 0 4 p p m upfield relative to the C-1 line of α-D-galactopyranose Usui *et al* [19] reported an upfield shift of 2 5 p p m for C-1 of α-kojibiose and for C-1 of 2-*O*-methyl-γ-D-glucopyranose, relative to C-1 in α-D-glucopyranose In the case of our disaccharide methyl glycoside, the chemical shift of one anomeric carbon atom (δ 100 35) was identical w th that of C-1 in **4** (δ 100 32), the other anomeric peak (δ 101 35) was shifted *downfield* relative to C-1 in **4** The former resonance was assigned to C-1 of our disaccharide and the latter to C-1′ Thus, *O*-substitution at C-2 was ruled out, and the unknown disaccharide methyl glycoside was identified as methyl 4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside (**1**)

We subsequently prepared by a separate procedure[1] a mixture containing both methyl 4-*O*-β-D-galactopyranosyl-α-D-galactopyranoside (**6**) and **1** We were able to verify the C-1 and C-1′ assignments for **1**, as the β-anomeric C-1′ resonance of **6** appeared downfield at δ 105 30 but the C-1 resonance of **6** coincided with the C-1 resonance of **1** at δ 100 35 The structure of **1** was also established by a chemical structure proof[1] However, as just described, the structure of **1** could be deduced from the ^{13}C-n m r data alone, even though six of the spectral assignments were ambiguous (C-2, C-3, C-5, C-2 , C-3′, and C-4)

The ^{13}C-n m r spectrum of methyl β-lactoside (**5**) has been published by Dorman and Roberts[20] and by Breitmaier *et al* [21] The chemical shifts determined by us, listed in Table I, agreed closely with those from the spectra reported Without the aid of isotopic labelling the complete spectrum of **5** could not be assigned with certainty by the earlier authors[20 21] or by us* In fact, only C-2′ (δ 71 85) C-4′ (δ 69 44), C-4 (δ 79 42), and the 1-OCH_3 (δ 58 03) lines were assigned unambiguously-Although ambiguous, the remaining tentative assignments were useful for the com parison of the spectrum of **5** with the spectrum of **2** in Table I

The problems of identification of our unknown trisaccharide methyl glycoside were similar to those found for the disaccharide derivative **1** As we considered the synthesis of **1** to be a reliable synthetic model for the trisaccharide synthesis[1 2], we confidently expected the trisaccharide to be methyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**) Comparison of the ^{13}C-n m r spectrum of the trisaccharide methyl glycoside with the spectra of **1** and **5** allowed the choice of **2** to the exclusion of the other possible, isomeric structures

At the two extremes of the trisaccharide spectrum (Fig 2 2 in Table I) the resonances for the three anomeric carbon atoms (δ 101 25, 103 94, and 104 17), for the three hydroxyl-bearing C-6 atoms (δ 61 47, 61 23, and 61 00), and for the 1-OCH_3 carbon atom (δ 58 03) were obvious The presence of six lines (δ 101 25, 71 86, 70 05 69 90, 69 49, and 61 47) that were nearly identical in shift to the six lines assigned to C-1′ through C-6′ of **1** virtually assured that the trisaccharide contained a terminal

*We have assigned the chemical shifts of **5** by comparison with the absolute assignments of the spectral lines of methyl β-D-galactopyranoside and of methyl β-D-glucopyranoside published by Gorin and Mazurek[11] Both the C-3 and the C-5 lines of **5** are definitely upfield of the C-3 and C-5 lines of methyl β-D-glucopyranoside which coincide[11] at 76 9

TABLE I

^{13}C-N M R CHEMICAL SHIFTS (P P M) IN D_2O

Compd [b]	4		1		2		5
C-1	100 32	C-1	100 35				
C-2	69 12	C-2	69 32[c]				
C-3	70 40	C-3[d]	71 89				
C-4	70 14	C-4	79 78				
C-5	71 60	C-5[d]	70 11[e]				
C-6	62 11	C-6	61 48				
		C-1′	101 35	C-1″	101 25		
		C-2′	69 50[c]	C-2″	69 49		
		C-3	70 03[e]	C-3″	70 05[f]		
		C-4	69 91[e]	C-4″	69 90[f]		
		C-5	71 89	C-5″	71 86[g]		
		C-6	61 48	C-6″	61 47[h]		
				C-1′	103 94[i]	C-1	103 87[m]
				C-2	71 77[g]	C-2	71 85
				C-3 [k]	76 29[j]	C-3′	73 69[n]
				C-4′	78 31	C-4	69 44
				C-5 [k]	73 76[i]	C-5′	76 23[o]
				C-6	61 23[h]	C-6	61 88[p]
				C-1	104 17[i]	C-1	103 99[m]
				C-2	73 14[i]	C-2	73 48[n]
				C-3	75 38[j]	C-3	75 33[o]
				C-4	79 71	C-4	79 42
				C-5	75 68[j]	C-5	75 65[o]
				C-6	61 00[h]	C-6	61 00[p]
1-OCH_3	55 93	1-OCH_3	56 07	1-OCH_3	58 03	1-OCH_3	58 03

[a]Downfield from tetramethylsilane 1,4-Dioxane (δ 67 45) was used as the internal standard in the calculation of chemical shifts [b]4 Methyl α-D-galactopyranoside, 1 Methyl4-*O*-α-D-galactopyranosyl-α-D-galactopyranoside, 2 methyl 4 -*O*-α-D-galactopyranosyl-β-lactoside and 5 Methyl β-lactoside [c–p]Groups of several peaks the assignments for which may be interchangeable

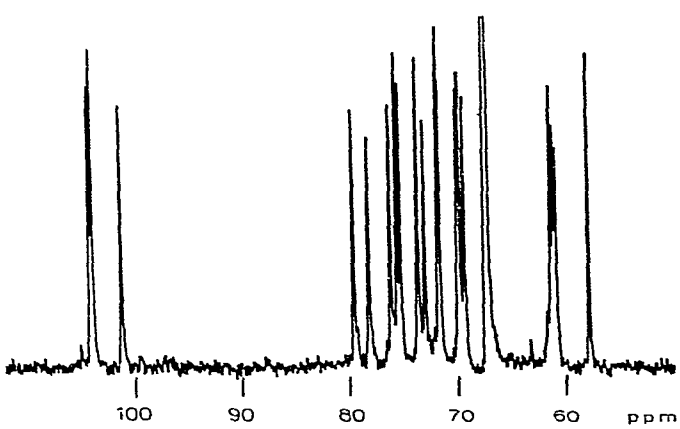

Fig 2 The ^{13}C-n m r spectrum of 2 in D_2O (off-scale peak at δ 67 45 is for 1,4-dioxane)

1,2-*cis*(α)-D-galactopyranosyl group These six lines were assigned to C-1″ through C-6″ The remaining thirteen lines in the spectrum were paired with resonances of **5** Significantly, each of twelve of the nineteen lines in the trisaccharide spectrum matched its pair in the spectra of **1** or **5** within 0 1 p p m

The C-4′ line of **5** at δ 69 44 was paired with the line at δ 78 31, which reflected the strong, downfield shift characteristic of *O*-substitution The possibility of *O*-substitution at C-6′ or C-6 was eliminated by the presence of three peaks between δ 61 0 and 61 5, which arose from three primary hydroxyl carbons atoms[13] As was argued in the structure proof of **1**, *O*-galactosylation at C-3′ would have been expected to effect a significant upfield shift of C-4′, relative to C-4′ of **5** (refs, 13–17) No line between δ 61 5 and 69 4 appeared in the spectrum of the trisaccharide and so *O*-galactosylation at C-3′ was ruled out

The C-1 and C-1′ lines in the trisaccharide spectrum were shifted only slightly, relative to the C-1 and C-1′ peaks of **5** The maximum possible upfield shift for each was 0 05 p p m (δ 103 99 to 103 94) and the maximum downfield shift for each was 0 30 p p m (δ 103 87 to 104 17) However, the possibility of *O*-substitution at C-2 or at C-2′ was not eliminated by these data For example, Usui *et al* [19] reported that the C-1 peak of methyl 2-*O*-α-D-glucopyranosyl-β-D-glucopyranoside (methyl β-koji-side) was shifted 0 5 p p m downfield from the C-1 peak of methyl β-D-gluco-pyranoside The effect of 2- or 2′-*O*-substitution on C-1 or C-1′ might have been equally small The possibility of 3-*O*-galactosylation was not immediately excluded, as C-4 might have been shifted upfield (δ 79 42 to 78 31) and C-3 shifted downfield (δ 75 33 to 79 71) relative to C-4 and C-3 in **5** However, none of the possible tri-saccharide isomers other than the one *O*-galactosylated at C-4′ would have been expected to reveal only three rather than four lines between δ 69 4 and 70 1 (corresponding to C-2 , C-3′, and C-4′ of **1** and C-4′ of **5**) Regardless of the point of *O*-substitution any significant downfield shift (at least 1 75 p p m) of the C-2″, C-3″, and C-4 resonances would have been unexpected Likewise, such a downfield shift at C-4′ would not have been predicted unless substitution had occurred at O 4′ Thus the identity of our trisaccharide glycoside was established as methyl 4 *O*-[4-*O*-(γ-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (**2**)

The effect of glycosylation at O-4 of a galactopyranoside on the C-3 and C-5 peaks cannot be stated with certainty The spectrum of **1** has been assigned as if the C-3 line were shifted 1 5 p p m downfield and the C-5 peak 1 5 p p m upfield relative to the C-3 and C-5 peaks of **4** If the assignments were reversed, the C-3 and C-5 lines of **1** would be a mere 0 3 p p m removed from their corresponding lines in **4** C-3 would be shifted slightly upfield and C-5 slightly downfield Similarly, the chemical shifts of **2** have been assigned as if *O*-galactosylation at O-4′ of **5** had caused a downfield shift of C-3′ (2 6 p p m) and an upfield shift of C-5′ (2 4 p p m) Again an alternative assignment (C-3′, C-5′ reversed) would leave the C-3′ and C-5′ lines virtually unaffected by the conversion of **5** into **2**

Methylation[19], glucosylation[19], or galactosylation at O-4 of either α- or β-D-glucose causes a significant upfield shift (~1 2 p p m) of the C-5 line Inter-

estingly, the C-3 line is shifted upfield upon *O*-methylation, *O*-β-glucosylation, or *O*-β-galactosylation at C-4, but it is shifted downfield upon *O*-α-glucosylation at C-4 of α- or β-D-glucose[19] The studies of Voelter *et al*[13] on *O*-methylated methyl galactopyranosides suggest indirectly that methylation at O-4 causes the C-3 line to be shifted downfield and the C-5 line to be shifted upfield The C-3 peak is shifted upfield, and the C-5 peak is shifted downfield in methyl 2,6-di-*O*-methyl-α-D-galactopyranoside, relative to the C-3 and C-5 lines of **4** In contrast, the C-3 line is shifted downfield, and the C-5 line is shifted upfield in methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside, relative to the C-3 and C-5 peaks of methyl 3-*O*-methyl-β-D-galactopyranoside[13] Thus it is not unlikely that *O*-α-galactosylation at C-4 of **1** and C-4′ of **2** caused upfield shifts of the C-5 line of **1** and the C-5′ line of **2**, and downfield shifts of the C-3 peak of **1** and the C-3′ peak of **2**

^{13}C-N m r spectroscopy has thus proved extremely useful in determination of the structures of the synthetic disaccharide **1** and of the synthetic trisaccharide **2** The spectra obtained for these pure methyl glycosides were simpler than those expected for the corresponding reducing sugars as only one anomer of each was present in solution Absolute assignment of the spectra was impossible, but the comparison of spectra of structurally related compounds yielded legitimate structure-proofs for **1** and **2** ^{13}C-N m r spectroscopic analysis is simple, quick, and nondestructive, yet it provides a wealth of structural information

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