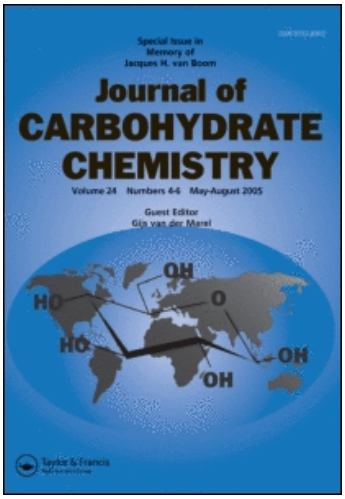


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of the 1-Amino-Alditols Derived from Cellobiose, Lactose and Maltose. A Comprehensive NMR Study of Some Alditols and Amino-Alditols

Ida Christiansen-Brams[a]; Morten Meldal[a]; Klaus Bock[a]
[a] Department of Chemistry, Carlsberg Laboratory, Gl. Carlsberg Vej, Valby, Denmark

To cite this Article Christiansen-Brams, Ida , Meldal, Morten and Bock, Klaus(1992) 'Synthesis of the 1-Amino-Alditols Derived from Cellobiose, Lactose and Maltose. A Comprehensive NMR Study of Some Alditols and Amino-Alditols', Journal of Carbohydrate Chemistry, 11: 7, 813 — 835
To link to this Article: DOI: 10.1080/07328309208018272
URL: http://dx.doi.org/10.1080/07328309208018272

J. CARBOHYDRATE CHEMISTRY, 11(7), 813-835 (1992)

SYNTHESIS OF THE 1-AMINO-ALDITOLS DERIVED FROM CELLOBIOSE, LACTOSE AND MALTOSE.

A comprehensive NMR study of some alditols and amino-alditols

Ida Christiansen-Brams, Morten Meldal and Klaus Bock*

Department of Chemistry, Carlsberg Laboratory, Gl. Carlsberg Vej 10, DK-2500 Valby, Denmark

Received January 10, 1992 - Final Form June 22, 1992

ABSTRACT

Gram quantities of the 1-amino-alditols derived from cellobiose, lactose and maltose were synthesized in 80-90% yield via a reductive amination with benzylamine and subsequent catalytic removal of the benzyl group at atmospheric hydrogen pressure. The 1-amino-alditols together with the products from direct reduction of cellobiose, lactose and six maltooligosaccharides (d.p. 2-7) were fully characterized by NMR. Assignment and analysis was done from a combination of 1D ^{1}H and ^{13}C spectra and 2D ^{1}H-^{1}H COSY and ^{13}C-^{1}H correlation experiments. ^{13}C chemical shift pH titration curves for the amino-alditols are presented.

INTRODUCTION

The reductive amination of reducing disaccharides yields compounds with a free amino group and with known stereochemical configuration, suitable for the study of different areas in which carbohydrates are involved as ligands for receptors, e.g., glycopeptides, glycoproteins or glycolipids. Thus the open chain part of the disaccharide amino-alditol may serve as a more flexible and yet restricted spacer moiety in the construction of artificial glycoconjugates, e.g., artificial ligands for multivalent lectins binding complex carbohydrates in biological membranes and cellular compartments.[1] Alternatively, they may be coupled to an insoluble matrix for affinity chromatography.[2]

A number of methods for the synthesis of 1-amino-alditols via reductive amination has previously been reported. Wayne and Adkins[3] prepared glucamine (1-amino-1-deoxy-**D**-glucitol) in low yield (26%) by treating **D**-glucose with methanolic ammonia followed by hydrogenation at 150 atm hydrogen pressure with Raney nickel as a catalyst. The low yield could be due to the formation of diglucosylamines as described by Isbell and Frush.[4] Holly et al.[5] used a similar approach with liquid ammonia to obtain app. 80% pure glycamines in 65% yield. Recently an industrial process in which glucose was treated with liquid ammonia and hydrogenated over a Ni-catalyzed fixed bed yielding 80% of crystalline glucamine has been reported.[6] Kagan et al.[7] introduced the reductive alkylation of benzylamine with galactose to give galactamine in 50% yield when hydrogenolyzed in the presence of palladium on charcoal. Lemieux[8] described the synthesis of 1-amino-alditols derived from the easily available monosaccharides by catalytic hydrogenation in the presence of hydrazine. This procedure has been used on a preparative scale (100 g) with yields exceeding 90%. Alditol amines derived from the disaccharides isomaltose, isomaltulose or *O*-α-**D**-glucopyranosyl-(1-1)-**D**-fructose by treatment with hydrazine or methanolic ammonia followed by suitable hydrogenation have been prepared by Klein et al.[9] in good to reasonable yields. Wiegandt and Ziegler[10] have prepared 1-amino-alditols from oligosaccharides in mg amounts. Thus 1-amino-1-deoxy-4-*O*-β-**D**-galactopyranosyl-**D**-glucitol (1-amino-1-deoxylactitol) was obtained in 55% yield by treatment of lactose with sodium cyanoborohydride and ammonium acetate in water. 1-Amino-1-deoxylactitol has also been prepared by Kallin et al.,[11] who synthesized the 1-deoxy-1-(4-trifluoroacetamidophenyl)aminolactitol (TFAN-derivative) in 68% yield by reacting lactose with 4-trifluoroacetamidoaniline and sodium cyanoborohydride at pH 6 followed by gel filtration for purification. This TFAN-aminolactitol was then transformed into the 1-amino-1-deoxylactitol by oxidation with cerium ammonium nitrate in 94% yield on a mg scale. We now describe a general approach to gram scale synthesis of the 1-amino-alditols derived from cellobiose, lactose and maltose using reductive amination with benzylamine and sodium borohydride followed by catalytic removal of the benzyl group at atmospheric hydrogen pressure.

In the assignment of ^{1}H and ^{13}C NMR resonances to molecules possessing basic sites, e.g., $-NH_2$, the method of monitoring the chemical shifts as a function of pH has proven useful in previous studies. Thus amines,[12,13] amino acids[14] and peptides,[15,16] all of which possess amino functionalities, have been studied through assignments based on characteristic effects on ^{1}H and ^{13}C shifts of the amine center, comparisons with related compounds and proton decoupling and INDOR experiments. In this work we present the first study of the effects of amino group protonation in the sugar alditol series. Complete assignments of the three

synthesized 1-amino-alditols were easily obtained by a combination of 1D ^{1}H and ^{13}C spectra and 2D ^{1}H-^{1}H COSY and ^{13}C-^{1}H correlation experiments.[17]

In contrast to the vast amount of conformational analysis done on pyranoid sugars and on acyclic sugar chains[18] there have appeared only few reports on systems, in which both of these structural features are present. This is probably due to the very complex NMR spectra that are obtained from glycosyl-alditols. Hawkes and Lewis[19] have interpreted the NMR spectra of some tetritols, pentitols and hexitols in deuterium oxide by computer simulation and used the resulting shifts and coupling constants for conformational analysis. Their results show that the free alditols prefer a planar carbon chain except when this results in a 1,3 parallel arrangement of two C-O bonds. This is consistent with the hypothesis established by Jeffrey and Kim[20] who relates conformation of the alditols to configuration in the solid state. Hoffman et al.[21,22] have assigned the ^{1}H and ^{13}C NMR spectra of glucitol and maltitol by a combination of 2D NMR experiments and extensive spin simulation of the 1D spectra and compared these results with crystallography data and low-energy conformations computed using the MM2CARB force field. The comparison showed good agreement between the crystallographic analysis and the low-energy computed conformations but little coherence with NMR measurements in solution. Their NMR measurements showed in additon that maltitol, having its bulky glucose substituent at the 4-position, is unexpectedly less restricted than glucitol as far as conformational mobility of the backbone around the C-4 centre is concerned. Shimamura et al.[23] have recently reported the assignment of ^{13}C signals for five reduced isomaltooligosaccharides (d.p. 2-6) by a combination of 1D ^{13}C NMR and 2D-INADEQUATE experiments. For use in a comparative NMR study we have synthesized the 1-4 linked alditols derived from cellobiose, lactose, maltose, maltotriose, maltotetrose, maltopentose, maltohexose and maltoheptose by direct reduction of the sugars with sodium borohydride.

RESULTS AND DISCUSSION

The first step in the synthesis of amino-alditols was a treatment of a concentrated solution of the sugar in water with benzylamine to form the imine intermediate which was then reduced with sodium borohydride to yield the 1-benzylamino-1-deoxy derivatives **1**, **3** and **5**. The ^{13}C and ^{1}H NMR data for the three compounds are presented in Tables 2, 3 and 4. Kagan et al.[7] used only brief heating to 60 °C when introducing the benzylamino group on galactose but with larger oligosaccharides prolonged heating was necessary. In the case of disaccharides the temperature was kept at 60 °C for 3 h to get complete conversion to the imine adduct.

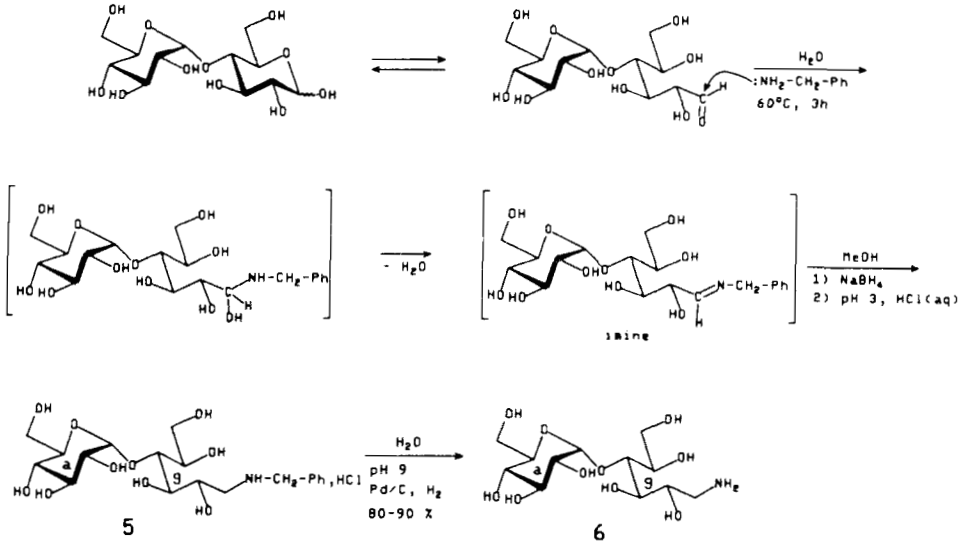
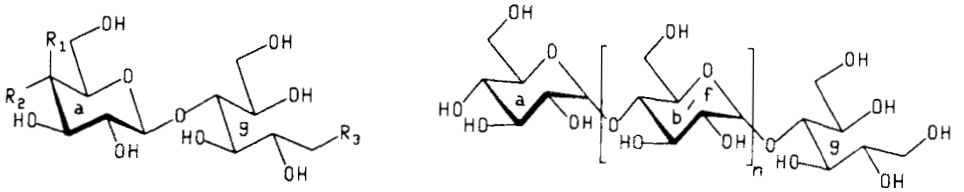


Fig. 1. Synthesis of 1-amino-1-deoxy-4-*O*-α-**D**-glucopyranosyl-**D**-glucitol, **6**.



1 : R_1 = H, R_2 = OH, R_3 = NH-CH_2-Ph,HCl
2 : R_1 = H, R_2 = OH, R_3 = NH_2
7 : R_1 = H, R_2 = OH, R_3 = OH
3 : R_1 = OH, R_2 = H, R_3 = NH-CH_2-Ph,HCl
4 : R_1 = OH, R_2 = H, R_3 = NH_2
8 : R_1 = OH, R_2 = H, R_3 = OH

9 : n = 0
10 : n = 1
11 : n = 2
12 : n = 3
13 : n = 4
14 : n = 5

Fig. 2. Structures synthesized and subjected to ^1H and ^{13}C NMR spectroscopy.

The reduction with sodium borohydride proceeded without problems and we detected no formation of the hydroxy-alditols formed via hydrolysis of the imine. To cleave the boron complexes formed during the reduction and to remove boric acid as its trimethylester (bp 67-69 °C) we acidified to pH 3 followed by co-evaporation with methanol. The second step was a catalytic removal of the benzyl group to give the free amines **2**, **4** and **6**. This reaction was

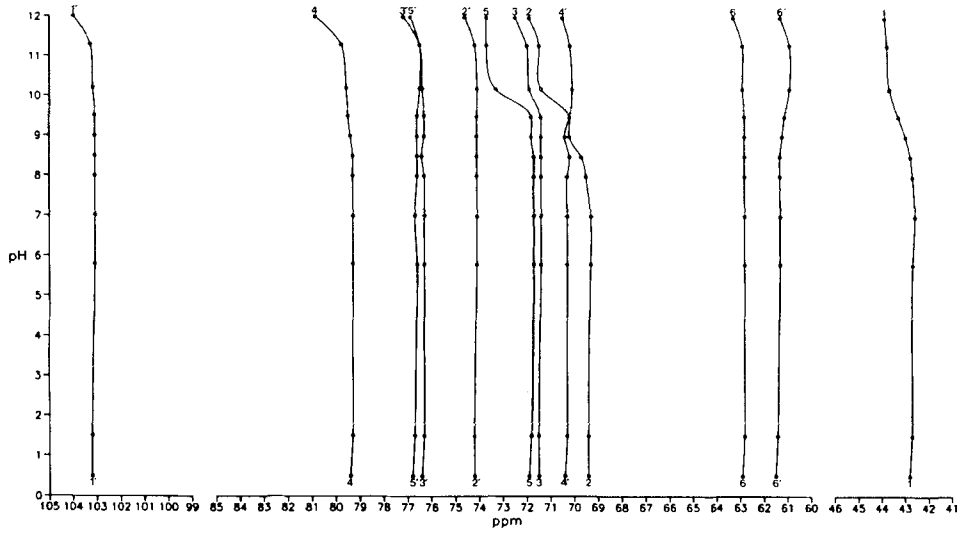


Fig. 3. ^{13}C chemical shifts of 1-amino-1-deoxy-4-*O*-β-**D**-glucopyranosyl-**D**-glucitol, **2** at various pH values.

carried out at pH 9 and room temperature with atmospheric hydrogen pressure and Pd/C as catalyst. In contrast to previous results[7] we detected no formation of the bis-amino-alditols upon removal of the benzyl group, as confirmed by FAB-MS. In Tables 5, 6 and 7 ^{13}C and ^{1}H NMR data for the hydrogenation products at three different pH values are presented.

All the alditols synthesized here have been fully characterized by NMR, including the alditols derived from maltotriose, maltotetrose, maltopentose, maltohexose and maltoheptose, compounds **10** - **14**. Spectra of the 1-amino-alditols **2**, **4** and **6** were recorded at different pH (pD) values to illustrate the effect of amino group protonation, and plots of the ^{13}C chemical shift values as a function of pH have been constructed, see Figures 3, 4 and 5.

The obtained curves resemble titration curves and implementing the Henderson-Hasselbach equation[24] an approximate pK value of 9.4 for the amino group can be reached from the δ values near the point of inflection. At high pH values (> 12) a beginning deprotonation of hydroxyl groups leading to downfield shifts is observed.

The protonation shifts (Δ_C = δ protonated form - δ free base) of ^{13}C nuclei for the three amino-alditols (**2**, **4** and **6**) investigated here indicate that protonation of the amino group leads to general upfield shifts of ^{13}C resonances. This is in agreement with results described by other groups.[12,13] Especially the C-2 position[25] to the protonated group is affected, mainly due to electric field effects[26] and chemical shift differences of $\Delta_{C\text{-}2}$ = -2.1 – -4.8 ppm (upfield)

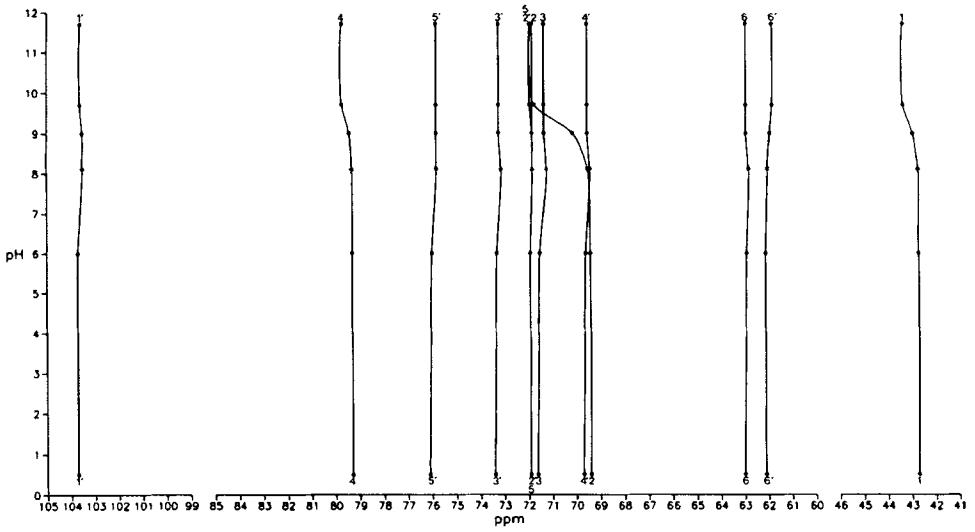


Fig. 4. ^{13}C chemical shifts of 1-amino-1-deoxy-4-*O*-β-**D**-galactopyranosyl-**D**-glucitol, **4** at various pH values.

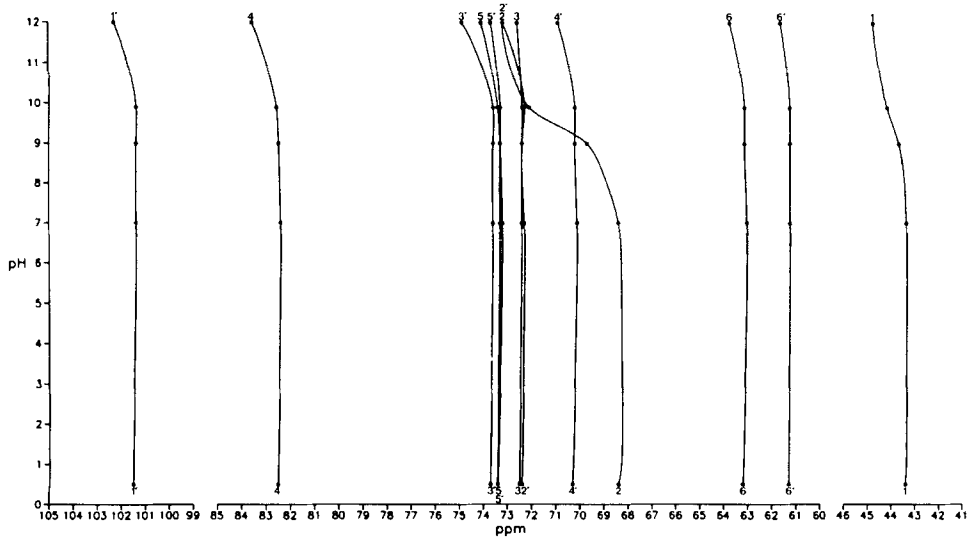


Fig. 5. ^{13}C chemical shifts of 1-amino-1-deoxy-4-*O*-α-**D**-glucopyranosyl-**D**-glucitol, **6** at various pH values.

TABLE 1. Coupling constants (in Hz) for the glucitol moiety in some reduced sugars.

	glucitol[a]	maltitol[a]	7	8	9	10	11
J / linkage		α	β	β	α	α	α
$J_{1A,2}$	4.3	4.9	3.6		4.7	4.7	
$J_{1B,2}$	6.6	6.6	6.9	7.0	6.7	6.2	
$J_{2,3}$	5.4	3.3	5.9	5.6			
$J_{3,4}$	2.5	4.8	2.3	2.4			
$J_{4,5}$	7.7	4.0	7.0		3.7		
$J_{5,6A}$	3.3	4.0	3.3	3.0	4.0	3.8	4.2
$J_{5,6B}$	6.2	7.1	6.2		7.3	7.3	7.0

a. Data taken from ref. 22.

are observed. The C-1 position is affected to a lesser extent with Δ_{C-1} = -0.6 - -1.3 ppm (upfield). Protonation shifts of C-3 carbons and carbon atoms further away are generally small (< 1 ppm) with one exception. C-5 of **2** is shifted -1.9 ppm upfield upon protonation possibly due to changes in the relative population of existing conformations. This is also reflected in the coupling constants of **2** with $J_{4,5}$ and $J_{5,6A}$ changing from 4.9 to 7.1 Hz and 5.5 to 2.1 Hz, respectively, going from basic to acidic pH. In Figures 3, 4 and 5 the curves corresponding to the different carbon atoms are numbered 1, 2, 3 etc., with C-1 being the amine carbon/anomeric carbon. Carbon atoms in the pyranosyl unit are marked with a prime.

Going from the free base to the protonated form ^{1}H chemical shifts of the amino-alditols exhibit the opposite effect from the shifts of the carbon atoms. Protonation of the amino group leads to downfield shifts of ^{1}H resonances in agreement with results described by others.[13,26] The downfield protonation shifts decrease as the distance from the site of protonation is increased, with Δ_H values in the range of $\Delta_{H-1} \approx 0.5$ ppm, $\Delta_{H-2} \approx 0.3$ ppm and $\Delta_{H-3} \approx 0.05$ ppm.

Assignment and analysis of the NMR spectra of the alditols **7** - **14** was done using a combination of 1D and 2D NMR experiments. The spectra became more and more complex as the size of the oligosaccharide increased as was expected. The results for maltitol, **9**, are identical within 0.2 ppm (^{13}C chemical shift) and 0.03 ppm (^{1}H chemical shift) with those published by Hoffman et al.[21,22] and as can be seen in Table 1 the coupling constants for the glucitol moiety are in good agreement, indicating an increased conformational mobility

around the C-4 centre on going from the free glucitol to the α1-4 linked glucopyranosyl unit in maltitol.

Comparing the coupling constants in the glucitol moiety of the reduced maltooligosaccharides, **10** - **14**, with maltitol, **9**, no significant effect can be seen originating from the increasingly larger glucopyranosyl moiety. The coupling constants of the glucitol moiety in cellobitol **7** and lactitol **8** are found to be almost identical and when compared to the free glucitol there is a marked agreement. This suggests a fundamental difference in the orientation of the pyranoid ring relative to the alditol chain in α and β linked sugar alditols. This has also been predicted by Lichtenthaler and Lindner[28] on the basis of crystal structure analysis, *Jeffrey's* alditol rule[20] and the exo-anomeric effect principles.[29]

In this work we have presented an easy way to obtain gram amounts of the 1-amino-alditols derived from the three readily available disaccharides and the method is most likely applicable to larger oligosaccharides. We have characterized the synthesized compounds fully by NMR providing a comprehensive set of data which can be useful in the studies of reduced sugars.

EXPERIMENTAL

General. Benzylamine was distilled prior to use. Sodium borohydride was purchased from FLUKA Chemie AG (Switzerland), trifluoroacetic acid (TFA) from MERCK (Germany), acetonitrile and methanol from LAB-SCAN (Ireland) and Pd/C (10%) from FERAK (Germany). Concentrations were conducted *in vacuo* at t < 40 °C. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 500 spectrometer. The ^{1}H and ^{13}C resonances were assigned by ^{1}H, ^{13}C, ^{1}H-^{1}H COSY and ^{13}C-^{1}H correlation experiments. The 2D NMR spectroscopy was performed as described earlier.[17] pH (pD) of NMR samples was adjusted with NaOD or D_2SO_4 and pH (pD) values were measured at room temperature using a PHM63 Digital pH Meter (Radiometer) equipped with an INGOLD electrode. HPLC was performed on a Waters system with a 600 controller, a 410 differential refractometer or a 991 photodiode array detector, both equipped with preparative flow cells, and a model 600 pump with modified 80 mL/min pump heads. The system was fitted with switchable analytical RCM (8×10) and Deltapak (19×300) columns and a preparative radial pack module for columns (50×300 mm) packed with reversed phase C_{18}. Elution with a buffer A (0.1% aqueous trifluoroacetic acid) and a buffer B (0.1% aqueous trifluoroacetic acid, 90% acetonitrile) was employed for both analytical (1 mL/min) and preparative (20 mL/min) separations. A gradient of 100% buffer A, 0% buffer B to 80% buffer A, 20% buffer B in 60 min. was used for purification and the wavelength for UV detection was 265 nm. Optical rotations were measured on a Perkin Elmer 141 Polarimeter.

TABLE 2

^{13}C NMR chemical shift data for 1-benzylamino-alditols **1**, **3** and **5**[a]

Compound[b]	C-1	C-2	C-3	C-4	C-5	C-6	CH_2(benzyl)
1							
Unit-a	103.0	74.1	76.3	70.3	76.7	61.4	-
Unit-g	49.6	68.6	71.3	79.2	71.8	62.8	51.9
3							
Unit-a	103.7	71.8	73.3	71.8	76.1	62.0	-
Unit-g	49.8	68.5	71.3	79.5	69.5	62.8	52.0
5							
Unit-a	101.4	72.3	73.6	70.2	72.3	61.3	-
Unit-g	50.3	67.7	73.4	82.4	73.3	63.1	51.9

a. Measured at 125.77 MHz on solutions in D_2O (pH 1) at 27 °C (reference: internal 1,4-dioxane, 67.4 ppm).
b. Unit identifications are defined in Figures 1 and 2.

TABLE 3

^{1}H NMR chemical shift data for 1-benzylamino-alditols **1**, **3** and **5**[a]

Compound[b]	H-1A	H-1B	H-2	H-3	H-4	H-5	H-6A	H-6B	CH_2A	CH_2B
1										
Unit-a	4.39	-	3.12	3.32	3.17	3.25	3.66	3.51	-	-
Unit-g	3.17	3.02	4.02	3.65	3.70	3.76	3.67	3.57	4.17	4.10
3										
Unit-a	4.30	-	3.33	3.45	3.71	3.49	3.55	3.53	-	-
Unit-g	3.16	2.96	4.02	3.58	3.66	3.71	3.66	3.54	4.12	4.08
5										
Unit-a	4.87	-	3.37	3.51	3.22	3.62	3.62	3.56	-	-
Unit-g	3.04	3.04	3.93	3.61	3.63	3.74	3.55	3.64	4.10	4.10

a. Measured at 500 MHz on solutions in D_2O (pH 1) at 27 °C (reference: internal 1,4-dioxane, 3.53 ppm).
b. Unit identifications are defined in Figures 1 and 2.

TABLE 4

$J_{H,H}$ values (Hz) for 1-benzylamino-alditols **1**, **3** and **5**[a]

Compound[b]	$J_{1A,1B}$	$J_{1A,2}$	$J_{1B,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$	$J_{HA,HB}$ (benzyl)
1										
Unit-a	-	7.8	-	9.5				5.5	12.5	-
Unit-g	12.5	3.0	10.0					5.5	12.0	13.0
3										
Unit-a	-	7.8	-	10.0	3.5					-
Unit-g	12.5	3.0	10.0	4.5	2.0	7.0	3.0		12.0	13.0
5										
Unit-a	-	4.0	-	10.0	9.7	9.5				-
Unit-g							4.0	7.0	12.0	0

a. Measured at 500 MHz on solutions in D_2O (pH 1) at 27 °C based on a first-order analysis (± 0.2 Hz).
b. Unit identifications are defined in Figures 1 and 2.

The syntheses of 1-benzylamino-1-deoxy-4-*O*-β-**D**-glucopyranosyl-**D**-glucitol, HCl, **1** and 1-amino-1-deoxy-4-*O*-β-**D**-glucopyranosyl-**D**-glucitol, **2** are described below in detail and will serve as general procedures ***a*** and ***b***. The synthesis of 4-*O*-β-**D**-glucopyranosyl-**D**-glucitol, **7** is described in detail in general procedure **c**. The syntheses of 4-*O*-β-**D**-galactopyranosyl-**D**-glucitol, **8**, 4-*O*-α-**D**-glucopyranosyl-**D**-glucitol, **9** and the alditols **10** - **14** derived from maltotriose, maltotetrose, maltopentose, maltohexose and maltoheptose are carried out according to general procedure **c**.

General procedure ***a***:

1-Benzylamino-1-deoxy-4-*O*-β-D-glucopyranosyl-D-glucitol, HCl, 1. **D**-Cellobiose (4.0 g, 11.7 mmol) was dissolved in water (4 mL) and benzylamine (2 mL, 18.3 mmol) was added with heating to 60 °C. When all material was dissolved stirring was continued for 3 h at 60 °C. Then methanol (15 mL) was added and the solution allowed to cool to room temperature. Sodium borohydride (0.89 g, 23.4 mmol) was added over a period keeping the temperature below 30 °C. After stirring overnight at room temperature the solution was concentrated, redissolved in methanol (25 mL) and pH was adjusted to 3 with 4 N HCl(aq) followed by concentration twice with methanol. Redissolving the residue in methanol (25 mL) and filtering of the methanolic solution followed by concentration of the filtrate afforded crude **1** (5.5 g, 100%) as a hygroscopic syrup. NMR data are presented in Tables 2, 3 and 4. A sample was purified by HPLC for optical rotation and elemental analysis as the TFA salt. $[\alpha]_D^{20}$ - 14.6° (*c* 9.2, water).

Anal. Calcd for $C_{21}H_{32}F_3NO_{12}$, 1.5 H_2O: C 43.90, H 6.14, N 2.44. Found: C 43.62, H 5.81, N 2.59.

General procedure ***b***:

1-Amino-1-deoxy-4-*O*-β-D-glucopyranosyl-D-glucitol, 2. The crude product **1** (5.5 g, 11.7 mmol) isolated as described above was dissolved in water (100 mL) and pH adjusted to 9 with 25% NH_3(aq). Pd/C (1.0 g) was added and the mixture was subjected to an atmospheric hydrogen pressure under vigourous stirring overnight. Filtration and concentration of the filtrate gave 4.0 g (90% - overall yield) of **2** as a very hygroscopic syrup which was more than 90% pure as estimated from ^{13}C NMR. NMR data are presented in Tables 5, 6 and 7. FAB-MS, m/z = 344.2 ($M+H^+$).

1-Benzylamino-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol, HCl, 3. **D**-Lactose (4.0 g, 11.1 mmol), water (4 mL) and benzylamine (2 mL, 18.3 mmol) was treated as described in general procedure ***a*** with addition of sodium borohydride (0.84 g, 22.2 mmol). Crude **3** (5.2 g, 100%) was obtained as a hygroscopic syrup. NMR data are presented in Tables 2, 3 and 4. A sample was purified by HPLC for optical rotation and elemental analysis as the TFA salt. $[\alpha]_D^{20}$ - 0.4° (*c* 9.5, water).

Anal. Calcd for $C_{21}H_{32}F_3NO_{12}$, 3 H_2O: C 41.93, H 6.37. Found: C 41.72, H 6.03.

TABLE 5

^{13}C NMR chemical shift data for 1-amino-alditols **2**, **4** and **6**[a]

Compound[b]	pH	C-1	C-2	C-3	C-4	C-5	C-6
2							
Unit-a	0.5	103.2	74.2	76.4	70.4	76.8	61.5
Unit-g		42.8	69.4	71.5	79.4	71.9	62.9
Unit-a	7	103.1	74.1	76.3	70.3	76.7	61.3
Unit-g		42.6	69.3	71.4	79.3	71.7	62.8
Unit-a	12	104.0	74.6	77.2	70.5	76.9	61.3
Unit-g		43.9	71.9	72.5	80.9	73.7	63.3
4							
Unit-a	0.5	103.7	71.9	73.4	69.7	76.1	62.1
Unit-g		42.7	69.4	71.6	79.3	71.9	63.0
Unit-a	6	103.7	71.9	73.3	69.6	76.0	62.1
Unit-g		42.7	69.4	71.5	79.3	71.9	62.9
Unit-a	12	103.6	71.8	73.2	69.5	75.8	61.8
Unit-g		43.3	71.8	71.3	79.7	71.9	62.9
6							
Unit-a	0.5	101.5	72.4	73.7	70.3	73.4	61.3
Unit-g		43.4	68.4	72.5	82.5	73.4	63.2
Unit-a	6	101.4	72.3	73.6	70.1	73.3	61.2
Unit-g		43.3	68.4	72.4	82.4	73.2	63.0
Unit-a	12	102.3	73.2	74.9	70.9	73.7	61.6
Unit-g		44.7	73.2	72.6	83.6	74.1	63.7

a. Measured at 125.77 MHz on solutions in D_2O at 27 °C (reference: internal 1,4-dioxane, 67.4 ppm).
b. Unit identifications are defined in Figures 1 and 2.

TABLE 6

^{1}H NMR chemical shift data for 1-amino-alditols **2**, **4** and **6**[a]

Compound[b]	pH	H-1A	H-1B	H-2	H-3	H-4	H-5	H-6A	H-6B
2									
Unit-a	0.5	4.36	-	3.11	3.28	3.19	3.26	3.70	3.55
Unit-g		3.14	2.84	3.90	3.61	3.65	3.72	3.65	3.52
Unit-a	7	4.35	-	3.10	3.27	3.18	3.24	3.69	3.53
Unit-g		3.12	2.83	3.89	3.60	3.64	3.71	3.64	3.51
Unit-a	12	4.31	-	3.07	3.20	3.13	3.19	3.70	3.52
Unit-g		2.62	2.46	3.58	3.55	3.65	3.68	3.53	3.44
4									
Unit-a	0.5	4.29	-	3.31	3.45	3.70	3.52	3.56	3.51
Unit-g		3.15	2.82	3.92	3.60	3.64	3.72	3.68	3.47
Unit-a	6	4.29	-	3.31	3.45	3.70	3.50	3.56	3.50
Unit-g		3.15	2.81	3.92	3.60	3.64	3.71	3.66	3.52
Unit-a	12	4.26	-	3.29	3.41	3.69	3.45	3.52	3.52
Unit-g		2.59	2.40	3.60	3.52	3.63	3.69	3.59	3.47
6									
Unit-a	0.5	4.90	-	3.38	3.51	3.22	3.66	3.56	3.47
Unit-g		3.00	2.95	3.89	3.68	3.67	3.77	3.58	3.63
Unit-a	6	4.89	-	3.36	3.50	3.21	3.63	3.61	3.54
Unit-g		2.98	2.93	3.86	3.65	3.65	3.75	3.55	3.45
Unit-a	12	4.83	-	3.32	3.49	3.19	3.62	3.67	3.57
Unit-g		2.56	2.51	3.55	3.64	3.58	3.72	3.55	3.44

a. Measured at 500 MHz on solutions in D_2O at 27 °C (reference: internal 1,4-dioxane, 3.53 ppm).
b. Unit identifications are defined in Figures 1 and 2.

TABLE 7

$J_{H,H}$ values (Hz) for 1-amino-alditols **2**, **4** and **6**[a]

Compound[b]	pH	$J_{1A,1B}$	$J_{1A,2}$	$J_{1B,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
2										
Unit-a	0.5	-	7.9	-	9.3			2.1	5.5	11.5
Unit-g		12.8	3.2	9.8	5.2	2.2	7.1	2.1	5.8	12.3
Unit-a	7	-	7.9	-	9.4	9.1	9.9	2.2	5.5	12.3
Unit-g		13.0	3.1	9.7	5.2	2.3	7.5	3.1	5.8	12.0
Unit-a	12	-	7.8	-	7.2	10.6	7.6	1.9	7.0	11.6
Unit-g		13.2	3.8	8.1	4.6	3.5	4.9	5.5	5.0	11.8
4										
Unit-a	0.5	-	7.8	-	9.9	3.4				
Unit-g		12.8	2.7	9.9	5.5	2.1				
Unit-a	6	-	7.8	-	9.4	3.2				
Unit-g		12.8	3.0	9.7	5.0	2.3	7.3	2.9		12.3
Unit-a	12	-	7.8	-	9.9	3.4				
Unit-g		13.2	3.7	8.3	5.4	2.6	6.3	3.9	5.8	11.7
6										
Unit-a	0.5	-	3.9	-	10.0		9.5			
Unit-g		13.1	3.6	8.7	2.0					
Unit-a	6	-	3.9	-	9.9	9.1	9.6	2.3	5.2	12.5
Unit-g		13.2	4.0	8.5	2.1		3.3	4.1	7.0	12.0
Unit-a	12	-	4.0	-	9.7	9.0	9.7	2.2	5.4	11.7
Unit-g		13.1	4.7	8.0	4.7	2.7	4.2	5.0	6.4	11.6

a. Measured at 500 MHz on solutions in D_2O at 27 °C based on a first-order analysis (± 0.2 Hz).
b. Unit identifications are defined in Figures 1 and 2.

TABLE 8

^{13}C NMR chemical shift data for alditols **7** - **8**[a] and **9** - **14**[b]

Compound[c]	C-1	C-2	C-3	C-4	C-5	C-6
7						
Unit-a	103.2	74.1	76.3	70.2	76.5	61.3
Unit-g	63.4	72.0	70.2	80.0	73.0	62.9
8						
Unit-a	103.8	71.8	73.3	69.5	75.8	61.8
Unit-g	63.4	73.0	70.2	80.2	72.0	62.9
9						
Unit-a	101.2	72.3	73.6	70.1	73.2	61.1
Unit-g	63.5	72.2	71.5	82.5	73.4	63.0
10						
Unit-a	100.5	72.5	73.6	70.1	73.4	61.2
Unit-b	101.0	72.1	74.1	77.4	71.7	61.1
Unit-g	63.5	72.3	71.2	82.6	73.4	63.0
11						
Unit-a	100.5	72.5	73.6	70.1	73.4	61.2
Unit-b	100.3	72.3	74.0	77.7	71.9	61.2
Unit-c	101.0	72.1	74.1	77.5	71.7	61.1
Unit-g	63.5	72.3	71.2	82.6	73.3	63.0

TABLE 8 cont.

Compound[c]	C-1	C-2	C-3	C-4	C-5	C-6
12						
Unit-a	100.5	72.5	73.6	70.1	73.4	61.2
Unit-b	100.4	72.3	74.0	77.7	72.0	61.2
Unit-c	100.3	72.3	74.0	77.6	71.9	61.2
Unit-d	101.0	72.1	74.0	77.5	71.7	61.1
Unit-g	63.5	72.3	71.2	82.6	73.4	63.0
13						
Unit-a	100.5	72.5	73.6	70.1	73.5	61.2
Unit-b	100.4	72.3	74.0	77.7	72.3	61.2
Unit-c	100.4	72.3	74.0	77.6	71.9	61.2
Unit-d	100.3	72.3	74.0	77.6	71.9	61.2
Unit-e	101.0	72.1	74.0	77.5	71.7	61.2
Unit-g	63.5	72.3	71.2	82.6	73.3	63.0
14						
Unit-a	100.5	72.5	73.6	70.1	73.5	61.2
Unit-b	100.4	72.3	74.1	77.7	72.3	61.2
Unit-c	100.4	72.3	74.1	77.6	71.9	61.2
Unit-d	100.4	72.3	74.1	77.6	71.9	61.2
Unit-e	100.4	72.3	74.1	77.6	71.9	61.2
Unit-f	101.0	72.2	74.1	77.5	71.7	61.1
Unit-g	63.5	72.3	71.2	82.6	73.3	63.0

a. Measured at 125.77 MHz on solutions in D_2O at 27 °C (reference: internal 1,4-dioxane, 67.4 ppm).
b. Measured at 125.77 MHz on solutions in D_2O at 27 °C (reference: α-**D**-Glc*p* (a): C-4, 70.1 ppm).
c. Unit identifications are defined in Figure 2.

TABLE 9

^{1}H NMR chemical shift data for alditols **7** - **8**[a] and **9** - **14**[b]

Compound[c]	H-1A	H-1B	H-2	H-3	H-4	H-5	H-6A	H-6B
7								
Unit-a	4.36	-	3.12	3.30	3.22	3.23	3.69	3.56
Unit-g	3.56	3.43	3.74	3.63	3.66	3.72	3.66	3.52
8								
Unit-a	4.29	-	3.32	3.44	3.70	3.47	3.54	3.54
Unit-g	3.54	3.40	3.75	3.62	3.66	3.72	3.66	3.52
9								
Unit-a	5.09	-	3.55	3.71	3.41	3.86	3.82	3.76
Unit-g	3.67	3.62	3.84	3.85	3.86	3.96	3.77	3.65
10								
Unit-a	5.40	-	3.58	3.68	3.41	3.72	3.85	3.76
Unit-b	5.12	-	3.61	4.00	3.66	3.99	3.85	3.82
Unit-g	3.68	3.64	3.86	3.89	3.87	3.97	3.78[d]	3.67[d]
11								
Unit-a	5.38	-	3.58	3.68	3.41	3.71	3.82	3.76
Unit-b	5.39	-	3.61	4.00	3.66	3.86	3.82	3.76
Unit-c	5.11	-	3.61	3.95	3.64	3.99	3.83	
Unit-g	3.67	3.64	3.86	3.90	3.88	3.98	3.76[d]	3.69[d]

TABLE 9 cont.

Compound[c]	H-1A	H-1B	H-2	H-3	H-4	H-5	H-6A	H-6B
12								
Unit-a	5.38	-	3.58	3.67	3.41	3.71	3.84	
Unit-b	5.38	-	3.61	3.93	3.64	3.83	3.84	
Unit-c	5.38	-	3.61	3.93	3.64	3.83	3.84	
Unit-d	5.11	-	3.61	3.93	3.64	3.99	3.83	
Unit-g		3.65	3.85	3.89	3.88	3.97		3.65
13								
Unit-a	5.38	-	3.57	3.70	3.41	3.72	3.85	
Unit-b	5.37	-	3.63	3.97	3.64	3.87	3.85	
Unit-c	5.37	-	3.63	3.97	3.68	3.86	3.85	
Unit-d	5.39	-	3.63	3.97	3.86	3.85	3.85	
Unit-e	5.11	-	3.62	3.97	3.65	4.01	3.85	
Unit-g	3.66		3.87	3.91	3.89	3.99	3.68	
14								
Unit-a	5.40	-	3.57	3.68	3.41	3.71	3.85	
Unit-b	5.39	-	3.63	3.96	3.67	3.85	3.85	
Unit-c	5.39	-	3.63	3.96	3.67	3.85	3.85	
Unit-d	5.39	-	3.63	3.96	3.67	3.85	3.85	
Unit-e	5.39	-	3.63	3.96	3.67	3.85	3.85	
Unit-f	5.11	-	3.63	3.96	3.67	4.01	3.85	
Unit-g	3.69		3.85	3.91	3.89	3.99	3.79	

a. Measured at 500 MHz on solutions in D_2O at 27 °C (reference: internal 1,4-dioxane, 3.53 ppm).
b. Measured at 500 MHz on solutions in D_2O at 27 °C (reference: α-**D**-Glc*p* (a): H-4, 3.41 ppm).
c. Unit identifications are defined in Figure 2.
d. Assignments may be reversed.

TABLE 10

$J_{H,H}$ values (Hz) for alditols **7** - **14**[a]

Compound[b]	$J_{1A,1B}$	$J_{1A,2}$	$J_{1B,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
7									
Unit-a	-	7.9	-	8.7	9.1		1.5	4.0	12.0
Unit-g	11.8	3.6	6.9	5.9	2.3	7.0	3.3	6.2	12.0
8									
Unit-a	-	7.8	-	10.0	3.4				
Unit-g	11.7		7.0	5.6	2.4		3.0		
9									
Unit-a	-	3.9	-	9.9	9.0	10.3	2.4	4.8	12.4
Unit-g	11.4	4.7	6.7			3.7	4.0	7.3	11.8
10									
Unit-a	-	3.9	-	9.9	8.5	10.0	2.4	4.9	12.2
Unit-b	-	3.9	-	9.9	8.8	10.0	2.4	4.7	12.2
Unit-g	11.8	4.7	6.2				3.8	7.3	12.2
11									
Unit-a	-	3.8	-	9.8	8.1	10.2			
Unit-b	-	3.8	-	9.8	8.3				
Unit-c	-	3.8	-	9.8	8.3				
Unit-g							4.2	7.0	11.7

TABLE 10 cont.

Compound[b]	$J_{1A,1B}$	$J_{1A,2}$	$J_{1B,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
12									
Unit-a	-	3.8	-	10.0					
Unit-b	-	3.8	-	10.0					
Unit-c	-	3.8	-	10.0					
Unit-d	-	3.8	-	10.0					
13									
Unit-a	-	3.6	-	10.2	8.9				
Unit-b	-	3.6	-	10.2					
Unit-c	-	3.8	-	10.2					
14									
Unit-f	-	3.9	-						

a. Measured at 500 MHz on solutions in D_2O at 27 °C based on a first-order analysis (± 0.2 Hz).
b. Unit identifications are defined in Figures 1 and 2.

1-Amino-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol, 4. The crude product **3** (5.2 g, 11.1 mmol) isolated above was treated according to general procedure ***b*** with an overall yield of 3.5 g (83%) of **4** as a very hygroscopic syrup which was more than 90% pure as estimated from ^{13}C NMR. NMR data are presented in Tables 5, 6 and 7. FAB-MS, m/z = 344.2 ($M+H^+$).

1-Benzylamino-1-deoxy-4-*O*-α-D-glucopyranosyl-D-glucitol, HCl, 5. D-Maltose (4.0 g, 11.1 mmol), water (2 mL) and benzylamine (2 mL, 18.3 mmol) was treated as described in general procedure ***a*** with addition of sodium borohydride (0.84 g, 22.2 mmol). Crude **5** (5.2 g, 100%) was obtained as a hygroscopic syrup. NMR data are presented in Tables 2, 3 and 4. A sample was purified by HPLC for optical rotation and elemental analysis as the TFA salt. $[\alpha]_D^{20}$ + 58.4° (*c* 9.6, water).

Anal. Calcd for $C_{21}H_{32}F_3NO_{12}$, 2.5 H_2O: C 42.57, H 6.29. Found: C 42.76, H 5.98.

1-Amino-1-deoxy-4-*O*-α-D-glucopyranosyl-D-glucitol, 6. The crude product **5** (5.2 g, 11.1 mmol) isolated above was treated according to general procedure ***b*** with an overall yield of 3.5 g (83%) of **6** as a very hygroscopic syrup which was more than 90% pure as estimated from ^{13}C NMR. NMR data are presented in Tables 5, 6 and 7. FAB-MS, m/z = 344.3 ($M+H^+$).

General procedure **c**:

4-*O*-β-D-glucopyranosyl-D-glucitol, 7. D-cellobiose (100 mg, 0.3 mmol) was dissolved in water (2 mL) and sodium borohydride (50 mg, 1.3 mmol) in water (1 mL) was added. The mixture was stirred at room temperature for 18 h. Then it was neutralized with ion exchange resin (Amberlite IR-120, H^+), filtered and concentrated three times with methanol. The yield was 90%. NMR data are presented in Tables 8, 9 and 10.

ACKNOWLEDGEMENTS

Elemental analyses were provided by LEO Pharmaceutical Products, Denmark. FAB-MS spectra were recorded by Carl Erik Olsen, Dept. of Chemistry, The Royal Veterinary and Agricultural University, Denmark. We thank Mr. Bent Ole Pedersen for assistance in recording and assigning the NMR spectra. This work was supported by a grant (ICB) from the Danish Technical Research Council.

REFERENCES AND NOTES

1. Y. C. Lee, R. T. Lee, K. Rice, Y. Ichikawa and T. C. Wong, *Pure Appl. Chem.*, **63**, 499 (1991).
2. G. Magnusson, S. Ahlfors, J. Dahmén, K. Jansson, U. Nilsson, G. Noori, K. Stenvall and A. Tjörnebo, *J. Org. Chem.*, **55**, 3932 (1990).

3. W. Wayne and H. Adkins, *J. Am. Chem. Soc.*, **62**, 3314 (1940).

4. H. S. Isbell and H. L. Frush, *J. Org. Chem.*, **23**, 1309 (1958).

5. F. W. Holly, E. W. Peel, R. Mozingo and K. Folkers, *J. Am. Chem. Soc.*, **72**, 5416 (1950).

6. H. Kelkenberg, *Tensides - Surfactants - Detergents*, **25**, 8 (1988).

7. F. Kagan, M. A. Rebenstorf and R. V. Heinzelman, *J. Am. Chem. Soc.*, **79**, 3541 (1957).

8. R. U. Lemieux, *U.S. Pat. 2,830,983* (1958); *Chem. Abstr.*, **52**, 14668c (1958).

9. J. Klein, W. Behrens and M. Kunz, *Ger. Offen. 3,625,931* (1986); *Eur. Pat. 255 033* (1987); *Chem. Abstr.*, **110**, 95711j (1989).

10. H. Wiegandt and W. Ziegler, *Hoppe-Seyler's Z. Physiol. Chem.*, **355**, 11 (1974).

11. E. Kallin, H. Lönn and T. Norberg, *Glycoconjugate J.*, **5**, 145 (1988) and *ibid.*, **3**, 311 (1986).

12. J. E. Sarneski, H. L. Surprenant, F. K. Molen and C. N. Reilley, *Anal. Chem.*, **47**, 2116 (1975).

13. J. G. Batchelor, J. Feeney and G. C. K. Roberts, *J. Magn. Res.*, **20**, 19 (1975).

14. W. J. Horsley and H. Sternlicht, *J. Am. Chem. Soc.*, **90**, 3738 (1968).

15. J. Feeney, P. Partington and G. C. K. Roberts, *J. Magn. Res.*, **13**, 268 (1974).

16. F. R. N. Gurd, P. J. Lawson, D. W. Cochran and E. Wenkert, *J. Biol. Chem.*, **246**, 3725 (1971).

17. K. Bock, J. Ø. Duus, B. Norman and S. Pedersen, *Carbohydr. Res.*, **211**, 219 (1991).

18. See for example: P. L. Durette and D. Horton, *Adv. Carbohydr. Chem. Biochem.*, **26**, 49 (1971).

19. G. E. Hawkes and D. Lewis, *J. Chem. Soc. Perkin Trans. II*, 2073 (1984).

20. G. A. Jeffrey and H. S. Kim, *Carbohydr. Res.*, **14**, 207 (1970).

21. R. E. Hoffman and D. B. Davies, *Magn. Reson. Chem.*, **26**, 425 (1988).

22. R. E. Hoffman, T. J. Rutherford, B. Mulloy and D. B. Davies, *Magn. Reson. Chem.*, **28**, 458 (1990).

23. A. Shimamura, H. Tsumori and H. Mukasa, *Carbohydr. Res.*, **220**, 243 (1991).

24. $pH = pK + \log((\delta_{max} - \delta)/(\delta - \delta_{min}))$. See J. S. Cohen, R. I. Shrager, M. McNeel and A. N. Schechter, *Nature*, **228**, 642 (1970).

25. The labels 1, 2, 3 etc. describe carbon nuclei one, two, three etc. bonds away from the amine nitrogen (N,C-1,C-2,C-3 etc.).

26. E. Breitmaier and W. Voelter, *Carbon-13 NMR Spectroscopy*, 3rd ed.; VCH: New York, 1990, p 121f and references cited therein.

27. J. L. Sudmeier and C. N. Reilley, *Anal. Chem.*, **36**, 1698 (1964).

28. F. W. Lichtenthaler and H. J. Lindner, *Liebigs Ann. Chem.*, 2372 (1981).

29. See R. U. Lemieux, S. Koto and D. Voisin, *ACS Symposium Series*, No. 87, on "Anomeric Effect, Origin and Consequences", p 17 ff (1979) for a summary.