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DETERMINATION OF THE POSITION OF LINKAGE OF 2-ACETAMIDO-2-DEOXY-D-GALACTOSE AND 2-ACETAMIDO-2-DEOXY-D-GLUCOSE RESIDUES IN OLIGOSACCHARIDES AND GLYCOPROTEINS. SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-D-XYLITOL AND 2-ACETAMIDO-2-DEOXY-L-THREITOL*

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ABSTRACT

A method has been studied for the determination of the position of the linkage of the 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-glucose residues in oligosaccharides and glycoproteins that is based on the borohydride reduction of the reducing terminal residues to the corresponding alditol derivatives, periodate oxidation, borohydride reduction, hydrolysis (eventually followed by borohydride reduction), separation of the fragments as per-*O*-(trimethylsilyl) or per-*O*-(trifluoroacetyl) derivatives, and identification of the fragments as derivatives of 2-acetamido-2-deoxyglycerol, 2-acetamido-2-deoxy-L-threitol, 2-acetamido-2-deoxy-L-arabinitol, 2-acetamido-2-deoxy-D-xylitol, 2-acetamido-2-deoxy-D-galactitol, and 2-acetamido-2-deoxy-D-glucitol by gas–liquid chromatography–mass spectrometry. New syntheses for the standard compounds 2-acetamido-2-deoxy-L-threitol and 2-acetamido-2-deoxy-D-xylitol are described.

INTRODUCTION

The most useful method for the determination of the chemical structure of O-glycoproteins (glycoproteins containing an O–glycosyl linkage between the carbohydrate reducing residue and a serine or threonine residue, generally known as "mucin-type") is based on a β-elimination reaction in the presence of alkaline

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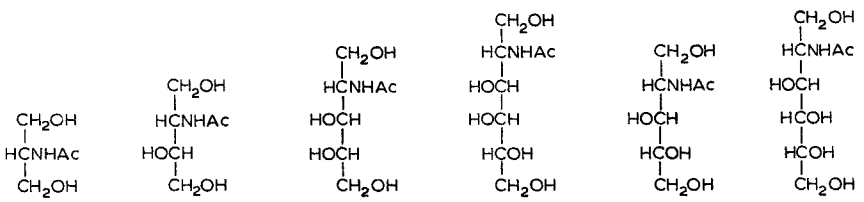
borohydride[1]. This method leads to oligosaccharides having a 2-acetamido-2-deoxy-D-galactitol end-residue that may be substituted at C-3, C-4, or C-6. Similarly, treatment of N-glycoproteins (glycoproteins containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-*β*-D-glucopyranosylamine linkage] with sodium or potassium borohydride under strongly alkaline conditions results in oligosaccharides having a 2-acetamido-2-deoxy-D-glucitol end-residue[2]. In addition, investigation of the chemical structure of oligosaccharides by periodate oxidation has shown that it is advantageous to reduce the terminal residue to the corresponding alditol derivative in order to avoid the formation of periodate-resistant, formic esters[3,4], and this procedure also may give oligosaccharides having 2-acetamido-2-deoxy-D-galactitol or -D-glucitol end-residues.

Sequential periodate oxidation–sodium borohydride–mild, acid hydrolysis (Smith degradation) of oligosaccharides containing a terminal 2-acetamido-2-deoxy-D-galactitol substituted at positions 6; 3 and 3,6; 4 and 3,4; and 4,6 and 3,4,6, respectively gives 2-acetamido-2-deoxyglycerol (**1**), 2-acetamido-2-deoxy-L-threitol (**2**), 2-acetamido-2-deoxy-L-arabinitol (**3**), and 2-acetamido-2-deoxy-D-galactitol (**4**). Similarly, application of the Smith degradation to oligosaccharides containing a terminal 2-acetamido-2-deoxy-D-glucitol residue substituted at positions 6; 3 and 3,6; 4 and 3,4; and 4,6 and 3,4,6 gives **1**, **2**, 2-acetamido-2-deoxy-D-xylitol (**5**), and 2-acetamido-2-deoxy-D-glucitol (**6**), respectively.



1 **2** **3** **4** **5** **6**

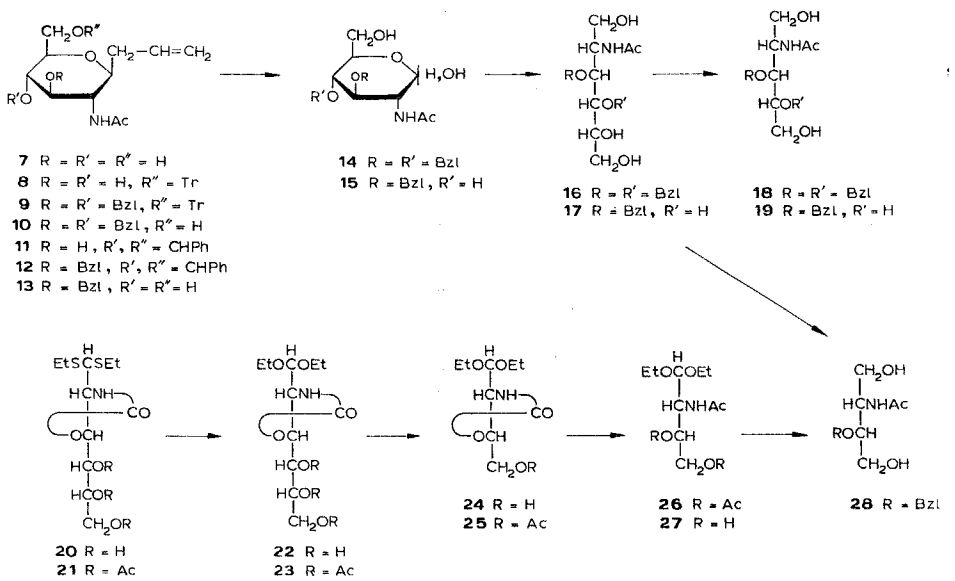
Identification of some of these 2-amino-2-deoxyalditols has been performed by paper[5] and cation-exchange chromatography[2,6], and by electrophoresis in borate buffer[7]. The present report describes convenient syntheses of the standard compounds **2** and **5**, the characterization by g.l.c. and m.s. of their per-*O*-(trimethylsilyl) derivatives, and the separation of compounds **1**, **2**, **3**, and **4**; **1**, **2**, **5**, and **6**; and **1**, **2**, **3**, **4**, **5**, and **6** by g.l.c. of their per-*O*-(trimethylsilyl) and per-*O*-(trifluoroacetyl) derivatives.

RESULTS AND DISCUSSION

2-Acetamido-2-deoxy-L-threitol (**2**) and 2-acetamido-2-deoxy-D-xylitol (**5**) were obtained in crystalline form by routes involving protective groups (allyl and benzyl) that may be removed under mild conditions. Compound **2** had been obtained in amorphous form by a route involving *O*-methyl derivatives as intermediates[5], but removal of the methyl protective group required drastic conditions.

The synthesis of compound **5** proceeded from allyl 2-acetamido-2-deoxy-β-D-glucopyranoside[8] (**7**) through the crystalline 6-*O*-trityl (**8**), 3,4-*O*-benzyl-6-*O*-trityl (**9**), and 3,4-di-*O*-benzyl (**10**) derivatives obtained by conventional methods. The allyl group of **10** was isomerized with tris(triphenylphosphine)rhodium chloride in the presence of 1,4-diazabicyclo[2.2.2]octane[9,10], and then hydrolyzed with mercuric chloride in the presence of mercuric oxide[11], to give 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-D-glucose (**14**). This compound was reduced with sodium borohydride, to give amorphous 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-D-glucitol (**16**). In a parallel route involving the crystalline 4,6-*O*-benzylidene (**11**), 3-*O*-benzyl-4,6-*O*-benzylidene (**12**), and 3-*O*-benzyl (**13** and **15**) derivatives, obtained by conventional methods, and similar removal of the allyl protective group, and the reductive step, 2-acetamido-3-*O*-benzyl-2-deoxy-D-glucitol (**17**) was obtained in crystalline form.

Oxidation of the 3,4-di-*O*-benzyl derivative **16** with sodium metaperiodate, followed by reduction with sodium borohydride, gave syrupy 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-D-xylitol (**18**). Removal of the benzyl groups by hydrogenolysis gave crystalline 2-acetamido-2-deoxy-D-xylitol (**5**). Periodate oxidation of the 3-*O*-benzyl derivative **17**, followed by reduction with sodium borohydride, gave crystalline 2-acetamido-3-*O*-benzyl-2-deoxy-L-threitol (**28**), and removal of the benzyl group by



hydrogenolysis gave crystalline 2-acetamido-2-deoxy-L-threitol (**2**). Periodate oxidation of the 3-*O*-benzyl derivative **17** for a brief period of time, followed by reduction with sodium borohydride, gave a mixture of the just-mentioned 3-*O*-benzyl derivative of L-threitol (**28**) and crystalline 2-acetamido-3-*O*-benzyl-2-deoxy-D-xylitol (**19**). After separation, removal of the benzyl group of **19** gave 2-acetamido-2-deoxy-D-xylitol (**5**), identical with the product described earlier.

2-Acetamido-2-deoxy-L-threitol (**2**) was synthesized by a second route, starting from 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-D-glucose diethyl dithioacetal[12] (**20**). Acetylation gave the triacetate **21**, and the diethyl dithioacetal group was exchanged with a diethyl acetal group[13] to give crystalline 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-D-glucose diethyl acetal (**22**), further characterized by its triacetate (**23**). Periodate oxidation of compound **22** followed by sodium borohydride reduction gave the L-threitol derivative **24** (which was characterized by the monoacetate **25**), and removal of the cyclic 2-*N*-,3-*O*-carbonyl group gave an amine that was directly acetylated to give 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-L-threose diethyl acetal (**26**). *O*-Deacetylation gave **27**, which was hydrolyzed, and the resulting product was directly reduced to give 2-acetamido-2-deoxy-L-threitol (**2**), indistinguishable from the compound previously described.

2-Acetamido-2-deoxy-L-arabinitol (**3**) was prepared from 2-amino-2-deoxy-L-arabinose hydrochloride[14] by total acetylation *O*-deacetylation, and borohydride reduction.

2-Acetamido-2-deoxyglycerol[15] (**1**), 2-acetamido-2-deoxy-L-threitol (**2**), 2-acetamido-2-deoxy-D-xylitol (**5**), and 2-acetamido-2-deoxy-D-glucitol[15,16] (**6**) were

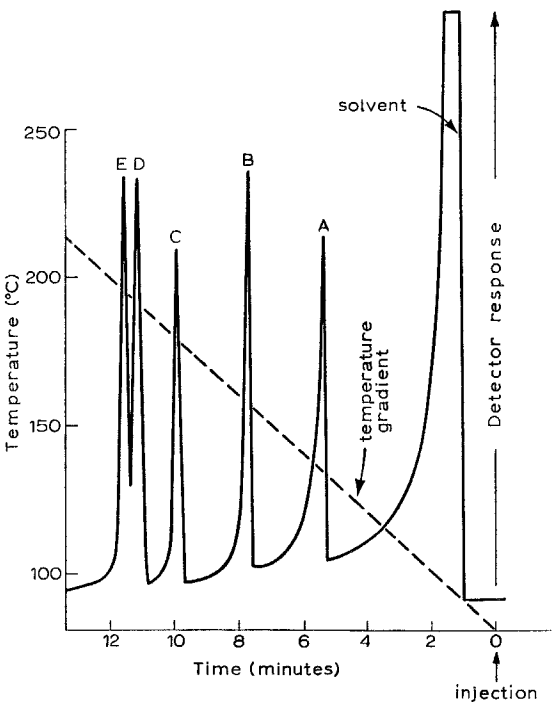


Fig. 1. Gas–liquid chromatography, on a column of 1% of OV-17 on Chromosorb GHP, of the per-*O*-(trimethylsilyl) derivatives of: (A) 2-acetamido-2-deoxyglycerol (**1**), (B) 2-acetamido-2-deoxy-L-threitol (**2**), (C) 2-acetamido-2-deoxy-D-xylitol (**5**) or -L-arabinitol (**3**), (D) *myo*-inositol (internal standard), and (E) 2-acetamido-2-deoxy-D-glucitol (**6**).

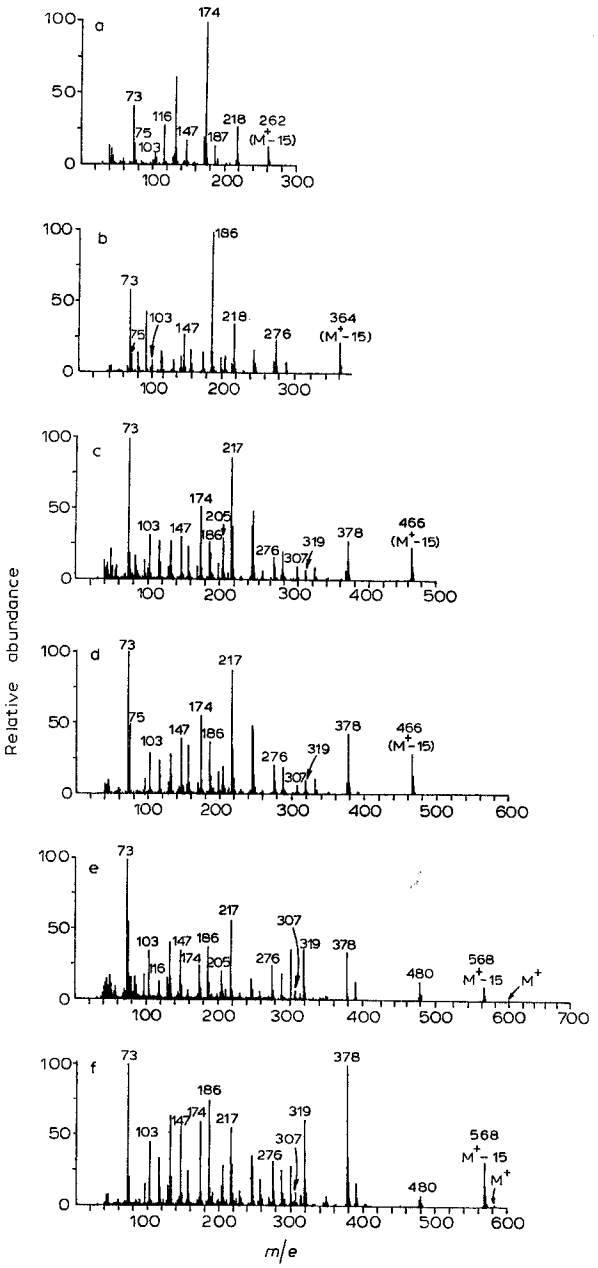


Fig. 2. Mass spectra of the per-*O*-(trimethylsilyl) derivatives of: (a) 2-acetamido-2-deoxyglycerol (**1**), (b) 2-acetamido-2-deoxy-L-threitol (**2**), (c) 2-acetamido-2-deoxy-L-arabinitol (**3**), (d) 2-acetamido-2-deoxy-D-xylitol (**5**), (e) 2-acetamido-2-deoxy-D-galactitol (**4**), and (f) 2-acetamido-2-deoxy-D-glucitol (**6**).

well separated as their per-*O*-(trimethylsilyl) derivatives, as well as from the standard *myo*-inositol derivative, by gas–liquid chromatography on a column of OV-17 on Chromosorb GHP (see Fig. 1). The mass spectra of the per-*O*-(trimethylsilyl) derivatives of **1**, **2**, **3**, **4**, **5**, and **6** (see Fig. 2) showed similarities, each compound exhibiting a peak corresponding to an $(M-15)^+$ fragment, as well as characteristic fragments containing the acetamido group (fragments A, B, C, D, E, and F and/or G, see Scheme 1). All derivatives showed a peak for the fragment at *m/e* 174 (fragment A) corresponding to a two-carbon chain containing an acetamido group. The mass

Scheme 1. Mass spectral fragmentation of per-*O*-(trimethylsilyl) derivatives of 2-acetamido-2-deoxy-alditols.

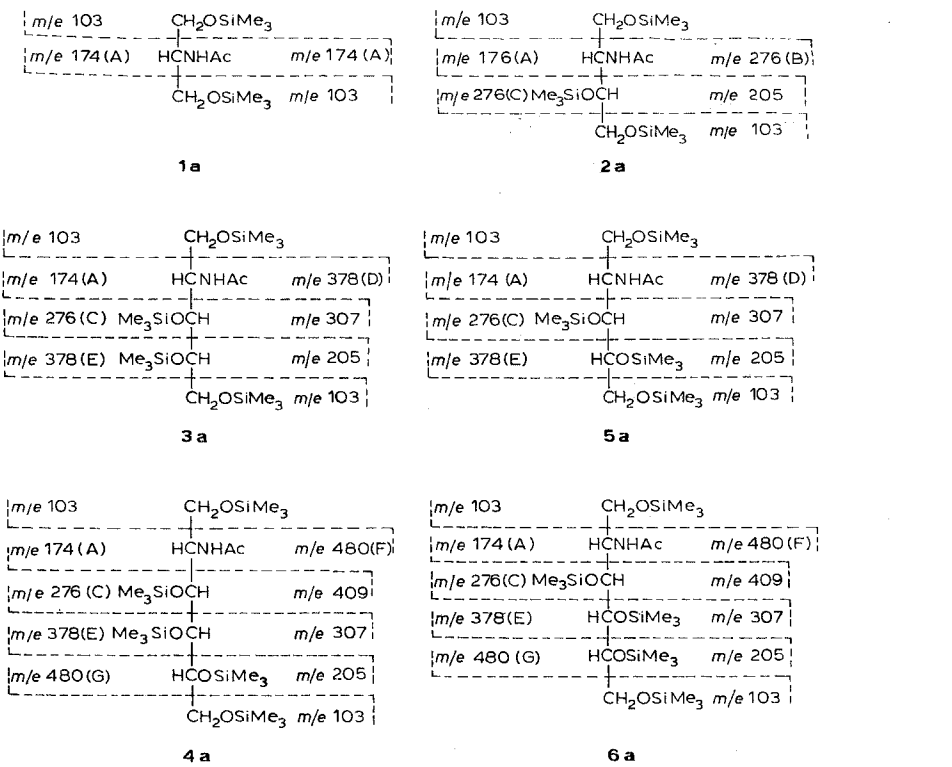


TABLE I

STRUCTURE AND RELATIVE INTENSITY OF THE MAJOR FRAGMENTS OBTAINED IN THE MASS SPECTROMETRY OF THE PER-*O*-(TRIMETHYLSILYL) DERIVATIVES OF 2-ACETAMIDO-2-DEOXYALDITOLS

Ion (m/e)	*Structure of fragment*	*Source of fragment*[a]					
		1a	**2a**	**3a**	**4a**	**5a**	**6a**
73	$(Me_3Si)^+$	57	57	100	100	100	100
103	$(CH_2{=}OSiMe_3)^+$	12	8	31	29	35	20
116	$(CHCH_2OSiMe_3)^+$	32	19	26	24	14	17
174	Fragment A (Scheme 1)	100	16	53	55	26	30
186	$(AcNHCHCH{=}CHOSiMe_3)^+$		100	27	36	36	41
205	$(Me_3OSiCH_2OSiMe_3)^+$		88	30	20	20	12
217	$(Me_3OSiCHCH{=}CHOSiMe_3)^+$		34	87	88	56	32
276	Fragment B or C (Scheme 1)		22	16	21	24	20
307	$[CH(OSiMe_3)CH(OSiMe_3)CH_2OSiMe_3)]^+$			9	7	9	5
319	$[CH(OSiMe_3)CH(OSiMe_3){=}CHOSiMe_3]^+$			9	9	33	36
378	Fragment D or E (Scheme 1)			27	42	34	66
480	Fragment F or G (Scheme 1)					14	5

[a]Relative intensity in percent.

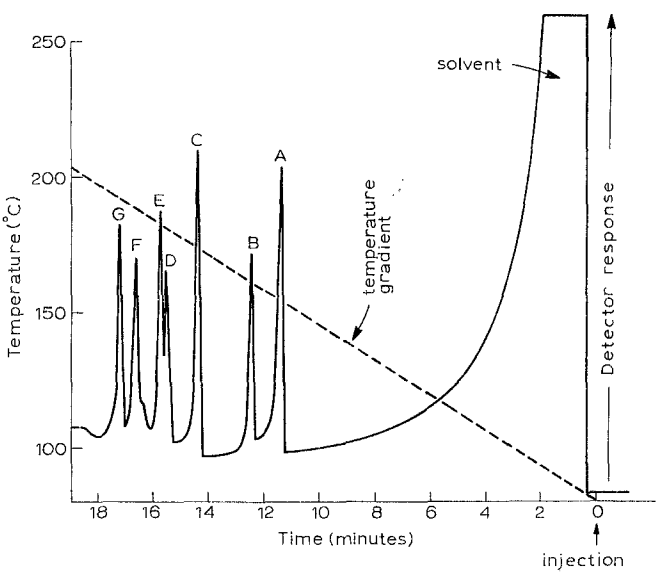
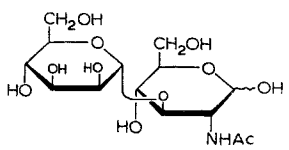


Fig. 3. Gas–liquid chromatography, on a column of 1:1 2% of OV-17–1% of OV-210 on Supelcoport, of the per-*O*-(trifluoroacetyl) derivatives of: (A) 2-acetamido-2-deoxyglycerol (**1**), (B) *myo*-inositol (internal standard), (C) 2-acetamido-2-deoxy-L-threitol (**2**), (D) 2-acetamido-2-deoxy-D-xylitol (**5**), (E) 2-acetamido-2-deoxy-L-arabinitol (**3**), (F) 2-acetamido-2-deoxy-D-glucitol (**6**), and (G) 2-acetamido-2-deoxy-D-galactitol (**4**).

spectra of the two derivatives **3** and **5**, and of the two derivatives **4** and **6**, respectively, were very similar.

Attempts at separating the per-*O*-(trimethylsilyl) derivatives of **3** and **5** on various columns and with various programs were unsuccessful, both derivatives being eluted at the same retention time. Finally, separation was achieved with the per-*O*-(trifluoroacetyl) derivatives of **3** and **5**, as well as those of **1**, **2**, **4**, and **6**, on a mixed-phase column of 2% of OV-17 and 1% of OV-210 on Supercoport (see Fig. 3).

The usefulness of the periodate oxidation–borohydride reduction–acid hydrolysis method for the structure identification of oligosaccharides was ascertained by its application to the following known disaccharides containing a 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) reducing end: 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose[17] (**29**), 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose[18] (**30**), 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-D-glucose[19] (**31**), and 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose[20] (di-*N*-acetylchitobiose) (**32**). After reduction with sodium borohydride, and periodate



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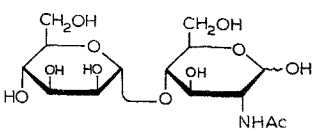

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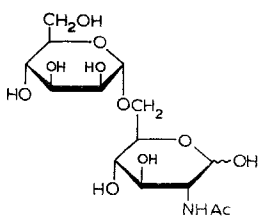

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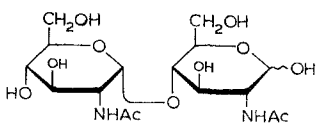

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oxidation, the iodate and periodate ions were removed by ion-exchange chromatography. This removal is necessary in order to avoid the need for a large amount of sodium borohydride in the next reducing step. As the mild, acid hydrolysis and reduction that follow the second borohydride reduction may split off some of the *N*-acetyl groups, a subsequent acetylation was performed with acetic anhydride and pyridine. In this step, the use of a solid catalyst, such as silver carbonate or a weakly basic resin, was avoided, as it is cumbersome to remove at the micro level, The *O*-acetyl groups introduced by this treatment were removed with sodium borohydride, which also transformed all the other liberated carbohydrate components into alditols. This step may be replaced by an *O*-deacetylation with ammonia (or sodium methoxide), if nonreducing 2-acetamido-2-deoxy-D-glucosyl residues are present in the oligosaccharide investigated; this would allow differentiation between a 2-acetamido-2-deoxy-D-hexose obtained from a 2-acetamido-2-deoxy-D-hexosyl residue

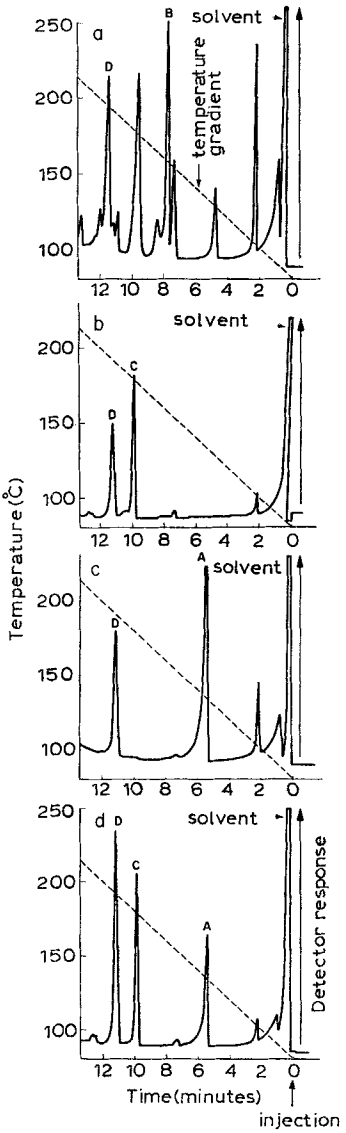

Fig. 4. Gas–liquid chromatography, on a column of 1% of OV-17 on Chromosorb GHP, of the per-*O*-(trimethylsilyl)ated products of the Smith degradation of: (a) 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose (**29**), (b) 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose (**30**), (c) 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-D-glucose (**31**), and (d) 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (di-*N*-acetylchitobiose) (**32**). [The peaks correspond to the per-*O*-(trimethylsilyl) derivatives of: (A) 2-acetamido-2-deoxyglycerol (**1**), (B) 2-acetamido-2-deoxy-L-threitol (**2**), (C) 2-acetamido-2-deoxy-D-xylitol (**5**), and (D) *myo*-inositol (internal standard).]

linked at O-3 or O-4 in the core of the chain from a reducing, terminal 2-acetamido-2-deoxy-D-hexose residue linked at O-3, O-4, and O-6.

In the case of the 4- (**30**) and 6-linked (**31**) 2-acetamido-2-deoxy-D-glucose residues (see Fig. 4, b and c), the g.l.c. separations of the trimethylsilyl derivatives were unambiguous, only 2-acetamido-2-deoxy-D-xylitol (**5**) and 2-acetamido-2-deoxyglycerol (**1**), respectively, being formed. Similarly, only **1** and **5** were observed after treatment of an oligosaccharide having both a nonreducing-terminal and a 4-linked (**32**) 2-acetamido-2-deoxy-D-glucose residue (see Fig. 4, d). The gas–liquid chromatogram (see Fig. 4, a) observed for a 3-linked residue (**29**) was, however, more complex, owing to the degradation of the (1→3)-linkage by the alkaline conditions of the first reductive step. The chromatographic peak corresponding to 2-acetamido-2-deoxy-L-threitol (**2**) was clearly identified by the presence, in the mass spectrum, of the peak at *m/e* 174 characteristic for an acetamido group, and all the other chromatographic peaks could be eliminated, because of the absence of the m.s. peak at *m/e* 174.

By paper chromatography, Foster *et al.*[5] qualitatively separated the 2-amino-2-deoxy-D-alditol derivatives produced by oxidation of a substituted 2-amino-2-deoxy-D-glucitol residue, whereas Tarentino *et al.*[7] compared, by electrophoresis in 10% borate buffer, two samples of 2-acetamido-2-deoxy-D-xylitol. Lee and Scocca[2] described a method of determining the linkage of the 2-amino-2-deoxy-D-glucitol residue of carbohydrate chains obtained by alkaline degradation and reduction; this method was quantitative and was based on cation-exchange, column chromatography, but the standards, 2-acetamido-2-deoxy-D-xylitol and 2-acetamido-2-deoxy-D-threitol, had been obtained by periodate oxidation of disaccharides and were not chemically characterized.

EXPERIMENTAL

General methods. — Melting points were determined on a Mettler FP2 hot-stage equipped with a microscope, and correspond to "corrected" melting points. Optical rotations were determined for solutions in 1-dm, semimicro tubes with a Perkin–Elmer Model 141 polarimeter. The chloroform used was analytical reagent grade, and contained ~0.75% of ethanol. Infrared spectra were recorded, for potassium bromide discs or for thin films, with a Perkin–Elmer Model 237 spectrometer. Nuclear magnetic resonance (n.m.r.) spectra were recorded at 60 MHz with a Varian T-60 spectrometer, with chloroform-*d* ("Silanor C") or pyridine-d_5 ("Silanor P") (containing 1% of tetramethylsilane, MSD Isotopic Products, Montreal, Canada, as the internal standard) as the solvent. Mass spectra were recorded with an analytical system consisting of a DuPont 21–491 mass spectrometer interfaced with a Hewlett–Packard 5700 A gas chromatograph and a DuPont 491–094 data-acquisition system; the gas chromatograph was equipped with a stainless-steel column (0.07 $cm^2 \times 300$ cm) of Chromosorb WHP (80–100 mesh) coated with 3% of OV-1 (Supelco Inc., Bellefonte, PA 16823), which was conditioned overnight at 300°, and the temperature of the column was programmed for a rise of 12°/min from 150 to 320°,

with helium as the carrier gas at a flow rate of 30 ml/min. All solvent mixtures were v/v. Evaporations were performed in a rotary evaporator under diminished pressure, with an outside bath-temperature kept below 45°. Solutions in volatile solvents (< 5 ml) were evaporated under a stream of nitrogen. The microanalyses were performed by Dr. M. Manser, Zurich, Switzerland.

Chromatographies. — Column chromatography on silica gel was performed on Silica gel Merck (70–325 mesh, E. Merck A. G., Darmstadt, Germany) used without pretreatment. The ratio of the diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2 to 3 ml/g of substance to be chromatographed. The proportion of weight of substance to weight of silica gel was 1:60 to 1:100. Thin-layer chromatography (t.l.c.) was performed on plates precoated with Silica gel G (layer thickness 0.25 mm, Merck) used without pretreatment. The distance of solvent-travel was 5 cm, and the zones were detected by spraying the chromatograms with 1:1:18 anisaldehyde–conc. H_2SO_4–ethanol[21] or with 20% H_2SO_4, followed by heating on a hot plate for a few minutes. Gas-liquid chromatography of the per-*O*-(trimethylsilyl) derivatives was performed with a Perkin–Elmer Model 900 gas chromatograph, equipped with a flame-ionization detector and a stainless-steel column (150 × 0.3 cm) packed with 1% of OV-17 on Chromosorb GHP (100–200 mesh, Supelco), with nitrogen as the carrier gas, and programmed for a rise of 10°/min from 80 to 250°. Gas chromatography of the per-*O*-(trifluoroacetyl) derivatives was performed on a column (150 × 0.3 cm) packed with 2% OV-17–1% OV-210 on Supelcoport (100–200 mesh, Supelco), and programmed for a rise of 6.5°/min from 80 to 225°. Retention times (t'_R) are given relative to that of hexa-*O*-(trimethylsilyl)- and hexa-*O*-(trifluoroacetyl)-*myo*-inositol, respectively.

Compounds previously synthesized. — The synthesis of 2-acetamido-2-deoxy-3- (**29**), -4- (**30**), and -6-*O*-α-D-mannopyranosyl-D-glucose (**31**) has been reported[17–19]. 2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (di-*N*-acetylchitobiose) (**32**) was prepared from the hexa-*O*-acetyl derivative according to a modification[22] of the method of Barker *et al.*[20]. 2-Acetamido-2-deoxyglycerol[15] (**1**) was prepared by conventional *N*-acetylation of 2-amino-2-deoxyglycerol (Sigma Chemical Co., St. Louis, MO 63178), 2-acetamido-2-deoxy-L-arabinitol (**3**) from 2-amino-2-deoxy-L-arabinose hydrochloride[14] by reduction with $NaBH_4$, 2-acetamido-2-deoxy-D-galactitol (**4**) as described by Crimmin[23], and 2-acetamido-2-deoxy-D-glucitol (**6**) as described by Bragg and Hough[16].

*Allyl 2-acetamido-2-deoxy-6-*O*-trityl-β-D-glucopyranoside* (**8**). — A solution of allyl 2-acetamido-2-deoxy-β-D-glucopyranoside[24] (**7**, 2.59 g) in dry pyridine (50 ml) was treated with chlorotriphenylmethane (3.5 g) for 72 h at room temperature. The mixture was poured onto a mixture of ice (500 g) and K_2CO_3 (10 g), and then extracted with chloroform (4 × 100 ml). The extracts were washed with water (3 × 75 ml), dried (K_2CO_3), and evaporated. The last trace of pyridine was removed by several additions and evaporations of toluene. The residue was chromatographed on a column of silica gel with 9:1 chloroform–ethanol containing 0.1% of triethylamine, to give 4.98 g (79%) of **8**, which crystallized from methanol; m.p. 126–127°, $[\alpha]_D^{22}$ −27°

(*c* 1.9, methanol); R_F 0.21 (9:1 chloroform–ethanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1650 (Amide I and allyl), 1550 (Amide II), 740, and 695 cm^{-1} (Ph); n.m.r. (pyridine-d_5): δ 7.30 (m, 15 H, CPh_3) and 2.10 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{30}H_{33}NO_6$: C, 71.55; H, 6.60; N, 2.78; O, 19.06. Found: C, 71.42; H, 6.67; N, 2.86; O, 19.15.

*Allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-trityl-β-*D*-glucopyranoside* (**9**). — α-Bromotoluene (benzyl bromide; 1.5 ml) and powdered KOH (3 g) were added to a solution of compound **8** (2.5 g) in anhydrous toluene (100 ml). The mixture was boiled under reflux while being stirred for 3 h, and filtered while hot on a Celite layer, and the inorganic residue was washed with hot toluene. The filtrate and washings were combined, washed with water (4 × 50 ml), dried (K_2CO_3), and evaporated. The residue was chromatographed on a column of silica gel with 49:1 chloroform–ethanol, to give 2.34 g (69%) of **8**, which crystallized from dichloromethane–ether; m.p. 207–208°, $[\alpha]_D^{22}$ +15° (*c* 1.7, chloroform); R_F 0.22 (chloroform) and 0.49 (49:1 chloroform–ethanol); ν_{max}^{KBr} 3280 (NH), 1650 (Amide I and allyl), 1560 (Amide II), 745, and 690 cm^{-1} (Ph); n.m.r. (chloroform-*d*): δ 7.37 (m, 25 H, 5 Ph) and 1.87 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{44}H_{45}NO_6$: C, 77.28; H, 6.63; N, 2.05; O, 14.04. Found: C, 77.22; H, 6.68; N, 2.18; O, 13.97.

*Allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**10**). — A solution of **9** (2 g) in methanol (50 ml) was treated with 4M HCl (10 ml) for 4 h at room temperature. Evaporation gave a residue that was dissolved in chloroform (200 ml). The solution was washed with water (4 × 50 ml), saturated $NaHCO_3$ solution (2 × 50 ml), and water (2 × 50 ml), dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 1.14 g (88%) of **10**, which crystallized from methanol; m.p. 199–201°, $[\alpha]_D^{22}$ −47° (*c* 2.2, methanol); R_F 0.38 (19:1 chloroform–ethanol); ν_{max}^{KBr} 3400 (OH), 3280 (NH), 1650 (Amide I and allyl), 1555 (Amide II), 740, 725, and 680 cm^{-1} (Ph); n.m.r. (pyridine-d_5): δ 7.33 (m, 10 H, 2 Ph) and 1.97 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.08; N, 3.17; O, 21.74. Found: C, 68.10; H, 7.04; N, 3.08; O, 21.65.

*2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-*D*-glucopyranose* (**14**). — A solution of **10** (1 g) and 1,4-diazabicyclo[2.2.2]octane (0.15 g; Eastman Kodak Co., Rochester, N.Y. 14650) in 90% methanol (50 ml) was heated to boiling. Tris(triphenylphosphine)-rhodium chloride (0.39 g; Alpha Products, Ventron Corporation, Danvers, MA 01923) was added, and the mixture was boiled under reflux for 4 h, cooled to room temperature, filtered, and the filtrate evaporated without being dried. A solution of the residue in chloroform (200 ml) was successively washed with 5% citric acid solution (2 × 50 ml) and water (2 × 50 ml), and evaporated without being dried. The residue was dissolved in 90% acetone (50 ml), and the solution was stirred with $HgCl_2$ (1 g) and yellow HgO (1 g) for 30 min at room temperature. The mixture was filtered on a Celite layer, the inorganic residue was washed with acetone, and the filtrate and washings were evaporated. Chromatography of the residue on a column

of silica gel with 9:1 chloroform–ethanol gave 573 mg (63%) of **14**, which crystallized from methanol–ether; m.p. 186–188°, $[\alpha]_D^{22}$ −5° (no mutarotation; *c* 2.6, methanol); R_F 0.30 (9:1 chloroform–ethanol) and 0.36 (9:1 chloroform–methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph); n.m.r. (pyridine-d_5): δ 7.30 (m, 10 H, 2 Ph) and 2.00 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{22}H_{27}NO_6 \cdot 0.5H_2O$: C, 64.38; H, 6.87; N, 3.41; O, 25.34. Found: C, 63.99; H, 6.74; N, 3.34; O, 25.64.

*2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-*D*-glucitol* (**16**). — A solution of **14** (402 mg) in methanol (50 ml) was treated with a solution of $NaBH_4$ (100 mg) in methanol (10 ml) for 2 h at room temperature. The solution was then cooled to 0°, and stirred with Dowex 50 (H^+) cation-exchange resin. The resin was filtered off, and washed with methanol, and the filtrate and washings were evaporated. $H_4B_2O_5$ was removed by several additions and evaporations of methanol. The residue was chromatographed on a column of silica gel with 9:1 chloroform–ethanol, to give 335 mg (83%) of **16**, which could not be crystallized. It was obtained as an amorphous solid by precipitation from a dichloromethane solution with pentane; $[\alpha]_D^{22}$ −13° (*c* 3.7, chloroform); R_F 0.22 (9:1 chloroform–ethanol), 0.28 (9:1 chloroform–methanol), and 0.68 (65:25:4 chloroform–ethanol–water); ν_{max}^{KBr} 3400 (broad, OH and NH), 1650 (Amide I), 1525 (Amide II), 725, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{22}H_{29}NO_6 \cdot H_2O$: C, 64.06; H, 7.33; N, 3.40; O, 25.51. Found: C, 63.81; H, 7.11; N, 3.21; O, 25.79.

*2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-*D*-xylitol* (**18**). — A mixture of **16** (750 mg) in 50% methanol (40 ml) with $NaIO_4$ (400 mg) in water (20 ml) was kept for 20 h at 4° and for 2 h at room temperature, diluted with water (110 ml), and extracted with chloroform (6 × 25 ml). The extracts were combined, washed successively with water (2 × 25 ml), saturated $NaHCO_3$ solution (2 × 25 ml), and water (2 × 50 ml), and evaporated without being dried. The syrupy residue (R_F 0.42 in 9:1 chloroform–ethanol) was dissolved in methanol (30 ml), and treated with $NaBH_4$ (100 mg) in methanol (10 ml) for 2 h at room temperature. The solution was deionized by stirring with Dowex 50 (H^+) cation-exchange resin, the resin was filtered off and washed with methanol, and the filtrate and washings were combined and evaporated. $H_4B_2O_5$ was removed by repeated addition and evaporation of methanol. The residue was chromatographed on a column of silica gel with 9:1 chloroform–ethanol, to give 514 mg (74%) of **18** as a syrup; $[\alpha]_D^{20}$ −42° (*c* 2.4, chloroform); R_F 0.32 (9:1 chloroform–ethanol); ν_{max}^{film} 3400, 3300 (OH and NH), 1650 (Amide I), 1550 (Amide II), 740, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{21}H_{27}NO_5$: C, 67.54; H, 7.29; N, 3.75; O, 21.42. Found: C, 67.46; H, 7.31; N, 3.67; O, 21.57.

*2-Acetamido-2-deoxy-*D*-xylitol* (**5**). — (*a*) *From* **18**. A solution of compound **18** (400 mg) in methanol (20 ml) was hydrogenated in the presence of 10% Pd-on-charcoal (200 mg) for 3 h at room temperature and 2 atm. The catalyst was filtered off on a Celite layer, and washed several times with methanol. The filtrate and washings were combined, and evaporated, and the residue was chromatographed on

a column of silica gel with 65:25:4 chloroform–methanol–water, to give 190 mg (92%) of **5**; m.p. 87–88°, $[\alpha]_D^{20}$ −14° (*c* 2.3, methanol); R_F 0.20 (65:25:4 chloroform–methanol–water); ν_{max}^{KBr} 3300 (OH and NH), 1640 (Amide I), and 1550 cm^{-1} (Amide II); g.l.c. of the 1,3,4,5-tetra-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 0.91; g.l.c. of the 1,3,4,5-tetra-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-}O\text{-}(trifluoroacetyl)\text{-}myo\text{-}inositol}$ 1.25; m.s. of the 1,3,4,5-tetra-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 466 $(M-15)^+$, 378 (fragments D and E), 307, 276 (fragments B and C), 218 $(Me_3SiOCH_2CH{=}CHOSiMe_3)^+$, 217 $(Me_3SiOCHCH{=}CHOSiMe_3)^+$, 174 (fragment A), 147 $(Me_2Si{=}OSiOMe_3)^+$, 103, 75 $(MeSi{=}OH)^+$, and 73 $(Me_3Si)^+$.

Anal. Calc. for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25; O, 41.41. Found: C, 43.40; H, 7.90; N, 7.17; O, 41.25.

(*b*) *From* **19**. A solution of **19** (70 mg) in methanol (5 ml) was hydrogenated in the presence of 10% Pd-on-charcoal (30 mg) as just described. Processing of the reaction mixture, followed by chromatography in the same way, gave 46 mg (89%) of **5** having the same properties as those prepared by method (*a*).

*Allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**11**). — A suspension of **7** (2.6 g) in benzaldehyde (50 ml) was treated with fused $ZnCl_2$ (3 g), and stirred under N_2 for 20 h at room temperature. The mixture was poured into a cold mixture of 10% NH_4Cl solution (200 ml) and hexane (500 ml). After being kept for 2 h in an ice bath, the solid product that separated was filtered off, and washed successively with water, ether, and hexane. Recrystallization from methanol gave 3 g (65%) of **11** as needles; m.p. 279–281° (dec.), $[\alpha]_D^{22}$ −86° (*c* 1.4, pyridine); R_F 0.36 (9:1 chloroform–ethanol); ν_{max}^{KBr} 3450 (OH), 3280 (NH), 1625 (Amide I and allyl), 1550 (Amide II), 750, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.63; N, 4.01; O, 27.48. Found: C, 61.70; H, 6.61; N, 4.06; O, 27.32.

*Allyl 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside* (**12**). — A solution of **11** (3.9 g) in *N*,*N*-dimethylformamide (75 ml) was treated with α-bromotoluene (2.5 ml), BaO (9 g), and powdered $Ba(OH)_2 \cdot 8H_2O$ (2.5 g), and heated for 2 h at 100° while being stirred. The mixture was filtered, and the filtrate evaporated to dryness. The residue was triturated with water, and the solid filtered off. Recrystallization from 1:1 chloroform–methanol gave 4.1 g (84%) of **12** as needles, m.p. 263–265°, $[\alpha]_D^{25}$ −54° (*c* 2.2, pyridine); R_F 0.60 (19:1 chloroform–ethanol) and 0.68 (9:1 chloroform–ethanol); ν_{max}^{KBr} 3270 (NH), 1650 (Amide I and allyl), 1570 (Amide II), 740, and 680 cm^{-1} (Ph); n.m.r. (pyridine-d_5): δ 7.30 (m, 10 H, 2 Ph) and 1.96 (s, 3 H, $NHCOCH_3$).

Anal. Calc. for $C_{25}H_{29}NO_6$: C, 68.32; H, 6.65; N, 3.19; O, 21.84. Found: C, 68.22; H, 6.62; N, 3.22; O, 21.75.

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**13**). — A suspension of **12** (2 g) in 60% acetic acid (100 ml) was heated for 2 h at 100° while being stirred. The mixture was evaporated, and the residue was dried by several additions and distillations of toluene. Chromatography on a column of silica gel with 9:1 chloro-

form–ethanol gave 1.3 g (82%) of **13**, which crystallized from methanol–ether: m.p. 190–191°, $[\alpha]_D^{22}$ −4° (*c* 2.4, pyridine); R_F 0.3 (9:1 chloroform–ethanol); ν_{max}^{KBr} 3300 (broad OH and HN), 1650 (Amide I and allyl), 1550 (Amide II), 725, and 680 cm^{-1} (Ph).

Anal. Calc. for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99; O, 27.32. Found: C, 61.47; H, 7.27; N, 3.99; O, 27.20.

*2-Acetamido-3-*O*-benzyl-2-deoxy-*D*-glucose* (**15**). — A solution of **13** (1 g) and 1,4-diazabicyclo[2.2.2]octane (0.15 g) in 90% methanol (50 ml) was heated to boiling and then treated with $(Ph_3P)_3RhCl$ (0.3 g). The mixture was boiled under reflux for 3 h, and filtered, while hot, on a Celite layer, and the inorganic residue was washed with hot methanol. The filtrate and washings were combined, stirred with Dowex 50 (H^+) cation-exchange resin, and the suspension filtered. Evaporation of the filtrate gave a residue which was dissolved in 90% acetone (50 ml), and treated with $HgCl_2$ (1 g) and yellow HgO (1 g) for 30 min at room temperature. The mixture was filtered, and the filtrate was evaporated to a residue which was chromatographed on a column of silica gel with 4:1 chloroform–methanol, to give 550 mg (62%) of **15**. T.l.c. with 65:25:4 chloroform–ethanol–water showed two zones for the two anomers: R_F 0.48 and 0.54, the latter preponderating. Crystallization from methanol–acetone gave 740 mg (53%) of the fast-moving anomer as needles, m.p. 199–201° (dec.), $[\alpha]_D^{25}$ +45 → +29° (*c* 1.6, methanol); ν_{max}^{KBr} 3440 (OH), 3290 (NH), 1650 (Amide I), 1550 (Amide II), 745, and 690 cm^{-1} (Ph); g.l.c. of the 1,4,6-tri-*O*-(trimethylsilyl) derivative: $t'_{hexa\text{-O-}(trimethylsilyl)\text{-myo-}inositol}$ 1.55.

Anal. Calc. for $C_{15}H_{21}NO_6$: C, 57.87; H, 6.80; N, 4.50; O, 30.83. Found: C, 57.78; H, 6.78; N, 4.49; O, 30.90.

*2-Acetamido-3-*O*-benzyl-2-deoxy-*D*-glucitol* (**17**). — A solution of **15** (311 mg) in methanol (50 ml) was treated with a solution of $NaBH_4$ (100 mg) in methanol (10 ml) for 2 h at room temperature. The solution was then cooled to 0°, stirred with Dowex 50 (H^+) cation-exchange resin, and the suspension filtered. The filtrate was evaporated, and the residue was repeatedly dissolved in methanol and the solution evaporated. Chromatography of the residue on a column of silica gel with 4:1 chloroform–methanol gave 272 mg (87%) of **17**, which crystallized from methanol–acetone–ether as needles, m.p. 127–129°, $[\alpha]_D^{25}$ −12° (*c* 1.95, methanol); R_F 0.27 (4:1 chloroform–methanol); ν_{max}^{KBr} 3520 (OH), 3310 (NH), 1640 (Amide I), 1550 (Amide II), 730, and 695 cm^{-1} (Ph); n.m.r. (pyridine-d_5): δ 7.53 (m, 5 H, Ph) and 2.07 (s, 3 H, $NHCOCH_3$); g.l.c. of the 1,4,5,6-tetra-*O*-(trimethylsilyl) derivative: $t'_{hexa\text{-O-}(trimethylsilyl)\text{-myo-}inositol}$ 1.45.

Anal. Calc. for $C_{15}H_{32}NO_6$: C, 57.50; H, 7.40; N, 4.47; O, 30.64. Found: C, 57.36; H, 7.34; N, 4.44; O, 30.52.

*2-Acetamido-3-*O*-benzyl-2-deoxy-*L*-threitol* (**28**). — A solution of **17** (150 mg) in 50% methanol (10 ml) was cooled to 0°, and treated with a solution of $NaIO_4$ (214 mg) in water (10 ml) during 30 min. After being kept for 4 h at room temperature, the mixture was stirred with Amberlite IR-45 (AcO^-) anion-exchange resin, and filtered. The filtrate was treated with a solution of $NaBH_4$ (100 mg) in water (10 ml)

for 2 h at room temperature, cooled to 0°, stirred with Dowex 50 (H^+) cation-exchange resin, and the suspension filtered. Evaporation, followed by repeated additions and distillations of methanol, gave a residue that was chromatographed on a column of silica gel with 4:1 chloroform–methanol. Fractions containing **28** were combined, and evaporated, to give 75 mg (62%) of **27**, which crystallized from ethyl acetate–ether; m.p. 137–139°, $[\alpha]_D^{25}$ −29° (*c* 1.7, methanol); R_F 0.52 (4:1 chloroform–methanol); ν_{max}^{KBr} 3300 (broad, OH and NH), 1650 (Amide I), 1560 (Amide II), 750, and 690 cm^{-1} (Ph); g.l.c. of the 1,4-di-*O*-(trimethylsilyl) derivative: $t'_{hexa\text{-O-}(trimethylsilyl)\text{-myo-}inositol}$ 1.22.

Anal. Calc. for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53; O, 25.27. Found: C, 61.54; H, 7.56; N, 5.50; O, 25.15.

*2-Acetamido-3-O-benzyl-2-deoxy-*D*-xylitol* (**19**). — A solution of **17** (300 mg) was oxidized with $NaIO_4$ (428 mg) in the same way as described in the previous experiment, except that the entire oxidation time was 30 min (at room temperature). After reduction with $NaBH_4$ and processing in the way just described, a residue was obtained that showed, in t.l.c. in 4:1 chloroform–methanol, two spots, one having R_F 0.52 and corresponding to the L-threitol derivative **28**, and the other, R_F 0.40, corresponding to **19**. Chromatography on a column of silica gel with 4:1 chloroform–methanol gave 81 mg (33.5%) of **27** and 108 mg (40%) of **19**. The latter compound crystallized from methanol–ether as needles, m.p. 131–132°, $[\alpha]_D^{25}$ −32° (*c* 0.4, methanol); ν_{max}^{KBr} 3320 (OH), 3290 (NH), 1645 (Amide I), 1560 (Amide II), 750, 725, and 680 cm^{-1} (Ph); g.l.c. of the 1,4,5-tri-*O*-(trimethylsilyl) derivative: $t'_{hexa\text{-O-}(trimethylsilyl)\text{-myo-}inositol}$ 1.35.

Anal. Calc. for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94; O, 28.83. Found: C, 59.19; H, 7.45; N, 4.80; O, 28.13.

*2-Acetamido-2-deoxy-*L*-threitol* (**2**). — (*a*) *From* **28**. A solution of **28** (126 mg) in methanol (20 ml) was treated with 10% Pd-on-charcoal (30 mg), and hydrogenated for 16 h at room temperature and 2 atm. The catalyst was filtered off on a Celite layer, and washed with methanol; the filtrate and washings were combined, and evaporated. Crystallization of the residue from methanol–ether gave 66 mg (81%) of **2**; m.p. 113–115° (lit.[5] solid obtained by lyophilization, m.p. 90–90.5°), $[\alpha]_D^{25}$ −44° (*c* 0.64, methanol) {lit.[5] $[\alpha]_D^{25}$ −42 ±4° (*c* 1, water)}; R_F 0.34 (65:25:4 chloroform–methanol–water); ν_{max}^{KBr} 3430, 3325 (OH), 1650 (Amide I), and 1555 cm^{-1} (Amide II); g.l.c. of the 1,3,4-tri-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-O-}(trimethylsilyl)\text{-myo-}inositol}$ 0.68, and of the 1,3,4-tri-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-O-}(trifluoroacetyl)\text{-myo-}inositol}$ 1.16; m.s. of the 1,3,4-tri-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 364 $(M-15)^+$, 276 (fragments C and B), 218 $(Me_3SiOCH_2CH{=}CHOSiMe_3)^+$, 186 $(Me_3SiOCH{=}CHCHNHAc)^+$, 174 (fragment A), 147 $(Me_2SiO{=}SiOMe_3)^+$, 103, 75 $(Me_2SiO{=}OH)^+$, and 73 $(Me_3Si)^+$.

Anal. Calc. for $C_6H_{13}NO_4$: C, 44.17; H, 8.03; N, 8.58; O, 39.22. Found: C, 44.04; H, 7.96; N, 8.57; O, 39.31.

(*b*) *From* **27**. A solution of compound **27** (11 mg) in 60% acetic acid (5 ml) was heated for 1 h at 80°, cooled, and evaporated, and the residue was dried by several

additions and distillations of toluene. A solution of the residue in water (2 ml) was treated with $NaBH_4$ (10 mg) for 2 h at room temperature, cooled to 0°, and stirred with Dowex 50 (H^+) cation-exchange resin. The resin was filtered off, and washed with water and methanol, and the filtrate and washings were combined, and evaporated to dryness. $H_4B_2O_5$ was removed by repeated additions and distillations of toluene, to give 5 mg (65.5%) of **2**, indistinguishable from that obtained by method (*a*).

4,5,6-Tri-O-*acetyl-2-amino-2*-N,*3*-O-*carbonyl-2-deoxy*-D-*glucose diethyl dithioacetal* (**21**). — A solution of compound[12] **20** (5 g) in dry pyridine (30 ml) was treated with acetic anhydride (25 ml) for 16 h at room temperature. Evaporation of the mixture, and drying of the residue by repeated additions and distillations of toluene, gave a syrup (6.5 g, 87%) that was homogeneous in t.l.c.: R_F 0.43 (19:1 chloroform–ethanol) and 0.54 (1:1 ethyl acetate–ether); $[\alpha]_D^{20}$ −26° (*c* 0.9, chloroform); ν_{max}^{film} 3300 (broad, NH), 1725–1700 (OAc and five-membered, cyclic –O–CONH–), and 750 cm^{-1} (C–S); n.m.r. (pyridine-d_5): δ 6.43 (1 H, NH), 2.17, 2.13, and 2.09 (3 s, 9 H, 3 OCOCH_3). An analytical sample was obtained by passing a solution of **21** in chloroform through a short column of silica gel, followed by evaporation of the solvent under a stream of nitrogen, and drying in high vacuum over P_2O_5.

Anal. Calc. for $C_{17}H_{27}NO_8S_2$: C, 46.66; H, 6.22; N, 3.20; S, 14.66. Found: C, 46.63; H, 6.26; N, 3.25; S, 14.54.

2-Amino-2-N,*3*-O-*carbonyl-2-deoxy*-D-*glucose diethyl acetal* (**22**). — To a solution of **21** (6 g) in absolute ethanol (70 ml) were added yellow HgO (11 g) and $CdCO_3$ (2 g). The suspension was heated to boiling, treated with a boiling solution of $HgCl_2$ (25 g) in absolute ethanol (50 ml), and boiled under reflux for 5 h while being stirred. The inorganic, insoluble salts were filtered off on a Celite layer, and washed with hot ethanol (50 ml). The filtrate and washings were combined, and evaporated to dryness, the residue was dissolved in chloroform (200 ml), and the solution was washed with water (4 × 50 ml), treated with a solution of disodium (ethylenedinitrilo)-tetraacetate (7.5 g) in hot water (50 ml), and vigorously stirred for 3 h at room temperature. After separation, the chloroform layer was washed with water (3 × 50 ml), dried (Na_2SO_4), and evaporated to a syrup which was dissolved in methanol (150 ml) and treated with M sodium methoxide solution in methanol (5 ml) for 16 h at 4°. The solution was then rapidly passed, while cold, through a column of Dowex 50 (H^+) cation-exchange resin, and evaporated. Chromatography of the residue on a column of silica gel with 4:1 chloroform–methanol gave 1.8 g (48%) of **22**, which crystallized from methanol–ether as prismatic needles, m.p. 115–118°, $[\alpha]_D^{20}$ −84° (*c* 0.5, methanol); R_F 0.42 (4:1 chloroform–ethanol); ν_{max}^{KBr} 3480, 3440, 3380 (OH), 3250 (NH), and 1730 cm^{-1} (five-membered, cyclic –O–CONH–).

Anal. Calc. for $C_{11}H_{21}NO_7$: C, 47.31; H, 7.58; N, 5.02; O, 40.10. Found: C, 47.03; H, 7.49; N, 5.03; O, 39.69.

4,5,6-Tri-O-*acetyl-2-amino-2*-N,*3*-O-*carbonyl-2-deoxy*-D-*glucose diethyl acetal* (**23**). — A solution of **22** (235 mg) in dry pyridine (3 ml) was treated with acetic anhydride (4 ml) for 16 h at room temperature. The mixture was evaporated, and the

residue dried by several additions and distillations of toluene. Crystallization from chloroform–pentane gave 312 mg (92%) of **23** as needles, m.p. 73–75°, $[\alpha]_D^{20}$ −41° (*c* 0.7, chloroform); R_F 0.38 (19:1 chloroform–ethanol); ν_{max}^{KBr} 3400 (NH), 1780, 1745 (OAc), and 1728 cm^{-1} (five-membered, cyclic O–CONH–); n.m.r. (chloroform-*d*): δ 6.70 (1 H, NH), 2.16, 2.13, 2.10 (3 s, 9 H, 3 $OCOCH_3$), and 1.30 (t, 6 H, 2 CH_2–CH_3).

Anal. Calc. for $C_{17}H_{27}NO_{10}$: C, 50.37; H, 6.71; N, 3.45; O, 39.47. Found: C, 50.38; H, 6.71; N, 3.48; O, 39.39.

*2-Amino-2-*N*,3-*O*-carbonyl-2-deoxy-*L*-threose diethyl acetal* (**24**). — A solution of **22** (3.8 g) in water (40 ml) was cooled to 0°, and alternately treated with solutions of $NaIO_4$ (8%; 80 ml) and $NaHCO_3$ (5%; 40 ml), the pH of the reaction mixture being kept almost at 7.0. After 4 h at room temperature, the mixture was stirred with Amberlite IR-45 (OAc^-) anion-exchange resin, and the suspension was filtered. The solute in the filtrate was reduced with a solution of $NaBH_4$ (4%; 10 ml) for 2 h at room temperature, and the mixture was cooled to 0°, and stirred with Rexyn 300 (H^+, HO^-) ion-exchange resin. The resin was filtered off, washed several times with water, and the filtrate and washings were combined, and evaporated to dryness. Chromatography on a column of silica gel with 9:1 chloroform–ethanol gave 1.9 g (64%) of **24** as a syrup; $[\alpha]_D^{20}$ −53° (*c* 0.6, methanol); R_F 0.33 (9:1 chloroform–ethanol); ν_{max}^{film} 3350 (broad, OH and NH) and 1735 cm^{-1} (five-membered, cyclic O–CONH–); n.m.r. (pyridine-d_5): δ 1.13 (t, 6 H, 2 CH_2CH_3).

Anal. Calc. for $C_9H_{17}NO_5$: C, 49.31; H, 7.82; N, 6.39; O, 36.49. Found: C, 49.24; H, 7.79; N, 6.24; O, 36.28.

*4-*O*-Acetyl-2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-*L*-threose diethyl acetal* (**25**). — A solution of **24** (100 mg) in dry pyridine (3 ml) was treated with acetic anhydride (5 ml) for 16 h at room temperature, and evaporated, and the residue was dried by additions and evaporations of toluene. Crystallization from ether–pentane gave 103 mg (87%) of needles, m.p. 86–87°, $[\alpha]_D^{20}$ −63° (*c* 0.6, chloroform); R_F 0.42 (19:1 chloroform–ethanol); ν_{max}^{KBr} 3250 (NH) and 1725 cm^{-1} (five-membered –O–CONH–); n.m.r. (chloroform-*d*): δ 6.53 (1 H, NH), 2.17 (3 H, $OCOCH_3$), and 1.27 (t, 6 H, 2 CH_2–CH_3).

Anal. Calc. for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36; O, 36.74. Found: C, 50.62; H, 7.31; N, 5.46; O, 36.71.

*2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-*L*-threose diethyl acetal* (**26**). — Compound **24** (400 mg) was treated with a solution of $Ba(OH)_2 \cdot 8H_2O$ (350 mg) in water (10 ml), and heated for 4 h at 80° under N_2. CO_2 was bubbled into the mixture, and the Ba salts were filtered off, and successively washed with water and methanol. The filtrate and washings were combined, and evaporated, and the residue was dried by additions and evaporations of toluene, taken up in methanol (10 ml), and the suspension filtered on a Celite layer. Evaporation of the filtrate gave a brownish residue that was acetylated with pyridine (7 ml) and acetic anhydride (10 ml) for 16 h at room temperature. The mixture was evaporated, and the residue was chromatographed on a column of silica gel with 19:1 chloroform–ethanol, to give 303 mg (52%) of **26**,

crystallized from ether–pentane, m.p. 134–135°; $[\alpha]_D^{20}$ −18° (*c* 0.7, chloroform); R_F 0.33 (19:1 chloroform–ethanol); ν_{max}^{KBr} 3260 (NH), 1735 (OAc), 1650 (Amide I), and 1560 cm^{-1} (Amide II).

Anal. Calc. for $C_{14}H_{25}NO_7$: C, 52.65; H, 7.89; N, 4.39; O, 35.07. Found: C, 52.67; H, 7.88; N, 4.46; O, 35.09.

*2-Acetamido-2-deoxy-*L*-threose diethyl acetal* (**27**). — A solution of **26** (200 mg) in methanol (20 ml) was treated with 0.1M sodium methoxide solution in methanol (1 ml) for 16 h at 4°. The solution was de-ionized by stirring with Dowex 50 (H^+) cation-exchange resin at 4°, and the resin was filtered off, and washed with methanol. Evaporation of the filtrate and washings gave a residue which crystallized from methanol–ether, to give plates (136 mg, 92%), m.p. 124°, $[\alpha]_D^{20}$ +15° (*c* 0.5, methanol); ν_{max}^{KBr} 3250 (broad OH and NH), 1640 (Amide I), and 1560 cm^{-1} (Amide II); m.s. of 3,4-di-*O*-(trimethylsilyl) derivative: *m/e* 364 $(M-15)^+$, 334 $(M-OEt)^+$, 318 $(Me_2Si{=}OCHOSiMe_3CHNHAcCH{=}OEt)^+$, 276 $[Me_3SiOCHCHNHAcCH(OEt)_2]^+$, 205 $(Me_3SiOCHCHCH_2OSiMe_3)^+$, 173 $(Me_3SiOCHCHNHAc)^+$, 147 $(Me_3SiO{=}SiMe_2)^+$, 75 $(MeSi{=}OH)^+$, and 73 $(Me_3Si)^+$; g.l.c. of the 3,4-di-*O*-(trimethylsilyl) derivative: $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 0.77.

Anal. Calc. for $C_{10}H_{21}NO_5$: C, 51.05; H, 9.00; N, 5.95; O, 34.00. Found: C, 51.00; H, 8.97; N, 6.04; O, 33.88.

2-Acetamido-2-deoxyglycerol (N-*acetylserinol*) (**1**). — A solution of 2-amino-2-deoxyglycerol (20 mg; 2-amino-1,3-propanediol, Sigma Chemical Co.) in methanol (5 ml) was treated with acetic anhydride (0.1 ml) for 1 h at room temperature, and then evaporated. The residue was crystallized from methanol–ethyl acetate (29 mg, 93%); m.p. 90–91°; (lit.[15] m.p. 89–90°); g.l.c. of the 1,3-di-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 0.45, and of the 1,3-di-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-}O\text{-}(trifluoroacetyl)\text{-}myo\text{-}inositol}$ 0.92; m.s. of the 1,3-di-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 262 $(M-15)^+$, 218 $(Me_3SiOCH_2CH{=}CHOSiMe_3)^+$, 187 $[Me_3SiOCH_2CH(NHAC){=}CH]^+$, 174 (fragment A), 147 $(Me_3SiO{=}SiMe_2)^+$, 116 $(MeSiOCH_2{=}CH)^+$, 103 $(Me_3SiO{=}CH_2)^+$, 75 $(MeSi{=}OH)^+$, and 73 $(Me_3Si)^+$.

*2-Acetamido-2-deoxy-*L*-arabinitol* (**3**). — A solution of 2-amino-2-deoxy-L-arabinose hydrochloride[2] (5 mg) in dry pyridine (1 ml) was treated with acetic anhydride (1 ml) for 16 h at room temperature, and then evaporated. A solution of the residue in methanol (2 ml) was treated with M sodium methoxide solution in methanol (5 drops) for 2 h at room temperature, and then with $NaBH_4$ (10 mg) for 2 h. The mixture was diluted with methanol (3 ml), passed through a column of Dowex 50 (H^+) cation-exchange resin, and the effluent evaporated, to give a residue (3.5 mg); g.l.c. of the 1,3,4,5-tetra-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 0.91, and of the 1,3,4,5-tetra-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-}O\text{-}(trifluoroacetyl)\text{-}myo\text{-}inositol}$ 1.28; m.s. of the 1,3,4,5-tetra-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 466 $(M-15)^+$, 378 (fragments D and E), 307, 277 $(Me_3SiOCH_2NHAcCH_2OSiMe_3)^+$, 276 (fragments B and C), 217 $(Me_3SiOCHCH{=}CHOSiMe_3)^+$, 205, 186 $(AcNHCHCH{=}CHOSiMe_3)^+$, 174 (frag-

ment A), 147 $(Me_2Si{=}OSiMe_3)^+$, 103 $(Me_3SiO{=}CH_2)^+$, 75 $(Me_3Si{=}OH)^+$, and 73 $(Me_3Si)^+$.

2-Acetamido-2-deoxy-D-*galactitol* (**4**). — This compound was prepared from 2-acetamido-2-deoxy-D-galactose by the method of Crimmin[23]; g.l.c. of the 1,3,4,5,6-penta-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 1.05, and of the 1,3,4,5,6-penta-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-}O\text{-}(trifluoroacetyl)\text{-}myo\text{-}inositol}$ 1.40; m.s. of the 1,3,4,5,6-penta-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 583 $(M)^+$, 568 $(M-15)^+$, 480 (fragments F and G), 378 (fragments D and E), 319 $(Me_3SiOCH_2CH{=}COSiMe_3CHOSiMe_3)^+$, 204 $(Me_3SiOCHCH{=}CHOSiMe_3)^+$, 186 $(Me_3SiOCH{=}CHCHNHAc)^+$, 174 (fragment A), 147 $(Me_3SiO{=}SiMe_2)^+$, 103 $(Me_3SiO{=}CH_2)^+$, 75 $(Me_3Si{=}OH)^+$, and 73 $(Me_3Si)^+$.

2-Acetamido-2-deoxy-D-*glucitol* (**6**). — This compound was prepared from 2-acetamido-2-deoxy-D-glucose by the method of Bragg and Hough[16]; g.l.c. of the 1,3,4,5,6-penta-*O*-(trimethylsilyl) derivative (see Fig. 1): $t'_{hexa\text{-}O\text{-}(trimethylsilyl)\text{-}myo\text{-}inositol}$ 1.04, and of the 1,3,4,5,6-penta-*O*-(trifluoroacetyl) derivative (see Fig. 3): $t'_{hexa\text{-}O\text{-}(trifluoroacetyl)\text{-}myo\text{-}inositol}$ 1.35; m.s. of the 1,3,4,5,6-penta-*O*-(trimethylsilyl) derivative (see Fig. 2 and Scheme 1): *m/e* 583 $(M)^+$, 568 $(M-15)^+$, 480 (fragments F and G), 378 (fragments D and E), 319 $(Me_3SiOCH_2CH{=}COSiMe_3CHOSiMe_3)^+$, 276 (fragments B and C), 217 $(Me_3SiOCHCH{=}CHOSiMe_3)^+$, 186 $(Me_3SiOCH{=}CHNHAc)^+$, 174 (fragment A), 147 $(Me_3SiO{=}SiMe_2)^+$, 103 $(Me_3SiO{=}CH_2)^+$, 75 $(Me_3Si{=}OH)^+$, and 73 $(Me_3Si)^+$.

Experiments with disaccharides. — A solution of one of the disaccharides **29**, **30**, **31**, or **32** (2 mg) in water (9 ml) was treated with 0.1% aqueous $NaBH_4$ solution (1 ml) for 2 h at room temperature, and then passed through a column of Dowex 50 (H^+) cation-exchange resin. The effluent was evaporated, and the residue treated by several additions and evaporations of methanol. The resulting disaccharide alditol was treated with 10% aqueous $NaIO_4$ (2 ml) for 20 h at 4°, and 4 h at room temperature. The solution was then passed through a column of Amberlite IR-45 (OAc^-) anion-exchange resin, and the effluent evaporated to dryness. The oxidation product was reduced with 0.1% $NaBH_4$ solution (10 ml) for 2 h at room temperature, and the solution was passed through a column of Dowex (H^+) cation-exchange resin, the effluent evaporated, and the residue treated by several additions and evaporations of methanol. A solution of the residue in 0.5M aqueous HCl (2 ml) was heated for 1 h at 80°, and evaporated. The residue was dissolved in pyridine (1 ml), treated with acetic anhydride (0.5 ml) for 2 h at room temperature, and the solution evaporated to dryness. The residue was treated with 0.1% $NaBH_4$ (1 ml) for 1 h at room temperature, the solution was passed through a column of Dowex 50 (H^+) cation-exchange resin, the effluent evaporated, and the residue treated by several additions and evaporations of methanol. The residue obtained was per-*O*-(trimethylsilyl)ated, and the product used for the g.l.c. analyses (see Fig. 4).

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