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AN IMPROVED METHOD FOR THE LIQUID CHROMATOGRAPHY OF THE 1-DEOXY-1-(2-PYRIDYLAMINO)ALDITOL DERIVATIVES OF OLIGOSACCHARIDES AND ITS APPLICATION TO STRUCTURAL STUDIES OF THE CARBOHYDRATE MOIETIES OF GLYCOPROTEINS

PING W TANG AND J. MICHAEL WILLIAMS*
Chemistry Department, University College of Swansea, Swansea SA2 8PP (Great Britain)
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ABSTRACT

The 1-deoxy-1-(2-pyridylamino)alditols prepared by reductive amination of lactose, 2-acetamido-2-deoxy-D-glucose, and 2,5-anhydro-D-mannose have been characterised, and the efficiency of the reductive amination procedure, especially with 2,5-anhydro-D-mannose and 2-amino-2-deoxy-D-glucose hydrazone as starting materials, has been studied. The latter compound, which is a model for the oligosaccharide hydrazones released from glycoproteins and glycopeptides by hydrazinolysis, was first *N*-acetylated and the hydrazone group was found to be removed hydrolytically when a cation-exchange resin was used for deionisation. Such loss of the hydrazone group is desirable because the *N*-acetylated hydrazone was not efficiently derivatised by reductive amination. An amine-modified silica column was used to separate the components of a mixture of the pyridylamino derivatives of oligosaccharides from mono- to dodeca-saccharide in 20 min. A neutral fluorescent by-product, formed in all reductive aminations, was identified as (2-amino-1-pyridyl)cyanoborane and was eluted well before monosaccharide derivatives and thus did not interfere with the analysis.

INTRODUCTION

Oligosaccharides have been separated by liquid chromatography (l.c.) on permanently bonded amine columns[1] and Partisil PAC columns[2]. More recently, Aitzetmuller *et al.*[3], White *et al.*[4], and Baust *et al.*[5] improved the separation of monosaccharides and oligosaccharides on silica columns modified with di- or poly-amines. Most carbohydrates have their u.v. absorption maxima below 200 nm and, while some use has been made of end absorption near 200 nm, refractometers are commonly used for detection. However, refractometers can only be used for isocratic elution, and they are relatively insensitive. Many analyses of oligosaccharides need to be carried out on very small quantities (<1 nmol), and increased sensitivity

*To whom correspondence should be addressed.

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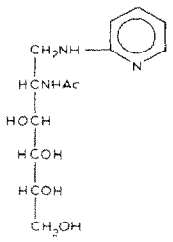
in l.c. analysis can be achieved by the introduction of u.v.-absorbing or fluorescent chromophores. For example, the perbenzoate derivatives of oligosaccharides were recently analysed[6] on the pmol scale using a reverse-phase C_8 column with a u.v. detector at 230 nm.

The reductive amination of reducing saccharides with 2-aminopyridine is a useful method for the introduction of a fluorescent label which enables 0.1-pmol quantities to be detected by l.c. with a fluorescence detector[7]. However, the yields of reductive amination products were sometimes as low as 10%, and two different l.c. columns were required for the analysis of oligosaccharide mixtures. We now report a detailed study of the preparation of the 2-aminopyridine derivatives, their isolation, their analysis by a single l.c. system, and applications of the procedure to the analysis of oligosaccharides derived from glycoproteins.

RESULTS AND DISCUSSION

The products of the reductive amination of saccharides using 2-aminopyridine and sodium cyanoborohydride in either aqueous 80% methanol[8] or aqueous 80% *N,N*-dimethylformamide[7] in a sealed tube were purified by Hase and collaborators by absorption on a cation-exchange resin followed by desorption with dilute ammonia[8]. In an attempt to simplify the procedure, reductive amination of D-glucose in refluxing aqueous 80% methanol was studied, but the only fluorescent product detected was a neutral compound which did not migrate in paper electrophoresis at pH 3.5. Similar reaction in a sealed tube, according to Hase and collaborators[8], gave the required D-glucose derivative in only 31% yield as determined by l.c. analysis. Higher yields were obtained when the reaction was carried out in *N,N*-dimethylformamide at 95° for 3 h, the D-glucose derivative, 1-deoxy-1-(2-pyridylamino)-D-glucitol, being isolated in almost quantitative yield. Reaction in a sealed tube was not necessary. 2-Acetamido-1,2-dideoxy-1-(2-pyridylamino)-D-glucitol (**1**) was similarly isolated in pure crystalline form in 77% yield from 2-acetamido-2-deoxy-D-glucose. Since 2-aminopyridine is present in large excess in these reactions, transamination could cause *N*-deacetylation of 2-acetamido-2-deoxy-D-glucose, but no *N*-deacetylated product could be detected by l.c. or paper electrophoresis.



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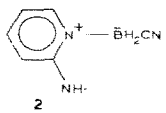

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The neutral by-product referred to above was formed in all reductive aminations, including a control experiment in which no saccharide was used. It was readily removed, and isolated crystalline, by elution of the cation-exchange resin with water. The compound was identified as (2-amino-1-pyridyl)cyanoborane (**2**) by ^{13}C-, ^{1}H-, and ^{11}B-n.m.r. spectroscopy, mass spectrometry, and i.r. and elemental analysis. The ^{11}B-n.m.r. spectrum contained a triplet ($^{1}J_{BH}$ 90 Hz), which established the presence of a BH_2 group, and the ^{13}C-n.m.r. spectrum contained signals for five carbon nuclei, that for the cyanide carbon being absent. This cyanide carbon atom gave a broad singlet ($W_{1/2}$ 16 Hz) when the noise-decoupled spectrum was measured at −60°. An attempt to observe a multiplet in which the boron–carbon coupling was resolved by increasing the probe temperature was unsuccessful[9]. The ring carbon atoms that were directly bonded to the ring nitrogen atom were shielded (by ~4 p.p.m.) relative to those of 2-aminopyridine. Similar shielding occurs in pyridine complexes of 9-borabicyclo[3.3.1]nonane and derivatives[10], and this observation excluded the alternative structure, (2-pyridylamino)cyanoborane. This is consistent with the report that the ring nitrogen atom is the stronger donor when 2-aminopyridine complexes with organoboranes[11]. Infrared absorptions at 2420 and 2190 cm^{-1} were assigned to B–H and C≡N stretching, respectively. The primary amino group was confirmed by the action of nitrous acid, which gave nitrogen. The compound was prepared in larger quantity by heating 2-aminopyridine and sodium cyanoborohydride in tetrahydrofuran in the presence of hydrochloric acid.

Some of the 1-deoxy-1-(2-pyridylamino)alditols could not be eluted from the cation-exchange resin in high yield without contamination by 2-aminopyridine. The products of the reductive amination of the oligosaccharides prepared from glycoproteins by hydrazinolysis followed by *N*-acetylation were purified by Hase and collaborators using three different ion-exchange columns, the overall yield being only 10%. A more convenient procedure for the separation of the 1-deoxy-1-(2-pyridylamino)alditols from inorganic salts and excess of 2-aminopyridine involved preparative electrophoresis as the only step*. When the quantities of oligosaccharide derivatives were so low that their fluorescence could not readily be detected on the paper strip, the excess of 2-aminopyridine could be detected, and the region between 2-aminopyridine and the origin was eluted with water. The (2-amino-1-pyridyl)cyanoborane did not interfere with the subsequent l.c. analysis since it was eluted well before all the monosaccharide derivatives.

The limitations of the C_{18} reverse-phase l.c. column for the analysis of the smaller oligosaccharide derivatives were confirmed using D-glucose, lactose, and maltotriose as a test mixture. This mixture was derivatised, and the products were isolated using a cation-exchange resin according to Hase and collaborators. L.c. analysis (C_{18} column) showed the presence of 2-aminopyridine, and the derivatives

*When derivatising the products of glycoprotein hydrazinolysis, amino acid hydrazides were first removed by gel-permeation chromatography.

of D-glucose and lactose co-eluted. The malto-oligosaccharide derivatives prepared from a starch hydrolysate were used in the search for a satisfactory l.c. system. A hypersil column, modified with 1,6-diaminohexane, and a linear gradient (80→60%) of aqueous acetonitrile gave an excellent separation of the oligosaccharide derivatives from mono- to dodeca-saccharide in 20 min. Fig. 1 shows the result obtained from a total sample weight of 4 μg using the least-sensitive setting of the fluorescence detector, with excitation at 230 nm and a 340-nm cut-off emission filter.

Since 2,5-anhydro-D-mannose is known[12] to be a somewhat labile compound, the yield of its pyridylamino derivative was determined by l.c. using 1-deoxy-1-(2-pyridylamino)-D-glucitol as the internal standard. The yield, after isolation *via*

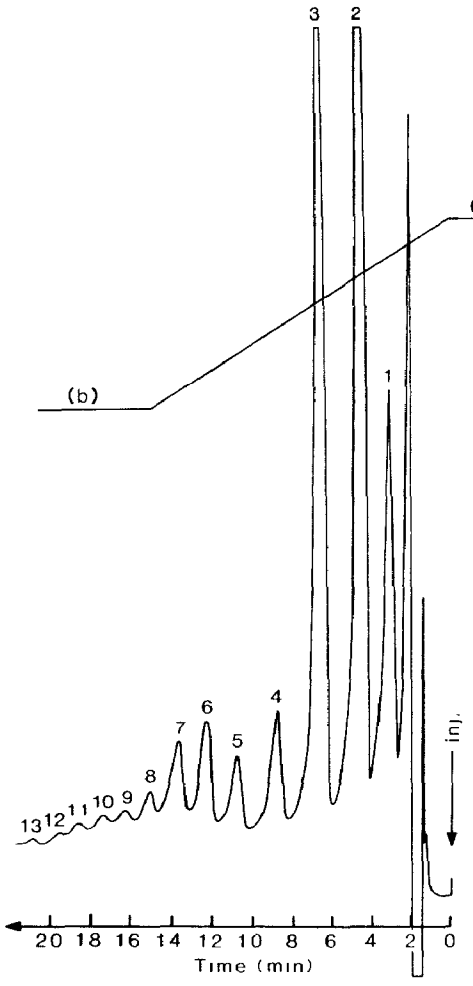
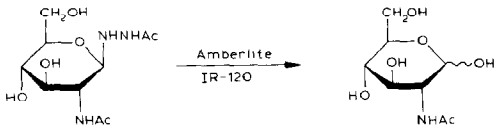


Fig. 1. L.c of the 1-deoxy-1-(2-pyridylamino)alditol derivatives of malto-oligosaccharides from starch The line (a)–(b) represents the solvent gradient (see Experimental).

preparative paper electrophoresis, was 92% based on 2-amino-2-deoxy-D-glucose hydrochloride. When the oligosaccharides derived from glycoproteins or glycopeptides by hydrazinolysis are nitrosated[13], the fate of the terminal 2-amino-2-deoxy-D-glucose hydrazone moiety is of interest. Nitrosation of 2-amino-2-deoxy-D-glucose hydrazone followed by reductive amination gave 2,5-anhydro-1-deoxy-1-(2-pyridylamino)-D-mannitol in 68% yield. When the nitrosation product was reduced with sodium cyanoborohydride, g.l.c.–m.s. of the trimethylsilyl ethers revealed glycerol as a minor product. This must have arisen by fragmentation of the intermediate diazonium ion to give glyceraldehyde and malonaldehyde. Reduction of the latter compound would give propane-1,3-diol, a trace of which was provisionally identified by g.l.c. It is likely that much of the malonaldehyde would not survive the conditions of nitrosation.

When reductive amination is applied to the glycan-amines that result from the hydrazinolysis of glycoproteins or glycopeptides, the amine constituents are first *N*-acetylated. 2-Amino-2-deoxy-D-glucose hydrazone was used as a model compound to determine the efficiency of the reductive amination and the fate of the hydrazone group. When 2-amino-2-deoxy-D-glucose hydrazone was *N*-acetylated using acetic anhydride in aqueous methanol containing sodium acetate, the product, after deionisation, was found by ^{13}C-n.m.r. analysis to be 2-acetamido-2-deoxy-D-glucose containing a little 1-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-acetylhydrazine (**3**). The latter compound was prepared from 2-acetamido-2-deoxy-D-glucose and acetylhydrazine.

When **3** was exposed to the conditions of *N*-acetylation and recovered by preparative paper electrophoresis without any contact with a cation-exchange resin, no change in structure had occurred as shown by ^{13}C-n.m.r. spectroscopy. When exposed to a cation-exchange resin (strong acid type), **3** was rapidly hydrolysed to 2-acetamido-2-deoxy-D-glucose. This hydrolysis of the hydrazone (or hydrazine) group is very desirable when reductive amination is to be used to convert the saccharides into fluorescent derivatives for further analysis, because **3** is not efficiently derivatised; the yield of the pyridylamino derivative **1** was only 30%.



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The oligosaccharide derivatives prepared from IgM glycopeptide by sequential hydrazinolysis, *N*-acetylation, and reductive amination were shown by l.c. analysis to be a very complex mixture (Fig. 2). A simpler mixture, which also provides structural information, is obtained by nitrosation of the oligosaccharide hydrazones released from IgM glycopeptide by hydrazinolysis[13]. Reductive amination of the nitrosation products and l.c. analysis gave the result shown in Fig. 3. The

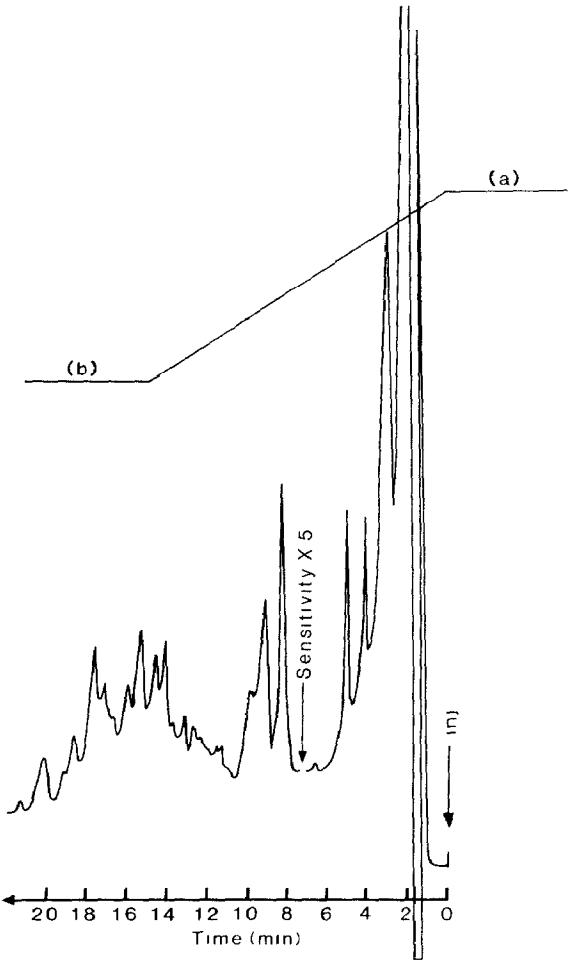


Fig. 2. L.c. of the pyridylamino derivatives of oligosaccharides from IgM glycopeptide.

tetrasaccharide with retention time (T) of 9.4 min could be assigned the structure **4** which arose from the $(Man)_2Man$-GlcNAc-GlcNAc core; the latter is a common feature in the oligosaccharides which are present in IgM and in other glycoproteins[14]. The only monosaccharide released in the nitrosation step was 2,5-anhydro-D-mannose (T 3.1 min), and two disaccharides (D-galactosyl-2,5-anhydro-D-mannose and L-fucosyl-2,5-anhydro-D-mannose) co-eluted (T 4.0 min). These disaccharide derivatives were separated under different l.c. conditions (Fig. 4). The smaller peak (2) was assigned to the fucosylanhydromannose (*cf.* ref. 13). Peak 3 represented the galactosylanhydromannose, and peak 1 represented 2,5-anhydro-D-mannose.

Finally, there is an additional useful feature of the reductive amination procedure when applied to the products of the hydrazinolysis of glycoproteins or

Fig. 3. L.c. of the pyridylamino derivatives of oligosaccharides formed by nitrosation of IgM oligosaccharides.

glycopeptides, or to their nitrosation products. The degradation products that can be formed in the hydrazinolysis give either very poor yields or no products in the reductive amination. 2-Amino-2-deoxy-D-glucose hydrazone is partially degraded to 1-deoxy-D-fructose hydrazone during hydrazinolysis, and subsequent nitrosation converts this into 1-deoxy-D-fructose[15]. Ketoses such as D-fructose gave a very poor yield of the pyridylamino derivative on reductive amination, and degradation products containing these end groups would therefore not be expected to be efficiently derivatised. The oligosaccharides released on hydrazinolysis of glycopeptides may contain a neutral aldose hydrazone as the terminal unit. The degradation of such a hydrazone gives either 1-deoxyketose hydrazone in the hydrazinium

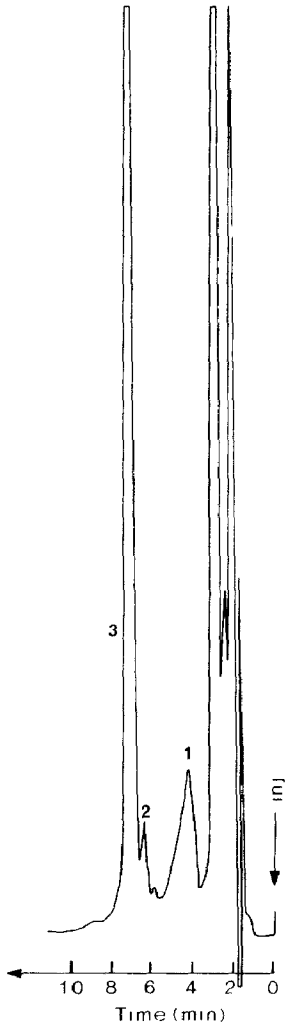
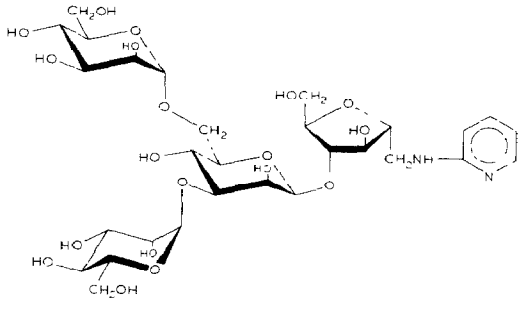


Fig. 4. As in Fig. 3, mono- to di-saccharide region.



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sulphate-catalysed hydrazinolysis, or a mixture of 1-deoxyalditol and 1,2-didehydro-1,2-dideoxyalditol in the uncatalysed hydrazinolysis[16]. The latter two degradation products would not react with 2-aminopyridine. Thus, oligosaccharides containing such residues would be excluded in the l.c. analysis, and such degradation products would interfere when the l.c. is carried out on underivatised oligosaccharides using a non-selective detector such as a refractometer.

EXPERIMENTAL

General. — Melting points were determined on a Kofler hot-stage and are corrected. N.m.r. spectra were recorded with Varian HA-100 (^{1}H) and XL-100 (^{13}C and ^{11}B) spectrometers on solutions in MeOD (internal Me_4Si) and D_2O (internal sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate). ^{11}B-N.m.r. spectra were obtained for solutions in 1,4-dioxane (external $BF_3.Et_2O$, coaxial cell). Analytical and preparative paper electrophoresis (p.e.) were performed at pH 3.5 with a pyridine–acetic acid–water (10:1:289) buffer at 2000 V and 15–30 mA for 1.5 h on Whatman No. 3 paper strips (57 cm long). Mass spectra were measured on an MS9 spectrometer.

Reductive aminations. — *(a) Preparative experiments.* *N,N*-Dimethylformamide (10 mL) containing 10% of glacial acetic acid was added to a mixture of D-glucose (1.0 g, 5.6 mmol), 2-aminopyridine (3.5 g, 37 mmol), and sodium cyanoborohydride (1.0 g, 16 mmol), and the mixture was heated at 95° for 3 h. P.e. then revealed three fluorescent spots; one corresponded to 2-aminopyridine and the other two had mobilities (M_A) relative to that of 2-aminopyridine of 0.55 and 0.0. Moist Amberlite IR-120 (H^+) resin (50 mL) was added to the solution and, when gas evolution had ceased (*caution:* HCN is formed), the mixture was transferred to a column containing more resin (150 mL). Elution of the resin with water (1.5 L) and concentration of the eluate gave a white solid which, on recrystallisation from water, afforded (2-amino-1-pyridyl)cyanoborane (30 mg), m.p. 101–102° (see below for identification).

Elution with 0.7M ammonia (1.5 L) and concentration of the eluate gave a solid (1.40 g), recrystallisation of which from ethanol gave 1-deoxy-1-(2-pyridylamino)-D-glucitol (1.21 g, 84%), m.p. 174–175°, $[\alpha]_D^{20}$ +67.5° (*c* 4.1, water); lit.[8] m.p. 173–174°; $\lambda_{max}^{H_2O}$ 237 nm (ε 11,500), emission max 391 nm (phosphate buffer, pH 3.8). ^{13}C-N.m.r. data (D_2O): δ 47.1 (C-1')*, 65.6 (C-6'), 73.3, 74.2 (×2), 74.6 (C-2',3',4',5'), 111.9 (C-3), 116.2 (C-5), 141.5 (C-4), 149.4 (C-6), and 161.4 (C-2). Mass spectrum: *m/z* 258 (M^+, 0.7%), 227 (0.9), 197 (1.4), 167 (2.4), 137 (26), and 107 (100).

2-Acetamido-2-deoxy-D-glucose was similarly derivatised to give 2-acetamido-1,2-dideoxy-1-(2-pyridylamino)-D-glucitol (77%), m.p. 162–163°, $[\alpha]_D^{20}$

*Primed locants refer to the numbering of the alditol moiety.

+47° (*c* 1.3, water), M_A 0.47 (fluorescent); $\lambda_{max}^{H_2O}$ 238 nm (ε 15,016). N.m.r. data (D_2O): ^1H, δ 1.94 (s, 3 H, Ac), 3.26–4.00 (m, 7 H), 4.3 (m, 1 H, H-2'), 6.74 (m, 2 H, H-3,5), 7.6 (m, 1 H, H-4), and 8.3 (m, 1 H, H-6); ^{13}C, δ 24.8 (CH_3), 45.2 (C-1'), 54.4 (C-2'), 65.4 (C-6'), 72.0, 74.1, 74.4, 112.0, 116.3, 141.5, 149.5, 161.4, and 177.1 (C=O). Mass spectrum: *m/z* 299 (M^+, 0.4%), 281 (0.7), 268 (0.4), 250 (0.6), 220 (1.2), 208 (1.0), 190 (1.2), 178 (2.6), 160 (9.9), 149 (17), and 107 (100).

Anal. Calc. for $C_{13}H_{21}N_3O_5$: C, 52.2; H, 7.0; N, 14.0. Found: C, 52.2; H, 7.4; N, 13.8.

The pyridylamino derivative of 2,5-anhydro-D-mannose was prepared as follows. A solution of 2-amino-2-deoxy-D-glucose hydrochloride (1.08 g, 5 mmol) and sodium nitrite (1.38 g, 20 mmol) in water (10 mL) was cooled in an ice-bath, and glacial acetic acid (0.84 mL) was added in three portions during 30 min. The solution was allowed to warm to room temperature (1 h), purged with N_2 (20 min), and concentrated to dryness under reduced pressure at 35°. The residue was heated with 2-aminopyridine (3 g, 32 mmol), sodium cyanoborohydride (0.8 g, 12.7 mmol), and acetic acid (0.8 mL) in *N,N*-dimethylformamide (10 mL) at 95° for 3 h. Isolation of the product via a cation-exchange resin, as described above, gave a syrup (1.5 g) which was shown by p.e. to contain 2-aminopyridine as well as the required derivative (M_A 0.48). Flash chromatography of an aliquot (1 g) on a column (6 × 15 cm) of Kieselgel 60 using ethanol gave a partial separation of 2-aminopyridine from 2,5-anhydro-1-deoxy-1-(2-pyridylamino)-D-mannitol which was obtained (0.515 g, 64%) free of 2-aminopyridine. ^{13}C-N.m.r. data (D_2O): δ 46.0 (C-1'), 64.0 (C-6'), 79.5, 81.1, 83.8, 85.4 (C-2',3',4',5'), 111.9 (C-3), 116.1 (C-5), 141.4 (C-4), 149.0 (C-6), and 161.0 (C-2). Mass spectrum: *m/z* 240 (0.1%, M^+), 205 (0.5), 191 (0.9), 133 (2.8), 107 (100), 94 (38), and 78 (44).

Reductive amination of lactose gave a crude solid product contaminated with 2-aminopyridine, and recrystallisation from water gave *N*-(2-pyridyl)-β-lactosylamine (45%) as the monohydrate, m.p. 217–219°, M_A 0.39; $\lambda_{max}^{H_2O}$ 233 nm (ε 12,236). ^{13}C-N.m.r. data (D_2O): δ 62.9, 63.9 (C-6',6''), 71.4, 73.8, 75.0, 75.4, 78.2 (×2), 78.3, 81.1, 85.1 (C-1'), 105.7 (C-1''), 112.5 (C-3), 118.2 (C-5), 142.0 (C-4), 149.7 (C-6), and 159.6 (C-2).

Anal. Calc. for $C_{17}H_{26}N_2O_{10} \cdot H_2O$: C, 46.8; H, 6.4; N, 6.4. Found: C, 46.6; H, 6.5; N, 6.0.

The residue (0.50 g) from the mother liquor was subjected to flash chromatography on Kieselgel 60 using ethanol to give 1'-deoxy-1'-(2-pyridylamino)lactitol as a syrup (0.45 g), free from 2-aminopyridine and the lactosylamine. ^{13}C-N.m.r. data (D_2O): δ 46.9 (C-1'), 63.3, 64.7 (C-6',6''), 71.1, 73.0 (×2), 73.7, 74.0, 75.2, 77.6, 82.4, 105.7, (C-1''), 111.9 (C-3), 115.9 (C-5), 141.3 (C-4), 149.0 (C-6), and 161.1 (C-2).

Anal. Calc. for $C_{17}H_{28}N_2O_{10}$: C, 48.6; H, 6.7; N, 6.7. Found: C, 48.7; H, 7.0; N, 6.4.

It is important to avoid using sodium cyanoborohydride which had deteriorated on storage. When the above experiment was repeated using a new batch of

reducing agent, ^{13}C-n.m.r. analysis of the product showed that reduction was complete.

(b) Small-scale experiments. A mixture of malto-oligosaccharides (1 mg, from starch hydrolysate*) was reductively aminated with 2-aminopyridine (5 mg) and sodium cyanoborohydride (1.5 mg) in *N,N*-dimethylformamide (0.2 mL) containing 10% of glacial acetic acid at 95° for 3 h. The products were isolated by preparative p.e., and a solution of the products in water (1 mL) was used for l.c. (see below).

2,5-Anhydro-D-mannose generated[13] from 2-amino-2-deoxy-D-glucose hydrochloride (5.12 mg) by nitrosation was isolated together with inorganic salts by concentration at 35° under reduced pressure of the reaction mixture after neutralisation with sodium carbonate. The resulting residue was reductively aminated with 2-aminopyridine (36 mg) and sodium cyanoborohydride (8 mg), and 1-deoxy-1-(2-pyridylamino)-D-glucitol (7.43 mg) was then added as the internal standard. One fifth of the solution was subjected to preparative p.e. and the fluorescent derivatives were isolated. A solution of the product in water (0.5 mL) was analysed by l.c. (see below), and the yield of the 2,5-anhydro-D-mannose derivative was found to be 92% using the response factor determined from standard mixtures.

The yield of the 2,5-anhydro-D-mannose derivative formed by sequential nitrosation and reductive amination of 2-amino-2-deoxy-D-glucose hydrazone was 68%. When the nitrosation product was reduced with sodium cyanoborohydride and subjected to g.l.c.–m.s. after trimethylsilylation, glycerol was identified as a minor product. Propane-1,3-diol was provisionally identified as a very minor product by g.l.c., but other minor products were not identified.

The oligosaccharide hydrazones (200 μg) isolated from IgM glycopeptide by hydrazinolysis were *N*-acetylated, using 4.5M sodium acetate (150 μL) and MeOH (80 μL) to which acetic anhydride (5.2 μL) was added in 5 portions during 1 h at room temperature. After 75 min, the solution was deionised with AG 50W-X2 (H^+) resin (3.5 mL). The resin was washed with water, and the eluate (30 mL) was extracted with ether and freeze-dried. The residue was reductively aminated with 2-aminopyridine (2.0 mg) and sodium cyanoborohydride (1.0 mg) in *N,N*-dimethylformamide (100 μL) containing 10% of acetic acid. A solution of the product (isolated as above) in water (500 μL) was analysed by l.c.

The oligosaccharide hydrazones (1.1 mg) from IgM glycopeptide were also nitrosated and the products were analysed by l.c. of the pyridylamino derivatives.

L.c. analysis. — The pyridylamino derivatives were analysed on a hypersil column (25 × 0.49 cm, 5 μ) which had been modified by equilibration with 50% aqueous acetonitrile (500 mL) containing 0.1% of 1,6-diaminohexane. For most analyses, a linear gradient from 80→60% aqueous acetonitrile during 15 min was used; all solvents contained 1,6-diaminohexane (0.01%). The flow rate was 2 mL/min, and a Kratos FS 970 l.c. fluorometer was used with excitation at 230 nm and

*Donated by Dr. J. F. Kennedy.

emission above 340 nm (cut-off filter). For the separation of disaccharides (Fig. 4), isocratic elution with aqueous 85% acetonitrile containing the diamine (0.01%) was used. For the quantitative measurements, isocratic elution with aqueous 80% acetonitrile containing the diamine (0.01%) and u.v. detection at 230 nm were used. Under these conditions, the pyridylamino derivatives of 2,5-anhydro-D-mannose and D-glucose had retention times of 2.1 and 2.8 min respectively.

1-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-2-acetylhydrazine (**3**). — A mixture of 2-acetamido-2-deoxy-D-glucose (2.0 g, 9.05 mmol) and acetylhydrazine (1.5 g, 20 mmol) in aqueous 95% ethanol (50 mL) and water (5 mL) was boiled under reflux overnight. T.l.c. (1:1 methanol–ethyl acetate) then showed that the reaction was complete (product R_F 0.31). Concentration of the solution gave a syrup which was crystallised from ethanol (2 mL) to give **3** (1.10 g, 44%), m.p. 60–61° (uncorr.). N.m.r. data (D_2O): ^1H, δ 4.23 (d, 1 H, *J* 9 Hz, H-1), 3.4–4.0 (m, 6 H), 2.04, and 1.93 (2 s, each 3 H, 2 Ac); ^{13}C, δ 22.8, 25.0 (Me), 54.9, 63.5, 72.5, 77.4, 79.6, 91.1 (C-1), 175.3, and 177.1 (C=O).

Anal. Calc. for $C_{10}H_{19}N_3O_6$: C, 43.3; H, 6.9; N, 15.1. Found: C, 43.1; H, 7.2; N, 14.4.

N-*Acetylation of 2-amino-2-deoxy-D-glucose hydrazone.* — A solution of 2-amino-2-deoxy-D-glucose hydrochloride (0.45 mmol) in hydrazine hydrate (2 mL) was kept overnight and then concentrated to a solid residue in a desiccator over P_2O_5 under reduced pressure. The residue was *N*-acetylated in aqueous MeOH containing sodium acetate, as described above for the oligosaccharide hydrazones. Deionisation was achieved using Amberlite IR-120 (H^+) and IRA-400 (AcO^-) resins. The residue after freeze-drying was 2-acetamido-2-deoxy-D-glucose [^{13}C-n.m.r. data (D_2O): δ 93.4 (C-1α), 97.6 (C-1β)] with the *N*-acetylated hydrazine **3** as a minor constituent [^{13}C-n.m.r. data: δ 91.1 (C-1); signals at δ 22.4 and 175.4 were assigned to 1,2-diacetylhydrazine, which originated from hydrazine that was difficult to remove completely from the hydrazone].

The *N*-acetylated hydrazine **3** was exposed to the above conditions of *N*-acetylation and then recovered by preparative p.e., using a pyridine–acetic acid buffer of pH 5.4 to avoid any hydrolysis. That no change in structure had occurred was indicated by ^1H- and ^{13}C-n.m.r. spectroscopy which also detected some acetic acid. When an aqueous solution of **3** was passed through Amberlite IR-120 (H^+) resin, hydrolysis occurred to give 2-acetamido-2-deoxy-D-glucose.

The *N*-acetylated hydrazine **3** was subjected to reductive amination and *N*-(2-pyridyl)-β-lactosylamine was added as the internal standard. The yield of 2-acetamido-1,2-dideoxy-1-(2-pyridylamino)-D-glucitol was 30% (determined by l.c. using u.v. detection).

(2-Amino-1-pyridyl)cyanoborane. — A solution of 2-aminopyridine (0.5 g, 5.32 mmol), sodium cyanoborohydride (0.335 g, 5.32 mmol), and 12M hydrochloric acid (0.44 mL) in tetrahydrofuran (20 mL) was stirred under reflux at 80° for 3 h. The solid was collected and washed with tetrahydrofuran (10 mL), and the combined filtrate and washings were concentrated to give a syrup which crystallised

upon trituration with water (5 mL) to yield the title compound (0.276 g, 40%), m.p. 102°; ν_{max}^{KBr} 3450, 3350, 3230 (N-H), 2400, 2430 (B-H), 2180 (C≡N), and 765 cm^{-1} (B-N). N.m.r. data (CD_3OD): ^{13}C, δ 113.6, 114.0 (C-3, C-5), 142.5 (C-4), 145.0 (C-6)*, and 157.8 (C-2); a singlet at δ 133.9 (CN) was detected when the measurement was performed at −60° with a 5-s pulse delay; ^{11}B (1,4-dioxane), δ 20.8 (t, J_{BH} 90 Hz); ^1H (CD_3OD), δ 7.91 (bd, 1 H, H-6), 7.66 (m, 1 H, H-4), 6.86 (bd, 1 H, H-3), 6.70 (m, 1 H, H-5), 1–4 [vb, BH_2; at −60°, the BH_2 signal was much narrower at δ 2.5 ($W_{1/2}$ 50 Hz)]; for a solution in CD_3SOCD_3, the NH_2 signal (bs) was detected at δ 7.3. Mass spectrum: *m/z* 133 (11%), 132 (32), 131 (12), 106 (51), 105 (100), 104 (26), 94 (57), 79 (45), 78 (59), and 67 (25) (Calc. for $C_6H_7BN_3$: 132.0733. Found 132.0737. Calc. for $C_5H_6BN_2$, 105.0624. Found: 105.0628). Reaction with nitrous acid gave nitrogen.

Anal. Calc. for $C_6H_8BN_3$: C, 54.1, H, 6.0; N, 31.6. Found: C, 54.2; H, 6.2; N, 31.8.

This compound was identical to the neutral fluorescent by-product formed in all reductive aminations.

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REFERENCES

1 R. SCHWARZENBACH, *J. Chromatogr.*, 117 (1976) 206–210.
2 F. M. RABEL, A. G. CAPUTO, AND E. T. BUTTS, *J. Chromatogr.*, 126 (1976) 731–740.
3 K. AITZETMULLER, B. B. WHEALS, AND P. C. WHITE, *J. Chromatogr.*, 176 (1979) 421–426.
4 C. A. WHITE, P. H. CORRAN, AND J. F. KENNEDY, *Carbohydr. Res.*, 87 (1980) 165–173.
5 J. G. BAUST, R. E. LEE, JR., R. R. ROJAS, D. L. HENDRIX, D. FRIDAY, AND H. JAMES, *J. Chromatogr.*, 261 (1983) 65–75.
6 P. F. DANIEL, D. F. DEFEUDIS, I. T. LOTT, AND R. H. MCCLUER, *Carbohydr. Res.*, 97 (1982) 161–180.
7 S. HASE, T. IKENAKA, AND Y. MATSUSHIMA, *J. Biochem. (Tokyo)*, 90 (1981) 407–414.
8 S. HASE, S. HARA, AND Y. MATSUSHIMA, *J. Biochem. (Tokyo)*, 85 (1979) 217–220.
9 B. R. GRAGG, W. J. LAYTON, AND K. NIEDENZU, *J. Organomet. Chem.*, 132 (1977) 29–36.
10 H. C. BROWN AND J. A. SODERQUIST, *J. Org. Chem.*, 45 (1980) 846–849.
11 B. R. GRAGG AND K. NIEDENZU, *J. Organomet. Chem*, 117 (1976) 1–11.
12 A. B. GRANT, *N. Z. J. Sci. Technol.*, B37 (1956) 509–521.
13 P. I. CLARK, S. NARASIMHAN, J. M. WILLIAMS, AND J. R. CLAMP, *Carbohydr. Res.*, 118 (1983) 147–155.
14 J. MONTREUIL, *Adv. Carbohydr. Chem. Biochem.*, 37 (1980) 170–182.
15 P. W. TANG AND J. M. WILLIAMS, *Carbohydr Res*, 121 (1983) 89–97.
16 J. M. WILLIAMS, *Carbohydr Res*, 128 (1984) 73–78.

*Assigned by specific decoupling.