Carbohydrate Research, 201 (1990) 135–144
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

1-Alkoxyamino-1-deoxy alditols, useful u.v.-absorbing derivatives of neutral and acidic oligosaccharides

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(Received July 26th, 1989; accepted for publication November 15th, 1989)

ABSTRACT

The feasibility of using 1-alkoxyamino-1-deoxy alditol derivatives to facilitate the purification and structural characterization of oligosaccharides was examined. 1-Benzyloxyamino-1-deoxy, 1-deoxy-1-*p*-nitrobenzyloxyamino, and 1-deoxy-1-[4-(*p*-nitrophenyl)butoxyamino] alditol derivatives of mono- and oligo-saccharides were prepared by reduction of the corresponding *O*-substituted oximes with sodium cyanoborohydride. The 1-alkoxyamino-1-deoxy alditol derivatives were separated by h.p.l.c. and characterized by ^{1}H-n.m.r. spectroscopy and f.a.b.-m.s. Per-*O*-methylation of the 1-benzyloxyamino-1-deoxy and 1-deoxy-1-*p*-nitrobenzyloxyamino alditol derivatives resulted in the elimination of a benzaldehyde moiety and formation of a quaternary ammonium salt. The positive mode f.a.b. mass spectra of these cationic per-*O*-methylated derivatives of oligosaccharides included very intense molecular ion signals, suggesting the possibility of a significant reduction in the amount of per-*O*-methylated oligosaccharide required for f.a.b.-m.s. analysis. Because the ionic nature of the derivatives hinders their analysis by g.c.-m.s. a new reagent, 4-(*p*-nitrophenyl)butoxyamine, was synthesized and used to prepare the 1-deoxy-1-[4-(*p*-nitrophenyl)butoxyamino] alditol derivatives of several mono- and oligo-saccharides. These could be separated by h.p.l.c., with detection by u.v. absorption, and they were stable under *O*-methylation conditions. The per-*O*-methylated derivatives of oligosaccharides can be analyzed by e.i.-m.s. as well as f.a.b.-m.s. *O*-Methylated 1-deoxy-1-[4-(*p*-nitrophenyl)butoxyamino] alditol derivatives of monosaccharides are amenable to processing by g.c.–m.s., allowing standard methylation analysis of derivatized oligosaccharides to be performed. Thus, the separation and structural analysis of oligosaccharides are facilitated by the physical and chemical properties (u.v. absorption, ease of separation by h.p.l.c., stability to methylation and hydrolysis conditions, and ease of analysis by g.c.–m.s. and f.a.b.-m.s.) imparted to oligosaccharides by forming the 1-deoxy-1-[4-(*p*-nitrophenyl)butoxyamino] alditol derivatives.

INTRODUCTION

A number of procedures for attaching u.v.-absorbing or fluorescent chromophores to carbohydrates have been developed in order to enhance the detection of carbohydrates after chromatographic separation[1–13]. The goal of such derivatization procedures is to decrease the amount of oligosaccharide that can be detected and to allow the use of solvent gradients during column elution. The most commonly used detection method, refractive-index, is incompatible with gradient elution. The ideal

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0008-6215/90/$03.50

derivative would also improve the chromatographic separation properties of oligosaccharides and enhance the sensitivity of their analysis by mass spectrometry. Methods which have been developed to achieve these aims include derivatization of the reducing end of oligosaccharides with ethyl *p*-aminobenzoate[1] or 2-aminopyridine, either by reductive amination[1-5] or by formation of aminoglycosides[6]. Methods involving the reaction of the reducing end with other nitrogen-containing compounds to form amines[7,8], aminoglycosides[9], hydrazones[10,11], or oximes[12,13] have also been described. However, none of these methods has all of the following desired attributes: *(i)* the derivatization should proceed in high yields for acidic as well as neutral oligosaccharides; *(ii)* each derivatized oligosaccharide should generate only one product; and *(iii)* the derivatized oligosaccharides, after chromatographic separation, should be amenable to standard analysis for glycosyl-linkage composition, *i.e.* the derivative has to be stable under alkaline methylation and acidic hydrolysis conditions. We now report on the preparation and characterization of 1-alkoxyamino-1-deoxy alditols, a new class of u.v.-absorbing derivatives of oligosaccharides that exhibits the above attributes.

RESULTS AND DISCUSSION

Preparation of hydroxylamine derivatives of mono- and oligo-saccharides. — The reaction of reducing sugars with *O*-substituted hydroxylamines was reported to yield the corresponding oximes in 10–20 minutes[13]. The utility of these derivatives during l.c. separations and u.v. detection was described[13]. However, the synthesis of oxime sugar derivatives has the disadvantage that both *syn* and *anti* isomers are formed, which complicates chromatographic separations. In order to obtain a single and more stable derivative, we converted the *O*-substituted oximes of sugars into 1-alkoxyamino-1-deoxy alditols by reduction with sodium cyanoborohydride. Both reactions of this two-step procedure can be carried out in a mixture of water and organic solvents (pyridine and methanol). Therefore, oligosaccharides that are not soluble in pure organic solvents can be derivatized.

The scope and limitations of the reduced-oxime method of labeling the reducing ends of saccharides were evaluated using standard carbohydrates, including glucose (**1**) and maltotriose (**2**). The reduction conditions (such as pH) were varied, and the reaction products were visualized by t.l.c. Quantitative reduction was obtained for all the alkoxyamino derivatives of neutral mono- and oligo-saccharides tested when the reaction mixture was kept near pH 3 by occasional additions of aqueous hydrochloric acid. The reductive amination of 2-aminopyridine derivatives is not quantitative under the same conditions[4]. The reaction of galacturonic acid-containing oligosaccharides with alkoxyamines yielded multiple products, probably lactones and methyl esters. However, after evaporation of the alkaline eluate from the cation exchange resin used for sample cleanup (see below), the galacturonic oligosaccharides were recovered as single products, as shown by h.p.l.c. Further work is required to determine whether the yields of these derivatives are reproducible enough to permit their use for accurate quantitation of acidic oligosaccharides.

The 1-benzyloxyamino-1-deoxy alditol derivative (**3**) of maltotriose was detected in l.c. effluents by u.v. absorbance at 254 nm. In order to improve u.v. sensitivity, the corresponding *p*-nitrobenzyl (PNBzl) derivative **4** was prepared by the same reaction sequence, providing a chromophore with a greater molar extinction coefficient[5,14].

The recovery of derivatized oligosaccharide alditols, after methylation and subsequent acid-catalyzed hydrolysis of their glycosidic linkages, would permit their use during glycosyl-linkage analysis. To evaluate the stability of the u.v.-absorbing label towards standard acid hydrolysis conditions **4** was treated for 30 min with M trifluoroacetic acid, and the u.v.-absorbing product was shown to be identical to the PNBzl derivative of glucose by l.c. However, methylation of either **3** or **4** under the conditions described by Hakomori[15] did not give the expected products. This was surprising, because no problems had been reported during the methylation analysis of 1-deoxy-1-methoxyamino alditols[16]. F.a.b.-m.s. analysis of the products formed by methylation of **3** or **4** showed a very intense ion at *m/z* 702, which is consistent with structure **7**. Although not intended in this case, the formation of **7** may offer a way to improve the ion intensity in positive-mode f.a.b. mass spectrometry of methylated oligosaccharides.

The loss, during methylation, of the aromatic moieties of **3** and **4** was confirmed by the following reaction sequence. Selective *N*-acetylation of **3** and **4** yielded **8** and **9**,

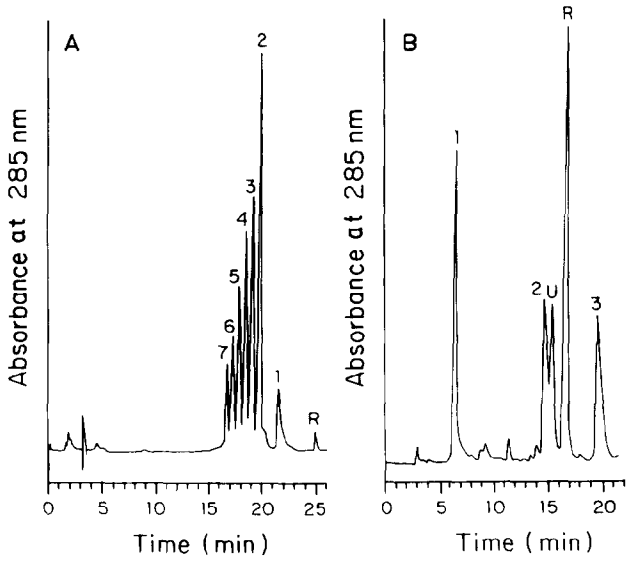


Fig. 1.H.p.l.c. separation of 4-(*p*-nitrophenyl)butoxyamino derivatives. Column, LiChrospher 100 CH-18 (250 × 5 mm, 5 μm); u.v. detection at 285 nm; flow rate 1 mL.min^{-1}; linear gradient over 20 minutes. *A*, PNPBut derivatives of maltodextrins. Peak 1 = glucose, 2 = maltose, 3–7 = maltotriose–maltoheptaose, R = reagent. Gradient elution from 9.5 to 40% aqueous CH_3CN, mixture 5mM in NH_4OAc. *B*, Mixture of the PNPBut derivatives of neutral and acidic oligosaccharides. Peak 1 = maltotriose, 2 = digalactosyluronic acid, 3 = trigalactosyluronic acid, R = reagent, U = unknown. Gradient elution from 26 to 43% aqueous CH_3CN, mixture 5mM in tetrabutylammonium phosphate.

respectively. Methylation of **8** and **9** yielded, in both cases, product **12**, which was analyzed by f.a.b.-m.s. The resulting spectrum of **12** included the expected $(M + Na)^+$ ion at *m/z* 738.

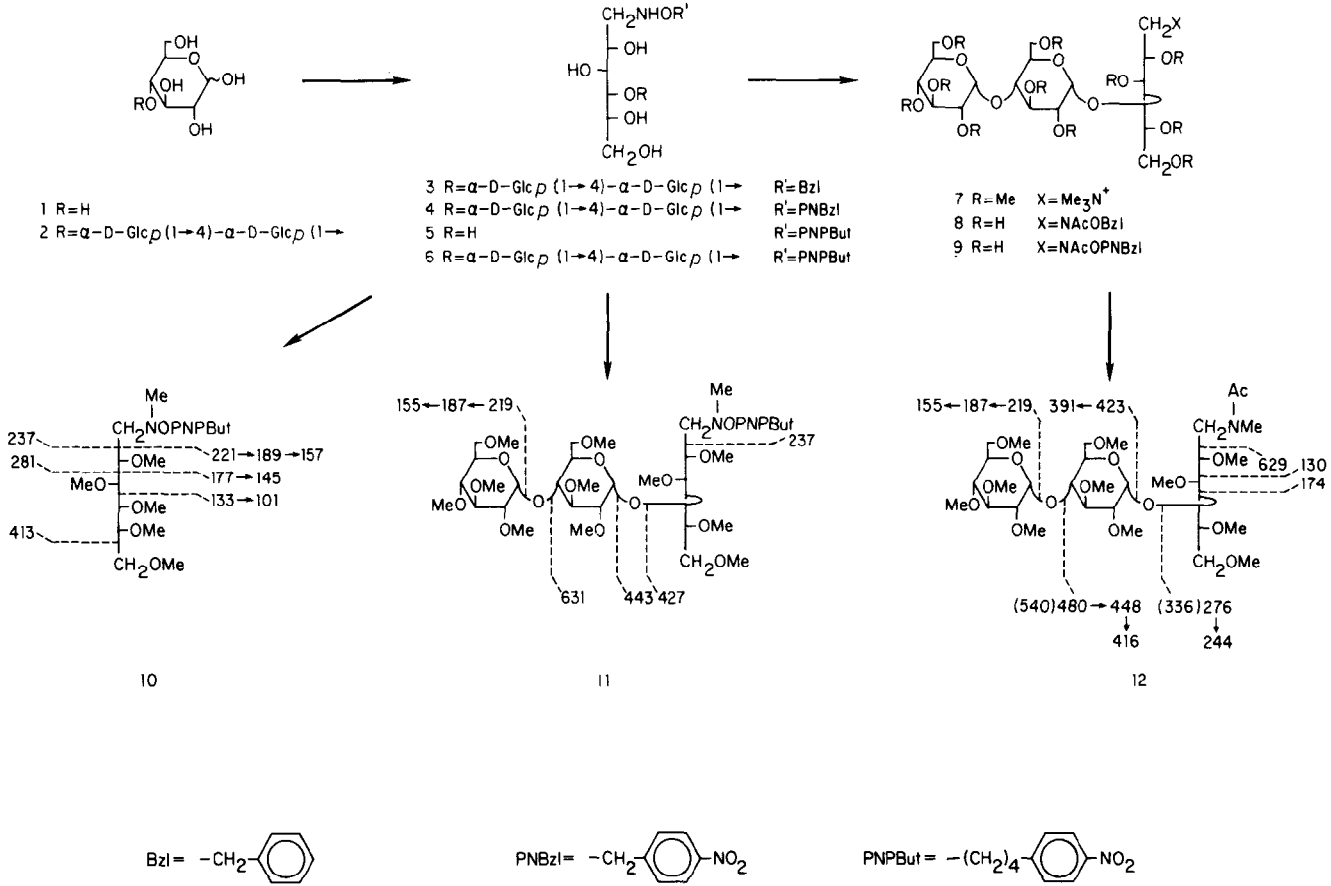
Product **12**, unlike **7**, was volatile and was therefore analyzed by g.l.c.-m.s. (e.i.). The observed fragmentations are depicted in Scheme 1. These included J-fragments[17] (*m/z* 540 = abJ_1, 480 = abJ_2, 336 = aJ_1, 276 = aJ_2) and some secondary-fragment ions (*m/z* 448, 416, 244) formed by the sequential elimination of methanol from J-fragments. Those ions, as well as the fragment ions generated by alditol cleavages (*m/z* 130, 174), contained the *N*-acetyl function. A-fragments, usually obtained from permethylated oligosaccharide alditols, were also present (*m/z* 219, 187; 423, 391) in the e.i. mass spectrum. There was no evidence of aromatic groups in any fragment ions.

The fact that both **3** and **4** yielded the same product after methylation indicated that the loss of the aromatic moiety was not caused by the *p*-nitro function. Rather, **7** and **12** are probably formed by abstraction of a benzylic proton under the strongly basic methylation conditions, leading to the elimination of a benzaldehyde (or *p*-nitrobenzaldehyde) moiety.

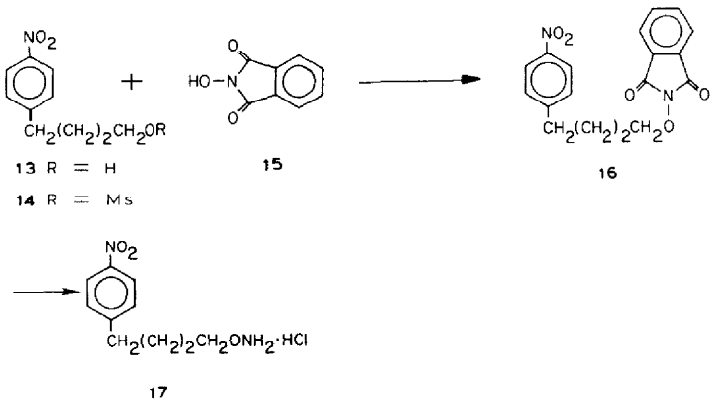
Two approaches were undertaken to test the foregoing hypothesis. We first attempted, unsuccessfully, to synthesize *O*-[1-methyl-1-(*p*-nitrophenyl)ethyl]hydroxylamine, in which the benzylic protons are replaced by methyl groups. We did succeed in preparing, by standard procedures[18], a derivatizing reagent [4-(*p*-nitrophenyl)butoxyamine hydrochloride (**17**) having an alkyl chain between the aromatic ring and the hydroxylamine function. The synthesis of **17** (Scheme 2) involved the conversion of commercially available 4-(*p*-nitrophenyl)butanol **13** into the mesylate **14**, which was reacted with *N*-hydroxyphthalimide (**15**) in the presence of base to form **16**. Cleavage of the phthalimide residue was achieved with hydrazine hydrate. The product was converted into the hydrochloride **17**.

Reductive alkoxyamination of glucose and maltotriose with **17** yielded the desired *p*-nitrophenylbutoxyamino (PNPBut) derivates **5** and **6** (Scheme 1). The identity of **5** was confirmed by ^{1}H-n.m.r. The nitrophenyl chromophore allowed sensitive detection of these derivatives by their u.v. absorption at 285 nm. Methylation of **5** and **6** according to Hakomori [15] led to dark-colored solutions and complex mixtures of products. However, a modified procedure using sodium hydroxide–dimethyl sulfoxide[19] gave the desired methylation products **10** and **11** (Scheme 1). The elimination of the benzaldehyde moiety from PNBzl derivatives observed under strongly basic methylation conditions does not occur with PNPBut compounds.

The derivative **10** and the corresponding derivatives of other monosaccharides are suitable for g.l.c.-m.s. analysis. In view of the low volatility of the oligosaccharide derivative **11**, its mass spectrum could best be obtained using the direct-insertion-probe election-impact (e.i.) technique. The mass spectra of such permethylated [*N*-methyl-4-(*p*-nitrophenyl)butoxyamino] alditols contain a dominant and diagnostic fragment ion at *m/z* 237, derived from the former reducing end including C-1. Other diagnostic ions are formed by the loss of 269 amu (*i.e.*, 237 + 32) from the molecular ion $(M)^+$, and are present in the e.i. mass spectra of the permethylated PNPBut derivatives of hexoses (*m/z*



Scheme 1. Reductive alkoxyamination of glucose and maltotriose and subsequent conversion of the products. Fragment ions obtained by e.i.-m.s. are given for the methylated products **10**, **11**, and **12**. J_1 ions are in parentheses.



Scheme 2.

189), deoxyhexoses (*m/z* 159), and pentoses (*m/z* 145). Chemical ionization (c.i.)-m.s. of **10** using methane gave the pseudomolecular $(M + H)^+$ ion at *m/z* 459. The only abundant ions in the high mass range of the e.i.-mass spectrum of **11** are those containing the PNPBut moiety [*m/z* 821 $= (M - 45)^+$, 631 = abJ_2]. The ion at *m/z* 443 is formed by cleavage of the *O*-glycosidic linkage at the glycosyl side. A similar cleavage has been described by Chaves das Neves and Riscado for permethylated deoxy(methoxyamino)alditol glycosides[16].

The *p*-nitrobenzyloxyamino and *p*-nitrophenylbutoxyamino derivatives are both useful u.v.-absorbing tags for saccharides. One may be more appropriate than the other, depending on whether the saccharide derivative is to be per-*O*-methylated, and how it is to be analyzed. The quarternary ammonium ion products (such as **7**) formed when PNBzl derivatives are methylated can be detected with very high sensitivity by positive-mode f.a.b.-m.s. On the other hand, when h.p.l.c. must be performed after methylation, the PNPBut derivative is more appropriate, because the chromophore is preserved, making it possible to detect the methylated products by u.v. absorption. Methylated PNPBut derivatives can be analyzed by either e.i.-m.s. or f.a.b.-m.s.

Liquid chromatography of PNPBut-alditol derivatives of oligosaccharides. — The PNPBut-alditol derivatives of oligosaccharides were partially purified prior to chromatography, in order to protect the h.p.l.c. columns. The comparatively small excess of reagent required to ensure complete reaction of the reducing glycoses facilitated this initial purification step, which involved applying the reaction mixture from the reductive alkoxyamination to a cartridge containing Dowex 50 cation-exchange resin. The loaded cartridge was washed with water to remove the borate salts. The derivatized products were eluted from the cartridge with M ammonia in 50% aqueous methanol, and further purified by passage through a C-18 cartridge. The alkoxyaminodeoxy alditol derivatives were eluted from the C-18 cartridge with a mixture of acetonitrile and water. The proportion of acetonitrile required depended on the size of the derivatized oligosaccharide and on the nature of the alkoxyamino part. The elution of PNPBut derivatives of neutral mono- and di-saccharides requires 30% aqueous acetonitrile, while the equivalent PNPBut derivatives of neutral trisaccharides and larger oligo-

saccharides are eluted with 20% aqueous acetonitrile. Because excess alkoxyamine reagent is eluted with 30% acetonitrile it is difficult to separate derivatized mono- and di-saccharides from the reagent using C-18 cartridges. Acidic oligosaccharides are eluted by 5–10% aqueous acetonitrile. Multiple samples are easily handled by using a vacuum manifold (Supelco) holding prepacked SCX ion-exchange and C-18 (Rainin) cartridges.

Attempts were made to separate the derivatives just described by l.c., using polar-bonded and reversed-phase columns. 1-Deoxy-1-(2-pyridylamino)alditol derivatives of oligosaccharides have been succesfully separated on amino-bonded columns[4]. In contrast, the PNPBut derivatives were not sufficiently well separated on amino- (Rainin Dynamax-60A NH_2) and diol- (LiChrospher DIOL) substituted silica columns. The peak shapes and resolution of the PNPBut derivatives were greatly improved with reversed-phase columns, but major differences were observed on the reversed-phase columns of different manufacturers (EM-Science, Beckman, Rainin, Phenomenex). Our best results were obtained with LiChrospher C-18 (EM-Science) and phenyl-bonded (Phenomenex) columns.

The separation of the PNPBut derivatives of mono- and oligo-saccharides is illustrated in Fig. 1. A mixture of malto-oligosaccharides (d.p. 1–7) was used to demonstrate the influence of molecular size on the separation (Fig. 1A). It should be noted that the order of elution is reversed from that of the underivatized oligosaccharides[20]. This provides evidence that the interaction between the PNPBut-derivatized oligosaccharides and the reversed-phase column material is enhanced by the nonpolar aromatic group. PNPBut derivatives of uronic acid-containing oligosaccharides elute, under the same conditions, as broad peaks. Good peak shape and separation of the PNPBut derivatives of both neutral and acidic oligosaccharides were achieved in the same chromatographic procedure (Fig. 1B) when a suitable amount (*e.g.* 5mM) of tetrabutylammonium phosphate, an ion-pair reagent[21], was added to the solvent. The PNPBut derivative of trigalactosyluronic acid eluted after the derivative of digalactosyluronic acid.

EXPERIMENTAL

Equipment. — H.p.l.c. was performed with either a Waters Model 6000A solvent delivery system controlled by a Model 660 solvent programmer or a Hewlett Packard 1090 liquid chromatograph. U.v. detection was accomplished with a Beckman UV163 variable-wavelength detector. Direct-insertion-probe e.i. mass spectra and g.c.-mass spectra were recorded with a Hewlett Packard 5985 mass spectrometer. F.a.b.-m.s. was performed using a VG Analytical ZAB-SE mass spectrometer. ^{1}H-n.m.r. spectra were recorded with either a Bruker AM-500 or a Bruker AM-250 spectrometer.

Materials. — The h.p.l.c. columns were silica-based and were variously substituted with amino groups (Dynamax-60A NH_2, Rainin), diol groups (LiChrospher Si100 DIOL, EM-Science), C_{18} groups (LiChrospher Si100 RP-18, EM-Science; Ultrasphere ODS, Beckman) or phenyl groups (Spherex 3 Phenyl, Phenomenex). All

solvents were l.c. grade. Tetrabutylammonium phosphate solution was purchased from Bodman Chemicals, and 4-(*p*-nitrophenyl)butanol, *N*-hydroxyphthalimide, and $NaCNBH_3$ were from Aldrich Chemical Company, Inc.

Standard procedure for the preparation of PNPBut derivatives. — The oligosaccharides (less than 3 μmol) were dissolved in water (100 μL) and treated with 10μmol ($\sim$2 mg) of 4-(*p*-nitrophenyl)butyloxyamine hydrochloride (**17**) in pyridine (200 μL) for 1 h at 60° in a sealed tube. The solvent was then evaporated under a stream of nitrogen, and a solution of sodium cyanoborohydride (5 mg) in methanol (250 μL) was added, followed by M aqueous HCl (20 μL). The solution was kept at 60° in a screw-capped test tube. At 15 minute intervals M HCl was added in 10 μL portions to maintain pH $\sim$3. When the reaction mixture remained acidic for 15 min without addition of HCl it was cooled and the solvent was evaporated under nitrogen. The residue was dissolved in 50% aqueous methanol and passed through a Dowex 50W-X2 cartridge (H^+ form, 1.5 mL). The resin was washed with water (3 mL) and the PNPBut derivatives eluted with M ammonia in 50% aqueous methanol. The solvent was evaporated from the eluate. The residue was dissolved in water (1 mL) and applied to a C-18 cartridge (Rainin; preflushed with excess ethanol, acetonitrile, and water). The derivatives of tri- and larger oligosaccharides were eluted with 20% aqueous acetonitrile; those of mono- and di-saccharides with 30% acetonitrile. The eluate was evaporated, the residue was redissolved in 10% acetonitrile (250 μL), the solution passed through a 0.2 μm filter, and aliquots (1–10μL) used for h.p.l.c.

Permethylation of 1-deoxy-1-[4-(p-nitrophenyl)butoxyamino] alditols. — A solution of 1-deoxy-1-[4-(*p*-nitrophenyl)butoxyamino] alditol (0.5–1.0 mg) and MeI (35 μL) in Me_2SO (300 μL) was added to finely powdered NaOH (16 mg). The mixture was stirred or sonicated for 30 min at room temperature. M Acetic acid (500 μL) and water (300 μL) were added, and the products were extracted into CH_2Cl_2 (3 $\times$ 500 μL). The organic phase was washed with water (3 mL) and dried with $MgSO_4$. The solvents were removed under a stream of nitrogen, and the dry residue was redissolved in acetone. A small aliquot was used for g.l.c.–m.s. and direct-insertion-probe (e.i.)-m.s. analyses. The major fragment ions of **10** and **11** are depicted in Scheme 1.

N-*Acetylation and methylation of 1-alkoxyamino-1-deoxy alditols.* — Acetic anhydride (100 μL) was added to a solution of **3** or **4** (0–5 mg) in methanol (500 μL) and pyridine (150 μL). The solution was kept for 6 h at room temperature and then concentrated in a stream of nitrogen. The residue was dissolved in Me_2SO (500 μL) and 4M potassium methylsulfinyl methanide solution (200 μL) was added[22]. The mixture was stirred for 1 h at room temperature, then MeI (75 μL) was added and the solution stirred for an additional 2 h. Water (3 mL) was added, and argon was passed through the solution for 5 min to remove excess methyl iodide. The mixture was applied to a C-18 cartridge (Rainin) that had been preconditioned with acetonitrile and water[23], and polar contaminants were eluted with water (2 $\times$ 3 mL) and then with 20% aqueous acetonitrile (3 mL). Aqueous 60% acetonitrile was sufficient to elute **12** from the cartridge.

N-*[4-p-Nitrophenyl)butoxy]phthalimide* (**16**). — 4-(*p*-Nitrophenyl)butanol (520 mg, 2.66 mmol) and pyridine (0.86 mL, 10.6 mmol) were dissolved in CH_2Cl_2 (10 mL)

and methanesulfonyl chloride (0.44 mL, 5.3 mmol) was added. The solution was stirred for 10 h at room temperature, quenched with water, and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases were washed with M aqueous HCl, saturated aqueous $NaHCO_3$, and water, then dried with $MgSO_4$ and evaporated under reduced pressure to give 4-(*p*-nitrophenyl)butyl methanesulfonate (**14**; 710 mg, 97.5%). The product was used without further purification. ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 8.19–8.13, 7.37–7.31 (2 m, 4 H, Ph-H), 4.27–4.23 (m, 2 H, CH_2O), 3.01 (s, 3 H, OSO_2CH_3), 2.80–2.75 (m, 2 H, NO_2PhCH_2), and 1.83–1.77 (m, 4 H, CH_2CH_2).

A suspension of *N*-hydroxyphthalimide (850 mg, 5.20 mmol) and potassium *tert*-butoxide (583 mg, 5.20 mmol) in Me_2NCHO (10 mL) was added to a stirred solution of **14** (710 mg, 2.60 mmol) in Me_2NCHO (3 mL). The mixture was stirred at 60° until the reaction was complete (10 h). The solvent was removed by co-evaporation with toluene and the product partitioned between CH_2Cl_2 and water. The organic phase was dried with $MgSO_4$ and concentrated. The product was chromatographed on a silica gel column with 20:1 toluene–ethyl acetate as eluent to yield **16** (600 mg, 68%), single spot on t.l.c. in the same solvent system; m.p. 115–116°. ^{1}H-N.m.r.-data (250 MHz, $CDCl_3$): δ 8.14–8.11 (m, 2 H, H-3,5 of NO_2Ph), 7.85–7.75 (m, 4 H, Phthal), 7.41-7.37 (m, 2 H, H-2, 6 of NO_2Ph), 4.26 (t, 2 H, *J* 6.0 Hz, CH_2O), 2.84 (t, 2 H, *J* 7.5 Hz, NO_2PhCH_2), and 1.94–1.84 (m, 4 H, CH_2CH_2).

*1-Deoxy-1-[4-(*p*-nitrophenyl)butoxyamino]-*D*-glucitol* (**5**). — Methanol (30 mL) and hydrazine hydrate (200 μL) were added to a solution of **16** (450 mg, 1.32 mmol) in Me_2NCHO (1 mL). The resulting solution was stirred for 3 h at 60°, then adjusted with M HCl to pH 2, and cooled to 0°. The precipitate was removed by filtration, the filtrate was evaporated, and the residue partitioned between aqueous M NaOH and ether. The ether phase was dried over $MgSO_4$ and filtered, and the filtrate was acidified with M methanolic HCl (2 mL). The solvent was evaporated to yield 4-(*p*-nitrophenyl)butoxyamine hydrochloride (**17**; 285 mg, 87%).

A mixture of D-glucose (15 mg, 0.083 mmol), **17**, (21 mg, 0.085 mmol), and sodium cyanoborohydride (30 mg, 0.47 mmol) was processed using the standard procedure described above. A column (1.0 × 8 cm) of Dowex 50W-X2 (H^+) was used for sample clean-up and the resulting solution was freeze dried. Analysis of this material by h.p.l.c. (see caption to Fig. 1 for conditions) indicated that it was primarily **5** (20.4 mg, 65%), plus a trace of **17**. ^{1}H-N.m.r. data (500 MHz, CD_3OD): δ 8.17–8.12, 7.44–7.42 (2 m, 4 H, Ph-H), 3.98 (ddd, 1 H, $J_{1b,2}$ 8.1, $J_{1a,2}$ 4.0, $J_{2,3}$ 4.6 Hz, H-2), 3.78 (dd, 1 H, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 11.1 Hz, H-6a), 3.76 (dd, 1 H, $J_{3,4}$ 2.1 Hz, H-3), 3.71 (t, 2 H, *J* 6.4 Hz, OCH_2), 3.70 (ddd, 1 H, $J_{4,5}$ 7.9, $J_{5,6}$ 5.8 Hz, H-5), 3.62 (dd, 1 H, H-6b), 3.62 (dd, 1 H, H-4), 3.11 (dd, 1 H, $J_{1a,1b}$ 13.2 Hz, H-1a), 2.91 (dd, 1 H, H-1b), 2.77 (t, 2 H, *J* 7.6 Hz, NO_2PhCH_2), 1.76–1.70, and 1.63–1.57 (2 m, 4 H, CH_2CH_2).

ACKNOWLEDGMENTS

This research was supported by Department of Energy (DOE) grant DE-FG09-85ER13424 and by DOE grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF

Plant Science Centers program. The authors wish to thank D. Warrenfeltz for mass spectral analysis and K. Moss for editorial assistance.

REFERENCES

1 J. W. Webb, K. Jiang, B. L. Gillec-Castro, A. L. Tarentino, T. H. Plummer, J. C. Byrd, S. J. Fisher, and A. L. Burlingame, *Anal. Biochem.*, 169 (1988) 337–349.
2 S. Hase, T. Ibuki, and T. Ikenaka, *J. Biochem.* (Tokyo), 95 (1984) 197 203.
3 P. W. Tang and J. M. Williams, *Carbohydr. Res.*, 136 (1985) 259–271.
4 E. Coles, V. N. Reinhold, and S. A. Carr, *Carbohydr. Res.*, 139 (1985) 1–11.
5 S. Satomura, K. Omichi, and T. Ikenaka, *Anal. Biochem.*, 154 (1986) 449–453.
6 G. R. Her, S. Santikarn, V. N. Reinhold, and J. C. Williams, J. *Carbohydr. Chem.*, 6 (1987) 129–139.
7 C. Prakash and I. K. Vijay, *Anal. Biochem.*, 128 (1983) 41–46.
8 E. Kallin, H. Lönn, and T. Norberg, *Glycoconjugate J.*, 3 (1986) 311–319.
9 O. Piskorska and J. Sokolowski, *J. Carbohydr. Chem.*, 5 (1986) 475–496.
10 W. F. Alpenfels, *Anal. Biochem.*, 114 (1981) 153–157.
11 K. Mopper and L. Johnson, *J. Chromatogr.*, 256 (1983) 27–38.
12 R. M. Thompson, *J. Chromatogr.*, 166 (1978) 201–212.
13 C.-C. Chen and G. D. McGinnis, *Carbohydr. Res.*, 122 (1983) 322–326.
14 M. S. Lawson and G. F. Russell, *J. Food Sci.*, 45 (1980) 1256–1258.
15 S. Hakomori. *J. Biochem.*, (Tokyo), 55 (1964) 205–208.
16 H. J. Chaves das Neves and A. M .V. Riscado, *J. Chromatogr.*, 367 (1986) 135–143.
17 N. K. Kochetkov and O. S. Chizov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–92.
18 Houben-Weyl, *Methoden der Organischen Chemie*, Bd. 10/1, G. Thieme Verlag, Stuttgart, 1971, p. 1192.
19 A. Gunnarsson, *Glycoconjugate J.*, 4 (1987) 239–245.
20 G. D. McGinnis, S. Prince, and J. Lowrimore, *J. Carbohydr. Chem.*, 5 (1986) 83–97.
21 A. G. Voragen, H. A. Schols, J. A. de Vries, and W. Pilnik, *J. Chromatogr.*, 244 (1982) 327–336.
22 W. S. York, A. G. Darvill, M. McNeil, T. T. Stevenson, and P. Albersheim, *Methods Enzymol.*, 118 (1985) 3 40.
23 T. J. Waeghe, A. G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.