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SYNTHESIS OF *p*-NITROPHENYL 6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-α-D-MANNOPYRANOSIDE*

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ABSTRACT

The reaction of *p*-nitrophenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside and 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline gave a crystalline, 6-*O*-substituted disaccharide derivative which, on de-isopropylidenation followed by saponification, produced the disaccharide *p*-nitrophenyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside. Synthesis of methyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside was also accomplished by a similar reaction-sequence. The structures of these disaccharides have been established by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Alteration of the levels of the activities of various glycosyltransferases in the sera of cancer patients has been reported by various research groups[2–6]. Among these glycosyltransferases, UDP-Gal . GlcNAc β-4-galactosyltransferaşe (EC 2.4.1 38) seems to be a tumor marker, particularly for monitoring the disease status of certain cancer patients[4,7]. Weiser *et al.*[6,8] detected an extra component (isoenzyme II) of galactosyltransferase by discontinuous, poly(acrylamide) electrophoresis of the sera of cancer patients, in addition to a major component (isoenzyme I) which also exists in normal human sera. Interestingly, these investigators have also isolated and purified an endogenous acceptor from pooled, malignant effusion[9]. The purified, cancer-associated galactosyltransferase acceptor termed CAGA seems to be a better acceptor for isoenzyme II than for isoenzyme I. This endogenous acceptor (CAGA) is a low-molecular-weight glycopeptide containing 70% of carbohydrates (mannose and 2-acetamido-2-deoxy-D-glucose) as the main constituents. As a result, a core region of an asparagine-linked type of glycoprotein has been suggested, as 2-acetamido-2-deoxy-D-galactose is absent. Based on these investigations, and studies reported by other investigators[10], we have initiated a program of synthesizing compounds in which a 2-acetamido-2-deoxy-β-D-glucopyranosyl group is glycosidically linked to a

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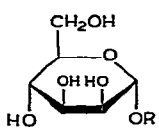
mannose residue (or residues) which may prove to be a specific acceptor for isoenzyme II.

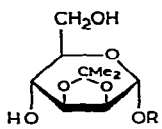
The activity of the β-6-*N*-acetylglucosaminyltransferase present in canine-submaxillary gland[11] has been examined with a variety of synthetic disaccharides prepared in our laboratory. Interestingly, the disaccharide β-Gal-(1→3)-α-GlcNAc-1→$OC_6H_4NO_2$-*o* was found to be a better acceptor than β-Gal-(1→3)-α-GalNAc-1→OMe. These observations further suggested to us the desirability of synthesizing the disaccharides β-D-GlcNAc-(1→6)-α-D-Man-1→OR (R = $C_6H_4NO_2$-*p* and Me) for testing the specificity of galactosyltransferase.

RESULTS AND DISCUSSION

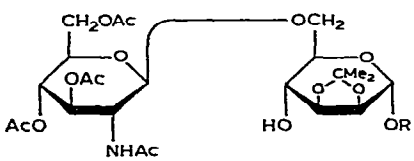
Reaction of *p*-nitrophenyl α-D-mannopyranoside (**1**) with 2,2-dimethoxypropane and anhydrous acetone in the presence of *p*-toluenesulfonic acid, under the conditions described for the preparation of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[12] (**4**) from methyl α-D-mannopyranoside (**2**), gave *p*-nitrophenyl



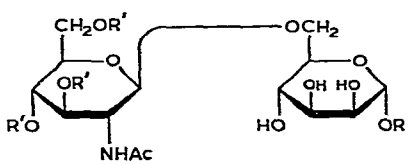
1 R = $C_6H_4NO_2$-*p*
2 R = Me



3 R = $C_6H_4NO_2$-*p*
4 R = Me



5 R = $C_6H_4NO_2$-*p*
6 R = Me



7 R = $C_6H_4NO_2$-*p*, R' = Ac
8 R = Me, R' = Ac
9 R = $C_6H_4NO_2$-*p*, R' = H
10 R = Me, R' = H

2,3-*O*-isopropylidene-α-D-mannopyranoside (**3**) as the major product. The assignment of acetal-ring size of isopropylidene acetals by ^{13}C-n.m.r. has recently been described[13] by Buchanan and co-workers. The ^{13}C-n.m.r. spectrum of **3** exhibited resonance for the acetal carbon atom at 108.49 p.p.m., and the chemical shifts for the methyl groups (26.03 and 27.68 p.p.m.) were slightly separated, thereby supporting the presence of a five-membered, cyclic ring. Similarly, we confirmed the structure of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**4**) by means of its ^{13}C-n.m.r. spectrum.

As reported earlier, reaction of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline gave the corresponding 6-*O*-substituted derivative in almost 30% yield[14], whereas treatment of the same oxazoline with *p*-nitrophenyl 2,3-di-*O*-acetyl-β-D-galactopyranoside gave the disaccharide product in ~54% yield[15]. According to Okuyama[16], reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-α-D-glucopyranose (instead of 2-acetamido-1,3,4-tri-*O*-acetyl-α-D-glucopyranose) gave the disaccharide product in almost double the yield. Based upon such observations, we have preferred the use of diols **3** and **4** for the synthesis of the title compounds. Moreover, it has become evident from investigations emanating

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS[a] (25.2 MHz) IN p.p.m. DOWNFIELD FROM Me_4Si

Atom	*Compound*					
	1	**3**	**9**	**2**	**4**	**10**
C-1	98.51	95.31	99.04	101.89	98.55	101.88
C-2	70.38	74.51	70.14[b]	71.68	76 31	71.09[c]
C-3	69.53	72 65	69.48	71 03	71.26	70 95[c]
C-4	66.40	67.16	66.65	67.88	69.40	67 92
C-5	75.29	77 98	73 21	73 63	78 98	72 43
C-6	60.80	59.95	68.83[d]	62 08	61.72	70.23[d]
C-1′			101.23			102 64
C-2′			55 25			56.69
C-3′			74 33			74.91
C-4′			70 37[b]			71.72[c]
C-5′			76.82			76.95
C-6′			60.86			61 91
$COCH_3$			23.00			23.40
C=O			168.84			175 47
OCH_3				55 84	55.97	55.77
$>C(CH_3)_2$		26.03			26 59	
		27 68			28.19	
$>C(CH_3)_2$		108.49			111.38	

[a]Solvent Me_2SO-d_6, except for D_2O for **2**, **4**, and **10**. The reference (Me_4Si) is internal for solutions in Me_2SO-d_6, and external for solutions in D_2O. [b]The assignments for C-2 and C-4′ may be reversed. [c]Indistinguishable. May be interchanged. [d]Inter-sugar aglyconic linkage.

from various laboratories[17,18] that, in such appropriately protected sugar alcohols having both the 4- and the 6-hydroxyl group free, the 4-hydroxyl group shows less reactivity towards glycosylation.

Reaction of diols **3** and **4** with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[14] in 1:1 nitromethane–toluene, in the presence of a catalytic amount of *p*-toluenesulfonic acid at 120–125°, proceeded readily, to give the disaccharide derivatives **5** and **6**, respectively. Removal of the isopropylidene group, particularly from **5**, with trifluoroacetic acid was found to be quite drastic. However, deacetonation was satisfactorily achieved with 65% acetic acid at 60°, to give **7** from **5**, and **8** from **6**. Deacetylation of compounds **7** and **8** in methanol in the presence of triethylamine and water provided the disaccharides **9** and **10**, respectively.

It is well established[19] that the carbon atoms of the hydroxymethyl group of aldohexopyranoses provide their signals in the region of 60–63 p.p.m. from Me_4Si (external) with D_2O as the solvent, and this region is generally free from signals from other types of carbon atoms that occur in carbohydrate structures. It is also well established[20] that alkylation of a hydroxyl group causes a 7–10-p.p.m. downfield shift in the resonance of the carbon atom bearing the hydroxyl group. Inspection of Table I shows that this is the case. Thus, the absence of a signal for C-6 at 60–63 p.p.m., and a downfield shift of 8.03 p.p.m. in the spectrum of **9**, and of 8.15 p.p.m. in that of **10**, relative to that of that carbon atom in **1** and **2**, confirm the position of the glycosidic linkage in disaccharides **9** and **10**, respectively. The complete absence of a C-6 signal in the region of 60–63 p.p.m. also confirmed that glycosylation had occurred only at the 6-hydroxyl group of acetals **3** and **4**. The ^{1}H-n.m.r. spectrum of **9** showed a clear doublet for an anomeric proton (H-1′) at δ 4.31 (*J* 7.5 Hz), thereby establishing the β-D configuration at the inter-sugar linkage. The C-1′ resonance at δ 101.23 in the ^{13}C-n.m.r. spectrum of **9** further supported the β-D configuration. Similarly, the ^{1}H- and ^{13}C-n.m.r. data are consistent with the structure assigned disaccharide **10**.

We have already reported a facile method for the preparation of 6-(benzyloxycarbonylamino)hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside[21], which, on hydrogenation, can provide 6-amino-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside, a ligand for the purification of galactosyltransferase[22].

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at room temperature. Ascending t.l.c. was conducted on plates coated with a 0.25-mm layer of Silica Gel 60 PF-254 (E. Merck, Darmstadt, Germany); the components were located by exposure to u.v. light, or by spraying the plate with 5% sulfuric acid in ethanol and heating. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ, U.S.A. N.m.r. spectra were recorded with

a Varian XL-100 instrument; ^{1}H-n.m.r. spectra at 100 MHz and ^{13}C-n m.r. spectra at 25.2 MHz were determined by the Fourier-transform (F.t.) mode; the positions of the peaks are expressed as δ values from the tetramethylsilane signal

p-*Nitrophenyl 2,3-O-isopropylidene-α-D-mannopyranoside* (**3**). — To a suspension of **1** (0.5 g) in dry acetone (5 mL) were added 2,2-dimethoxypropane (5 mL) and *p*-toluenesulfonic acid (100 mg). The mixture was stirred at room temperature until dissolution occurred, and then water (10 mL) was added, and stirring was continued for 3 h. The mixture was made neutral with M $NaHCO_3$ solution and evaporated. A solution of the residue in chloroform was washed with water, dried, and evaporated, to give a syrup that crystallized from chloroform–ether–hexane to afford **3** in 69% yield (0.391 g); m.p. 150–151°, $[\alpha]_D$ +110.3° (*c* 1, chloroform); R_F 0.65 in 3:2 chloroform–acetone; n.m.r. data (Me_2SO-d_6): δ 1.36 and 1.48 (s each, 2 × 3 H, isopropylidene methyls), 3.50 (m, 4 H, H-4,5,6,6'), 4.18 (t, 1 H, $J_{2,3} = J_{3,4}$ = 6 Hz, H-3), 4.39 (dd, 1 H, $J_{1,2}$ ~1, $J_{2,3}$ 6 Hz, H-2), 4.60 (t, 1 H, *J* 5.5 Hz, D_2O-exchangeable, OH-6), 5.36 (d, 1 H, *J* 4.5 Hz, D_2O-exchangeable, OH-4), 6.01 (d, 1 H, $J_{1,2}$ ~1 Hz, H-1), and 7.38 and 8.28 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{15}H_{19}NO_8$: C, 52.78; H, 5.61; N, 4.11. Found: C, 52.82, H, 5.52; N, 4.02.

p-*Nitrophenyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-O-isopropylidene-α-D-mannopyranoside* (**5**). — A solution of oxazoline[14] (790 mg, 2.4 mmol), **3** (682 mg, 2 mmol), and *p*-toluenesulfonic acid (10 mg) in 1:1 nitromethane–toluene (30 mL) was stirred at 120–125°, and the reaction was monitored by t.l.c. After ~1.5 h, the acid was neutralized with a few drops of pyridine, and the solution was evaporated to a brown residue which was dissolved in chloroform (100 mL), and the solution washed with water (2 × 20 mL), dried, and evaporated. The solid residue was purified by chromatography on a column of silica gel, with elution with 2:1 (v/v) chloroform–acetone, to give **5** (0.792 g, 51%), m.p. 115–118° (dec.), $[\alpha]_D$ +34.3° (*c* 1, chloroform); t.l.c. in 3:2 chloroform–acetone: R_F 0.46; n.m.r. data (Me_2SO-d_6): δ 1.34 and 1.46 (s each, 2 × 3 H, isopropylidene methyls), 1.78 (s, 3 H, NAc), 1.92, 1.98 and 2.02 (s each, 3 × 3 H, Ac), 4.40 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 6 Hz, H-2), 5.93 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 7.36 and 8.30 (2 m, 2 × 2 H, aromatic), and 7.84 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

Anal. Calc. for $C_{29}H_{38}N_2O_{16}$: C, 51.94; H, 5.71; N, 4.18. Found: C, 51.69; H, 5.61; N, 4.09.

p-*Nitrophenyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside* (**7**). — A mixture of **5** (300 mg) with 65% acetic acid (30 mL) was stirred for 1.5 h at 60°, cooled, and evaporated. Several additions and evaporations of toluene gave a solid mass which was purified by chromatography on a column of silica gel, with elution with 6:1 (v/v) chloroform–ethanol, to give **7** (0.228 g, 81%), m.p. 121–122° (acetone–ether), $[\alpha]_D$ +55.3° (*c* 1, Me_2SO); n.m.r. data (Me_2SO-d_6): δ 1.80 (s, 3 H, NAc), 1.95, 1.98 and 2.02 (s each, 3 × 3 H, 3 Ac), 5.52 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 7.30 and 8.31 (2 m, 2 × 2 H, aromatic), and 7.86 (d, 1 H, $J_{NH,2}$ 9 Hz, NH).

Anal. Calc. for $C_{26}H_{34}N_2O_{16}$: C, 49 52; H, 5.43; N, 4.44. Found: C, 49.36; H, 5.59; N, 4.34.

p-*Nitrophenyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside* (**9**). — A solution of **7** (0.3 g) in a mixture of methanol (9 mL), triethylamine (3 mL), and water (2.5 mL) was kept for 24 h at 4°, and then evaporated to dryness. Toluene was added to, and evaporated from, the residue, and the resulting material crystallized from methanol–ether to afford disaccharide **9** (0.202 g, 84%), m.p. 189–191°, $[\alpha]_D$ +71.5° (*c* 1, Me_2SO): n.m.r. data (Me_2SO-d_6): δ 1.83 (s, 3 H, NAc), 4.31 (d, 1 H, *J* 7.5 Hz, H-1′), 5.50 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 7.32 and 8.32 (2 m, 2 × 2 H, aromatic), and 7.60 (d, 1 H, $J_{NH,2}$, 9 Hz, NH).

Anal. Calc. for $C_{20}H_{28}N_2O_{13}$: C, 47.62; H, 5.59; N, 5.55. Found: C, 47.44; H, 5.84; N, 5.49.

Methyl 2,3-O-isopropylidene-α-D-mannopyranoside (**4**). — Compound **4** was prepared from **2** as described by Evans and Parrish[12].

Methyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3-O-isopropylidene-α-D-mannopyranoside (**6**). — Compound **6** was prepared from **4** (0.468 g, 2 mmol) as described for **5**, and was used as such for the next reaction.

Methyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**8**). — The isopropylidene group of compound **6** was removed as described for the preparation of **7**, to give a solid residue which was purified by chromatography on a column of silica gel, with elution with 9:1 (v/v) chloroform–methanol, to afford amorphous **8** in 48% yield (from **4**); $[\alpha]_D$ +10.3° (*c* 1, MeOH); n.m.r. data (D_2O): δ 2.46 (s, 3 H, NAc), 2.54, 2.57 and 2.61 (s each, 3 × 3 H, 3 Ac), and 3.86 (s, 3 H, OMe).

Methyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**10**). — Deacetylation of compound **8** (0.4 g) as described for **9** gave amorphous **10** (0.21 g, 70%), $[\alpha]_D$ +11.2° (*c* 1, water); n.m.r. data (D_2O): δ 2.5 (s, 3 H, NAc) and 3.85 (s, 3 H, OMe).

Anal. Calc. for $C_{15}H_{27}NO_{11} \cdot H_2O$: C, 43.37; H, 7.04; N, 3.37. Found: C, 43.67; H, 6.87; N, 3.40.

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REFERENCES

1 K. L. Matta, C. F. Piskorz, and J. J. Barlow, *Carbohydr. Res.*, 90 (1981) c1–c3.
2 C. Bauer, E. Köttgen, and W. Reutter, *Biochem. Biophys. Res. Commun.*, 76 (1977) 488–494.
3 R. J. Bernacki and U. Kim, *Science*, 195 (1977) 577–580.
4 S. K. Chatterjee, M. Bhattacharya, and J. J. Barlow, *Cancer Lett.*, 5 (1978) 239–244.

5 P. KHILANANI, T. H. CHOU, V. RATANATHARATHORN, AND D. KESSEL, *Cancer*, 41 (1978) 701–705.
6 D. K. PODOLSKY AND M. M. WEISER, *Biochem. Biophys. Res. Commun*, 65 (1975) 545–551
7 K. L MATTA, C. F. PISKORZ, R. A DICIOCCIO, AND J. J. BARLOW, *Proc Am Assoc. Cancer Res*, 21 (1980) 326.
8 M WEISER, D. K PODOLSKY, AND K. J. ISSELBACHER, *Proc Natl. Acad Sci. U S A*, 73 (1976) 1319–1322.
9 D. K. PODOLSKY AND M. M. WEISER, *Biochem. J*, 178 (1979) 279–287.
10 J P. PRIEELS, M. DOLMANS, M. SCHINDLER, AND N. SHARON, *Eur. J. Biochem*, 66 (1976) 579–582.
11 D. WILLIAMS, G. LONGMORE, K. L. MATTA, AND H. SCHACHTER, *J. Biol. Chem*, 255 (1980) 11,253–11,261.
12 M. E. EVANS AND F. W. PARRISH, *Carbohydr. Res.*, 54 (1977) 105–114.
13 J. G. BUCHANAN, M. ENCARNACION CHACÓN-FUERTES, A. R. EDGAR, S. J MOORHOUSE, D. I RAWSON, AND R. H. WIGHTMAN, *Tetrahedron Lett*, (1980) 1793–1796
14 K. L. MATTA AND O P BAHL, *Carbohydr. Res*, 21 (1972) 460–464
15 K. L. MATTA AND J. J. BARLOW, *Carbohydr. Res.*, 53 (1977) 209–216.
16 T. OKUYAMA, *Tohoku J. Exp. Med.*, 68 (1958) 319–320.
17 M. SHABAN AND R. W. JEANLOZ, *Carbohydr. Res.*, 17 (1971) 411–417.
18 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4063–4068
19 H J. JENNINGS AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 95 (1973) 606–608.
20 D. E. DORMAN AND J. D. ROBERTS, *J. Am. Chem. Soc*, 93 (1971) 4463–4472
21 K. L. MATTA AND J. J. BARLOW, *Carbohydr. Res.*, 51 (1976) 215–222.
22 R. BARKER, K. W. OLSEN, J. H. SHAPER, AND R L. HILL, *J. Biol. Chem*, 247 (1972) 7135–7147.