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SYNTHESIS OF 6-AMINO-1-HEXYL 2-ACETAMIDO-2-DEOXY-3-, -4-, AND -6-*O*-β-D-GALACTOPYRANOSYL-β-D-GLUCOPYRANOSIDES*

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ABSTRACT

6-Aminohexyl glycosides of β-D-Gal→β-D-GlcNAc disaccharides having β-(1→3)-, β-(1→4)-, and β-(1→6)-linkages were prepared. 6-(Benzyloxycarbonylamino)-1-hexanol was glycosylated with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride, to yield (80%) a crystalline β-glycoside acetate (**3**). Deacetylation of **3**, followed by isopropylidenation (2,2-dimethoxypropane–TsOH) gave a crystalline 4,6-*O*-isopropylidene derivative (**5**) in 84% overall yield. Benzylation of **5** [benzyl bromide–BaO–$Ba(OH)_2$ in *N*,*N*-dimethylformamide] gave its 3-*O*-benzyl derivative, which was converted into 6-(benzyloxycarbonylamino)-1-hexyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**7**) in 69% overall yield. Glycosylation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**12**) and silver trifluoromethanesulfonate in dry CH_2Cl_2 at 0° yielded the β-(1→6)-disaccharide glycoside (**17**) in 66% yield. Glycosylation of **5** with **12**, using mercuric cyanide in 1:1 (v/v) benzene–nitromethane at 60°, gave the β-(1→3)-disaccharide (86% yield). Limited benzylation of **7**, followed by chromatography, gave its 3,6-di-*O*-benzyl derivative (**9**) in 47% yield. Glycosylation of **9** with **12** yielded the β-(1→4)-disaccharide (89%). Removal of the protecting groups under standard conditions yielded the desired 6-aminohexyl glycosides of the disaccharides.

INTRODUCTION

We have recently published a general procedure[1] for preparation of 6-aminohexyl D-aldopyranosides. These glycosides proved to be extremely useful in studies of biological interactions involving carbohydrates. For example, freshly prepared, rat hepatocytes were found to bind specifically[2] to poly(acrylamide) gels containing the β-D-galactopyranosyl residue, and chicken hepatocytes specifically[3] bound to the gels containing the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue. In order to

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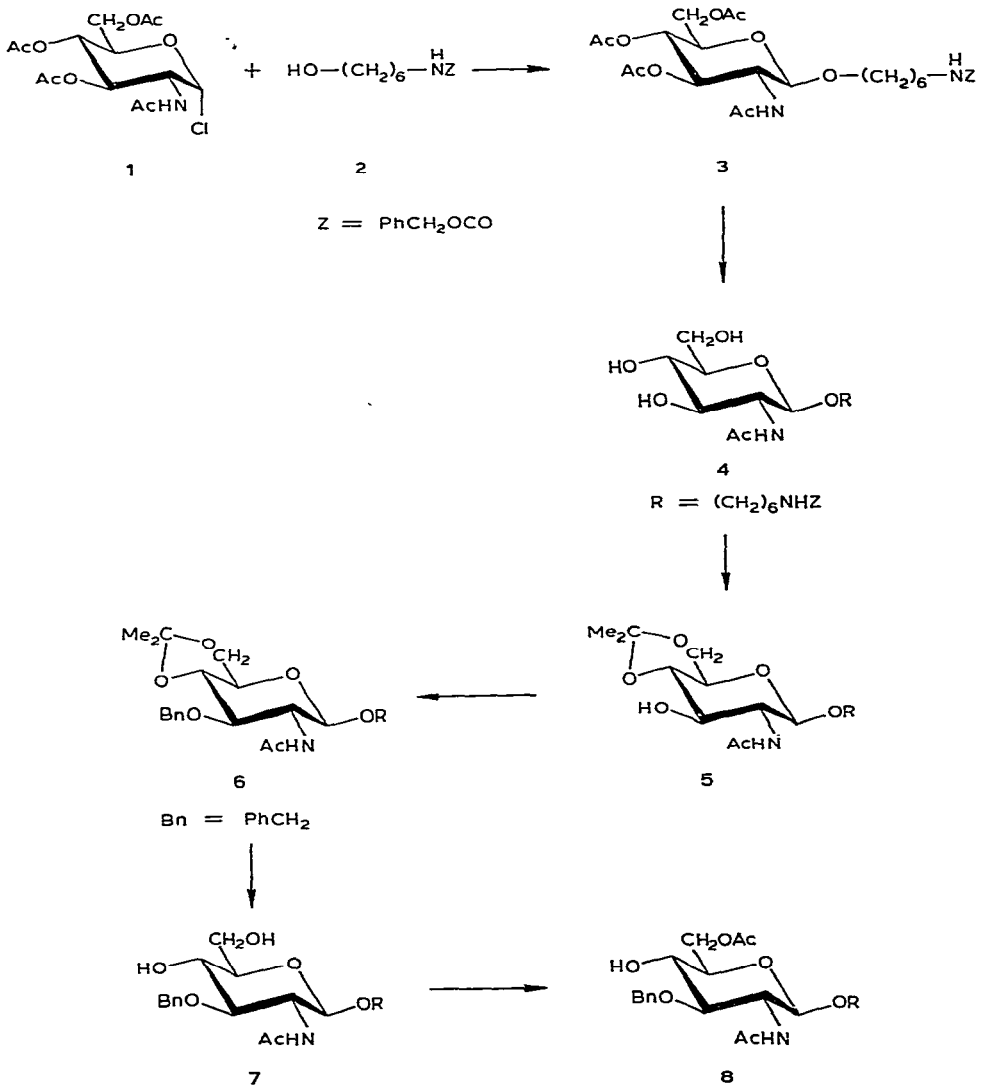
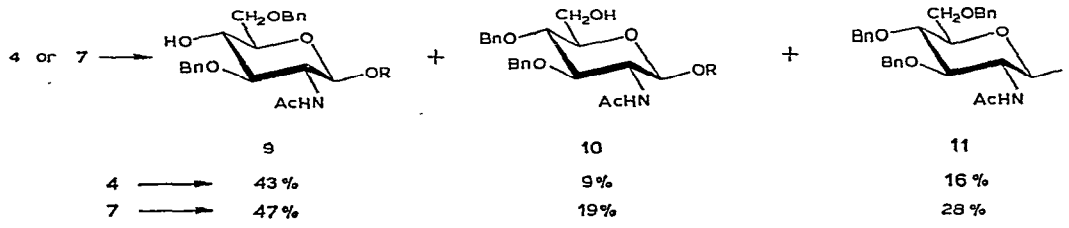
probe systematically the possible role of the sugar residues at the penultimate position of the nonreducing terminal, and to examine the effect of positional isomerism, we extended our synthesis of 6-aminohexyl glycosides to a series of disaccharides having the *O*-β-D-galactopyranosylated 2-acetamido-2-deoxy-β-D-glucopyranose sequence. D-Galactosylation of appropriately protected 6-(benzyloxycarbonylamino)-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranosides yielded the desired glycosides with this sequence containing (1→3), (1→4), and (1→6) linkages.

EXPERIMENTAL

Materials. — The following materials were obtained from the sources indicated,

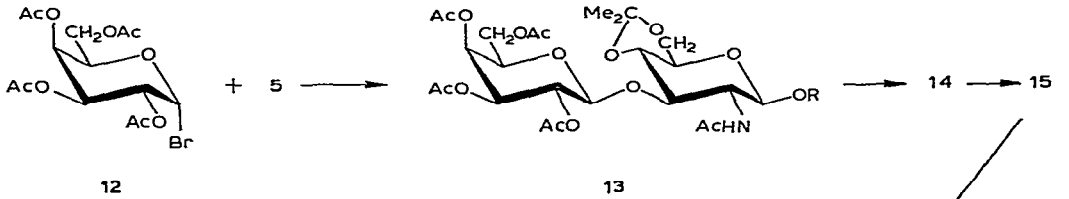




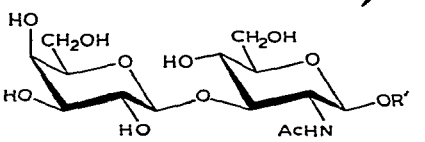
9 10 11

4 → 43% 9% 16%

7 → 47% 19% 28%

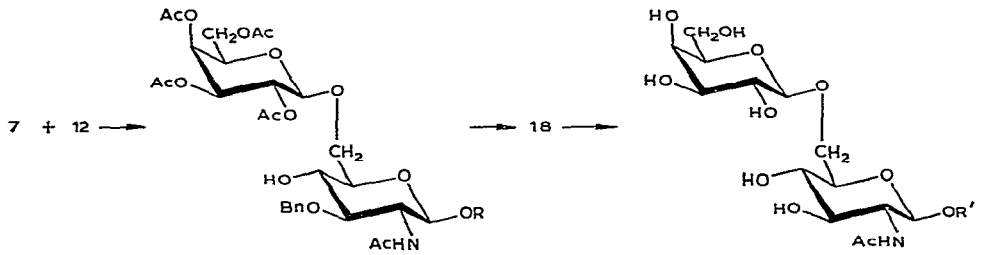


12 13

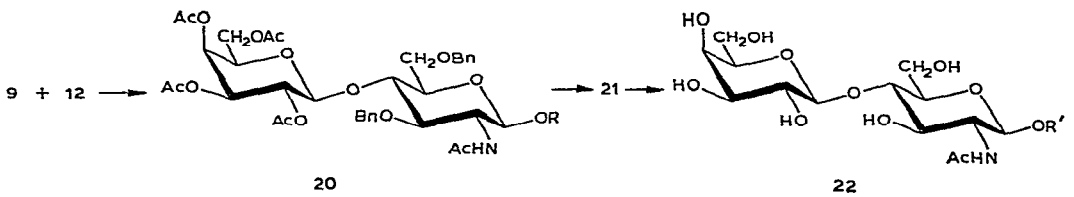


16

R = $(CH_2)_6NH_2$



17 19



20 22

and were used without further treatment: Rexyn 101, 16–50 mesh (Fisher), Amberlite IR-45, 20–50 mesh (Mallinkrodt), and Dowex AG1-X8, 200–400 mesh (Bio-Rad) ion-exchange resins; Sephadex G-15 and LH-20 (Pharmacia); silica gel Type 60, 70–230 mesh (Merck), silica gel Type LP-1, 10–20 μm (Quantum Industries, Fairfield, NJ); molecular sieves (Type 4A, Davison Chem.); α-bromotoluene (benzyl bromide; Aldrich or Fluka); palladium, 10% on charcoal (J. T. Baker); and silver trifluoromethanesulfonate (Fluka).

The following compounds were prepared as previously described: 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[1,4] (**1**), 6-(benzyloxycarbonylamino)-1-hexanol[4] (**2**), and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[1] (**12**).

General methods. — Unless otherwise stated, all evaporations were performed in a rotary evaporator at 30–40°. Melting points (uncorrected) were measured on a Fisher–Johns apparatus. Thin-layer chromatography (t.l.c.) was conducted on silica gel F-254 precoated on aluminum sheets (Merck). Solvent systems for the t.l.c. were: (*A*) 4:1 (v/v) ethyl acetate–acetone, (*B*) 1:1 (v/v) ethyl acetate–ether, (*C*) 1:1 (v/v) benzene–ethyl acetate, (*D*) 4:1 (v/v) chloroform–methanol, and (*E*) 3:2:1 (v/v) ethyl acetate–acetic acid–water. Components on t.l.c. plates were detected by fluorescent quenching of u.v. absorption, and also by spraying with 10% sulfuric acid in 50% ethanol followed by charring at 140°. All organic solvents were dried over molecular sieves (Type 4A). Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

Proton magnetic resonance (p.m.r.) spectra were recorded with a JEOL NMH-100 spectrometer. All compounds containing hydroxyl groups were treated three times with deuterium oxide, to effect exchange, prior to recording of the n.m.r. spectra. A Perkin–Elmer gas chromatograph Model 990 and a DuPont mass spectrometer Model 491 were used for analysis by gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.), using a column (4 mm × 2 m) of 3% of SE-30 on Gas-Chrom Q (100–120 mesh) at 150°.

*6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside* (**3**). — A mixture of **1** (30.7 g, 84 mmol), **2** (13.2 g, 56 mmol), and mercuric cyanide (14.2 g, 56 mmol) in 1:1 (v/v) benzene–nitromethane (250 mL) was stirred for 48 h at room temperature. At this time, another portion of **1** (10.2 g, 28 mmol) was added, and the reaction was allowed to proceed for an additional 24 h (72 h total). The mixture was evaporated to a syrup, which was dissolved in chloroform (500 mL); the solution was washed with M sodium chloride (3 × 100 mL), dried (sodium sulfate), and evaporated to a syrup which crystallized from acetone–ether to give pure **3** (26.0 g, 80% yield); m.p. 112–114° (lit.[5] m.p. 96–98°); p.m.r. data ($CDCl_3$): δ 1.20–1.76 [m, 8 H, $C(CH_2)_4C$], 1.92 (s, 3 H, NAc), 2.00 (s, 6 H, 2 OAc), 2.06 (s, 3 H, OAc), 4.67 (d, 1 H, *J* 8 Hz, anomeric proton), 5.13 (s, 2 H, CH_2Ph), and 7.35 (s, 5 H, Ph).

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**4**). — *O*-Deacetylation of **3** (59.9 mmol, 34.8 g) was accomplished with sodium

methoxide (0.6 mmol) in dry methanol (400 mL). The mixture was stirred for 24 h, de-ionized with Rexyn 101 (H^+) ion-exchange resin, filtered, and the resin washed with methanol. Evaporation of the filtrate and washings gave an oil that crystallized from ethanol–petroleum ether (b.p. 30–60°), to yield **4** (23.9 g, 88%); m.p. 180–182°. The p.m.r. spectrum of **4** was consistent with the structure assigned.

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**5**). — A solution of **4** (24.7 g, 54.4 mmol), 2,2-dimethoxypropane (22.6 g, 218 mmol), and *p*-toluenesulfonic acid (0.2 g, 1.16 mmol) in dry *N,N*-dimethylformamide[6] (400 mL) was stirred for 12 h at room temperature. At this time, no **4** remained (as determined by t.l.c., solvent *A*), and the acid was neutralized with Amberlite IR-45 (OH^-) resin, the suspension filtered, and the solid washed with acetone. Evaporation of the filtrate plus washings to a syrup, followed by crystallization from ethanol–ether, gave **5** (25.7 g, 96% yield); m.p. 144–146°; p.m.r. data (Me_2SO-d_6): δ 1.12–1.66 [m, 14 H, $C(CH_3)_2$ and $C(CH_2)_4C$], 1.82 (s, 3 H, NAc), 4.42 (d, 1 H, *J* 7 Hz, anomeric proton), 5.03 (s, 2 H, CH_2Ph), and 7.36 (s, 5 H, Ph).

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (**7**). — A mixture of **5** (3.56 g, 7.21 mmol), barium oxide (9 g, 58.7 mmol), and barium hydroxide octahydrate (3.69 g, 11.4 mmol) in dry *N,N*-dimethylformamide (60 mL) was cooled to 0°. The mixture was stirred, and three portions of benzyl bromide (9, 3, and 3 mL) were added, initially and then after 30 and 60 min, respectively. No starting material **5** remained (t.l.c., solvent *A*) after 4 h, and the reaction was stopped by the addition of methanol (20 mL). Dichloromethane (250 mL) was then added, the suspension was filtered, and the filtrate was washed with water (3 × 40 mL), dried (sodium sulfate), and evaporated. The resultant oil was purified on a column (2.5 × 35 cm) of silica gel Type 60, using solvent *C* as eluant. A product was isolated (R_F 0.63, solvent *A*) whose p.m.r. spectrum was in agreement with structure **6**. However, it was evident from analysis of the column effluent (t.l.c., solvent *A*) that a considerable proportion of **6** had been converted into **7** during the purification. For this reason, **7** was routinely prepared without isolation of **6**. Typically, the reaction mixture containing **6** was stirred with 60% acetic acid (7 mL) for 10 h at 55°. Evaporation of the mixture gave an oil which was fractionated on a column (2.5 × 35 cm) of silica gel Type 60 by elution with solvent *A*. Fractions containing **7** (homogeneous by t.l.c., solvent *A*) were combined, and evaporated, to give a syrup which crystallized from a mixture of ethanol and petroleum ether (b.p. 30–60°). The overall yield of **7** (2.69 g) from **5** was 69%; m.p. 154–156°. The p.m.r. spectrum of **7** was consistent with the structure assigned.

Anal. Calc. for $C_{29}H_{40}N_2O_8$ (544.63): C, 63.95; H, 7.40; N, 5.14. Found: C, 63.70; H, 7.39; N, 5.05.

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-6-O-acetyl-3-O-benzyl-2-deoxy-β-D-glucopyranoside (**8**). — To a stirred solution of imidazole (2.0 mmol, 0.14 g) in dry chloroform (20 mL), cooled to 0°, was added a solution of acetyl chloride (78.6 mg, 1.0 mmol) in dry chloroform (5 mL). Filtration of the precipitated imidazole hydrochloride was followed by addition, to the filtrate, of **7** (0.5 g, 919 μmol). The

solution was heated for 24 h at 80°, allowed to cool to room temperature, and washed with water (15 mL); the aqueous phase was separated, and back-extracted with chloroform (10 mL). The extracts were combined, dried (sodium sulfate), and evaporated to a syrup which was purified on a column (25 × 35 cm) of silica gel Type 60 using solvent *A*. Effluent fractions containing pure **8** (R_F 0.75; t.l.c., solvent *A*) were combined, and evaporated, to give amorphous **8** (0.35 g) in 61% yield; p.m.r. data ($CDCl_3$): δ 1.10–1.68 [m, 8 H, $C(CH_2)_4C$], 1.84 (s, 3 H, NAc), 2.00 (s, 3 H, OAc), and 7.14 (s, 5 H, Ph).

Anal. Calc. for $C_{31}H_{42}N_2O_9$ (586.66): C, 63.46; H, 7.22; N, 4.77. Found: C, 63.52; H, 7.21; N, 4.75.

Limited O-*benzylation of* **7**. — A mixture of **7** (1.2 g, 2.2 mmol), barium oxide (1.36 g, 8.84 mmol), barium hydroxide octahydrate (0.7 g, 2.2 mmol), and molecular sieves (type 4A) in dry *N*,*N*-dimethylformamide (25 mL) was cooled to 0° in an ice bath, and benzyl bromide (0.432 g, 2.52 mmol) was slowly added to the mixture during 2.5 h. After 24 h, no **7** remained (t.l.c., solvent *B*), and the reaction was stopped by the addition of methanol (10 mL). After filtration, and evaporation of the filtrate, an oil was obtained which was dissolved in chloroform (100 mL); the solution was washed with water (2 × 20 mL), dried (sodium sulfate), and evaporated, to give a syrup which was fractionated on a column (2.5 × 35 cm) of silica gel Type 60, using solvent *A*. Resolution between the three major products (R_F 0.91, 0.78, and 0.59; solvent *B*) was unsatisfactory, so the fractions containing these components were combined, and evaporated to an oil which was rechromatographed on a column (4 × 35 cm) of silica gel Type LP-1, eluted with solvent *B* at the rate of 15 mL/h. Fractions containing the pure components were combined and evaporated. The three compounds were, in part, identified by their p.m.r. spectra. The fastest-moving component (R_F 0.91, solvent *B*) contained 4 benzyl groups to 1 acetyl group, and was thus assigned structure **11**. Likewise, **9** and **10** were found to be di-*O*-benzyl derivatives. Tritylation was attempted on both of the unidentified di-*O*-benzyl glycosides (**9** and **10**) under reaction conditions identical to those used by Warren and Jeanloz[7]. No tritylated product from **9** or **10** could be detected (t.l.c., solvent *B*) after one week. The material having R_F 0.78 was tentatively assigned structure **9**, as we expected this material to be formed in higher yield than isomer **10**, and also to have greater mobility in t.l.c. The identity of the structure of **9** was later confirmed by methylation analysis of the disaccharide glycoside **22**.

Syrup **11** crystallized from acetone–ether, yield 0.446 g (28%); m.p. 151–152°.

Anal. Calc. for $C_{43}H_{52}N_2O_8$ (724.86): C, 71.25; H, 7.23; N, 3.87. Found: C, 71.37; H, 7.40; N, 3.77.

Crystallization of **10** occurred from acetone–ether, yield 0.265 g (19%); m.p. 143–145°.

Anal. Calc. for $C_{36}H_{46}N_2O_8$ (634.74): C, 68.12; H, 7.31; N, 4.41. Found: C, 68.41; H, 7.49; N, 4.21.

Preparation of **9**, **10**, *and* **11** *from* **4**. — A mixture of **4** (6 g, 13.2 mmol), freshly ground barium oxide (16.3 g, 10.6 mmol), barium hydroxide octahydrate (8.34 g,

26.4 mmol), and molecular sieves (Type 4A) in dry *N,N*-dimethylformamide (105 mL) was stirred. Three portions of benzyl bromide (each 1 mL, 8.3 mmol) were added to this mixture, initially, and then after 15 and 30 min (24.9 mmol total). After 2 h, methanol (20 mL) was added, and the mixture was filtered. The viscous filtrate was diluted with chloroform (600 mL), and the solution was washed with water (2 × 150 mL), dried (sodium sulfate), and evaporated to a syrup which was fractionated on a column (36 × 4.5 cm) of silica gel Type 60, with solvent *B* as the eluant. Fractions containing products having R_F values identical to those of **9**, **10**, and **11** were combined and evaporated. This mixture was further purified on a column of silica gel Type LP-1 as previously described for the preparation of **9**, **10**, and **11** from **7**. The three reaction products were identified as structures **9**, **10**, and **11** from a comparison of p.m.r., melting point, and t.l.c. data from previously identified materials synthesized from **6**. The yields of **9**, **10**, and **11** were 43, 9, and 16%, respectively.

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-2-deoxy-4,6-O-isopropylidene-3-O-(2,3,4,6,tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**13**). — A mixture of **5** (1.26 g, 2.54 mmol) with dry 1:1 (v/v) benzene–nitromethane (10 mL) was heated to 60° with stirring, and mercuric cyanide (0.64 g, 2.54 mmol) and **12** (1.05 g, 2.54 mmol) were added. After 1 h, the same quantities of **12** and mercuric cyanide were again added. After 3 h, the mixture was cooled to room temperature, benzene (100 mL) was added, and the mixture was washed with cold, saturated solutions of sodium hydrogencarbonate (25 mL) and sodium chloride (twice, 30 mL each). Each aqueous wash was back-extracted with chloroform (30 mL). The extracts were combined, dried (sodium sulfate), and evaporated, to give an oil which was placed on a column (2.5 × 35 cm) of silica gel Type 60, and eluted with solvent *C*. Two products were present in the eluate, as shown by t.l.c. (R_F 0.74 and 0.38, solvent *A*). The p.m.r. spectrum of the material of higher R_F value was in agreement with structure **13**.

Treatment of **13** with 60% acetic acid at 55° for deacetalation gave a product (**14**) that had R_F 0.38 (solvent *A*). The n.m.r. spectrum of **14** was identical to that of the material with R_F 0.38 that had been isolated during the purification of **13**. Complete removal of the protecting groups from this material, followed by methylation analysis, showed that this disaccharide was linked to C-3 of the 2-amino-2-deoxyglucose. The yield of **13** was 1.28 g (1.55 mmol, 61%); p.m.r. data ($CDCl_3$): δ 1.12–1.80 [m, 14 H, $C(CH_3)_2$ and $C(CH_2)_4C$], 1.98 (s, 3 H, NAc), 2.04 (s, 3 H, OAc), 2.09 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), and 7.40 (s, 5 H, Ph). The yield of **14** (0.50 g, 0.64 mmol) was 25%; p.m.r. ($CDCl_3$): δ 1.24–1.68 [m, 8 H, $C(CH_2)_4C$], 1.92–2.26 (m, 15 H, 5 Ac), and 7.24 (s, 5 H, Ph).

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranoside (**15**). — A solution of **13** (21 mg, 25 μmol) in 60% acetic acid (5 mL) was heated for 1.5 h at 55°. At this time, no **13** remained (t.l.c., solvent *A*), and the mixture was cooled to room temperature and evaporated, to give oily **14** in quantitative yield (20.6 mg).

A solution of **14** (40 mg, 51 μmol) and sodium methoxide (0.03 mmol) in dry

methanol (20 mL) was stirred for 1 h, made neutral with Rexyn 101 (H^+) resin, the suspension filtered, and the solid washed with methanol. The filtrate and washings were combined, and evaporated, to give oily **15** (30 mg, 95%); p.m.r. data (Me_2SO-d_6): δ 1.06–1.66 [m, 8 H, $C(CH_2)_4C$], 1.81 (s, 3 H, Ac), 5.00 (s, 2 H, CH_2Ph), and 7.32 (s, 5 H, Ph).

6-Aminohexyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranoside (**16**). — Hydrogenolysis of **15** (63 mg, 102 μmol) was accomplished with hydrogen in the presence of 10% palladium-on-charcoal (120 mg) in 60% acetic acid (15 mL), the mixture being stirred. The reaction was monitored by t.l.c., using solvent *D* or *E* (developed twice). After 1 h, the reaction was stopped, the mixture was filtered, and the filtrate was evaporated. The resultant oil was purified on a column (2.5 × 142 cm) of Sephadex G-15, using 0.1M acetic acid as the eluant. The effluent was analyzed by the phenol–sulfuric acid method[1] and by t.l.c. in solvent *E* (developed twice). Fractions containing pure **16** were combined and evaporated. This purification method gave the acetic salt of **16** in 93% yield. Removal of the acetate from the salt of **16** (and **19** and **22**) was readily accomplished with Bio-Rad AG1-X8 (OH^-) resin (200–400 mesh). Removal of the acetate was confirmed by p.m.r. spectroscopy; p.m.r. (of the acetate) (D_2O): δ 1.64–2.28 [m, 8 H, $C(CH_2)_4C$], 2.33 (s, 3 H, OAc), 2.44 (s, 3 H, NAc), 4.86 (d, 1 H, *J* 7 Hz, GlcNAc anomeric proton), and 4.94 (width at half-height ~8 Hz, Gal anomeric proton).

Anal. Calc. for $C_{22}H_{42}N_2O_{13} \cdot 0.5\ H_2O$ (551.58): C, 47.90; H, 7.86; N, 5.08. Found: C, 47.80; H, 7.98; N, 4.92.

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-3-O-benzyl-2-deoxy-6-O-β-galactopyranosyl-β-D-glycopyranoside (**18**). — Silver trifluoromethanesulfonate (0.177 g, 0.46 mmol) and **12** (0.15 g, 0.5 mmol) were added to a stirred, cooled (0°) solution of **7** (0.5 g, 0.92 mmol) in dry dichloromethane[8] (18 mL). The same quantities of silver trifluoromethanesulfonate and **12** were added three more times, at 30-min intervals. After 2 h, the mixture was warmed to room temperature, Celite was added, the mixture was filtered, and the precipitate was washed with dichloromethane (70 mL). The filtrate and washings were combined, washed with water (2 × 30 mL), dried (sodium sulfate), and evaporated. The resultant oil was purified on a column (2.5 × 35 cm) of silica gel Type 60 which was eluted with solvent *C* for the first 80 fractions (6 mL each), and then with solvent *A*. Fractions 93–104 were collected, combined, and evaporated, to yield **17** (0.53 g, 66%); p.m.r. data ($CDCl_3$): δ 1.16–1.73 [m, 8 H, $C(CH_2)_4C$], 1.92 (s, 3 H, NAc), 1.98 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 5.14 (s, 2 H, CH_2Ph), and 7.36 (s, 5 H, Ph).

O-Deacetylation of **17** to give **18** was accomplished by the procedure used in preparing **15** from **14**. The yield of **18** was 94%.

6-Aminohexyl 2-acetamido-2-deoxy-6-O-β-D-galactopyranosyl-β-D-glucopyranoside (**19**). — Hydrogenolysis of **18** to give **19** was accomplished as described for converting **15** into **16**. Compound **19** was isolated, as the acetic salt, in 95% yield;

p.m.r. data (D_2O): δ 1.70–2.44 [m, 8 H, $C(CH_2)_4C$], 2.51 (s, 3 H, OAc), 2.64 (s, 3 H, NAc), and 4.90–5.14 (m, 2 H, anomeric protons).

Anal. Calc. for $C_{22}H_{42}N_2O_{13} \cdot 0.5\ H_2O$ (551.58): C, 47.90; H, 7.86; N, 5.08. Found: C, 47.90; H, 7.98; N, 4.92.

6-(Benzyloxycarbonylamino)-1-hexyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**20**). — A mixture of **9** (120 mg, 189 μmol) and mercuric cyanide (47.7 mg, 189 μmol) in dry 1:1 (v/v) benzene–nitromethane (8 mL) was heated (85°) in an oilbath, with stirring, until the volume decreased to ~3 mL. Molecular sieves (Type 4A) were added, and the temperature was lowered to 60°. A total of three equivalents of **12** (each 105 mg, 189 μmol) was added to the mixture during 3 h. After 24 h, the mixture was cooled to room temperature, and filtered, and the filtrate was evaporated to a syrup. Chloroform (50 mL) was added, and the solution was successively washed with cold, saturated sodium hydrogencarbonate (35 mL) and water (2 × 40 mL), the aqueous layers being each back-extracted with chloroform (30 mL). The extracts were combined, dried (sodium sulfate), and evaporated, to yield an oil which was chromatographed on a column (2.5 × 35 cm) of silica gel Type 60 with solvent *C*. Fractions containing pure **20** were combined, and evaporated, to yield **20** (163 mg, 89%); p.m.r. data ($CDCl_3$): δ 1.14–1.74 [m, 8 H, $C(CH_2)_4C$], 1.98–2.39 (m, 15 H, NAc, 4 OAc), and 7.32 (s, 15 H, 3 Ph).

6-Aminohexyl 2-acetamido-2-deoxy-4-O-β-D-galactopyranosyl-β-D-glucopyranoside (**22**). — *O*-Deacetylation of **20** (to give **21**) and hydrogenolysis of **21** (to give **22**) was accomplished by the methods already described. The p.m.r. spectrum of **21** was consistent with the structure assigned. Compound **22** was isolated as the acetic salt. The overall yield in the preparation of **22** from **20** was 93%; p.m.r. data (D_2O): δ 1.50–2.38 [m, 8 H, $C(CH_2)_4C$], 2.47 (s, 3 H, OAc), 2.58 (s, 3 H, NAc), and 4.93–5.14 (m, 2 H, anomeric protons).

Anal. Calc. for $C_{22}H_{42}N_2O_{13}$ (542.57): C, 48.70; H, 7.80; N, 5.16. Found: C, 48.56; H, 7.67; N, 5.05.

Methylation analysis of the glycosides. — Methylation analyses of **16**, **19**, and **22** were performed as previously described[9], and gave the results expected. Thus, **16**, **19**, and **22** produced *O*-peracetylated 4,6-di-*O*-methyl, 3,4-di-*O*-methyl, and 3,6-di-*O*-methyl derivatives, respectively, of 2-deoxy-2-(*N*-methylacetamido)-D-glucitol.

DISCUSSION

Disaccharide glycosides may be prepared by one of two methods: formation of a monosaccharide glycoside followed by glycosylation to afford the disaccharide glycoside or, alternatively, synthesis of the disaccharide prior to coupling to the aglycon to yield the disaccharide glycoside. The latter approach requires three additional reactions once the disaccharide is formed (derivatization at the anomeric carbon atom of the reducing terminus, followed by coupling of the disaccharide to

the aglycon, and deprotection). Glycosylation with disaccharide derivatives tends to give lower yields than an equivalent reaction with monosaccharide derivatives. Therefore, we chose the former synthetic approach, involving preparation of the monosaccharide glycoside (example **3**) as an intermediate in the formation of the desired disaccharides (**16**, **19**, and **22**).

Coupling of a monosaccharide to a monosaccharide glycoside to form specifically linked disaccharides requires the appropriate protection of hydroxyl groups at which glycosylation is not desired. For this purpose, the unsubstituted glycoside **4** was converted into its 4,6-*O*-isopropylidene derivative **5** (96%), following a method[6] that gives an excellent yield. Glycosylation of **5** with **12** in the presence of mercuric cyanide at 60° gave the β-(1→3)-linked disaccharide glycoside (**14**) in 86% yield (see Experimental). Benzylation of **5**, followed by removal of the 4,6-*O*-isopropylidene group with acid under mild conditions afforded the 3-*O*-benzyl glycoside **7**, a key intermediate in the route to both the (1→4)- and (1→6)-linked disaccharide glycosides. Glycosylation of **7** with silver trifluoromethanesulfonate[9] at 0° produced the (1→6)-linked disaccharide **17** in 66% yield.

Selective protection of the 6-hydroxyl group of **7** was accomplished with 1-acetylimidazole, following a previously published method[10]. The resulting 6-*O*-acetyl-3-*O*-benzyl glycoside, **8**, was then glycosylated with **12**, to afford the (1→4)-linked disaccharide. A variety of reaction conditions were studied, all of which resulted in impractically low yields. In an effort to increase the yield in this difficult glycosylation reaction, the 6-hydroxyl group of **7** was protected with a (more stable) benzyl group. Partial benzylations of 2-acetamido-3-*O*-benzyl-2-deoxyglucosides have been reported where the aglycon is α-attached, and either benzyl[11] or allyl[7]. Partial benzylation of the β-glycoside **7** produced the desired 3,6-di-*O*-benzyl glycoside **9** in 47% yield, along with its 3,4-di-*O*-benzyl isomer **10** (19%) and the per-*O*-benzylated product **11** (28%).

The 3,6-di-*O*-benzyl compound **9** was also prepared (43%) by partial benzylation of the unsubstituted glycoside **4**. This route to **9** offers the advantages of having three fewer steps, and a higher overall yield, than the method starting from the 3-*O*-benzyl glycoside intermediate **7**. Compounds **9**, **10**, and **11** were identified by comparison (p.m.r. spectra and t.l.c.) with previously prepared samples. Finally, the 3,6-di-*O*-benzyl glycoside **9** was glycosylated with **12** to afford the β-(1→4)-linked disaccharide **20** in excellent yield (89%). In summary, this report describes the preparation of the 6-aminohexyl glycosides of all possible positional isomers of β-D-Gal→β-D-GlcNAc. These derivatives should prove useful in further studies on cell-carbohydrate recognition.

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