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Chemical synthesis of a hexasaccharide comprising the Lewis^x determinant linked β-(1 → 6) to a linear trimannosyl core and the precursor pentasaccharide lacking fucose [1]

Rakesh K. Jain, Khushi L. Matta *

Department of Gynaecologic Oncology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA

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Abstract

Phenyl 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-1-thio-α,β-mannopyranoside (**5**) was condensed with benzyl *O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**12**) in the presence of NIS-triflic acid to give, after removal of the chloroacetyl group, the key intermediate, benzyl *O*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**14**). A similar condensation of **6** and **7** with acceptor **14**, followed by the removal of protecting groups, afforded **16** and **18**, respectively. These compounds are expected to be useful in specificity studies of an antibody raised against a related, synthetic antigen that we are currently investigating.

Keywords: Lewis^x; *N*-Acetyllactosamine; Tumor antigens; Fucopyranosyl glycans

1. Introduction

Tumor-associated glycoproteins and glycolipids are known to carry Lewis^x (β-Gal-(1 → 4)[α-Fuc-(1 → 3)] β-GlcNAc) and 3′-sialyl Lewis^x determinants. The X-determinant was found to occur on carbohydrate chains in β-(1 → 6)-linkage with Man, GalNAc or Gal, in β-(1 → 2)-linkage with Man, or in β-(1 → 3)-linkage with Gal and

* Corresponding author.

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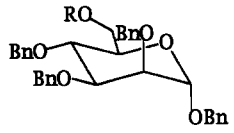
GalNAc [2–5]. The availability of synthetic saccharides containing the β-D-Gal-(1 → 4)-[α-L-Fuc-(1 → 3)]-GlcNAc moiety will be helpful in defining the fine specificities of various monoclonal and polyclonal antibodies raised against Lewis^x-containing membrane structures. Furthermore, these synthetic molecules when linked to protein, can be used as immunogens for the generation of useful antibodies.

Oligosaccharides with a reducing sugar can be directly attached to protein. However, under the coupling conditions, the monosaccharide moiety on the reducing end of the carbohydrate chain loses its pyranose form [6]. In order to preserve the pyranose form of the reducing terminal sugar of the core structure bearing the Lewis^x determinant, we extended the desired core structure with an additional sugar in order to leave the targeted core containing Lewis^x moiety intact when attached to protein for immunological studies. This strategy affords a simpler synthetic route to the desired hapten. For example, the title compound **18** contains an additional Man which when attached to the protein carrier will leave the desired carbohydrate structure, β-Gal-(1 → 4)-[α-Fuc-(1 → 3)]β-GlcNAc-(1 → 6)-α-Man-(1 → 6)-β-Man-(1 →), intact.

2. Results and discussion

A common intermediate, namely, benzyl *O*-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 6)-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**14**), was used for the synthesis of pentasaccharide (**16**) and hexasaccharide (**18**). For the synthesis of **14** we employed phenyl 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-1-thio-α,β-mannopyranoside (**5**) as a glycosyl donor and benzyl *O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**12**) as a glycosyl acceptor. Treatment of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose [7] with (phenylthio) trimethylsilane and trimethylsilyl triflate [8] afforded **4** in 79% yield after silica gel column chromatography. The ^{1}H NMR spectrum as well as ^{13}C NMR spectrum of **4** showed the mixture of α- and β-anomers in the ratio of 1:1.5 (on the basis of *O*-acetyl intensities). Chloroacetylation of **4** with chloroacetic anhydride–$NaHCO_3$–DMF [9] gave **5** in 78% yield. In the ^{13}C NMR spectrum, the resonance for C-6 displayed a downfield shift [δ 64.65 (α-anomer); δ 64.40 (β-anomer)] as compared to compound **4** [δ 61.94 (β-anomer); 61.57 (α-anomer)]. Compound **12** was obtained by condensation of known 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide [10] with benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside [11] (**2**). The latter was obtained from easily accessible benzyl α-D-mannopyranoside [12] in three steps. Thus, benzylation of this compound with benzyl bromide in THF in the presence of potassium hydroxide and 18-crown-6 ether [13] gave fully benzylated derivative **1** in 79% yield after silica gel column chromatography. Treatment of **1** with trimethylsilyl triflate in acetic anhydride [14] at −65 °C followed by de-*O*-acetylation with methanolic sodium methoxide afforded known **2** in 91% yield. In the ^{13}C NMR spectrum the C-6 resonance was observed at δ 69.83 (compound **1**) and δ 62.59 (compound **2**), which is a clear indication of the removal of O-6 benzyl group. The condensation of bromide **3** with **2** in chloroform in the presence of silver oxide gave the β-D-mannopyranosyl linked disaccharide **8** in 78% yield after purification through a silica gel column.



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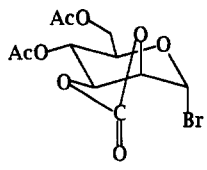
2 R = H



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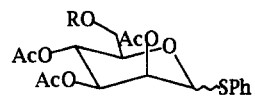
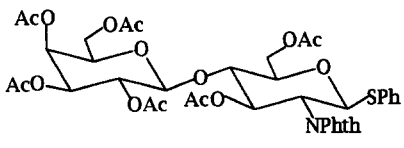


4 R = H
5 R = ClAc



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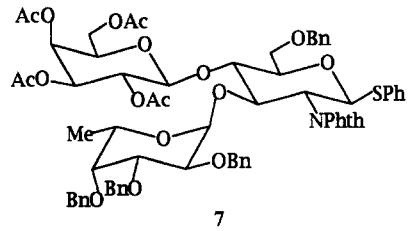

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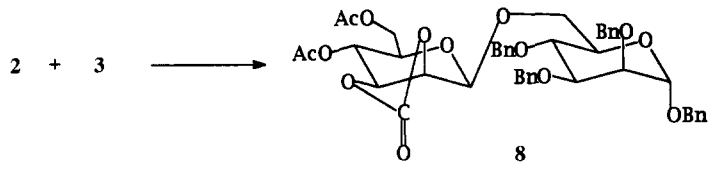
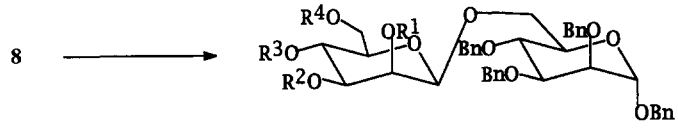
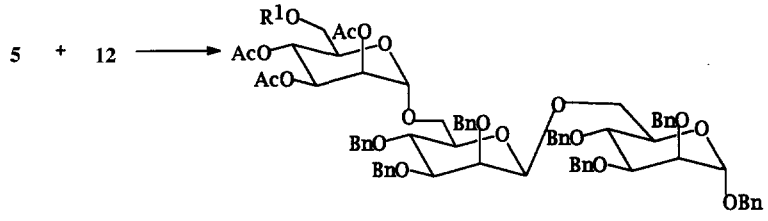
The ^{1}H NMR spectrum of **8** displayed characteristic signals for H-1′, H-1 and OAc at δ 5.03 (d, J 2.6 Hz), 4.96 (d, J 1.3 Hz), 2.00 and 1.92, respectively. Other signals were consistent with the structure assigned. The ^{13}C NMR spectrum contained two anomeric carbon signals at δ 97.50 ($^1J_{C,H}$ 159.23 Hz, C-1′) and 95.72 ($^1J_{C,H}$ 172.49 Hz, C-1), which confirmed a β-linkage for the newly incorporated mannopyranosyl residue. De-*O*-acetylation of **8** with methanolic sodium methoxide followed by benzylation, as described for the preparation of **2** (from **1**), gave the fully protected disaccharide **10** in 97% yield. A similar reaction sequence was employed for the synthesis of **12** from **10** as that described for the preparation of **2** from **1**. The ^{1}H NMR spectrum of **12** showed characteristic signals for H-1′ and H-1 at δ 5.24 (bs, H-1′) and 4.98 (bs, H-1), respectively. The ^{13}C NMR spectrum contained two anomeric carbon signals at δ 102.27 (C-1′) and 97.11 (C-1) and two C-6 signals at δ 69.27 (C-6) and 62.57 (C-6′). A regioselective glycosidation procedure with **12** through utilization of **5** in the presence of NIS-triflic acid [15] afforded the fully protected trisaccharide **13** in 80% yield (Scheme 1). The ^{1}H NMR spectrum of **13** displayed characteristic signals at δ 5.31 (dd, J 3.3 Hz, H-2″), 5.30 (d, J 1.5 Hz, H-1′), 5.29–5.28 (m, H-3″), 5.21 (t, J 10.0 Hz, H-4″), 4.99 (bs, H-1″), 4.98 (d, J 1.7 Hz, H-1), 4.09 (s, $COCH_2Cl$), 2.08, 1.92 and 1.88 (each s, 3 × OAc). The ^{13}C NMR spectrum displayed three anomeric carbons at δ 102.61 (C-1′), 98.13 (C-1″), 97.86 (C-1) and three C-6 carbons at δ 68.24 (C-6′), 66.49 (C-6) and 64.43 (C-6″). Removal of the chloroacetyl group from **13** with a thiourea–pyridine–ethanol procedure [16] provided an important intermediate **14** in 76% yield for further manipulation. Similarly, *N*-iodosuccinimide-triflic acid catalyzed glycosylation of **14** with known donor **6** [17] afforded, in 54% yield, the protected pentasaccharide derivative **15** (Scheme 2). The conversion of **15** into pentasaccharide **16** was then carried out in two steps: (a) treatment with methanolic sodium methoxide (de-*O*-acetylation), (b) 10% Pd–C/H_2 (hydrogenolysis for the removal of *O*-benzyl groups). The ^{1}H NMR





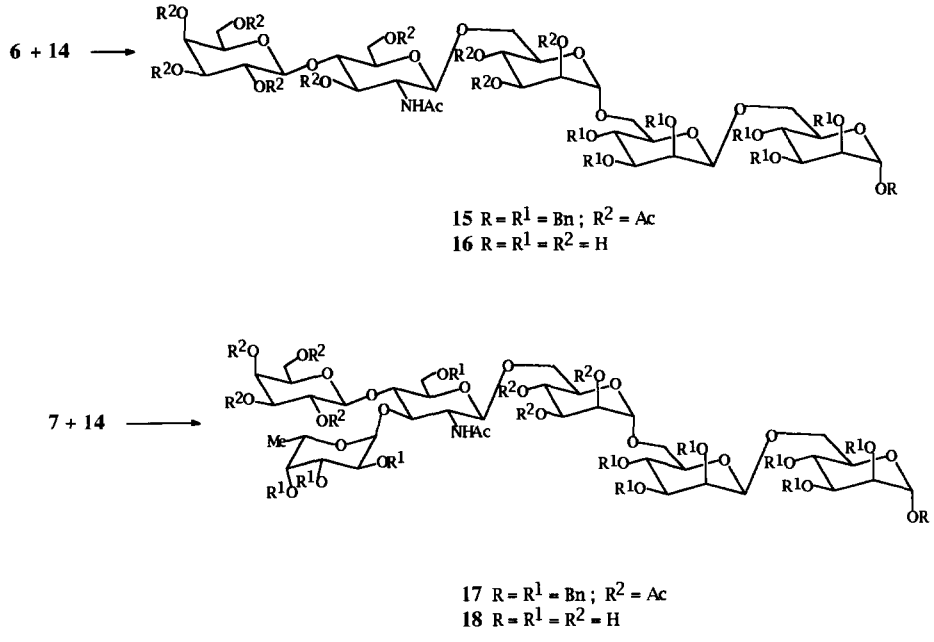
9 $R^1 = R^2 = R^3 = R^4 = H$
10 $R^1 = R^2 = R^3 = R^4 = Bn$
11 $R^1 = R^2 = R^3 = Bn$; $R^4 = Ac$
12 $R^1 = R^2 = R^3 = Bn$; $R^4 = H$



13 R^1 = ClAc
14 R^1 = H

Scheme 1.

spectrum of **16** showed five anomeric protons at δ 5.21 (bs, H-1′), 4.94 (bs, H-1″), 4.75 (bs, H-1), 4.63 (d, *J* 7.8 Hz, H-1‴), 4.52 (d, *J* 7.7 Hz, H-1⁗) and methyl acetamido protons at δ 2.10. The ^{13}C NMR spectrum showed five anomeric carbons at δ 101.88 (C-1⁗), 100.40 (C-1‴), 99.64 (C-1′), 98.57 (C-1″) and 93.08 (C-1). The structure of **16** was confirmed by ^{13}C NMR (see Table 1) and FAB mass spectroscopy (see Experimental section). For the synthesis of hexasaccharide **18**, compound **7** [18] was used as the glycosyl donor. A similar glycosylation of **14** with **7** at −75 °C followed by removal of the phthalimido group and *N*-,*O*-acetylation with pyridine–acetic anhydride afforded compound **17** in 66% yield. The ^{13}C NMR spectrum of **17** exhibited six anomeric carbons at δ 102.68 (C-1⁗), 102.14 (C-1‴), 100.39 (C-1′), 98.20 (C-1⁗), 97.85 (C-1″),



Scheme 2.

97.71 (C-1). The ^{13}C NMR (see Table 1) and FAB mass spectra of **18** were consistent with the structure assigned (see Experimental section).

3. Experimental

General methods.—Optical rotations were measured at ~ 25 °C with a Perkin–Elmer 241 Polarimeter. TLC was conducted on glass plates precoated with a 0.25 mm layer of

Table 1
^{13}C NMR chemical shifts [a] (ppm) (proposed assignments)

Residue	Compound no.	C-1	C-2	C-3	C-4	C-5	C-6	NAc
β-Gal-(1 → 4)	**16**	101.88	70.31	71.41	69.12	74.32	59.99	
β-GlcNAc-(1 → 6)		100.40	54.09	70.31	77.53	77.53	59.09	21.27
α-Man-(1 → 6)		98.57	69.58	69.96	65.66	73.31	67.93	
β-Man-(1 → 6)		99.64	69.12	69.96	64.65	73.74	68.84	
α-D-Man		93.08	69.34	69.58	65.66	71.99	67.55	
β-Gal-(1 → 4)	**18**	100.83	70.29	70.90	67.67	73.33	60.44	
α-Fuc-(1 → 3)		97.60	67.97	68.22	70.14	67.97	14.28	
β-GlcNAc-(1 → 6)		100.23	54.77	74.38	73.90	73.90	58.82	21.34
α-Man-(1 → 6)		98.58	69.34	69.64	67.33	71.99	65.59	
β-Man-(1 → 6)		99.64	68.84	70.05	65.79	72.44	65.76	
α-D-Man		93.09	69.12	69.58	66.72	71.48	64.66	

[a] Solutions in D_2O with Me_4Si as the external standard.

Silica Gel 60F-254 (Analtech GHLF uniplates). The compounds were located by exposure to UV light and (or) by spraying with 5% H_2SO_4 in EtOH and charring on a hot plate. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). NMR spectra were recorded at ~ 25 °C, ^{1}H NMR spectra with a Varian EM-390 at 90 MHz and with a Bruker AM-400 at 400 MHz and ^{13}C NMR spectra with a Bruker AM-400 at 100.6 MHz. All chemical shifts were referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhydrous sodium sulfate. Dichloromethane, *N,N*-dimethylformamide and 1,2-dichloroethane were dried over 4 Å molecular sieves. Elemental analyses were performed by Robertson Laboratory, Madison, New Jersey, USA. All new compounds gave satisfactory elemental analysis.

Benzyl 2,3,4,6-tetra-O-*benzyl*-α-D-*mannopyranoside* (**1**).—To a stirred mixture of benzyl α-D-mannopyranoside (15 g; 55.6 mmol), powdered KOH (22.5 g; 402 mmol), and 18-crown-6 (2.5 g; 9.3 mmol) in oxolane (400 mL) was added benzyl bromide (40 mL; 333 mmol) dropwise, and stirring was continued for 16 h at room temperature. The mixture was diluted with methylene chloride (600 mL) and washed with water, dried and evaporated. The residue was applied to a column of silica gel. Elution with 4:1 (v/v) hexane–ethyl acetate and evaporation of the fractions corresponding to the product yielded **1** (29 g; 79%), $[\alpha]_D$ +31° (*c* 1.6, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.35–7.19 (m, 25 H, arom.), 4.96 (d, *J* 1.7 Hz, 1 H, H-1), 4.86 (d, *J* 10.8 Hz, 2 H, OCH_2Ph), 4.73–4.62 (m, 8 H, 4 × OCH_2Ph), 3.94 (dd, *J* 8.4, *J* 2.1 Hz, 1 H, H-3); ^{13}C NMR: δ 98.04 (C-1), 80.86 (C-3), 76.01 (C-2), 75.83, 73.90, 73.54 (4 × OCH_2Ph), 72.71 (C-5), 69.97 (C-4), 69.63 (C-6). Anal. Calc. for $C_{41}H_{42}O_6$: C, 78.07; H, 6.71. Found: C, 78.29; H, 6.63.

Benzyl 2,3,4-tri-O-*benzyl*-α-D-*mannopyranoside* (**2**).—To a solution of **1** (18 g) in acetic anhydride (180 mL) was added 1:1 (v/v) trimethylsilyltrifluoromethane sulfonate–methylene chloride (11.6 mL) dropwise at −65 °C and the solution was stirred for 1 h at the same temperature. The mixture was poured onto 1:1 (v/v) methylene chloride-saturated $NaHCO_3$ solution (2000 mL) and stirred for 0.5 h. The organic layer was washed with water, dried and concentrated under reduced pressure. The crude product was de-*O*-acetylated with methanolic sodium methoxide for 2 h and applied to a column of silica gel. The column was eluted with 15–20% ethyl acetate in hexane. Evaporation of the fractions corresponding to the product gave **2** as a syrup (14.1 g; 91%); $[\alpha]_D$ +45° (*c* 1.7, $CHCl_3$); lit. [11] $[\alpha]_D$ +46° (*c* 1.8, $CHCl_3$): ^{1}H NMR (CD_2Cl_2): δ 7.33–7.20 (m, 20 H, arom.), 4.92 (d, *J* 1.6 Hz, 1 H, H-1), 4.88 (d, *J* 11.0 Hz, 2 H, OCH_2Ph), 4.70–4.58 (m, 6 H, 3 × OCH_2Ph), 3.96 (dd, *J* 8.5 and 2.0 Hz, 1 H, H-3); ^{13}C NMR: δ 98.27 (C-1), 80.82 (C-3), 76.06 (C-2), 75.60, 75.45, 73.69, and 73.53 (4 × OCH_2Ph), 72.45 (C-5), 69.68 (C-4), 62.59 (C-6).

Phenyl 2,3,4-tri-O-*acetyl-1-thio*-α,β-D-*mannopyranoside* (**4**).—To a stirred solution of 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose (7.0 g, 23.3 mmol) in methylene chloride (80 mL) was added trimethyl(phenylthio)silane (20 mL, 105.8 mmol) and trimethylsilyl triflate (6.4 mL, 33.2 mmol). Stirring was continued for 48 h at room temperature. The organic layer was washed with cold water, aq $NaHCO_3$, dried and concentrated. The residue was applied to a column of silica gel and the column was eluted with a 10–15% gradient of acetone in chloroform. Evaporation of product fractions gave **4** (7.2 g, 79%); ^{1}H NMR (CD_2Cl_2): δ 7.50–7.46 (m, 2 H, arom.), 7.36–7.28 (m, 3 H, arom.),

5.64–5.63 (m, 0.4 H, H-2α), 5.50 (bs, 0.6 H, H-1β), 5.49 (bs, 0.4 H, H-1α), 5.49–5.33 (m, 0.6 H, H-3β), 5.27–5.19 (m, 0.6 H, H-2β), 5.08 (dd, *J* 3.5 Hz, 0.4 H, H-3α), 2.15, 2.02 and 1.95 (3 × OAc α-isomers), 2.11, 2.06 and 1.99 (3 × OAc, β-isomer); ^{13}C NMR: δ 86.35 (C-1β), 85.76 (C-1α), 61.94 (C-6β), 61.57 (C-6α). Anal. Calcd for $C_{18}H_{22}O_8S$: C, 54.26; H, 5.57. Found: C, 55.22; H, 5.60.

Phenyl 2,3,4-tri-O-*acetyl-6*-O-*chloroacetyl-1-thio-*α,β-D-*mannopyranoside* (**5**).—To a stirred solution of **4** (7.0 g; 17.6 mmol) in dry DMF (70 mL) containing $NaHCO_3$ (5.2 g; 62 mmol) was added chloroacetic anhydride (7.0 g, 41 mmol), and the stirring was continued for 2 h at room temperature. The reaction mixture was poured into ice–water and extracted with chloroform, dried, and concentrated *in vacuo*. The residue was purified on a column of silica gel with 9:1 (v/v) $CHCl_3$–acetone as the eluent to give **5** (6.5 g; 78%); ^{1}H NMR (CD_2Cl_2): δ 7.50–7.47 (m, 2 H, arom.), 7.36–7.29 (m, 3 H, arom.), 5.64–5.63 (m, 0.4 H, H-2α), 5.50 (d, *J* 1.2 Hz, 0.6 H, H-1β), 5.48 (bs, 0.4 H, H-1α), 5.34–5.26 (m, 0.6 H, H-3β), 5.27–5.21 (m, 0.6 H, H-2β), 5.08 (dd, *J* 3.6 Hz, 0.4 H, H-3α), 4.11 (s, 0.8 H, $COCH_2Cl$-α), 4.03 (s, 1.2 H, $COCH_2Cl$-β), 2.16, 2.02 and 1.95 (3 × OAc α-isomer), 2.12, 2.06 and 1.99 (3 × OAc β-isomer); ^{13}C NMR: δ 86.03 (C-1β), 85.78 (C-1α), 64.65 (C-6α), 64.40 (C-6β), 41.35 ($COCH_2Cl\alpha$), 41.27 ($COCH_2Cl\beta$). Anal. Calcd for $C_{20}H_{23}O_9ClS$: C, 50.58; H, 4.88. Found: C, 50.45; H, 4.91.

Benzyl O-*(4,6-di*-O-*acetyl-2,3*-O-*carbonyl-*β-D-*mannopyranosyl)-(1* → *6)-2,3,4-tri*-O-*benzyl-*α-D-*mannopyranoside* (**8**).—A solution of **2** (9.0 g; 16.7 mmol) in chloroform (100 mL) was stirred for 0.5 h with silver oxide (12 g; 52 mmol) and $CaSO_4$ (13 g) under protection from light and moisture. A solution of bromide **3** (9.0 g; 25.5 mmol) in chloroform (150 mL) was then added dropwise during 3 h, and stirring was continued for an additional 0.5 h. The mixture was filtered through Celite, the solids were thoroughly washed with chloroform, and the filtrate and washings combined and concentrated to a small volume. The concentrate was applied to a column of silica gel and eluted with a solvent gradient consisting of 50–60% ethyl acetate in hexane. On evaporation, the fractions corresponding to the product gave a syrup which was dissolved in methylene chloride. The addition of hexane precipitated **8** (11.5 g; 78%); $[\alpha]_D$ +6° (*c* 1.6, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.34–7.23 (m, 20 H, arom.), 5.71–5.67 (m, 1 H, H-2′), 5.03 (d, *J* 2.6 Hz, H-1′), 4.96 (d, *J* 1.3 Hz, 1 H, H-1), 2.00 and 1.92 (each s, 6 H, 2 × OAc); ^{13}C NMR: δ 97.50 ($^1J_{C,H}$ 159.23 Hz, C-1′), 95.72 ($^1J_{C,H}$ 172.49, C-1), 80.76 (C-3), 77.02 (C-2), 67.75 (C-6), 63.86 (C-6′), 21.04, and 21.01 (2 × $COCH_3$). Anal. Calcd for $C_{45}H_{48}O_{14}$: C, 66.49; H, 5.95. Found: C, 66.62; H, 5.70.

Benzyl O-*(2,3,4,6-tetra*-O-*benzyl-*β-D-*mannopyranosyl)-(1* → *6)-2,3,4-tri*-O-*benzyl-*α-D-*mannopyranoside* (**10**).—A solution of **8** (20 g) in methanol (350 mL) and oxolane (50 mL) was treated with 0.01 M sodium methoxide for 16 h at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin, the resin suspension was filtered, and the filtrate concentrated to give a solid residue. A stirred solution of this solid in dry oxolane (150 mL) was treated with KOH (8.0 g; 142.8 mmol), 18-crown-6 (1.0 g) and benzyl bromide (11.35 mL, 95.5 mmol), as described for the preparation of **1**, to give **10** (25.5 g; 97%); $[\alpha]_D$ −0.2° (*c* 1.1, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.36–7.20 (m, 40 H, arom.), 5.25 (bs, 1 H, H-1′), 4.98 (d, *J* 1.3 Hz, 1 H,

H-1); ^{13}C NMR: δ 138.54–127.21 (C-arom.), 102.17 (C-1′), 96.89 (C-1), 69.77 (C-6), 68.67 (C-6′). Anal. Calcd for $C_{68}H_{70}O_{11}$: C, 76.68; H, 6.64. Found: C, 76.59; H, 6.71.

Benzyl O-*(6-O-acetyl-2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside* (**11**).—To a cold (−65 °C bath), stirred solution of **10** (12 g) in acetic anhydride (120 mL) was added 1:5 (v/v) trimethylsilyltriflate–CH_2Cl_2 (18 mL), and stirring was continued for 0.5 h. After processing as described for the preparation of **2** and by column chromatographic purification with 15–20% ethyl acetate in hexane as the eluent, compound **11** (7.8 g; 72%) was obtained; $[\alpha]_D$ +5° (*c* 0.3, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.38–7.21 (m, 35 H, arom.), 5.26 (bs, 1 H, H-1′), 4.97 (d, *J* 1.1 Hz, 1 H, H-1), 2.00 (s, 3 H, OAc). Anal. Calcd for $C_{63}H_{66}O_{12}$: C, 74.53; H, 6.55. Found: C, 74.61; H, 6.63.

Benzyl O-*(2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside* (**12**).—De-*O*-acetylation of **11** (7.6 g) with 0.02 M methanolic sodium methoxide for 2 h followed by silica gel column chromatography (solvent gradient consisting of 25–30% ethyl acetate in hexane) afforded a quantitative yield of **12**; $[\alpha]_D$ +9° (*c* 0.5, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.39–7.22 (m, 35 H, arom.), 5.24 (bs, 1 H, H-1′), 4.98 (bs, 1 H, H-1); ^{13}C NMR: δ 102.27 (C-1′), 97.11 (C-1), 69.27 (C-6), 62.57 (C-6′). Anal. Calcd for $C_{61}H_{64}O_{11}$: C, 72.45; H, 7.39. Found: C, 72.38; H, 7.41.

General procedure for glycosidation.—A solution of donors [**5** or **6** or **7** (1.2 mmol)], acceptors (**12** or **14**) and *N*-iodosuccinimide (2.5 mmol) in CH_2Cl_2 (25 mL) was stirred for 0.5 h with 4 Å molecular sieves (5 g) under an argon atmosphere at 0 °C (for compounds **13** and **15**) or at −70 °C (for compound **17**). A dilute solution of trifluoromethanesulfonic acid (0.1 mL in 15 mL CH_2Cl_2) was then added dropwise. Stirring was continued at the same temperature for another 1 h after which time the acid was neutralized with saturated aq $NaHCO_3$ solution. The mixture was filtered through Celite and the solids were thoroughly washed with CH_2Cl_2. The filtrate and washings were combined, successively washed with water, saturated $NaHCO_3$, 10% aq sodium thiosulfate, dried and then concentrated in vacuo.

Benzyl O-*(2,3,4-tri-O-acetyl-6-O-chloroacetyl-α-D-mannopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside* (**13**).—Glycosylation of **12** (4.5 g; 4.6 mmol) with donor **6** (2.8 g; 5.9 mmol) afforded **13** (5.0 g; 80%) after silica gel column chromatography (25–35% ethyl acetate in hexane); $[\alpha]_D$ +27° (*c* 1.7, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.38–7.21 (m, 35 H, arom.), 5.31 (dd, *J* 3.3 Hz, 1 H, H-2″), 5.30 (d, *J* 1.5 Hz, 1 H, H-1′), 5.29–5.28 (m, 1 H, H-3″), 5.21 (t, *J* 10.0 Hz, 1 H, H-4″), 4.99 (bs, 1 H, H-1″), 4.98 (d, *J* 1.7 Hz, 1 H, H-1), 4.09 (s, 2 H, $COCH_2Cl$), 2.08, 1.92, and 1.88 (each s, 9 H, 3 × OAc); ^{13}C NMR: δ 102.61 (C-1′), 98.13 (C-1″), 97.86 (C-1), 68.24 (C-6′), 66.49 (C-6), 64.43 (C-6″), 41.58 ($COCH_2Cl$), 21.17, 21.02, and 20.99 (3 × $COCH_3$). Anal. Calcd for $C_{75}H_{81}O_{20}Cl$: C, 67.33; H, 6.10. Found: C, 67.39; H, 6.12.

Benzyl O-*(2,3,4-tri-O-acetyl-α-D-mannopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzyl-β-D-mannopyranosyl)-(1 → 6)-2,3,4-tri-O-benzyl-α-D-mannopyranoside* (**14**).—A solution of **13** (2.5 g; 1.88 mmol) and thiourea (0.2 g; 3.3 mmol) in 4:1 (v/v) pyridine–ethanol (50 mL) was stirred for 1 h at 90 °C. The solvent was removed under reduced pressure and the residue dissolved in CH_2Cl_2. The organic layer was washed with water, dried,

and concentrated to a small volume. The concentrate was applied to a column of silica gel and the column eluted with a 20–25% gradient of ethyl acetate in hexane. On concentration, the fraction afforded **14** (1.6 g; 76%) amorphous; $[\alpha]_D$ +31° (*c* 1.2, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.38–7.19 (m, 35 H, arom.), 5.35 (dd, *J* 3.4 Hz, 1 H, H-2″), 5.30 (t, *J* 9.9 Hz, 1 H, H-3″), 5.17 (t, *J* 9.9 Hz, 1 H, H-4″), 4.99 (bs, 1 H, H-1′), 4.97 (d, *J* 1.9 Hz, 1 H, H-1″), 4.91 (d, *J* 1.4 Hz, 1 H, H-1), 2.06, 1.93 and 1.92 (each s, 9 H, 3 × OAc); ^{13}C NMR: δ 102.66 (C-1′), 98.27 (C-1″), 97.78 (C-1), 68.15 (C-6′), 66.88 (C-6), 61.69 (C-6″). Anal. Calcd for $C_{73}H_{80}O_{19}$: C, 66.30; H, 7.07. Found: C, 66.52; H, 7.29.

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)*-O-*(2-acetamido-3,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzyl-β*-D-*mannopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzyl-α*-D-*mannopyranoside* (**15**).—Glycosidation of **14** (0.3 g; 0.26 mmol) with **6** (0.3 g; 0.37 mmol) was followed by treatment with 4:1 (v/v) ethanol–hydrazine hydrate (4:1; v/v) at 100 °C for 2 h. The solvent was evaporated to give a residue which was dissolved in pyridine (50 mL) and acetic anhydride (25 mL) and stirred overnight at room temperature. Pyridine and acetic anhydride were removed under reduced pressure. The residue was applied to a column of silica gel and eluted with 5% methanol in chloroform to give **15** (0.25 g; 54%); $[\alpha]_D$ +14° (*c* 0.4, $CHCl_3$); ^1H NMR (CD_2Cl_2): δ 7.37–7.21 (m, 35 H, arom.), 6.06 (d, *J* 9.2 Hz, 1 H, NH), 5.34 (dd, *J* 2.8 Hz, 1 H, H-2″), 5.32 (bs, 1 H, H-1″), 5.31 (d, *J* 1.0 Hz, 1 H, H-4‴), 5.06 (d, *J* 7.7 Hz, 1 H, H-1‴), 5.03 (t, *J* 9.4 Hz, 1 H, H-4″), 4.98 (t, *J* 3.1 Hz, 1 H, H-3″), 4.94 (d, *J* 2.0 Hz, 1 H, H-1″), 4.88 (d, *J* 2.3 Hz, 1 H, H-1), 2.11–1.26 (cluster of s, 30 H, 9 × OAc and NAc). Anal. Calcd for $C_{99}H_{115}NO_{35}$: C, 63.28; H, 6.17; N, 0.75. Found: C, 63.49; H, 6.25; N, 0.72.

O-*(β*-D-*Galactopyranosyl)-(1 → 4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1 → 6)*-O-*(α*-D-*mannopyranosyl)-(1 → 6)*-O-*(β*-D-*mannopyranosyl)-(1 → 6)-α*-D-*mannopyranose* (**16**).—A solution of compound **15** (0.24 g) in 0.02 M sodium methoxide in methanol (20 mL) was stirred for 16 h at room temperature. The solution was deionized with Amberlite IR-120 (H^+) cation-exchange resin, filtered and concentrated under reduced pressure. The residue was dissolved in glacial acetic acid (50 mL) and shaken with 10% Pd–C (1 g) under hydrogen at ~ 345 kPa for 4 days at room temperature. The suspension was filtered through a bed of Celite, the solids were thoroughly washed with glacial acetic acid, and the combined filtrate and washings were concentrated under reduced pressure. The crude product was applied to a column of silica gel and eluted with 4:5:1 (v/v) $CHCl_3$–MeOH–H_2O. The fractions corresponding to **16** were concentrated and lyophilized to give an amorphous solid (0.042 g; 36%); $[\alpha]_D$ −9° (*c* 0.7, H_2O); ^1H NMR (D_2O): δ 5.21 (bs, 1 H, H-1′), 4.94 (bs, 1 H, H-1″), 4.75 (bs, 1 H, H-1), 4.63 (d, *J* 7.8 Hz, 1 H, H-1‴), 4.52 (d, *J* 7.7 Hz, 1 H, H-1⁗), 2.10 (s, 3 H, NAc); For ^{13}C NMR data see Table 1; *m/z* 868.5 $(M - H)^-$, 891.9 $(M + Na)^+$. Anal. Calcd for $C_{32}H_{55}NO_{26} \cdot 2\ H_2O$: C, 42.45; H, 6.57; N, 1.55. Found: C, 42.31; H, 6.61; N, 1.49.

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 4)*-O-*[2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl-(1 → 3)*-O*]-(2-acetamido-6*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*acetyl-α*-D-*mannopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzyl-β*-D-*mannopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzyl-α*-D-*mannopyranoside* (**17**).—

Glycosidation of **14** (1.1 g; 9.6 mmol) with donor **7** (2.2 g; 12.1 mmol) followed by processing as described for the preparation of **15** gave **17** (1.3 g; 66%) after silica gel column chromatography (solvent gradient consisting of 1:1 to 2:3 (v/v) hexane–ethyl acetate); $[\alpha]_D$ +5° (*c* 1.5, $CHCl_3$); ^{1}H NMR (CD_2Cl_2): δ 7.38–7.20 (m, 55 H, arom.), 5.93 (d, *J* 8.7 Hz, 1 H, NH), 5.33 (dd, *J* 3.6 and 2.4 Hz, 1 H, H-2″), 5.31 (bs, 1 H, H-4‴″), 5.29 (d, *J* 1.0 Hz, 1 H, H-1′), 5.27 (t, *J* 8.8 and 3.3 Hz, 1 H, H-3″), 5.00 (d, *J* 2.1 Hz, 1 H, H-1⁗), 4.98 (d, *J* 1.7 Hz, 1 H, H-1‴), 4.96 (d, *J* 1.7 Hz, 1 H, H-1), 2.17, 1.99, 1.98, 1.97, 1.93, 1.91, 1.90, and 1.89 (each s, 24 H, 7 × OAc and NAc), 1.23 (d, *J* 6.5 Hz, 3 H, H-6⁗); ^{13}C NMR: δ 102.68 (C-1⁗), 102.14 (C-1‴), 100.39 (C-1′), 98.20 (C-1⁗), 97.85 (C-1″), 97.71 (C-1), 61.11 (C-6⁗), 57.26 (C-2‴), 17.06 (C-6⁗). Anal. Calcd for $C_{122}H_{138}NO_{37}$: C, 66.29; H, 6.29; N, 0.63. Found: C, 66.58; H, 6.12; N, 0.71.

O-(β-D-*Galactopyranosyl*)-(1 → 4)-O-[α-L-*fucopyranosyl*-(1 → 3)]-(2-*acetamido*-2-*deoxy*-β-D-*glucopyranosyl*)-(1 → 6)-O-(α-D-*mannopyranosyl*)-(1 → 6)-O-(β-D-*mannopyranosyl*)-(1 → 6)-α-D-*mannopyranose* (**18**).—Compound **17** (0.4 g) was stirred in 0.05 M methanolic sodium methoxide (50 mL) for 16 h at room temperature. The solution was deionized with Amberlite IR-120 (H^+) cation exchange resin, filtered and concentrated under reduced pressure. The residue was dissolved in glacial acetic acid (40 mL) and shaken with 10% Pd–C (1.5 g) under hydrogen at ~ 345 kPa as described for the preparation of **16**. After purification over a silica gel column with 4:5:1 (v/v) $CHCl_3$–MeOH–H_2O as the eluent, **18** (0.09 g; 49%) was obtained as an amorphous solid; $[\alpha]_D$ −4° (*c* 0.7, H_2O); ^{1}H NMR (D_2O): δ 5.21 (d, *J* 1.7 Hz, 1 H, H-1′), 5.16 (d, *J* 4.0 Hz, 1 H, H-1⁗), 4.94 (bs, 1 H, H-1″), 4.76 (d, *J* 2.7 Hz, 1 H, H-1), 4.64 (d, *J* 7.8 Hz, 1 H, H-1), 4.50 (d, *J* 7.8 Hz, 1 H, H-1‴), 2.10 (s, 3 H, NAc), 1.22 (d, *J* 6.6 Hz, 3 H, H-6⁗); For ^{13}C NMR data see Table 1; *m*/*z* 1014.2 $(M - H)^-$. Anal. Calcd for $C_{38}H_{65}NO_{30}.H_2O$: C, 44.14; H, 6.53; N, 1.36. Found: C, 44.05; H, 6.62; N, 1.21.

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