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Synthesis of oligosaccharide substrates for *N*-linked glycoprotein processing enzymes ☆

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Abstract

The stereoselective syntheses of one pentasaccharide and one tetrasaccharide containing the Glc-α-(1 → 3)-Man-α moiety as their terminal unit, as well as one tetrasaccharide and one trisaccharide containing the Man-α-(1 → 2)-Man-α terminal unit were accomplished through the utilization of two key glycosyl donors, namely, 4-pentenyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside and ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside.

Keywords: Oligosaccharide substrates; *N*-linked glycoprotein

1. Introduction

The biosynthesis of the *N*-linked class of glycoproteins involves block transfer of the $Glc_3Man_9GlcNAc_2$ precursor oligosaccharide from its dolichol pyrophosphoryl derivative to nascent polypeptide chains, followed by a sequential removal of the three glucose residue [2–4]. Glucose removal takes place in the rough endoplasmic reticulum through the action of α-glucosidases I and II. The release of mannose is achieved in the Golgi complex by α-mannosidases I and II [5]. Lubas and Spiro [6,7] discovered a Golgi-situated endo-α-mannosidase which converts $Glc_{1-3}Man_{4-9}GlcNAc$ to $Man_{3-8}GlcNAc$ with the release of $Glc_{1-3}Man$, and thus demonstrated a glucosidase-independent pathway for the formation of complex *N*-linked oligosaccharides [8]. The above studies

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used as their substrates radiolabeled oligosaccharides isolated from tissue slices or cultured cells which had been incubated with D-[UL-^{14}C] glucose or another desired radio labeled sugar.

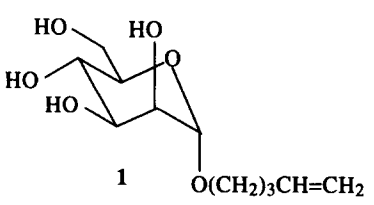
The non-availability of chemically synthesized substrates precluded any meaningful kinetic and specificity studies of these processing enzymes. The present paper represents our effort to initiate studies along these lines and reports the chemical synthesis of Man-α-(1 → 2)-Man-α-(1 → 2)-Man-α-OMe (**11**), Glc-α-(1 → 3)-Man-α-(1 → 2)-Man-α-(1 → 2)-Man-α-OMe (**13**), Man-α-(1 → 2)-Man-α-(1 → 2)-Man-α-(1 → 3)-Man-α-OMe (**20**) and Glc-α-(1 → 3)-Man-α-(1 → 2)-Man-α-(1 → 2)-Man-α-(1 → 3)-Man-α-OMe (**22**). The availability of these compounds will also facilitate specificity studies of Calnexin [9], a lectin-like protein which binds to monoglucosylated *N*-linked glycoproteins containing Glc-α-(1 → 3)-Man-α as the terminal sequence.

2. Results and discussion

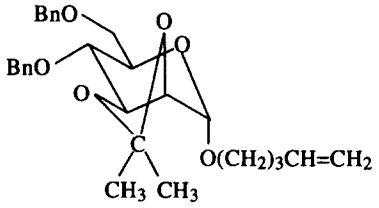
The synthesis and use of mannopyranosyl donors containing a permanent protecting group (benzyl) at O-3, O-4 and O-6 and a temporary protecting group (acetyl) at O-2 have been reported in the literature [10–17]. Our strategy involved the utilization of ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside [15,16] (**6**) as a key glycosyl donor. This glycosylating reagent, after condensation with an appropriately protected alcohol followed by de-*O*-acetylation, provides the desired intermediate with its C-2′ hydroxy free for further glycosylation and elongation. Similarly, our desire to introduce an α-D-glucopyranosyl residue at the O-3 position of the last mannopyranosyl residue resulted in the development and utilization of 4-pentenyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**5**) as an important glycosyl donor.

Isopropylidenation of 4-pentenyl-α-D-mannopyranoside [18] (**1**) with 2,2-dimethoxypropane–acetone (1 : 1, v/v) in the presence of 4-toluenesulfonic acid followed by a selective hydrolysis of the 4,6-*O*-isopropylidene afforded 4-pentenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside which on treatment with benzyl bromide in THF in the presence of potassium hydroxide and 18-crown-6 ether [19] gave 4-pentenyl 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranoside (**2**) in 65% yield (Scheme 1). Treatment of compound **2** with 70% aq acetic acid at 70°C provided the diol **3** in 56% yield (Scheme 1). The reaction of **3** with benzyl bromide in a mixture of methylene chloride and aqueous sodium hydroxide in the presence of tetrabutylammonium hydrogen sulfate under phase transfer catalysis [20] provided the major 2-*O*-benzylated derivative **4** in 62% yield along with the minor 3-*O*-benzylated derivative. Acetylation of **4** with pyridine–acetic anhydride furnished the glycosyl donor **5** in 84% yield (Scheme 1). The ^{1}H NMR spectrum of **5** exhibited two low-field chemical shifts at δ 5.24 (dd, $J = 3.3$ Hz, H-3) and 5.01 (d, $J = 1.6$ Hz, H-1), confirming that compound **5** had been acetylated at O-3.

Synthesis of **11** and **13**. — A regioselective glycosidation procedure [21] with methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside through utilization of **6** in the presence of NIS-triflic acid afforded the fully protected disaccharide **7** in 78% yield (Scheme 2). The ^{1}H NMR spectrum of **7** displayed the characteristic signals for H-2′, H-1′ and H-1 at δ



1



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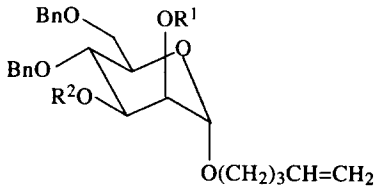
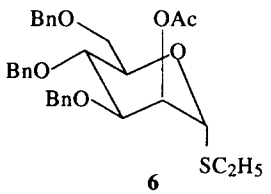


3 $R^1 = R^2 = H$

4 R^1 = Bn, R^2 = H

5 R^1 = Bn, R^2 = Ac



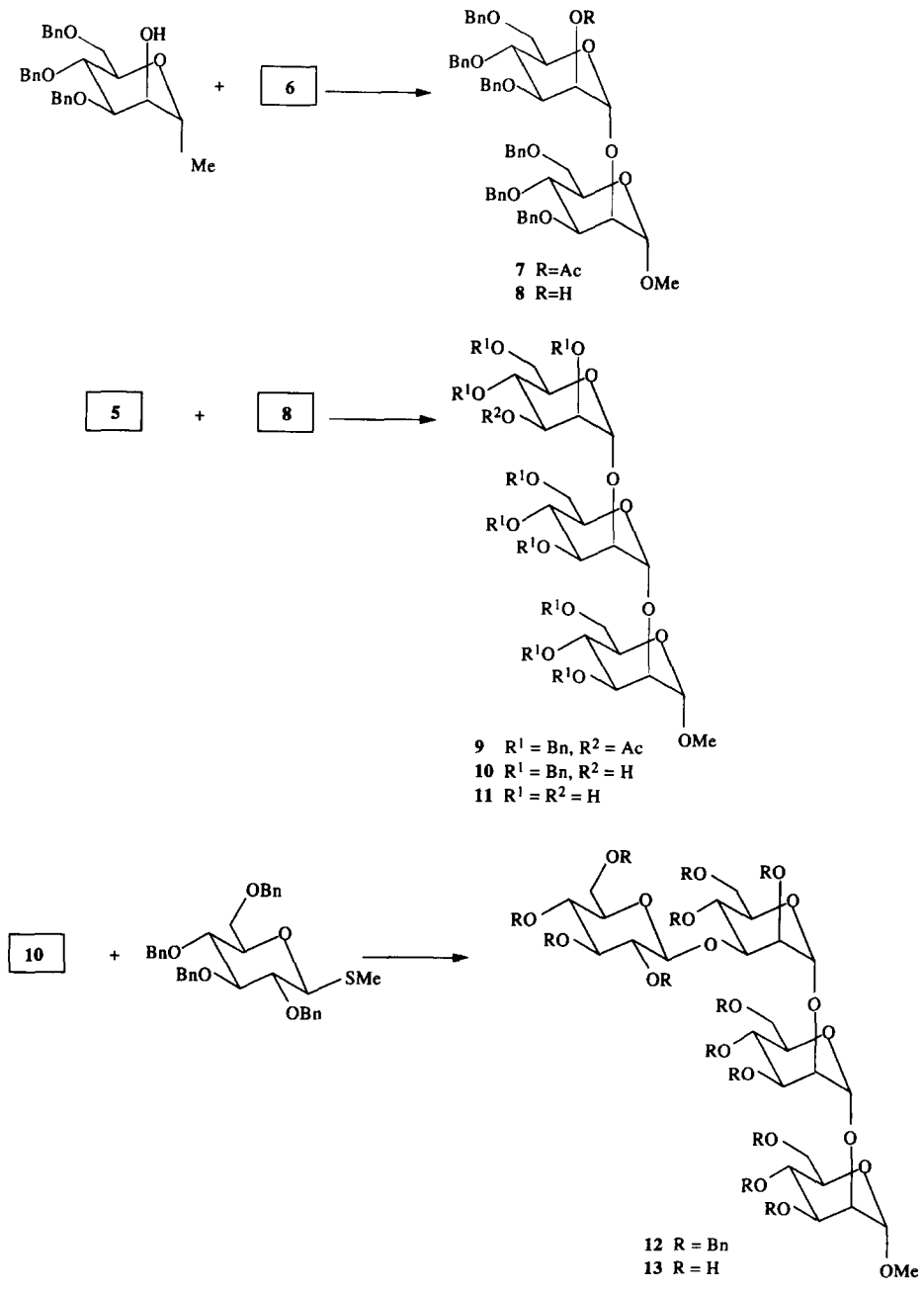
6

Scheme 1.

5.53 (m), 5.07 (bs) and 4.78 (bs), respectively. Other signals were consistent with the structure assigned. The ^{13}C NMR spectrum contained two anomeric carbon signals at δ 99.66 (C-1′) and 99.52 (C-1). De-*O*-acetylation of compound **7** with methanolic sodium methoxide provided the acceptor **8** in 94% yield for further manipulation.

Similarly, *N*-iodosuccinimide-triflic acid-catalyzed glycosylation of **8** with donor **5** afforded, in 64% yield, the protected trisaccharide derivative **9** (Scheme 2). The conversion of **9** into trisaccharide **11** was then carried out in 2 steps: (1) treatment with methanolic sodium methoxide to give compound **10** (de-*O*-acetylation) and (2) 10% Pd–C/H_2 (hydrogenolysis for the removal of O-benzyl groups). The ^1H NMR spectrum of methyl *O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 2)-α-D-mannopyranoside (**11**) showed three doublets at δ 5.33 (J = 1.5 Hz, H-1″), 5.09 (d, J = 1.6 Hz, H-1′) and 5.02 (d, J = 1.3 Hz). The ^{13}C NMR spectrum showed three anomeric carbons at δ 101.2 (C-1″), 99.59 (C-1′) and 98.27 (C-1).

The condensation of trisaccharide acceptor **10** with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside [22] was performed in the presence of copper bromide–tetrabutylammonium bromide [23] to furnish the protected tetrasaccharide **12** in 39% yield (Scheme 2). The ^1H NMR spectrum of **12** exhibited characteristic signals for H-1″ (δ 5.26), H-1′ (δ 5.19), H-1 (δ 4.85) and a doublet at δ 5.16 ($J_{1,2}$ = 3.4 Hz), which confirmed an α-linkage for the newly incorporated glucopyranosyl moiety. Hydrogenolytic cleavage of the *O*-benzyl groups of **12** in glacial acetic acid and in the presence of 10% palladium-on-carbon furnished the desired tetrasaccharide, methyl *O*-(α-D-glucopyranosyl)-(1 → 3)-*O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-D-mannopyranosyl (**13**), in 42% yield. The ^{13}C NMR spectrum of


BnO
OH
BnO
BnO
O
Me
+
6
BnO
OR
BnO
BnO
O
BnO
O
BnO
O
BnO
OMe
7 R=Ac
8 R=H
5
+
8
R¹O
R¹O
R¹O
R²O
O
R¹O
O
R¹O
R¹O
R¹O
O
R¹O
O
R¹O
R¹O
OMe
9 R¹ = Bn, R² = Ac
10 R¹ = Bn, R² = H
11 R¹ = R² = H
10
+
OBn
BnO
O
BnO
SMe
OBn
OR
RO
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O
RO
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O
OR
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RO
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OMe
12 R = Bn
13 R = H

Scheme 2.

13 exhibited four anomeric carbons at δ 101.0 (C-1″), 99.58 (C-1′), 99.63 (C-1) and 98.26 (C-1‴).

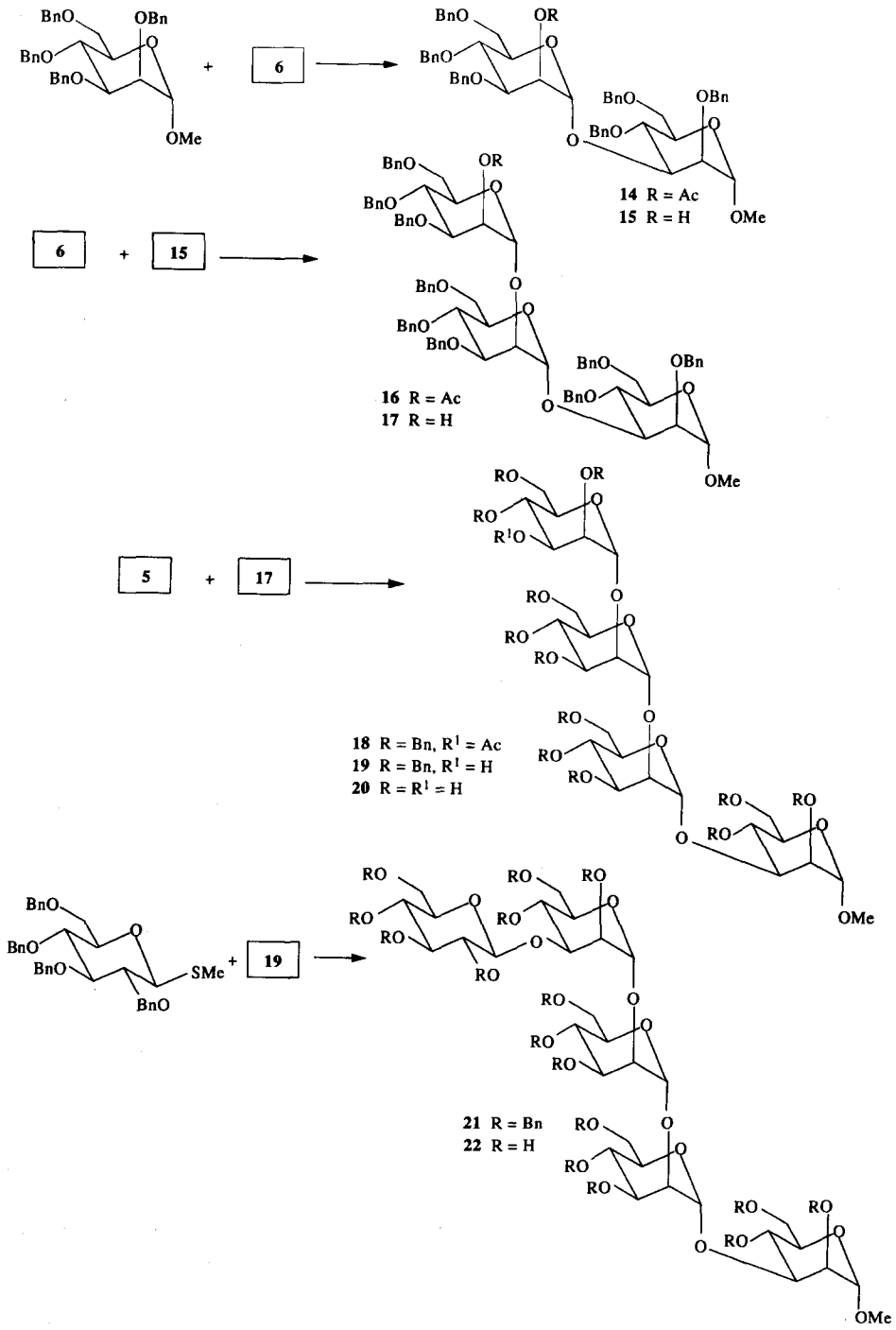
For the synthesis of methyl *O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 3)-α-D-mannopyranoside (**20**) and methyl *O*-(α-D-glucopyranosyl)-(1 → 3)-*O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 2)-*O*-(α-D-mannopyranosyl)-(1 → 3)-α-D-mannopyranoside (**22**), methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside [24] was used as the starting material. Glycosidation of this compound with **6** afforded, in 83% yield, the disaccharide derivative **14** (Scheme 3). The ^{1}H NMR spectrum of **14** displayed low field chemical shifts at δ 5.49–5.48 (m, 1 H, H-2′), 5.18 (d, J = 1.6 Hz, 1 H, H-1′) and 4.71 (d, J = 1.7 Hz, 1 H, H-1), confirming an α-configuration for the newly introduced glycosidic bond. A reaction sequence similar to that described for the preparation of **10** from **7** was performed for the synthesis of **19** from **14**(Scheme 3). Hydrogenolysis of the benzyl groups of **19** furnished amorphous **20**. The structure of **20** was confirmed by ^{13}C NMR and FAB mass spectroscopy (see Experimental section). A similar glycosylation of **19** with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside afforded, in 76% yield, the fully protected pentasaccharide derivative **21** (Scheme 3). The ^{1}H NMR spectrum showed five anomeric protons at δ 5.28 (bs, H-1‴), 5.21 (bs, H-1″), 5.18 (d, J = 3.0 Hz, H-1⁗), 4.90 (bs, H-1′) and 4.75 (bs, H-1), indicating an α-configuration for all residues.

Compound **21** was hydrogenolyzed in glacial acetic acid in a manner analogous to that described for 12 (to give 13) to furnish compound 22. The ^{13}C NMR and FAB mass spectra of 22 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 0.25 mm layers of silica gel 60F-254 (Analtech GHLF uniplates). The compounds were located by exposure to UV light or by spraying with 5% H_2SO_4 in EtOH and charring, or by both techniques. Column chromatography was performed on silica gel, Baker Analyzed (60–200 mesh). NMR spectra were recorded at ~ 25°; ^{1}H spectra with a Varian EM-390 at 90 MHz and with a Bruker AM-400 at 400 MHz and ^{13}C spectra with a Bruker AM-400 at 100.6 MHz. All chemical shifts are referenced to tetramethylsilane. Solutions in organic solvents were generally dried with anhydrous Na_2SO_4. Dichloromethane, *N*,*N*-dimethylformamide (DMF), 1,2-dichloroethane, acetone and 2,2-dimethoxypropane were dried over 4 Å molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, New Jersey, USA.

*4-Pentenyl 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-α-D-mannopyranoside* (**2**). — A solution of **1** (10.5 g) and *p*-toluenesulfonic acid (2.09 g) in dry acetone (105 mL) and dimethoxypropane (105 mL) was stirred at room temperature for 16 h. Water (209 mL) was then added to the reaction mixture and stirring was continued for 3 h at room temperature. The mixture was neutralized with 1 *M* $NaHCO_3$ solution (50 mL), the solvent was removed under reduced pressure, $CHCl_3$ (500 mL) was added, and the insoluble substance was filtered off. The organic solution was washed twice with water,


6
14 R = Ac
15 R = H
16 R = Ac
17 R = H
18 R = Bn, R¹ = Ac
19 R = Bn, R¹ = H
20 R = R¹ = H
21 R = Bn
22 R = H

Scheme 3.

dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography using a solvent gradient consisting of 2–6% MeOH in $CHCl_3$ to give 4-pentenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (7.5 g), which was used directly in the next step.

To a solution of the isopropylidene derivative (7.5 g) in THF (100 mL) were added KOH (6.13 g), 18-crown-6 (0.67 g) and benzyl bromide (8.45 mL). After the mixture was stirred at room temperature for 2 days, the solvent was evaporated and $CHCl_3$ was added. The organic solution was washed twice with water, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using a solvent gradient consisting of 5–10% ethyl acetate in hexane to give **2** (16 g, 65%); $[\alpha]_D$ + 27.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.22 (m, 10 H, arom.), 5.80 (m, 1 H,–C*H*=CH_2), 5.04 (d, *J* = 1.6 Hz, 1 H, H-1), 2.09 (m, 2 H, –CH_2–), 1.66 (m, 2 H, –CH_2–), 1.50 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3); ^{13}C NMR: 128.37–127.42 (m, arom.), 109.23 (*C*Me_2), 97.12 (C-1), 30.25 (CH_2), 28.58 (CH_2), 27.96 (CH_3), 26.32 (CH_3). Anal. Calcd for $C_{28}H_{36}O_6$: C, 71.77; H, 7.74. Found: C, 71.63; H, 7.63.

*4-Pentenyl 4,6-di-*O*-benzyl-α-*D*-mannopyranoside* (**3**). — A solution of **2** (12.5 g, 26.7 mmol) in 200 mL of 70% aq glacial acetic acid was stirred at 70°C for 1.5 h. After solvent removal under diminished pressure and co-distillation with several added portions of toluene, the product residue was precipitated from ethyl acetate–hexane to afford **3** (6.4 g, 56%); $[\alpha]_D$ + 62° (*c* 2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.24 (m, 10 H, arom.), 5.81–5.74 (m, 1 H, C*H*=CH_2), 5.02 (d, *J* = 1.5 Hz, 1 H, H-1), 3.41 (m, 2 H, –CH_2–), 2.10 (m, 2 H, –CH_2–), 1.65 (m, 2 H, CH_2); ^{13}C NMR: 138.3–127.7 (m, arom.), 99.53 (C-1), 30.20 (CH_2), 28.57 (CH_2). Anal. Calcd for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 69.69; H, 7.51.

*4-Pentenyl 2,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**4**). — A mixture of **3** (6 g, 14 mmol) in methylene chloride (218 mL), 5% sodium hydroxide (21 mL), benzyl bromide (3.2 mL, 27 mmol), and tetrabutylammonium hydrogen sulfate (1.08 g) was refluxed for 6 days, cooled, and the two layers were separated. The organic layer was washed with water 3 ×, dried over $MgSO_4$, and evaporated to give a crude product which was purified by column chromatography using hexane–ethyl acetate (4 : 1, v/v) to afford **4** (4.5 g, 62%). $[\alpha]_D$ + 21° (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.23 (m, 15 H, arom.), 5.77 (m, 1 H, C*H*=CH_2), 5.05 (d, *J* = 1.6 Hz, 1 H, H-1), 3.35 (m, 2 H, CH_2), 2.07 (m, 2 H, CH_2), 1.64 (m, 2 H, CH_2); ^{13}C NMR: 128.51–127.45 (m, arom.), 96.85 (C-1), 30.26 (CH_2), 28.62 (CH_2). Anal. Calcd for $C_{32}H_{38}O_6$: C, 74.10; H, 7.38. Found: C, 73.94; H, 7.28.

*4-Pentenyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**5**). — A solution of **4** (4.4 g, 8.5 mmol) in acetic anhydride (30 mL) and pyridine (60 mL) was stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was chromatographed using a solvent gradient consisting of 20–30% ethyl acetate in hexane to give **5** (4.0 g, 84%). $[\alpha]_D$ + 15° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.16 (m, 15 H, arom.), 5.77 (m, 1 H, C*H*=CH_2), 5.24 (dd, *J* = 3.3 Hz, 1 H, H-3), 5.01 (d, *J* = 1.6 Hz, 1 H, H-1), 3.41 (m, 2 H, –CH_2–), 2.08 (m, 2 H, –CH_2–), 1.95 (s, 3 H, OAc), 1.65 (m, 2 H, –CH_2–); ^{13}C NMR: 170.1 (CO), 138.2–127.5 (arom.), 97.80 (C-1), 69.03 (C-6), 67.11 (CH_2), 30.23 (CH_2), 28.55 (CH_2), 21.05 (OAc). Anal. Calcd for $C_{34}H_{40}O_7$: C, 72.83; H, 7.19. Found: C, 72.59; H, 7.06.

General procedure for glycosidation. — A solution of **6** (1.0–1.2 mmol), acceptor sugars (1 mmol), and *N*-iodosuccinimide (3 mmol) in CH_2Cl_2 (10 mL) was stirred for 0.5 h with 4 Å molecular sieves (2 g) under an Ar atmosphere at −10°C. A dilute solution of trifluoromethanesulfonic acid (0.2 mL in 20 mL CH_2Cl_2) was then added dropwise. Stirring was continued at the same temperature for another 0.5 h and the acid was neutralized with saturated aq $NaHCO_3$ solution. The mixture was filtered through Celite, the solids were thoroughly washed with saturated $NaHCO_3$ solution, water and 10% $Na_2S_2O_7$ solution, dried and concentrated in vacuo.

Methyl O-*(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**7**). — Glycosylation of methyl 3,4,6-tri-*O*-benzyl-α-D-*mannopyranoside (8.4 g, 18 mmol) with* **6** *(11.4 g, 22 mmol) gave* **7** *(12.8 g, 77.8%) after silica gel column chromatography (5–20% ethyl acetate in hexane).* $[\alpha]_D$ *+ 19° (c 1.8,* $CHCl_3$*);* ^{1}*H NMR (*$CDCl_3$*):* δ *7.32–7.15 (m, 30 H, arom.), 5.53 (bs, 1 H, H-2), 5.07 (bs, 1 H, H-1′), 4.78 (bs, 1 H, H-1), 3.25 (s, 3 H,* OCH_3*), 2.11 (s, 3 H, OAc);* ^{13}*C NMR: 99.66 (C-1′), 99.52 (C-1), 69.10 (C-6), 68.71 (C-6′), 54.63 (OMe), 21.06 (OAc). Anal. Calcd for* $C_{57}H_{62}O_{12}$*: C, 72.90; H, 6.65. Found: C, 72.72; H, 6.48.*

Methyl O-*(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**8**). — A solution of **7** (11.9 g, 12.9 mmol) in 10 m*M* methanolic NaOMe (200 mL) was stirred overnight at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite bed) and thoroughly washed with MeOH. The combined filtrate was concentrated to give compound **8** (10.7 g, 94%). $[\alpha]_D$ +26.8 (*c* 1.7, $CHCl_3$); ^{1}H NMR (CDl_3): δ 7.34–7.17 (m, 30 H, arom.), 5.13 (d, *J* = 1.2 Hz, 1 H, H-1′), 4.79 (bs, 1 H, H-1), 3.23 (s, 3 H, OMe); ^{13}C NMR: 101.1 (C-1′), 99.77 (C-1), 69.21 (C-6), 68.50 (C-6′), 54.63 (OMe). Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.94; H, 7.01.

Methyl O-*(3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**9**). — The reaction of compound **8** (5.0 g, 5.7 mmol) with **5** (3.2 g, 5.7 mmol) and NIS (4.1 g, 7 mmol) in CH_2Cl_2 (100 mL) for 20 min, by the method used for the synthesis of **7**, gave **9** (5.0 g, 64%). $[\alpha]_D$ +10.9 (*c* 2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.34–7.13 (m, 45 H, arom.), 5.29–5.26 (m, 1 H, H-3″), 5.20 (d, *J* = 1.5 Hz, 1 H, H-1″), 5.16 (d, *J* = 2.3 Hz, 1 H, H-1′), 4.8 (d, *J* 1.4 Hz, 1 H, H-1), 3.20 (s, 3 H, OMe), 2.92 (s, 3 H, OAc); ^{13}C NMR: 100.7 (C-1″), 99.79 (C-1′), 99.59 (C-1), 69.69 (C-6), 69.35 (C-6″), 68.87 (C-6′), 54.62 (OMe), 21.05 (OAc). Anal. Calcd for $C_{84}H_{90}O_{17}$: C, 73.55; H, 6.61. Found: C, 73.35; H, 6.47.

Methyl O-*(2,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-*O*-(3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**10**). — De-*O*-acetylation of **9** (4.6 g, 3.3 mmol) as described for the preparation of **8** gave compound **10** (4.2 g, 95%); $[\alpha]_D$ +15.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.34–7.14 (m, 45 H, arom.), 5.20 (bs, 1 H, H-1″), 5.17 (bs, 1 H, H-1′), 3.22 (s, 3 H, OMe); ^{13}C NMR: 100.7 (C-1″), 99.80 (C-1′), 98.65 (C-1), 69.44 (C-6), 69.11 (C-6 and C-6″), 54.65 (OMe). Anal. Calcd for $C_{82}H_{88}O_{16}$: C, 74.07; H, 6.67. Found: C, 73.98; H, 6.81.

Methyl O-*(α-*D*-mannopyranosyl)-(1 → 2)-*O*-(α-*D*-mannopyranosyl)-(1 → 2)-*O*-α-*D*-mannopyranoside* (**11**) — A mixture of **10** (0.7 g, 0.53 mmol) and 10% Pd–C (1 g) in glacial acetic acid (70 mL) was shaken under H_2 at 354 kPa for 2 days at room

temperature. The suspension was filtered off (Celite bed), solids were thoroughly washed with MeOH, and the filtrate and washings were combined and concentrated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (13:6:1 → 5:4:1) as the solvent to afford a syrupy product. The syrup was lyophilized to give amorphous compound **11** (0.2 g, 74%). $[\alpha]_D$ +73.1 (*c* 1.2, H_2O); ^1H NMR (D_2O): δ 5.33 (d, *J* = 1.5 Hz, 1 H, H-1″), 5.09 (d, *J* = 1.6 Hz, 1 H, H-1′), 5.02 (d, *J* = 1.3 Hz, 1 H, H-1), 3.45 (s, 3 H, OMe); ^{13}C NMR: 101.2 (C-1″), 99.59 (C-1′), 98.27 (C-1), 60.04 (C-6), 59.90 (C-6 and C-6″), 53.82 (OMe); *m/z* 520 $[M + 1]^+$, 542 $[M + Na]^+$ and 558 $[M + K]^+$. Anal. Calcd for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.61. Found: C, 43.83; H, 6.87.

Methyl O-*(2,3,4,6-tetra*-O-*benzyl-α-*D-*glucopyranosyl)-(1 → 3)*-O-*(2,4,6-tri*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 2)-3,4,6-tri*-O-*benzyl-α-*D-*mannopyranoside* (**12**). — A mixture of **10** (1.7 g, 1.28 mmol), methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (1.5 g, 2.6 mmol) and pulverized 4 Å molecular sieves (6 g) in 60 mL of dichloroethane–DMF (5:1, v/v) were added tetrabutylammonium bromide (1.2 g, 3.8 mmol) and copper bromide (0.86 g, 3.8 mmol). After stirring at room temperature for 4 days, the solid was filtered off (Celite bed) and washed with $CHCl_3$. The filtrate and washings were combined and washed five times with aq $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using a solvent gradient consisting of 8–16% ethyl acetate in hexane to give **12** (0.93 g, 39%); $[\alpha]_D$ +26° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.35–7.09 (m, 65 H, arom.), 5.26 (bs, 1 H, H-1″), 5.19 (d, *J* = 1.6 Hz, 1 H, H-1′), 5.16 (d, *J* = 3.4 Hz, 1 H, H-1‴), 4.85 (d, *J* = 1.6 Hz, 1 H, H-1), 3.21 (s, 3 H, OMe); ^{13}C NMR: 128.4–127.1 (m, arom.), 101.0 (C-1″ and C-1′), 99.76 (C-1 and C-1‴), 69.57 (C-6), 69.37 (C-6′ and C-6″), 68.36 (C-6‴) 54.63 (OMe). Anal. Calcd for $C_{116}H_{122}O_{21}$: C, 75.22; H, 6.64. Found: C, 75.30; H, 6.80.

Methyl O-*(α-*D-*glucopyranosyl)-(1 → 3)*-O-*(α-*D-*mannopyranosyl)-(1 → 2)*-O-*(α-*D-*mannopyranosyl)-(1 → 2)-α-*D-*mannopyranoside* (**13**). — Hydrogenolysis of **12** (0.89 g) by the method used for the synthesis of **11** gave **13** (0.16 g, 42%) as an amorphous solid. $[\alpha]_D$ +164° (*c* 1.1, H_2O); ^1H NMR (D_2O): δ 5.33 (bs, 1 H, H-1″), 5.30 (d, *J* = 3.8 Hz, 1 H, H-1‴), 5.08 (bs, 1 H, H-1′), 5.02 (bs, 1 H, H-1), 3.44 (s, 3 H, OMe); ^{13}C NMR (D_2O): 101.0 (C-1″), 99.58 (C-1′), 99.33 (C-1), 98.26 (C-1‴), 77.61 (C-2″), 77.46 (C-2′), 77.29 (C-2), 60.10 (C-6), 59.90 (C-6′ and C-6″), 59.66 (C-6‴), 53.81 (OMe); *m/z* 681 $[M + 1]^+$ and 703 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{44}O_{21}$: C, 44.11; H, 6.52. Found: C, 43.98; H, 6.79.

Methyl O-*(2-*O-*acetyl-3,4,6-tri*-O-*benzyl-α-*D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α-*D-*mannopyranoside* (**14**). — Glycosylation of methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (6.8 g, 14.8 mmol) with **6** (9.3 g, 17.8 mmol) gave compound **14** (11.3 g, 83.4%) after silica gel column chromatography (solvent gradient consisting of 10–20% ethyl acetate in hexane); $[\alpha]_D$ +29° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.35–7.17 (m, 30 H, arom.), 5.49–5.48 (m, 1 H, H-2′), 5.18 (d, *J* = 1.6 Hz, 1 H, H-1′), 4.71 (d, *J* = 1.7 Hz, 1 H, H-1), 3.28 (s, 3 H, OMe), 2.07 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): 99.44 (C-1′), 98.30 (C-1), 78.08 (C-3), 71.65 (C-6), 69.13 (C-6′), 54.64 (OMe) and 20.84 (OAc). Anal. Calcd for $C_{57}H_{62}O_{12}$: C, 72.90; H, 6.65. Found: C, 72.81; H, 6.71.

Methyl O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**15**). — De-*O*-acetylation of compound **14** (10.6 g) with methanolic sodium methoxide as described for the preparation of **8** afforded compound **15** (9.6 g, 93%); $[\alpha]_D$ +33° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.17 (m, 30 H, arom.), 5.21 (d, *J* = 1.3 Hz, 1 H, H-1′), 4.71 (d, *J* = 1.7 Hz, 1 H, H-1), 3.29 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$): 99.52 (C-1′), 98.51 (C-1), 71.80 (C-6), 69.36 (C-6′), 54.78 (OMe). Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.52; H, 6.72.

Methyl O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**17**). — Glycosylation of **15** (8.6 g) with **6** (6.1 g) afforded compound **16** which was treated with methanolic sodium methoxide in MeOH–THF (2 : 1, v/v) for 16 h as described for the preparation of **15** to give **17** (7.5 g, 70%); $[\alpha]_D$ +39° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.33–7.13 (m, 45 H, arom.), 5.23 (d, *J* = 1.6 Hz, 1 H, H-1″), 5.05 (d, *J* = 1.5 Hz, 1 H, H-1′), 4.60 (d, *J* = 1.5 Hz, 1 H, H-1), 3.26 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$): 100.99 (C-1″ and C-1′), 98.19 (C-1), 79.91 (C-3), 77.69 (C-2′), 71.80 (C-6), 71.64 (C-6′), 69.73 (C-6″), 54.76 (OMe). Anal. Calcd for $C_{82}H_{88}O_{16}$: C, 74.07; H, 6.67. Found: C, 74.15; H, 6.91.

Methyl O-*(3*-O-*acetyl-2,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**18**). — Compound **17** (1.5 g) was treated with **5** exactly as described for the preparation of **16** (from **15**) to give **18** (1.2 g, 59%) after silica gel column chromatography (solvent gradient consisting of 20–30% ethyl acetate in hexane); $[\alpha]_D$ +19° (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.28–7.08 (m, 60 H, arom.), 5.35–5.34 (m, 1 H, H-3‴), 5.28 (bs, 1 H, H-1‴), 5.15 (bs, 1 H, H-1″), 5.11 (bs, 1 H, H-1′), 3.23 (s, 3 H, OMe), 1.91 (s, 3 H, OAc). Anal. Calcd for $C_{111}H_{118}O_{22}$: C, 73.89; H, 6.59. Found: C, 73.75; H, 6.71.

Methyl O-*(2,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-*mannopyranoside* (**19**). — De-*O*-acetylation of compound **18** (1.0 g) with methanolic NaOMe afforded compound **19** (0.8 g, 94%); $[\alpha]_D$ +24° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.31–7.14 (m, 60 H, arom.), 5.26 (bs, 1 H, H-1‴), 5.21 (bs, 1 H, H-1″), 5.14 (bs, 1 H, H-1′), 4.70 (bs, 1 H, H-1), 3.25 (s, 3 H, OMe). Anal. Calcd for $C_{109}H_{116}O_{21}$: C, 74.29; H, 6.64. Found: C, 74.51; H, 6.59.

Methyl O-*(α*-D-*mannopyranosyl)-(1 → 2)*-O-*(α*-D-*mannopyranosyl)-(1 → 2)*-O-*(α*-D-*mannopyranosyl)-(1 → 3)-α*-D-*mannopyranoside* (**20**). — A mixture of **19** (0.3 g) and 10% Pd–C (1.0 g) in glacial acetic acid (20 mL) was shaken under H_2 using conditions similar to those described for the preparation of **11** (from **10**) to provide compound **20** (0.07 g, 55%); $[\alpha]_D$ +83° (*c* 0.8, H_2O); ^{1}H NMR (D_2O): δ 5.32 (bs, 1 H, H-1‴), 5.07 (bs, 1 H, H-1″), 5.04 (bs, 1 H, H-1′), 4.82 (bs, 1 H, H-1), 3.35 (s, 3 H, OMe); ^{13}C NMR (D_2O): 101.16 (C-1‴), 99.72 (C-1″), 99.62 (C-1′ and C-1), 77.50 (C-3), 77.43 (C-2″), 77.29 (C-2′), 59.99 and 59.84 (each for 2 × C-6), 53.72 (OMe); *m/z*: 679.3 $(M - H)^-$. Anal. Calcd for $C_{25}H_{44}O_{21} \cdot H_2O$: C, 42.98; H, 6.64. Found: C, 43.12; H, 6.71.

Methyl O-*(2,3,4,6-tetra*-O-*benzyl-α*-D-*glucopyranosyl)-(1 → 3)*-O-*(2,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1 → 3)-2,4,6-tri*-O-*benzyl-α*-D-

mannopyranoside (**21**). — Compound **19** (0.8 g, 0.47 mmol) was condensed with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (0.49 g, 1 mmol) in dichloroethane-*N*,*N*-dimethylformamide (5 : 1, 60 mL) in the presence of $CuBr_2$ (0.29 g, 1.3 mmol), tetrabutylammonium bromide (0.4 g, 1.2 mmol) and 4 Å molecular sieves (6 g), for 24 h at room temperature. The same amounts of glycosyl donor, $CuBr_2$ and tetrabutylammonium were added again, and stirring was continued for another 2 days. After processing as described above, the crude reaction product was purified by silica gel column chromatography using a solvent gradient consisting of 20–30% ethyl acetate in hexane. Evaporation of the fractions corresponding to the product yielded **21** (0.8 g, 76%); $[\alpha]_D$ +33° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–7.03 (m, 80 H, arom.), 5.28 (bs, 1 H, H-1‴), 5.21 (bs, 1 H, H-1″), 5.18 (d, J = 3.0 Hz, 1 H, H-1⁗), 4.90 (bs, 1 H, H-1′), 4.75 (bs, 1 H, H-1), 3.24 (s, 3 H, OMe). Anal. Calcd for $C_{143}H_{150}O_{26}$: C, 75.17; H, 6.62. Found: C, 75.31; H, 6.59.

Methyl O-(α-D-*glucopyranosyl*)-(1 → 3)-O-(α-D-*mannopyranosyl*)-(1 → 2)-O-(α-D-*mannopyranosyl*)-(1 → 2)-O-(α-D-*mannopyranosyl*)-(1 → 3)-α-D-*mannopyranoside* (**22**). — A solution of **21** (0.7 g) in glacial acetic acid (50 mL) was shaken under H_2 at ~ 345 kPa for 4 days at room temperature in the presence of 10% Pd–C (2 g). After processing as described for **19** (to give **20**), the crude product was purified by silica gel column chromatography using $CHCl_3$–MeOH–H_2O (4 : 5 : 1, v/v) to furnish **22** (0.12 g, 50%); $[\alpha]_D$ +101° (*c* 0.6, H_2O); ^1H NMR (D_2O): δ 5.19 (bs, 1 H, H-1‴), 5.12 (bs, 1 H, H-1″), 5.07 (d, J = 3.4 Hz, 1 H, H-1⁗), 4.84 (bs, 1 H, H-1′), 4.71 (bs, 1 H, H-1), 3.26 (s, 3 H, OMe); ^{13}C NMR (D_2O): 101.03 (C-1‴), 99.72 (C-1″), 99.61 (C-1′ and C-1), 99.35 (C-1⁗), 77.48 (C-3‴), 77.42 (C-3), 77.29 (C-2′ and C-2″), 59.85 (C-6, C-6′, C-6″ and C-6‴), 59.66 (C-6⁗), 53.71 (OMe); *m*/*z*: 841.1 $(M - H)^-$. Anal. Calcd for $C_{31}H_{54}O_{26} \cdot 1.5H_2O$: C, 42.81; H, 6.60. Found: C, 42.63; H, 6.75.

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