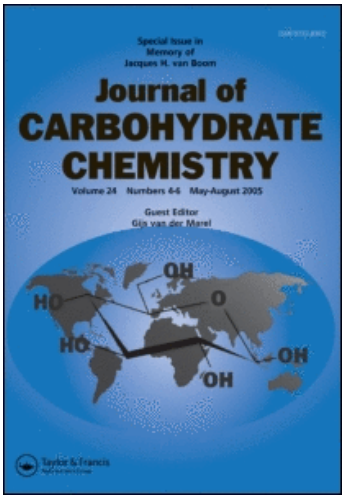

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A SHORT, EFFICIENT SYNTHESIS OF THE OCTAMANNAN RESIDUE OF HIGH MANNOSE TYPE SUGAR CHAINS

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ABSTRACT

An efficient route for the synthesis of octamannan **1**, found in high mannose type sugar chains, is described. To construct octasaccharide **1** by as few synthetic steps as possible, we employed a chemoenzymatic strategy: the enzymatic synthesis of oligosaccharide blocks using glycosidases followed by chemical coupling to form a branched structure. By use of this methodology, many synthetic steps were eliminated and **1** was easily synthesized.

INTRODUCTION

Recently, high mannose type sugar chains have attracted special attention due to the discovery of gp120 in the conserved V3 loop of the viral coat of HIV. This glycoprotein plays an important role during HIV infection of cells and is responsible not only for attachment and penetration of the target cell, but also for the antiviral immune response.[1] About 30% of the molecular mass of gp120 consists of high mannose type sugar chains. It is thought that high mannose type sugar chains are essential at various stages of HIV

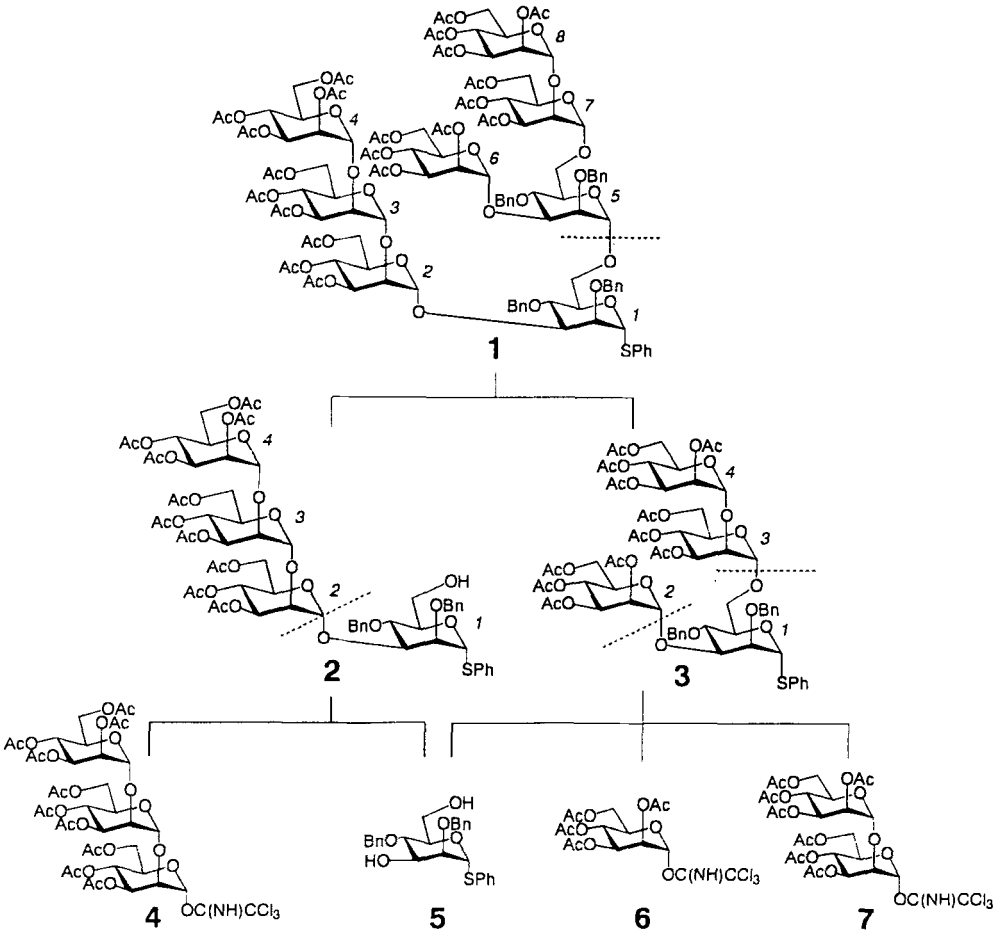
infection.[2] To study the function of these high mannose type sugar chains, large amounts of material are necessary. For this reason, an efficient synthetic technology is necessary to enable large scale preparation. The chemical synthesis of high mannose type sugar chains has been intensively studied since the mid-1980's[3] and as a result the synthetic technology for complex carbohydrates has also benefited.[4] Synthesis of the major segments of high mannose type glycoproteins has been achieved both by use of a novel protecting group[5] and by the chemistry of *n*-pentenyl glycosides.[6] Nevertheless, the synthesis of high mannose type sugar chains on a multigram scale remains difficult, mainly due to the numerous protection and deprotection steps required. In contrast, enzymatic oligosaccharide synthesis can decrease the number of necessary synthetic steps by eliminating the need for many protection and deprotection steps.[7] We have accumulated a great deal of experience on the synthesis of oligosaccharides using glycosidases.[8] Recently, we reported the preparation of the key intermediate for the synthesis of N-linked sugar chains by combining the enzymatic synthesis of oligosaccharide blocks using glycosidases and the chemical synthesis of the branching oligosaccharides.[9]

In this report, we describe the synthesis of a partial structure of the high mannose type sugar chain octamannan **1** which has a triantennary structure, based on our chemoenzymatic strategy (glycosidase assisted synthesis of oligosaccharide blocks and construction of branching oligosaccharides by chemical glycosylation).

RESULTS AND DISCUSSION

Our synthetic approach to the construction of octasaccharide **1** is based on the assembly of various enzymatically and/or chemically prepared synthetic blocks. The retrosynthetic analysis of octasaccharide **1** is shown in Scheme 1. Disconnection at the indicated bonds leads to mannotetraose acceptor **2**[9] and mannotetraose **3**.[9] Compound **2** corresponds to mannotriose donor **4**[9] and acceptor **5**.[9] Further retrosynthetic analysis of **3** leads to mannose derivative **5**, mannose donor **6**[9] and mannobiose donor **7**.[9] Oligosaccharide blocks such as mannobiose[8c] (Manα1-2Man) and mannotriose[8c] (Manα1-2Manα1-2Man) can be prepared enzymatically.

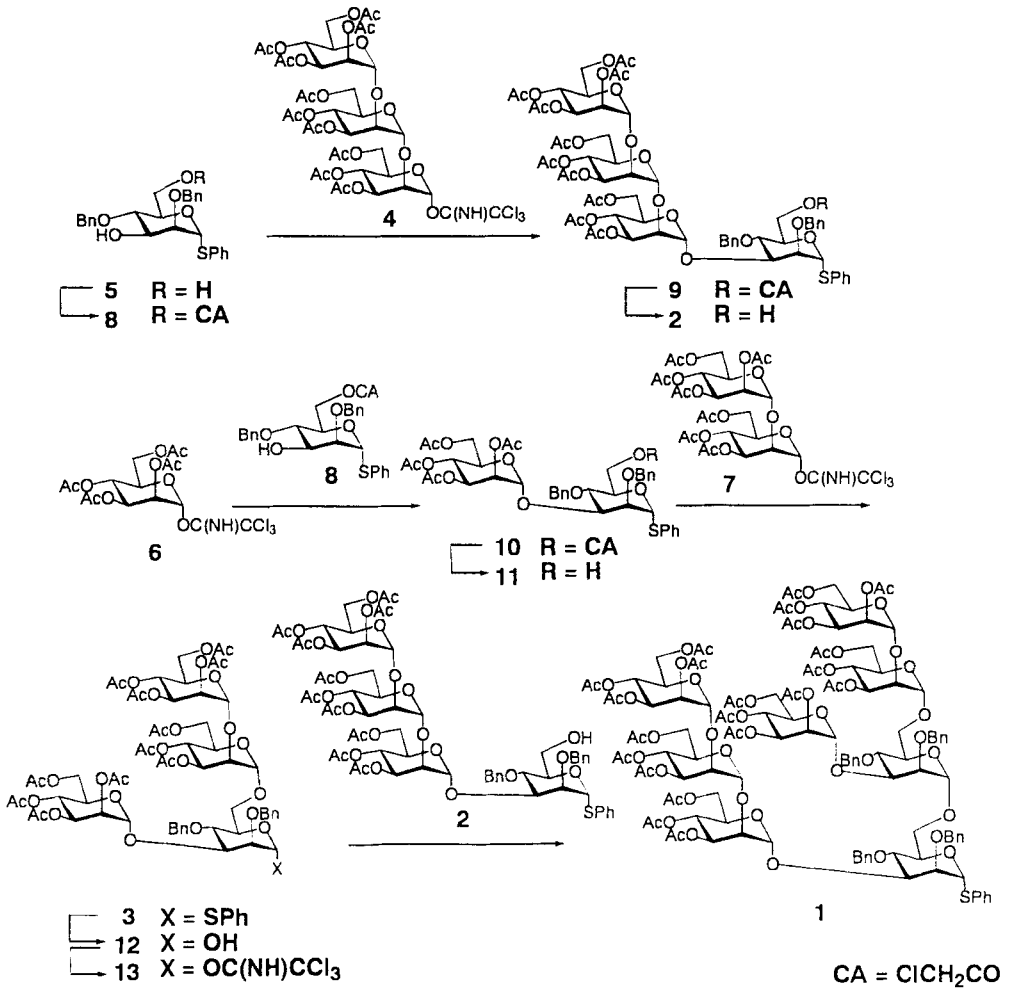
The oligosaccharide blocks were prepared enzymatically as reported previously.[8c] The reverse hydrolysis of mannose in the presence of α-mannosidase from *Aspergillus*



Scheme 1

niger afforded Manα1-2Man and Manα1-2Manα1-2Man as well as other mannooligosaccharides. These two oligosaccharides were then converted into the corresponding donors **4** and **7** as follows: 1) acetylation using Ac_2O and pyridine, 2) selective deacetylation of the anomeric position by ammonium carbonate, and 3) treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[9]

For the construction of compound **2**, we have examined the coupling of glycosyl donor **4** and mannose acceptor **5**, in which the C-3 and C-6 positions are free, to give the C-3 glycosylated compound (**2**) as a minor product.[9] In order to increase the yield of **2**, we



Scheme 2

examined the use of a C-6 protected acceptor to prevent reaction with the primary hydroxyl group during the glycosylation reaction. To synthesize the C-6 protected acceptor, we first tried a selective chloroacetylation of compound **5** using chloroacetic anhydride and pyridine at low temperature, but this reaction resulted in a mixture of the mono- and diacylated compounds. Regioselective chloroacetylation of mannose derivative **5** to obtain compound **8** was accomplished by treatment with chloroacetic anhydride in a mixture of toluene/ dichloromethane (1:1) at 60 °C (82% yield).

Glycosylation of acceptor **8** and donor **4** afforded tetrasaccharide **9** in 80% yield. The structure of **9** was confirmed by ^{1}H, ^{13}C and 2D NMR spectroscopy. The α1-3 mannosidic linkage of **9** is supported by the coupling constant between C-1 and H-1 (172 Hz). Hydrolysis of the chloroacetyl group in compound **9** by heating with thiourea and $NaHCO_3$ in EtOH gave compound **2** in 86% yield.

For the preparation of branched mannotetraose **3**, we coupled C-6 protected acceptor **8** and donor **6** in the presence of TfOH at -20 °C to afford disaccharide **10** in 93% yield. Removal of the chloroacetyl group on compound **10** provided disaccharide acceptor **11** in 86% yield. Glycosylation of acceptor **11** with donor **7** gave branched tetrasaccharide **3** in 79% yield.

In order to construct octasaccharide **1**, the thiophenyl group at the anomeric position of **3** was converted into the corresponding trichloroacetimidate group.[10] Treatment of compound **3** with NBS in acetone/water (9:1)[11] gave hemiacetal **12** in quantitative yield and trichloroacetimidation of **12** using trichloroacetonitrile and DBU afforded tetrasaccharide donor **13** in 80% yield. Condensation of **13** with **2** in CH_2ClCH_2Cl, in the presence of TfOH at -20 °C, gave octamannan **1** as a single product in 74% yield. The structure of the newly formed α1-6 mannosidic linkage of **1** is supported by the coupling constant between C-1 and H-1 (170 Hz).

In conclusion, we have developed a short synthesis of the mannan residue of one of the high mannose type oligosaccharides present on the viral coat of HIV. This synthesis efficiently combines enzymatic techniques with standard chemical glycosylation methods to greatly reduce the number of necessary synthetic steps.

EXPERIMENTAL

General methods. Optical rotations were measured at 25 °C with a HORIBA polarimeter SEPA-300. ^{1}H and ^{13}C NMR spectra were measured on a UNITY 500 spectrometer in $CDCl_3$ and were referenced to Me_4Si. Silica gel column chromatography was performed using BW300 (Fuji Silisia Co., Ltd., Aichi, Japan). Analytical TLC was performed on aluminum plates coated with silica gel 60 F_{254} (Merck). Gel for size exclusion chromatography (Bio-Beads) was a product of Bio-Rad. Acid washed molecular sieves (AW300) was purchased from Aldrich and activated at 180 °C under vacuum immediately

prior to use. All glycosylation reactions were performed in anhydrous solvents under an atmosphere of dry Ar.

Phenyl 2,4-Di-*O*-benzyl-6-*O*-chloroacetyl-1-thio-α-D-mannopyranoside (8). A mixture of **5**[9] (4.58 g, 0.01 mol), chloroacetic anhydride (10.0 g, 0.06 mol) in dry toluene:CH_2Cl_2 (1:1, 20 mL) was stirred at 60 °C for 3 h. The reaction mixture was diluted with EtOAc and washed with brine, sat. aq $NaHCO_3$, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc, 4:1) to give **8** (4.40 g, 82%): Rf 0.56 (hexane:EtOAc, 3:1); $[\alpha]_D$ + 121° (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.45-7.24 (m, 15H, arom), 5.62 (bs, 1H, H-1^1), 4.38 (dd, 1H, $J_{5\text{-}6a}$ = 5.5 Hz, J_{gem} =11.5 Hz, H-6a), 4.43 (dd, 1H, $J_{5\text{-}6b}$ = 2.5 Hz, H-6b), 3.93-3.91 (m, 2H, CH_2Cl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 79.57 (C-1), 64.95 (C-6) 40.58 (CH_2Cl).

Anal. Calcd for $C_{28}H_{29}O_6SCl$: C, 63.57; H, 5.52; S, 6.06. Found: C, 63.33; H, 5.63; S, 5.80.

Phenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-6-*O*-chloroacetyl-1-thio-α-D-mannopyranoside (9). A mixture of **8** (79 mg, 0.149 mmol), TfOH (5 μL) and molecular sieves (600 mg, AW 300) in dry CH_2ClCH_2Cl (2 mL) was stirred at -20 °C for 30 min. The glycosyl donor **4**[9] (145 mg, 0.136 mmol) in dry CH_2ClCH_2Cl (2 mL) was added dropwise for 10 min. The reaction was quenched with sat. aq $NaHCO_3$. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by size exclusion chromatography (25mmϕ × 650mm, Bio-Beads S-X3, toluene) to give **9** (160 mg, 80%): Rf 0.58 (toluene:EtOAc, 1:2); $[\alpha]_D$ + 70° (*c* 1.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.65 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^1), 5.02 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1^3), 4.94 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1^4), 3.95-3.88 (CH_2Cl);^{13}C NMR (125 MHz, $CDCl_3$) δ 100.57 (C-1^2), 100.08 (C-1^3), 99.28 (C-1^4) 84.36 (C-1^1), 40.58 (CH_2Cl).

Anal. Calcd for $C_{66}H_{80}O_{31}SCl$: C, 55.17; H, 5.61; S, 2.23. Found: C, 54.87; H, 5.33; S, 2.00.

Phenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (2).

To a stirred solution of **9** (66 mg, 0.046 mmol) in EtOH (2 mL) was added thiourea (18 mg, 0.236 mmol) and $NaHCO_3$ (4 mg, 0.048 mmol). The mixture was stirred at 70 °C for 1 h. The reaction mixture was diluted with EtOAc and washed with brine, sat. aq $NaHCO_3$, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene:EtOAc, 1:1) to give **2** (54 mg, 86%). Physical data are in agreement with a known compound.[9]

Phenyl *O*-(3,4,6-Tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (11). A mixture of **8** (716 mg, 1.35 mmol), TfOH (10 μL) and molecular sieves (7.8 g, AW 300) in dry CH_2Cl_2 (5 mL) was stirred at 0 °C for 30 min, then cooled at -20 °C. The glycosyl donor **6**[9] (953 mg, 1.93 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise for 10 min. The reaction was quenched with sat. aq $NaHCO_3$. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene:EtOAc, 5:1~4:1) to give **10** (1.18 g, 93%):Rf 0.59 (toluene:EtOAc, 3:1); $[\alpha]_D$ + 79° (*c* 1.03, $CHCl_3$); ^1H NMR (500MHz, $CDCl_3$) δ 5.64 (d, $J_{1,2}$ = 1.0 Hz, 1H, H-1^1), 5.17 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^2), 2.10, 2.09, 2.04, 2.01 (Ac); ^{13}C NMR (125MHz, $CDCl_3$) δ 99.56 (C-1^2), 84.52 (C-1^1), 79.59 (C-3^1), 40.62 (CH_2Cl). To a stirred solution of **10** (485 mg, 0.56 mmol) in EtOH (10 mL) was added thiourea (140 mg, 1.83 mmol) and $NaHCO_3$ (52 mg, 0.61 mmol). The mixture was stirred at 70 °C for 12 h. The solvent was evaporated *in vacuo*. The residue was diluted with $CHCl_3$ and washed with sat. aq $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene:EtOAc, 4:1) to give **11** (379 mg, 86%). Physical data are in agreement with a known compound.[9]

Phenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)]-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (3). A mixture of **11** (81 mg, 0.103 mmol), TfOH (5 μL) and molecular sieves (2 g, AW 300) in dry CH_2ClCH_2Cl (2 mL) was stirred at -20 °C for 30 min. The glycosyl donor **7**[9] (108 mg, 0.138 mmol) in dry CH_2ClCH_2Cl (2 mL) was added dropwise for 5 min. The reaction was quenched with sat. aq $NaHCO_3$. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was washed with brine, dried ($MgSO_4$) and

concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene:EtOAc, 2:1~1:1) to give **3** (114 mg, 79%). Physical data are in agreement with a known compound.[9]

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)]-2,4-di-*O*-benzyl-D-mannopyranose (12).** To a stirred solution of **3** (431 mg, 0.31 mmol) in acetone:water (9:1, 4.6 mL) was added NBS (246 mg, 1.38 mmol). The mixture was stirred at room temperature for 30 min. The residue was diluted with EtOAc and washed with sat. aq $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (toluene:EtOAc, 1:2) to give **12** (390 mg, quant.): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.10 (m, 10H, arom), 5.16, 5.05, 4.82 (each anomeric H-1), 2.10, 2.06, 2.05, 2.03, 2.03, 2.02, 1.99 (33H, Ac).

Anal. Calcd for $C_{60}H_{76}O_{32}.H_2O$: C, 54.04; H, 5.85. Found: C, 53.95; H, 5.99.

***O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)]-2,4-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (13).** To a mixture of **12** (390 mg, 0.03 mmol) and trichloroacetonitrile (0.3 mL) in CH_2Cl_2 (1.5 mL) was added DBU (0.05 mL) at 0 °C. The mixture was stirred for 5 h. The reaction mixture was purified by silica gel column chromatography (hexane:EtOAc, 1:1) to afford the compound **13** (341 mg, 80%): Rf 0.31 (toluene:EtOAc, 2:3); $[\alpha]_D$ + 61° (*c* 1.04, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (bs, 1H, H-1^1), 5.16, 5.06, 4.76 (H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.72, 99.34, 98.62, 94.62 (C-1).

Anal. Calcd for $C_{62}H_{76}O_{32}NCl_3$: C, 51.23; H, 5.27; N 0.96. Found: C, 50.98; H, 5.23; N, 1.21.

Phenyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)]-2,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→3)]-2,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (1). A mixture of

2 (32 mg, 0.023 mmol), TfOH (5 μL) and molecular sieves (1 g, AW 300) in dry CH_2ClCH_2Cl (1.5 mL) was stirred at 0 °C for 30 min, then cooled at -20 °C. The glycosyl donor **13** (40 mg, 0.027 mmol) was dissolved in dry CH_2ClCH_2Cl (3 mL) which was added dropwise to the glycosyl acceptor solution for 1 h. The reaction was quenched with sat. aq $NaHCO_3$. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by size exclusion chromatography (25mmϕ × 650mm, Bio-Beads S-X1, toluene) to give **1** (45 mg, 74%): Rf 0.33 (toluene:EtOAc, 1:2); $[\alpha]_D$ + 60° (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.58 (bs, 1H, H-1^1), 5.09 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^7), 5.04 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 5.00 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-13), 4.96 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1^5), 4.93 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.66 (bs, 1H, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 100.61 (C-1^2), 100.06 (C-1^3), 99.34 (C-1), 99.27 (C-1), 98.71 (C-1^7), 97.38 (C-1^5), 84.77 (C-1^1).

Anal. Calcd for $C_{124}H_{152}O_{61}S$: C, 56.19; H, 5.78. Found: C, 56.15; H, 5.79.

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