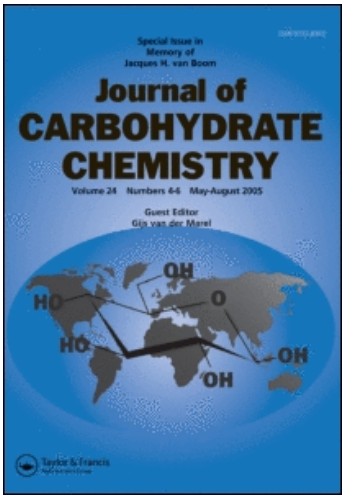

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Synthesis of *Cryptococcus neoformans* Capsular Polysaccharide Structures. IV. Construction of Thioglycoside Donor Blocks and Their Subsequent Assembly†

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ABSTRACT

Di- and trisaccharide thioglycoside building blocks, ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside, ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-1-thio-α-D-mannopyranoside and ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside, corresponding to repetitive structures in the capsular polysaccharide (CPS) of *Cryptococcus neoformans* have been synthesised using silver triflate-promoted couplings between benzobromoxylose and properly protected mannose ethyl thioglycosides. The blocks contain an orthogonal allyl group in the 3-position of the mannose residue to allow continued formation of the (1 → 3)-linked mannan backbone of the CPS. They have benzyl ethers as persistent protecting groups to facilitate access to the acetylated target structures. Assembly of the blocks employing DMTST as promoter in diethyl ether afforded in high yield and complete stereoselectivity penta- and hexasaccharide motifs from *C.*

†This paper is dedicated to Professor Gérard Descotes on the occasion of his 70th birthday.

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neoformans serotype A–C. The latter were deallylated into new acceptors to allow synthesis of larger CPS-fragments.

Key Words: Convergent synthesis; Modular approach; Block synthesis; Glycoconjugate vaccines.

INTRODUCTION

The fungi *Cryptococcus neoformans* is an opportunistic species causing severe diseases, i.e., meningitis, and death, especially in immunodeficient patients.[1,2] *C. neoformans* is divided into different serotypes, A–D, depending on the structure of the surface capsular polysaccharide (CPS), which is an important virulence factor.[3–5] The major polysaccharide is a heteropolymer made of an α-D-(1 → 3)-mannan backbone substituted with β-D-xylose residues in the 2 and 4-positions, β-D-glucuronic acid residues in the 2-positions and acetyl groups in the 6-positions.[6,7] The structures are more or less repeated in triads (Figure 1) and the different serotypes are defined by the amount and position of the xylose substituents (Table 1). The acetylation pattern is believed to be a major immunological determinant[7] but little else is known about the distribution of the 6-*O*-acetyl groups.

To investigate in more detail the nature of the immunological determinants and the biosynthesis of the *C. neoformans* CPS, well-defined synthetic oligosaccharides corresponding to fragments of the native CPS are in demand.

An attractive synthetic pathway to various CPS structures would be to combine di- and trisaccharide thioglycoside building blocks, corresponding to the substituted mannose motifs, through the formation of the internal α-(1 → 3) linkages of the mannan backbone. With four disaccharide blocks (**I**, **II**, **IV** and **V**) and two trisaccharide blocks (**III** and **VI**) (Figure 2), all possible structures could theoretically be constructed. In initial attempts, however, this approach met with severe difficulties, regarding not only the synthesis of the building blocks but also their coupling.[8,9] Therefore another approach was employed to manufacture a number of *C. neoformans* CPS structures including tetrasaccharides.[10–12] In this, the mannan backbone was built up first and the xylose, glucuronic acid and acetyl residues were subsequently introduced. The same strategy has recently been used for the synthesis of a type A hexasaccharide structure.[13] Although so far successful, the synthesis of larger fragments

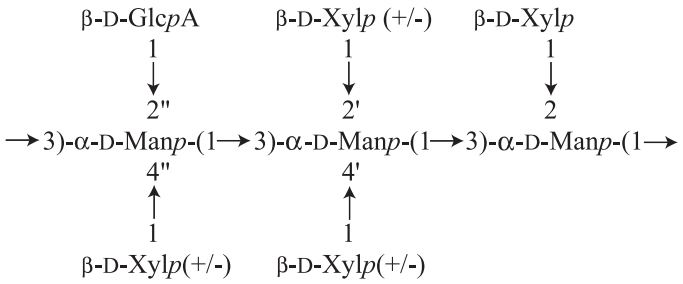


Figure 1. Schematic structure of deacetylated *C. neoformans* CPS.

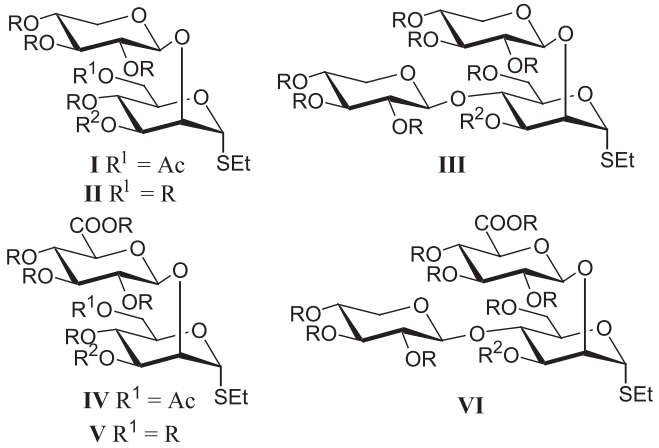
Table 1. Correlation between Xyl*p* substitution and serotype.

	Serotype			
Xyl*p* pos.	A	B	C	D
2′	+	+	+	−
4′	−	−	+	−
4″	−	+	+	−

using this technique would probably be limited because of the number of rather complex reactions involved on large structures. Thus, the block approach has therefore always been pursued. The successful formation and subsequent assembly of xylose-containing di- and trisaccharide building blocks (corresponding to structures **I**–**III** in Figure 2) are described herein.

RESULTS AND DISCUSSION

First, to allow later formation of immunogenic glycoconjugates, the mannose acceptor **7** was constructed from the known peracetylated spacer-bearing glycoside **1**.[14] Thus, deacetylation of **1** (→**2**), and subsequent 4,6-*O*-benzylidene acetal formation(→**3**), tin activation and regioselective 3-*O*-*p*-methoxybenzylation[15] (→**4**), debenzylidenation (→**5**), and benzylation (→**6**) gave the key acceptor **7** in 27% overall yield after final removal of the *p*-methoxybenzyl group (Scheme 1).



R = Persistent protecting group, removable in the presence of acetyl groups
R^2 = Temporary protecting group, removable in the presence of R and Ac groups

Figure 2. Desired thioglycoside building blocks.

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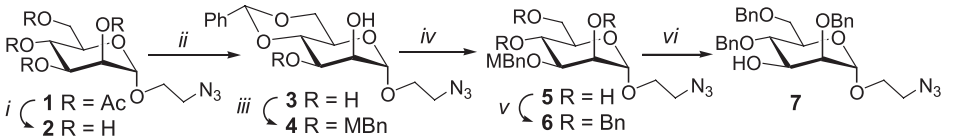
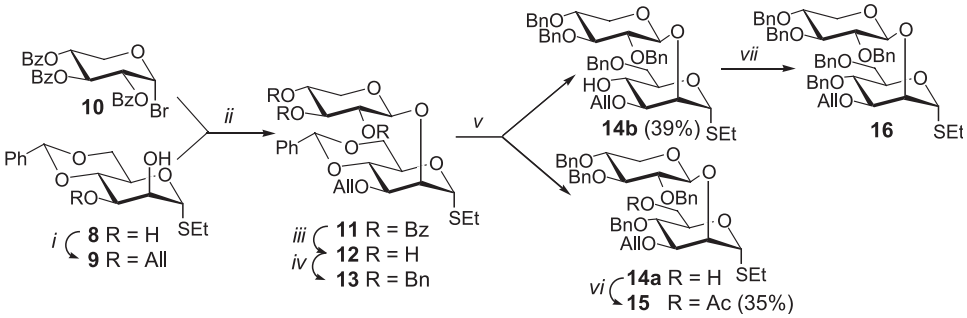
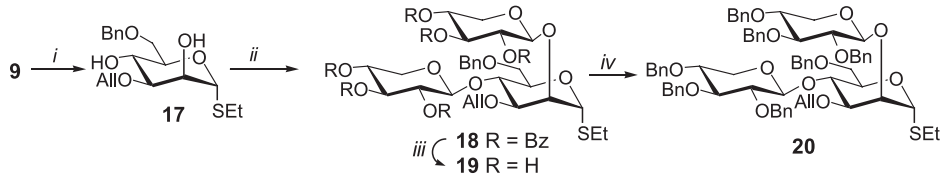


Scheme 1. *i*: NaOMe, MeOH, 99%; *ii*: $PhCH(OMe)_2$, *p*-TsOH, MeCN, 70%; *iii*: a) Bu_2SnO, MeOH, reflux; b) MBnCl, CsF, DMF, 64%; *iv*: HOAc (aq 70%), 70°C, 85%, *v*: BnBr, NaH, DMF, 88%; *vi*: DDQ, CH_2Cl_2/H_2O, 0°C, 82%.

The building blocks were prepared from the thioglycoside derivative **8**,[16] which was regioselectively allylated in the 3-position using tin activation to give **9** (86%) (Scheme 2). Analogously to earlier syntheses,[10,11] silver triflate-promoted glycosylation of the latter with benzobromoxylose **10**[17] gave the β-linked disaccharide **11** in excellent yield (≈95%). Since the continued use of acyl protecting groups was prohibited, due to the presence of acetyl substituents in the target compounds, the benzoyl groups in **11** were changed into benzyl groups by Zemplén deacylation (→**12**, ^{1}H NMR: 4.58 (d, 1H, J = 9 Hz, H-1′)) followed by conventional benzylation (→**13**, 71% from **9**). This sequence could be performed on a large scale and gram quantities of disaccharide **13** were efficiently synthesised. To allow for the introduction of a 6-*O*-acetyl group and also to release torsional strains in the donor,[18] which could explain earlier low coupling yields,[8,9] the benzylidene acetal in **13** was opened under reductive conditions that normally yield mainly the 4-*O*-benzyl derivative (BH_3–Me_3N, $AlCl_3$, CH_2Cl_2/Et_2O).[19] Here, however, an inseparable 1:1-mixture of the 4-*O*- and the 6-*O*-benzyl derivatives **14a** and **14b** was obtained. Since both the 6-*O*-acetylated and benzylated derivates were desired this was not a disadvantage. Regioselective acetylation[20] of the obtained mixture afforded an easily separable mixture of the first thioglycoside donor **15** (35%, compare **I** in Figure 2) and **14b**. The latter was subsequently benzylated to give the donor **16** (compare **II** in Figure 2) in 34% yield from **13**. Notably, **14b** also comprises the possibility to construct a trisaccharide donor block upon introduction of another xylose residue in the 4-position.

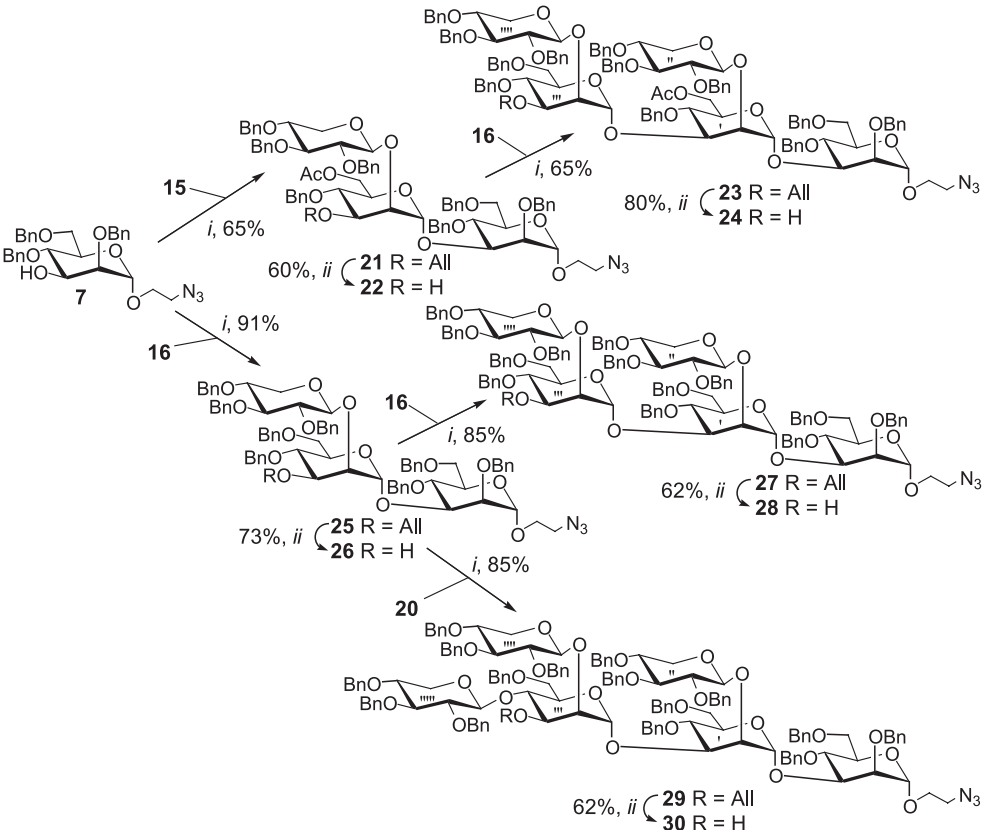


Scheme 2. *i*: a) Bu_2SnO, MeOH, reflux; b) AllBr, CsF, DMF, 86%; *ii*: AgOTf, DTBP, CH_2Cl_2, −40°C; *iii*: NaOMe, MeOH, 82%; *iv*: BnBr, NaH, DMF, 87%; *v*: BH_3–Me_3N, $AlCl_3$, CH_2Cl_2/Et_2O, 0°C; *vi*: AcCl, collidine, CH_2Cl_2, −70°C; *vii*: BnBr, NaH, DMF, 91%.



Scheme 3. *i*: $NaCNBH_3$, HCl/Et_2O, THF, 75%; *ii*: **10**, AgOTf, DTBP, CH_2Cl_2, −40°C; *iii*: NaOMe, MeOH, 65% over two steps; *iv*: BnBr, NaH, DMF, 61%.

However, a more efficient route to a trisaccharide building block (compare **III** in Figure 2) was to introduce the two xylose moieties at the same time (Scheme 3). Reductive opening of the benzylidene acetal in **9** yielded the 2,4-diol **17** (75%), which was glycosylated with donor **10** using silver triflate as promoter to yield the trisaccharide **18**. Subsequent deacylation (→**19**), and benzylation, then afforded the desired block **20** in 40% yield from **17**.



Scheme 4. *i*: DMTST, Et_2O; *ii*: $PdCl_2$, EtOH/MeOH.

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Assembly of the available blocks was then investigated. Dimethyl(methylthio)-sulfonium triflate (DMTST)-promoted glycosylations in diethyl ether of acceptor **7** with disaccharide donors **15** and **16** gave the α-linked trisaccharides **21** and **25**, respectively, in high yields (Scheme 4). Furthermore, after removal of the 3′-*O*-allyl group with $PdCl_2$, both the obtained trisaccharide acceptors **22** and **26**, could be efficiently elongated by new stereoselective DMTST-promoted glycosylations proving the assembly concept. Glycosylation of acceptor **22** with donor **16** afforded pentasaccharide **23** (65%, 95% calculated on consumed acceptor), whereas coupling of acceptor **26** with donor **16** or trisaccharide donor **20** yielded pentasaccharide **27** (85%) and hexasaccharide **29** (85%), respectively. Subsequent deallylations then afforded new acceptors, the pentasaccharides **24** (80%) and **28** (62%) and hexasaccharide **30** (62%), into which glucuronic acid-containing building blocks can be introduced to produce structures corresponding to *Cryptococcus* serotypes A–C.

In conclusion, xylose-containing di- and trisaccharide motifs of the mannan backbone in the *C. neoformans* CPS have been efficiently synthesised as thioglycoside blocks, which were shown to be good donors in glycosylation reactions. This modular approach allows an iterative selective deprotection/glycosylation scheme to synthesize larger CPS oligosaccharide structures including acetylated target molecules. Access to similar glucuronic acid-containing building blocks should allow the synthesis of a large variety of CPS structures from the different serotypes found in *C. neoformans*.

EXPERIMENTAL

General methods. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and/or 8% aq sulfuric acid for visualization. Column chromatography was performed on silica gel (0.040–0.063 mm, Amicon). NMR spectra were recorded in $CDCl_3$ (internal Me_4Si, δ = 0.00) at 25°C on a Varian 300 MHz or 400 MHz instrument. MALDI-TOF spectra were recorded on a Bruker Biflex III instrument using 2′,4′,6′-trihydroxyacetophenone trihydrate (THAP) as matrix. Organic phases were dried over Na_2SO_4 before evaporation, which was performed under reduced pressure.

2-Azidoethyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (7). A solution of **1**[14] (1.26 g, 3.02 mmol) in MeOH (25 mL) was treated with a catalytic amount of 1 M methanolic NaOMe at rt overnight. Dowex 50 (H^+) ion-exchange resin was added and the mixture was filtered, and concentrated to give 2-azidoethyl α-D-mannopyranoside[21] (**2**, 742 mg, 99%) as a white solid; NMR data ($CDCl_3/CD_3OD$): ^{13}C, δ 50.8, 61.3, 66.5, 66.7, 71.0, 71.6, 72.9 and 100.4. α,α-Dimethoxytoluene (536 μL, 3.57 mmol) and *p*-toluenesulfonic acid (40 mg) were added to a solution of **2** (742 mg, 2.98 mmol) in MeCN (25 mL), and the resulting mixture was stirred at 60°C overnight. The mixture was neutralised with Et_3N, concentrated and purified by silica gel chromatography (toluene–EtOAc 1:1) to yield 2-azidoethyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**3**, 707 mg) as a mixture containing some unreacted starting material and also dibenzylidenated material, which was used without further purification in the next step; NMR data: ^{13}C, δ 50.5, 63.5, 66.7, 68.4, 68.7, 70.8, 78.7, 100.5, 102.3, 126.3–137.1. A solution of this mixture and dibutyltin oxide (626 mg, 2.52 mmol) in MeOH (50 mL) was refluxed for

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1 h, then concentrated and dried in vacuum. The residue was dissolved in DMF (50 mL) and *p*-methoxybenzyl chloride (341 μL, 2.52 mmol) and CsF (414 mg, 2.72 mmol) were added, and the mixture was stirred at rt overnight. The mixture was diluted with toluene, washed twice with a sat aq KF solution, dried and concentrated. Silica gel chromatography (toluene–EtOAc 3:1) yielded 2-azidoethyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (**4**, 612 mg, 45% from **2**) as a syrup; NMR data: ^{13}C, δ 50.7, 55.5, 63.9, 67.0, 69.0, 70.0, 73.1, 75.4, 78.8, 100.4, 101.9, 114.1–159.7. The position of the *p*-methoxybenzyl group is proven by the downfield shift of H-2 after acetylation of **4** [δ 5.4 (dd, 1H, H-2)]. A solution of **4** (227 mg, 0.50 mmol) in 70% aq AcOH (10 mL) was heated at 70°C for 3 h, then concentrated and co-concentrated twice with toluene. Purification by silica gel chromatography (toluene–EtOAc 1:3) gave 2-azidoethyl 3-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (**5**, 155 mg, 85%); NMR data: ^{13}C, δ 50.3, 55.1, 61.0, 64.7, 66.5, 67.7, 71.6, 72.7, 78.9, 99.7, 113.7–159.2. A 60% oil dispersion of sodium hydride (75 mg, 1.89 mmol) was washed once with dry light petroleum. DMF (3 mL) was added, and thereafter a solution of **5** (155 mg, 0.42 mmol) in DMF (4 mL) was added dropwise at 0°C. After 15 min, benzyl bromide (210 μL, 1.76 mmol) in DMF (3 mL) was added, and the solution was allowed to reach rt. MeOH (0.5 mL) was carefully added and the mixture was diluted with toluene, washed three times with H_2O, dried and concentrated. Purification on a silica gel column (toluene–EtOAc 9:1) gave 2-azidoethyl 2,4,6-tri-*O*-benzyl-3-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (**6**, 235 mg, 88%) as a syrup; NMR data: ^{13}C, δ 50.4, 55.2, 66.3, 69.2, 71.8, 72.0, 72.7, 73.2, 74.6 (2C), 74.9, 79.6, 98.1, 113.6–158.9. DDQ (173 mg, 0.76 mmol) was added at 0°C to a solution of **6** (374 mg, 0.58 mmol) in CH_2Cl_2:H_2O (19:1, 20 mL). After stirring for 3 h, the mixture was washed twice with aq $NaHCO_3$ and water. Concentration of the organic phase followed by silica gel chromatography (toluene–EtOAc 6:1) afforded **7** (248 mg, 82%); $[\alpha]_D$ + 22° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 50.4, 66.3, 69.0, 71.1, 71.5, 72.9, 73.3, 74.6, 76.3, 78.1, 97.1, 127.3–138.1.

Anal. Calcd for $C_{29}H_{33}N_3O_6$: C, 67.04; H, 6.40. Found: C, 66.85; H, 6.23.

Ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-1-thio-α-D-mannopyranoside (15) and ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (14b). A solution of **8**[16] (3.00 g, 9.60 mmol) and dibutyltin oxide (2.87 g, 11.53 mmol) in MeOH (200 mL) was refluxed for 1 h, then concentrated and dried in vacuum. The residue was dissolved in DMF (200 mL). Allyl bromide (979 μL, 11.57 mmol) and CsF (1.89 g, 12.44 mmol) were added, and the mixture was stirred at rt overnight. The reaction mixture was diluted with toluene and washed twice with a sat aq KF solution, dried and concentrated. Silica gel chromatography (toluene–EtOAc 3:1) yielded ethyl 3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**9**, 2.92 g, 86%); NMR data: ^{13}C, δ 14.9, 25.0, 63.8, 68.7, 71.5, 71.9, 75.4, 79.1, 84.2, 101.6, 117.6, 126.1–128.9, 134.4, 137.5. Silver triflate (5.47 g, 21.28 mmol) dissolved in dry toluene was added at −40°C to a stirred solution of **9** (3.00 g, 8.51 mmol), **10**[17] (11.18 g, 21.28 mmol) and 2,6-di-*tert*-butylpyridine (3.80 mL, 17.02 mmol) in distilled CH_2Cl_2 (250 ml) containing crushed molecular sieves (4 Å). After 1 h, Et_3N (2 mL) was added, and stirring was continued for 15 min. The mixture was diluted with CH_2Cl_2, filtered through a pad of

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Celite, concentrated, and purified by silica gel chromatography (toluene–EtOAc 12:1) to give crude ethyl (2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**11**). The crude disaccharide was dissolved in MeOH (250 mL), 1 M methanolic NaOMe (10 mL) was added, and the mixture was stirred for 4 h at rt. Dowex 50 (H^+) ion-exchange resin was added, and the stirring was continued for 30 min. Filtration and concentration of the mixture followed by silica gel chromatography (toluene–EtOAc 1:3) gave ethyl (β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**12**, 3.37 g, 82% over two steps) as a white foam; NMR data: ^{13}C, δ 15.2, 25.9, 64.6, 65.5, 68.7, 69.7, 70.4, 73.2, 74.1, 74.8, 74.9, 79.8, 84.7, 101.1, 101.8, 118.9, 126.3–133.8, 134.1, 137.7; ^{1}H, δ 4.58 (d, 1 H, $J_{1,2}$ = 9 Hz, H-1′), 5.32 (s, 1 H, H-1). A 60% oil dispersion of sodium hydride (1.28 g, 31.85 mmol) was washed once with dry light petroleum. DMF (15 mL) was added followed by dropwise addition at 0°C of a solution of **12** (3.43 g, 7.08 mmol) in DMF (60 mL). After 15 min, benzyl bromide (3.54 mL, 29.73 mmol) in DMF (60 mL) was added and the solution was stirred for 1 h. MeOH (5 mL) was carefully added and the mixture was diluted with toluene and washed three times with H_2O, dried and concentrated. Purification on a silica gel column (toluene–EtOAc 15:1) gave ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**13**, 4.67 g, 87%) as a syrup; NMR data: ^{13}C, δ 15.0, 25.6, 64.0, 64.5, 68.7, 70.9, 73.3, 74.1, 75.1, 75.5, 77.7, 77.8, 78.9, 81.3, 83.2, 83.7, 101.5, 103.0, 117.0, 126.0–128.8, 134.7, 137.5–138.6. MALDI-TOF MS: *m/z* calcd for $C_{44}H_{50}NaO_9S$ ($[M + Na]^+$): 777.31. Found 776.72.

A solution of $AlCl_3$ (707 mg, 5.30 mmol) in diethyl ether (50 mL) was added dropwise during 30 min to a stirred mixture of **13** (1.00 g, 1.32 mmol), BH_3–trimethylamine complex (3.86 g, 52.98 mmol) and 4 Å molecular sieves in CH_2Cl_2:diethyl ether (5:1, 120 mL) at 0°C. After 1 h, the mixture was filtered through a pad of Celite and 1M H_2SO_4 was added to the filtrate, which then was stirred for 30 min. The phases were separated and the organic layer was washed with aq $NaHCO_3$ and H_2O, dried and concentrated. A short silica gel column gave a mixture of **14a** and **14b**, which was acetylated at the primary hydroxyl group before further purification. The crude disaccharide mixture and *sym*-collidine (286 μL, 2.15 mmol) were dissolved in CH_2Cl_2 (30 mL) and the solution was cooled to −70°C. Acetyl chloride (92 μL, 1.28 mmol) was added and the reaction was stirred for 10 min at −70°C, then quenched with MeOH, and allowed to attain rt. Concentration of the mixture, followed by silica gel chromatography (toluene–EtOAc 15:1), afforded **15** (366 mg, 35% over two steps) together with 390 mg of ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**14b**). **15**: $[\alpha]_D$ + 37° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.0, 20.5, 25.7, 63.3, 64.1, 69.9, 70.5, 73.4, 74.0, 75.1, 75.2, 75.6, 76.9, 77.3, 78.4, 81.2, 82.5, 83.9, 103.5, 118.1, 127.6–128.9, 134.6, 138.0–138.8, 170.7; MALDI-TOF MS: *m/z* calcd for $C_{46}H_{54}NaO_{10}S$ ($[M + Na]^+$): 821.33. Found 820.91.

Anal. Calcd for $C_{46}H_{54}O_{10}S$: C, 69.15; H, 6.81. Found: C, 68.91; H, 6.61.

Ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-3-*O*-allyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (16). A 60% oil dispersion of sodium hydride (41 mg, 1.03 mmol) was washed once with dry light petroleum. DMF (2 mL) was added, and a solution of **14b** (390 mg, 0.52 mmol) in DMF (10 mL) was added dropwise at 0°C.

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After 15 min, benzyl bromide (110 μL, 0.93 mmol) in DMF (5 mL) was added and the solution was stirred for 4 h. MeOH (1 mL) was carefully added and the mixture was diluted with toluene and washed three times with H_2O, dried and concentrated. Purification on a silica gel column (toluene–EtOAc 15:1) gave **16** (378 mg, 34% over two steps) as a syrup; $[\alpha]_D$ + 48° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.0, 25.5, 64.1, 69.4, 70.5, 71.9, 73.1, 73.4, 74.7, 74.9, 75.2, 75.5, 77.0, 77.3, 78.5, 81.0, 82.3, 84.0, 103.5, 117.7, 127.3–129.0, 134.8, 138.3–138.8; MALDI-TOF MS: *m/z* calcd for $C_{51}H_{58}NaO_9S$ ($[M + Na]^+$): 869.37. Found 868.92.

Anal. Calcd for $C_{51}H_{58}O_9S$: C, 72.31; H, 6.90. Found: C, 72.09; H, 7.11.

Ethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (20). A solution of **9** (832 mg, 2.36 mmol), $NaCNBH_3$ (1.56 g, 23.6 mmol) and 3 Å molecular sieves in distilled THF (100 mL) was stirred at rt under argon for 30 min. HCl in diethyl ether was added until pH = 1. After 30 min, the reaction mixture was filtered through a layer of Celite, concentrated and purified on silica gel (toluene–EtOAc, 4:1) to give ethyl 3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**17**, 624 mg, 1.76 mmol, 75%); NMR data: ^{13}C, δ 15.2, 25.4, 68.6, 69.8, 70.5, 71.1, 71.3, 74.0, 79.6, 84.0, 118.6, 128.1–128.8, 134.5, 138.2. Silver triflate (362 mg, 1.41 mmol) dissolved in dry toluene was added at −40°C to a stirred solution of **17** (100 mg, 0.28 mmol), **10** (741 mg, 1.41 mmol) and 2,6-di-*tert*-butylpyridine (253 μL, 1.13 mmol) in distilled CH_2Cl_2 (50 ml) containing crushed molecular sieves (4 Å). After 2h, Et_3N (1 mL) was added and stirring was continued for 15 min. The mixture was diluted with CH_2Cl_2, filtered through a pad of Celite, concentrated, and purified by silica gel chromatography (toluene–EtOAc 10:1) to give crude ethyl (2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1 → 2)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**18**). The crude trisaccharide was dissolved in MeOH (100 mL). 5 mL of 1 M methanolic NaOMe was added and the mixture was stirred for 2 h at rt. Dowex 50 (H^+) ion-exchange resin was added, and stirring was continued for 30 min. Filtration and evaporation, followed by silica gel chromatography (CH_2Cl_2:MeOH 3:1), then gave ethyl (β-D-xylopyranosyl)-(1 → 4)-[(β-D-xylopyranosyl)-(1 → 2)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**19**, 113 mg, 65% over two steps); NMR data: ^{13}C, δ 15.0, 25.8, 65.6, 65.7, 68.7, 69.5, 69.8, 71.4, 71.5, 72.1, 73.6, 73.7, 74.1, 75.3, 75.6, 76.4, 76.9, 82.7 (C-1), 102.2, 103.4 (C-1′, 1″), 118.3, 128.1–133.1, 134.4, 137.6; MALDI-TOF MS: *m/z* calcd for $C_{29}H_{42}NaO_{13}S$ ($[M + Na]^+$): 641.22. Found 640.91.

A solution of **19** (113 mg, 0.18 mmol) and BnBr (260 μL, 2.19 mmol) in dry DMF (10 mL) was added dropwise to a cold (0°C) suspension of NaH (110 mg, 2.75 mmol) in DMF (10 mL). The mixture was stirred at rt for 4 h before the addition of MeOH (2 mL). Toluene was added, and the mixture was washed with sat aq $NaHCO_3$ and water. The organic layer was dried, concentrated and purified by silica gel chromatography (toluene–EtOAc, 10:1) to give **20** (129 mg, 0.11 mmol, 61%); $[\alpha]_D$ + 52° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.0, 25.5, 63.7, 64.0, 68.9, 71.3, 71.9, 72.9, 73.1, 73.3, 74.7, 74.9, 75.5, 76.5, 76.7, 77.0, 77.5, 78.2, 81.1, 82.0, 82.3, 84.0, 84.2, 103.1, 103.3, 116.8, 127.3–129.0, 135.3, 138.2–138.9; MALDI-TOF MS: *m/z* calcd for $C_{70}H_{78}NaO_{13}S$ ($[M + Na]^+$): 1181.51. Found 1180.95.

Anal. Calcd for $C_{70}H_{78}O_{13}S$: C, 72.51; H, 6.78. Found: C, 72.30; H, 6.91.

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2-Azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (24). A solution of **7** (102 mg, 0.20 mmol) and **15** (235 mg, 0.29 mmol) in dry diethyl ether (10 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an argon atmosphere for 30 min. DMTST (203 mg, 0.78 mmol) was added to the mixture, and stirring was continued for 6 h. After neutralization with Et_3N, the mixture was filtered through a pad of Celite and concentrated. The residue was purified on a silica gel column (toluene–EtOAc 6:1) to yield crude 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-α-D-mannopyranoside) (**21**, 163 mg, 65%). $PdCl_2$ (60%, 3 mg, 9.8 μmol) was added to a solution of **21** (123 mg, 0.098 mmol) in EtOH:MeOH (1:1, 6 mL). The mixture was stirred for 7 h, then filtered through a pad of Celite, concentrated and purified on a silica gel column (toluene–EtOAc 5:1) to yield 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**22**, 71 mg, 60%); NMR data: ^{13}C, δ 20.8, 50.6, 63.7, 64.1, 66.8, 69.2, 70.0, 71.1, 72.6, 72.8, 73.6, 73.7, 74.6, 74.9, 75.0, 75.2, 75.8, 76.4, 77.5, 77.5, 78.9, 80.4, 81.3, 83.3, 98.1 (C-1), 100.5 (C-1′), 104.0 (C-1″), 126.9–138.9, 170.9; ^1H, δ 5.15 (H-1′). A solution of **22** (65 mg, 0.053 mmol) and **16** (68 mg, 0.080 mmol) in dry diethyl ether (5 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an argon atmosphere for 30 min. DMTST (55 mg, 0.21 mmol) was added to the mixture, and the stirring was continued for 5 h. After neutralization with Et_3N, the mixture was filtered through a pad of Celite and concentrated. The residue was purified on a silica gel column (toluene–EtOAc 9:1) to yield 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(6-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside [**23**, 69 mg, 65% (95% based on consumed acceptor)] together with 21 mg of unreacted **22**; NMR data **23**: ^{13}C, δ 20.3, 50.2, 62.9–83.1, 97.4 (C-1), 99.8, 100.3 (C-1′, 1‴), 103.4 (2C, C-1″, 1⁗), 116.4, 126.4–128.5, 135.0, 137.6–139.1, 170.4. $PdCl_2$ (60%, 1 mg, 3.2 μmol) was added to a solution of **23** (60 mg, 0.030 mmol) in EtOH:MeOH (1:1, 6 mL). The mixture was stirred for 5 h, filtered through a pad of Celite, concentrated and purified on a silica gel column (toluene–EtOAc 6:1) to yield **24** (47 mg, 80%); $[\alpha]_D$ + 10° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, 20.5, 50.4, 63.1–83.2, 97.6 (C-1), 100.3, 100.7 (C-1′, 1‴), 103.6, 103.9 (C-1″,1⁗), 126.6–139.2, 170.6; ^1H, δ 5.14, 5.28 (H-1′, 1‴); MALDI-TOF MS: *m/z* calcd for $C_{116}H_{125}N_3NaO_{25}$ ($[M + Na]^+$): 1982.85. Found 1983.05.

Anal. Calcd for $C_{116}H_{125}N_3O_{25}$: C, 71.04; H, 6.42. Found: C, 70.84; H, 6.62.

2-Azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (26). A solution of **7** (44 mg, 0.085 mmol) and **16** (100 mg, 0.12 mmol) in dry diethyl ether (5 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an argon atmosphere for 30 min. DMTST (87 mg, 0.34 mmol) was added to the mixture, and stirring was continued for 3 h. After neutralization with Et_3N, the mixture was filtered through a pad of Celite and concentrated. The residue was purified on a silica gel column (toluene–EtOAc 12:1) to yield 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**25**, 100

mg, 91%); NMR data: ^{13}C, δ 50.4, 63.9–83.5, 97.8 (C-1), 99.9 (C-1′), 103.6 (C-1″), 117.2, 126.8–129.1, 135.1, 138.2–139.0. $PdCl_2$ (60%, 1 mg, 3.2 μmol) was added to a solution of **25** (41 mg, 0.031 mmol) in EtOH:MeOH (1:1, 6 mL). The mixture was stirred for 5 h, filtered through a pad of Celite, concentrated and purified on a silica gel column (toluene–EtOAc 9:1) to yield **26** (29 mg, 73%); $[\alpha]_D$ + 33° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 50.4, 63.9, 66.6, 69.0, 69.4, 70.7, 72.2, 72.4, 72.5, 73.3, 73.3, 73.4, 74.4, 74.6, 74.7, 74.9, 75.5, 77.2, 77.5, 80.4, 80.7, 83.2, 97.9 (C-1), 100.5 (C-1′), 103.9 (C-1″), 126.8–128.7, 138.1–138.9; ^{1}H, δ 5.22 (H-1′); MALDI-TOF MS: *m/z* calcd for $C_{75}H_{81}N_3NaO_{15}$ ($[M + Na]^+$): 1286.56. Found 1286.17.

Anal. Calcd for $C_{75}H_{81}N_3O_{15}$: C, 71.24; H, 6.46. Found: C, 71.03; H, 6.67.

2-Azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (28). A solution of **26** (76 mg, 0.060 mmol) and **16** (74 mg, 0.087 mmol) in dry diethyl ether (10 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an argon atmosphere for 30 min. To the mixture was added DMTST (63 mg, 0.24 mmol) and the stirring was continued for 2 h. After neutralization with Et_3N, the mixture was filtered through a pad of Celite, and concentrated. The residue was purified on a silica gel column (toluene–EtOAc 9:1) to yield crude 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)-(3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**27**, 105 mg, 85%). $PdCl_2$ (60%, 1 mg, 3.2 μmol) was added to a solution of **27** (105 mg, 0.051 mmol) in EtOH:MeOH (1:1, 6 mL). The mixture was stirred for 5 h, filtered through a pad of Celite, concentrated and purified on a silica gel column (toluene–EtOAc 9:1) to yield **28** (64 mg, 62%); $[\alpha]_D$ + 9° (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 50.6, 63.4–83.5, 97.9 (C-1), 100.7, 100.8 (C-1′, 1‴), 103.9, 104.1 (C-1″, 1⁗), 126.4–128.7, 138.4–139.5; ^{1}H, δ 5.18, 5.28 (H-1′, 1‴); MALDI-TOF MS: *m/z* calcd for $C_{121}H_{129}N_3NaO_{24}$ ($[M + Na]^+$): 2030.89. Found 2030.73.

Anal. Calcd for $C_{121}H_{129}N_3O_{24}$; C, 72.33; H, 6.47. Found: C, 72.19; H, 6.62.

2-Azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(6-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (30). A solution of **20** (33 mg, 29 μmol) and **26** (25 mg, 20 μmol) in dry diethyl ether (5 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an argon atmosphere for 30 min. To the mixture was added DMTST (20 mg, 79 μmol), and stirring was continued for 30 min. After neutralization with Et_3N, the mixture was filtered through a pad of Celite and concentrated. The residue was purified on a silica gel column (toluene–EtOAc 10:1) to yield 2-azidoethyl (2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(3-*O*-allyl-6-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 2)]-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside side (**29**, 39 mg, 17 μmol, 85%); Selected NMR data: ^{13}C, δ 97.8 (C-1), 99.6, 100.2 (C-1′, 1‴), 103.2, 103.3, 103.5 (C-1″, 1⁗, 1⁗′); MALDI-TOF MS: *m/z* calcd for $C_{143}H_{153}N_3NaO_{28}$ ($[M + Na]^+$): 2383.05, Found 2383.35. $PdCl_2$ (60%, 5 mg, 17 μmol) was added to a solution of **29**

(18 mg, 7.6 μmol) in EtOH:MeOH (1:1, 4 mL). The mixture was stirred for 2 h, filtered through a pad of Celite, concentrated and purified on a silica gel column (toluene–EtOAc 10:1) to give **30** (11 mg, 62%); $[\alpha]_D$ + 25° (*c* 1.0, $CHCl_3$); Selected NMR data: ^{13}C, δ 97.7 (C-1), 98.6, 100.2 (C-1′, 1‴), 103.1, 103.4, 104.0 (C-1″, 1⁗, 1⁗′); ^{1}H, δ 5.17, 5.29 (H-1′, 1‴).

Anal. Calcd for $C_{140}H_{149}N_3O_{28}$: C, 72.43; H, 6.47. Found: C, 72.20; H, 6.61.

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