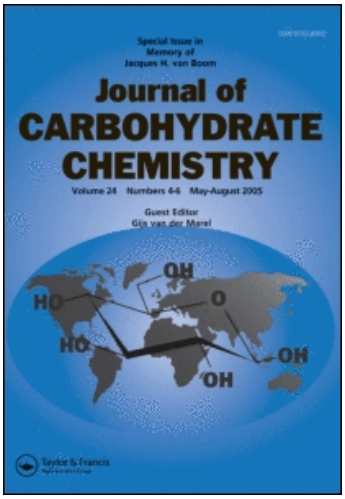

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Synthesis of Oligosaccharides Corresponding to Structures Found in Capsular Polysaccharides of *Cryptococcus neoformans*. Part 1

Per J. Garegg[a]; Lars Olsson[a]; Stefan Oscarson[a]
[a] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

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SYNTHESIS OF OLIGOSACCHARIDES CORRESPONDING TO STRUCTURES FOUND IN CAPSULAR POLYSACCHARIDES OF *CRYPTOCOCCUS NEOFORMANS*. PART 1.

Per J. Garegg, Lars Olsson and Stefan Oscarson

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

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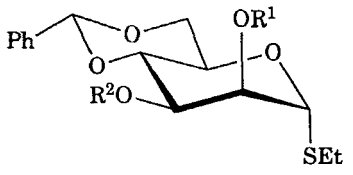
ABSTRACT

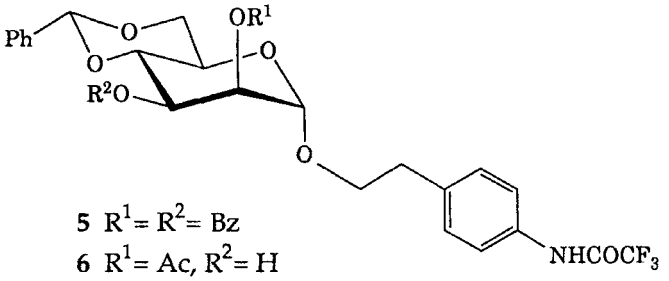
Syntheses are described of two trisaccharides, 2-(*p*-trifluoroacetamidophenyl)ethyl *O*-(β-D-xylopyranosyl)-(1→2)-*O*-(α-D-mannnopyranosyl)-(1→3) α-D-mannopyranoside (**10**) and 2-(*p*-trifluoroacetamidophenyl)ethyl *O*-(α-D-mannopyranosyl)-(1→3)-[*O*-(β–D-xylopyranosyl)-(1→2)]-α-D-mannopyranoside (**12**), and of the tetrasaccharide 2-(*p*-trifluoroacetamidophenyl)ethyl *O*-(β-D-xylopyranosyl)-(1→2)-*O*-(α-D-mannno-pyranosyl)-(1→3)-[*O*-(β-D-xylopyranosyl)-(1→2)]-α-D-mannopyranoside (**15**). These correspond to parts of the repeating unit of the major structure of the capsular polysaccharide of *Cryptococcus neoformans* serogroup A depicted below:

β-D-Xyl*p* β-D-Xyl*p* β-D-Glc*p*A
1 1 1
↓ ↓ ↓
2 2 2
3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→

INTRODUCTION

Cryptococcus neoformans is the major etiologic agent of cryptococcosis, often meningitis. The organism has emerged as a primary cause of opportunistic infections associated with AIDS. Its capsular polysaccharide is thought to be a virulence factor and governs the serotype specificity. At least four different serotypes, A–D, are known.[1] These consist mainly of a polysaccharide composed of D-mannose, D-xylose, D-glucuronic acid and *O*-acetyl groups. A common feature to all serotype structures is an α-D-Man*p*-(1→3)-α-D-Man*p* backbone with xylopyranosyl and glucuronopyranosyl groups linked to the 2-positions of the mannosyl residues. The 6-position of the mannosyl residues are often acetylated. Structures of the *Cryptococcus neoformans* serotypes have been proposed, based mainly on NMR studies.[2-4] In order to provide model compounds for these studies and in order to obtain synthetic immunogens for the production of epitope and serotype specific antibodies as well as competitive inhibitors for their characterization, we have now started to synthesize various oligosaccharides corresponding to the *Cryptococcus neoformans* capsular structures.





1 $R^1 = R^2 = H$
2 $R^1 = H$, R^2 = TBDMS
3 $R^1 = p$MBn, R^2 = TBDMS
4 $R^1 = R^2$ = Bz

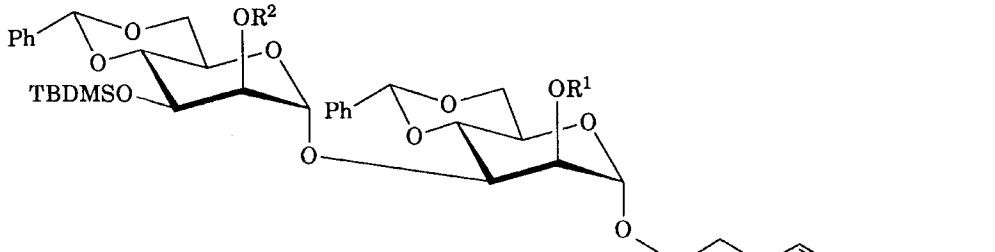
5 $R^1 = R^2$ = Bz
6 R^1 = Ac, $R^2 = H$

RESULTS AND DISCUSSION

Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[5] (**1**) was stannylated with one equivalent of dibutyltin oxide and the product was treated with *tert*-butyldimethylsilyl chloride to give the 3-silyl ether **2** (95%). The 3-substitution was confirmed by acetylation of **2**, which gave the characteristic down-field shift of H-2 in the ^1H NMR spectrum. *p*-

Methoxybenzylation of *O*-2 then gave the fully substituted thioglycoside donor **3** (89%). The glycosyl acceptor **6** was also constructed from **1**. It would seem advantagous to convert **1** into a 2,3-orthoacetate and then open this under mild acidic conditions to obtain methyl 4,6-*O*-benzylidene-2-*O*-acetyl-1-thio-α-D-mannopyranoside and proceed from that intermediate, but this route was not pursued due to the risk of rearrangement of the thioglycoside.[6] Instead, **1** was benzoylated in the 2,3-positions and the product **4** (94%) was condensed with 2-(*p*-trifluoroacetamidophenyl)ethanol using dimethyl(thiomethyl)sulfonium triflate as promoter.[7] The product **5** (87%), containing a linking arm suitable for attachment to a protein,[8] was deacetylated and then converted into the 2,3-orthoacetate, which was treated directly with aqueous trifluoroacetic acid to give the expected axial 2-acetate **6** (68%).[9] The 2-*O*-substitution was once more proven by the down-field shift of H-2 in ^{1}H NMR spectrum.

The above thioglycoside **3** was converted into the corresponding glycosyl bromide in the presence of the glycosyl acceptor **6** and silver triflate.[10] The disaccharide **7** thus produced (57%) was oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[11] to give the glycosyl acceptor **8** (98%). Cerium(IV)ammonium nitrate (CAN)[12] was much less efficient for this conversion. Compound **7** was also deacetylated into the 2-hydroxy compound which was then oxidized with DDQ to give the 2,2'-dihydroxy compound **13** (79%). The sequence of protecting group removal is important here, the reverse procedure, i.e. oxidation of **7** to the 2'-hydroxy compound followed by deacetylation gives rise to base-catalyzed silyl migration.[13]



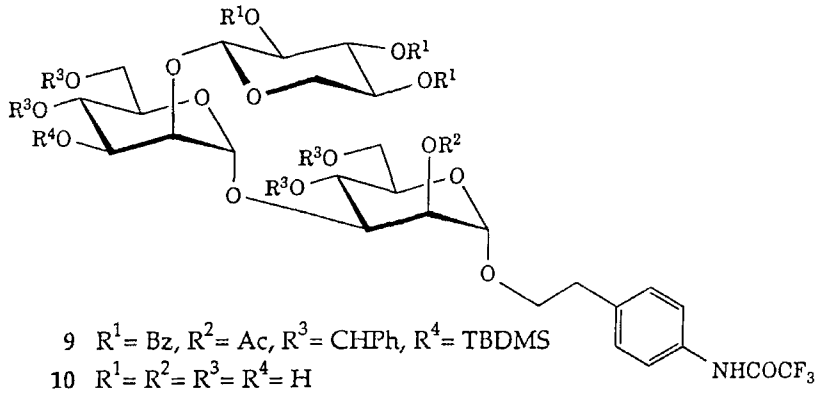
7 R^1= Ac, R^2= *p*MBn
8 R^1= Ac, R^2= H
13 R^1= R^2= H

2,3,4-Tri-*O*-benzoyl-α-D-xylopyranosyl bromide[14] was condensed with the above glycosyl acceptor **8** in the presence of silver triflate,[15,16] to give the

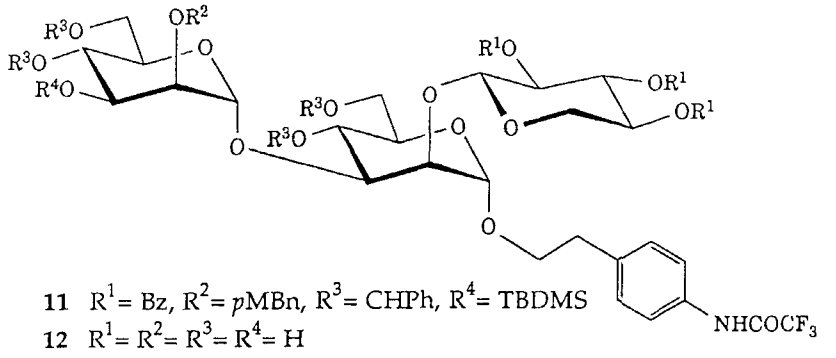
trisaccharide **9** (72%). Deprotection of this, first by treatment with aqueous acetic acid to remove the benzylidene and silyl groups, followed by deacylation produced the target trisaccharide **10** (61%).

The same xylosyl bromide was also condensed in the presence of silver triflate with the 2-hydroxy compound obtained by deacetylation of **7** (above) to give the trisaccharide **11** (78%). This was then deprotected as described for the synthesis of **10** followed by catalytic hydrogenolysis to remove the *p*-methoxybenzyl group to give **12** (22%).

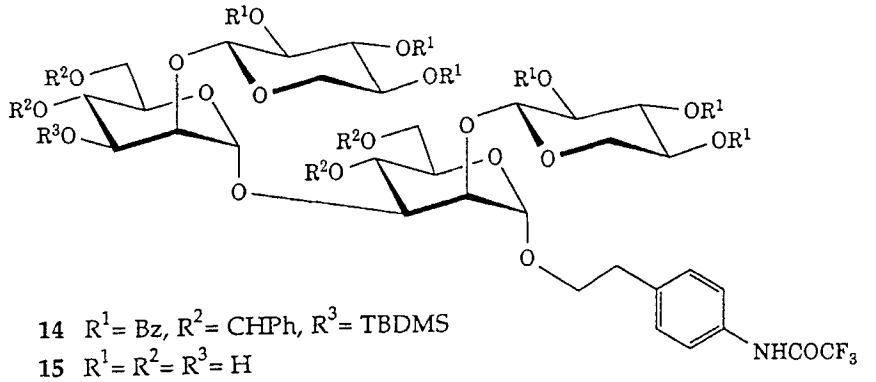
The above glycosyl acceptor **13** was similarly xylosylated to give the tetrasaccharide **14** (71%). This was then deprotected as described for the synthesis of **10** to give **15** (44%). The β-configuration of the xylosyl residues in **10**, **12** and **15** is shown by the $^{3}J_{H\text{-}1,H\text{-}2}$-coupling constants (7-8 Hz), and the α-configuration of the mannose residues in **15** (and thereby in the other derivatives) is shown by the $^{1}J_{C\text{-}1,H\text{-}1}$-coupling constants (169 and 172 Hz).



9 R^1= Bz, R^2= Ac, R^3= CHPh, R^4= TBDMS
10 $R^1 = R^2 = R^3 = R^4$= H



11 R^1= Bz, R^2= *p*MBn, R^3= CHPh, R^4= TBDMS
12 $R^1 = R^2 = R^3 = R^4$= H



14 R^1= Bz, R^2= CHPh, R^3= TBDMS
15 $R^1 = R^2 = R^3$= H

Thus, the present pathway has proven to be effective for the syntheses of the described oligosaccharides. It is also designed to make further syntheses of other oligosaccharides found in *Cryptococcus neoformans* from the same precursors possible. Built-in is the possibility to introduce either xylose or glucuronic acid in the various 2-positions as well as the possibility to elongate the mannan backbone *via* selective removal of the silyl group in the 3-position. These syntheses are now under way.

EXPERIMENTAL

General methods. Melting points are corrected. Concentrations were performed under reduced pressure at <40 °C (bath) except for concentrations of solutions in *N,N*-dimethylformamide for which 50 °C was used. NMR spectra were recorded in $CDCl_3$ (internal Me_4Si, δ=0.00) or D_2O (internal acetone ^{13}C δ=31.0, ^1H δ=2.21) at 25 °C unless otherwise stated, using a JEOL GX-270 instrument. Optical rotations were recorded at room temperature with a Perkin-Elmer 241 polarimeter. TLC was performed on Silica Gel F_{254} (Merck) with detection by UV light and/or by charring with 8% sulfuric acid. Silica gel (0.040-0.063 mm, Amicon) was used for column chromatography. Organic solutions were dried over Na_2SO_4 prior to concentrations. Semipreparative HPLC was carried out on a Dynamax 60-A silica gel column with a flow rate of 2 ml/min and detection was achieved by measuring the absorbance at 260 nm, the elution system used was a 10 min isocratic flow of hexane-ethyl acetate (20% ethyl acetate) followed by a 40

min linear gradient of hexane-ethyl acetate (20 to 80% ethyl acetate) unless otherwise stated.

Ethyl 4,6-*O*-Benzylidene-3-*O*-*t*-butyldimethylsilyl-1-thio-α-D-manno-pyranoside (2). A solution of ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**1**) (2.31 g, 7.4 mmol) and dibutyltin oxide (1.84 g, 7.4 mmol) in methanol (100 mL) was refluxed. After 30 min when the reaction mixture became clear, the solution was concentrated and the residue dried in vacuum and then dissolved in *N,N*-dimethylformamide (50 mL). To this solution *t*-butyldimethylsilyl chloride (1.38 g, 9.3 mmol) was added, and the mixture was stirred for 2 h. Toluene (excess) was added and the reaction mixture was washed twice with water and then concentrated. Flash chromatography (toluene-ethyl acetate 6:1) gave **2** (3.00 g, 7.0 mmol, 95%), $[\alpha]_D$ +122° (*c* 0.5, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -5.1, -4.5 (CH_3-Si), 14.8 (S-CH_2-$\underline{C}H_3$), 18.0 (C *t*-butyl), 24.8 (S-CH_2-$\underline{C}H_3$), 25.8 (CH_3 *t*-butyl), 63.7, 68.5, 70.2, 73.3, 79.1 (C-ring), 83.7 (C-1), 101.7 ($\underline{C}$H-Ar), 126.1-137.4 (aromatic C).

Anal. Calcd for $C_{21}H_{34}O_5SSi$: C, 59.1; H, 8.0. Found: C, 59.1; H, 8.1.

Acetylation of **2** gave the 2-*O*-acetylated compound with NMR data: ^1H, δ 5.21 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 1.5 Hz), 5.27 (H-2, dd, $J_{H\text{-}2,H\text{-}3}$ 3.7 Hz), 5.59 ($C\underline{H}$-Ar, s).

Ethyl 4,6-*O*-Benzylidene-2-*O*-*p*-methoxybenzyl-3-*O*-*t*-butyl-dimethylsilyl-1-thio-α-D-mannopyranoside (3). A solution of **2** (684 mg, 1.60 mmol) and *p*-methoxybenzyl bromide (0.42 mL, 3.2 mmol) in *N,N*-dimethylformamide (5 mL) was added dropwise to a concentrated slurry of sodium hydride (120 mg, 5.0 mmol) and *N,N*-dimethylformamide. After 30 min, the reaction mixture was partitioned between toluene and water. The organic phase was dried and concentrated. Flash chromatography (toluene-ethyl acetate 25:1) yielded **3** (776 mg, 1.42 mmol, 89%). HPLC was used to purify an analytical sample for optical rotation and elementary analysis. The elution system was a 10 min isocratic flow of hexane-ethyl acetate (20% ethyl acetate) followed by a 40 min linear gradient of hexane-ethyl acetate (20 to 50% ethyl acetate), $[\alpha]_D$ +77° (*c* 0.8, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -4.8, -4.4 (CH_3-Si), 14.9 (S-CH_2-$\underline{C}H_3$), 18.6 (C *t*-butyl), 25.3 (S-$\underline{C}H_2$-CH_3), 25.9 (CH_3 *t*-butyl), 55.2 (CH_3-O), 64.8, 68.6, 70.9, 73.5, 79.3, 80.5 (C-ring, O-$\underline{C}H_2$-Ar), 84.0 (C-1), 101.8 ($\underline{C}$H-Ar), 113.8, 126.2-137.6, 159.3 (aromatic C)

Anal. Calcd for $C_{29}H_{42}O_6SSi$: C, 63.7; H, 7.7. Found: C, 63.8; H, 7.7.

Ethyl 2,3-di-*O*-Benzoyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4). Benzoyl chloride (6.7 mL, 58 mmol) was added to a solution of **1** (4.5 g, 14 mmol) in pyridine (150 mL). After 2 h at room temperature, the reaction

mixture was partitioned between 1M hydrochloric acid and dichloromethane. The organic layer was washed with 1M hydrochloric acid, aqueous sodium hydrogencarbonate, water (twice), dried and concentrated. Crystallization (ethyl acetate-hexane) of the syrup gave **4** (3.5 g, 6.7 mmol, 47%). Flash chromatography (toluene-ethyl acetate 25:1) of the remaining residue yielded another 47% of **4**, $[\alpha]_D$ -44° (*c* 1.1, chloroform), mp 139-140 °C. NMR data ($CDCl_3$): ^{13}C, δ 14.9 (S-CH_2-$\underline{C}H_3$), 25.6 (S-$\underline{C}H_2$-CH_3), 64.5, 68.6, 69.3, 72.7 (C-ring), 83.2 (C-1), 101.9 ($\underline{C}$H-Ar), 126.1-137.0 (aromatic C) 165.3 (C=O benzoyl)

Anal. Calcd for $C_{29}H_{28}O_7S$: C, 66.9; H, 5.4. Found: C, 67.1; H, 5.6.

2-(*p*-Trifluoroacetamidophenyl)ethyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (5). A solution of dimethyl(thiomethyl)sulfonium triflate (DMTST) (1.25 g, 4.8 mmol) in dichloromethane (2 mL) was added to a stirred mixture of **4** (800 mg, 1.54 mmol), 2-(*p*-trifluoroacetamidophenyl)-ethanol (585 mg, 2.31 mmol) and 4Å molecular sieves in dichloromethane (30 mL) at room temperature. After 1 h, triethylamine (2 mL) was added and the mixture was stirred for another 15 min before it was filtered through Celite and concentrated. Flash cromatography (toluene-ethyl acetate 14:1) gave **5** (927 mg, 1.34 mmol, 87%), [α]$_D$ -54° (*c* 1.1, chloroform). NMR data ($CDCl_3$): ^{13}C, δ 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 63.8, 68.7, 69.0, 70.9, 76.6 (C-ring, O-$\underline{C}H_2$-Ar, O-$\underline{C}H_2$-CH_2-Ar), 98.1, 101.8 (C-1, $\underline{C}$H-Ar), 121.0-137.0 (aromatic C), 165.5, 165.6 (C=O benzoyl).

Anal. Calcd for $C_{37}H_{32}NO_9F_3$: C, 64.2; H, 4.7; N, 2.0. Found: C, 64.0; H, 4.6; N, 2.0.

2-(*p*-Trifluoroacetamidophenyl)ethyl 2-*O*-Acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (6). Methanolic sodium methoxide (0.5 mL, 1M) was added to a solution of **5** (430 mg, 0.62 mmol) in methanol (40 mL). The mixture was stirred overnight and then neutralized with Dowex 50 (H^+) resin, filtered, concentrated and dried in a vacuum. The dried residue was dissolved in dry acetonitrile (40 mL) and trimethyl orthoacetate (0.31 mL, 2.40 mmol) was added. After 30 min the reaction mixture was concentrated and the residue again dissolved in acetonitrile (40 mL). Aqueous trifluoroacetic acid (90%, 50 μL) was added and after 15 min the mixture was partitioned between ethyl acetate and aqueous sodium hydrogencarbonate. The organic phase was washed with water, dried and concentrated. Column chromatography (toluene-ethyl acetate 3:1) gave **6** (223 mg, 0.42 mmol, 68%). Crystallization of the syrup from diethyl ether-light

petroleum bp 60-70 °C gave crystals having mp 152-153 °C, $[\alpha]_D$ +42° (*c* 1.0, chloroform). NMR data ($CDCl_3$): ^{13}C, δ 21.0 (CH_3 acetyl), 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 63.3, 67.1, 68.3, 68.6, 72.1, 78.8 (C-ring, O-$\underline{C}H_2$-Ar), 98.1, 102.1 (C-1, $\underline{C}$H-Ar), 121.0-137.0 (aromatic C), 170.7 (C=O acetyl); ^1H, δ 4.76 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 1.5 Hz), 5.18 (H-2, dd, $J_{H\text{-}2,H\text{-}3}$ 3.7 Hz), 5.54 ($C\underline{H}$-Ar, s).

Anal. Calcd for $C_{25}H_{26}NO_8F_3$: C, 57.1; H, 5.0; N, 2.7. Found: C, 57.4; H, 5.2; N, 2.6.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(4,6-*O*-Benzylidene-3-*O*-*t*-butyldimethylsilyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (7). A solution of **3** (226 mg, 0.41 mmol), **6** (150 mg, 0.27 mmol), silver triflate (280 mg, 1.09 mmol) and 2,6-di-*tert*-butylpyridine (75 μL, 0.35 mmol) in dichloromethane (15 mL) was stirred with molecular sieves for 30 min. Bromine (22 μL, 0.41 mmol) was added and after 30 min the reaction was quenched with triethylamine (1 mL). After another 15 min the reaction mixture was applied directly to flash chromatography (toluene-ethyl acetate 15:1) which yielded **7** (163 mg, 0.160 mmol, 57%), $[\alpha]_D$ +24° (*c* 0.9, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -4.9, -4.5.(CH_3Si), 18.4 (C *t*-butyl), 20.9 (CH_3 acetyl), 25.9 (CH_3 *t*-butyl), 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 55.3 (CH_3-O), 63.5, 65.0, 68.4, 68.7, 69.8, 70.0, 71.6, 73.2, 78.2, 78.9, 79.0 (C-ring, O-$\underline{C}H_2$-Ar, O-$\underline{C}H_2$-CH_2-Ar), 98.1, 100.0, 101.6, 102.1 (C-1,1′, 2 x $\underline{C}$H-Ar), 113.6, 121.0-137.6, 159.1 (Aromatic C),169.7 (C=O acetyl).

Anal. Calcd for $C_{52}H_{62}NO_{14}SiF_3$: C, 61.8; H, 6.2; N, 1.4. Found: C, 61.5; H, 6.2; N, 1.6.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(4,6-*O*-Benzylidene-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (8). A solution, saturated with water, of **7** (77 mg, 0.076 mmol) and DDQ (19 mg, 0.086 mmol) in dichloromethane (15 mL) was stirred overnight. The reaction mixture was then washed with aqueous sodium hydrogencarbonate, water and concentrated. After flash chromatography (light petroleum bp 60-70 °C-ethyl acetate 3:1) **8** (67 mg, 0.075 mmol, 98%) was collected. HPLC was used to purify an analytical sample for optical rotation and elementary analysis. $[\alpha]_D$ +32° (*c* 0.4, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -5.2, -4.4 (CH_3-Si), 18.2 (C *t*-butyl), 20.9 (CH_3 acetyl), 25.7 (CH_3 *t*-butyl), 35.3 (O-CH_2-$\underline{C}H_2$-Ar), 63.6, 64.0, 68.4, 68.7, 69.4, 70.6, 71.7, 71.9, 78.7 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 98.0, 100.5, 101.5, 102.0 (1,1′, 2 x $\underline{C}$H-Ar), 121.1-137.1 (aromatic C), 169.9 (C=O acetyl).

Anal. Calcd for $C_{44}H_{54}O_{13}NSiF_3$: C, 59.4; H, 6.1; N, 1.6. Found: C, 59.8; H, 5.9; N, 1.8.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(2,3,4-Tri-*O*-benzoyl-β-D-xylopyranosyl)-(1→2)-*O*-(4,6-*O*-benzylidene-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-α-D-mannopyranoside (9). A mixture of 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl bromide (32 mg, 0.060 mmol), **8** (22mg, 0.025 mmol), 2,6-di-*tert*-butylpyridine (7 μL, 0.031 mmol) and molecular sieves in dichloromethane (7 mL) was cooled to -40 °C and silver triflate (20 mg, 0.078 mmol) dissolved in toluene was added. After 1 h triethylamine was added (1 mL) the mixture was then allowed to attain room temperature before it was directly applied to flash chromatography (light petroleum bp 60-70 °C-ethyl acetate 7:2) to give **9** (24 mg, 0.18 mmol, 72%). HPLC was used to purify an analytical sample for optical rotation and elementary analysis. $[\alpha]_D$ -12° (*c* 1.0, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -4.5, -4.4 (CH_3-Si), 18.6 (C *t*-butyl), 20.9 (CH_3 acetyl), 26.0 (CH_3 *t*-butyl), 35.3 (O-CH_2-$\underline{C}H_2$-Ar), 59.1, 63.6, 65.0, 67.0, 67.8, 68.0, 68.4, 68.5, 68.6, 68.7, 70.6, 71.5, 75.7, 78.2, 78.9 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 95.3, 98.0, 98.2, 101.4, 102.0 (C-1,1′,1″, 2 x $\underline{C}$H-Ar), 121.0-137.5 (aromatic C), 164.9, 165.0, 165.6 (C=O benzoyl), 169.6 (C=O acetyl).

Anal. Calcd for $C_{70}H_{74}O_{20}NSiF_3$: C, 63.0; H, 5.6; N, 1.1. Found: C, 62.8; H, 5.4; N, 1.2.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(β-D-Xylopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (10). A solution of **9** (95 mg, 0.071 mmol) in aqeuous acetic acid (25 mL, 70%) was stirred at 65 °C for 3 h. The mixture was then concentrated and co-concentrated once with toluene. Flash chromatography (chloroform-methanol 9:1) of the residue gave (56 mg, 0.054 mmol, 75%) of product. NMR data ($CDCl_3$): 13C, δ 20.8 (CH_3 acetyl), 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 62.0-78.6 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 97.0, 99.8, 100.7 (C-1,1′,1″), 121.0-136.8 (aromatic C), 165.7, 165.9 (C=O benzoyl), 170.7 (C=O acetyl). Further deprotection was accomplished by dissolving the compound in methanol (25 mL) and treating it with methanolic sodium methoxide (catalytic amount, 1M) overnight at room temperature. After neutralisation with Dowex 50 (H^+), filtration and concentration, the residue was dissolved in water and washed with diethyl ether. The water phase was freeze-dried, purified on a column of Bio-Gel P-2 and freeze-dried again to give **10** (30 mg, 0.044 mmol, 81%), $[\alpha]_D$ +44° (*c* 0.9, chloroform). NMR data (D_2O): ^{13}C, δ 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 61.2, 66.0, 66.5, 67.5, 68.8, 70.0, 70.2, 70.5,

73.3, 73.5, 73.9, 76.2, 78.1, 79.2 (C-2-6,2′-6′,2′′-5′′, O-$\underline{C}H_2$-CH_2-Ar), 100.1, 101.1, 103.2 (C-1,1′,1′′), 123.1, 130.6, 133.9, 138.8 (aromatic-C), ^1H (70 °C), δ 4.43 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 7.7 Hz), 4.78 (H-1), 5.19 (H-1).

Anal. Calcd for $C_{27}H_{38}NO_{16}F_3 \cdot 3\ H_2O$: C, 43.6; H, 5.9; N, 1 .9. Found: C, 43.7; H, 5.6; N, 2.1

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(4,6-*O*-Benzylidene-3-*O*-*t*-butyldimethylsilyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl)-(1→3)-[*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1→2)]-4,6-*O*-benzylidene-α-D-mannopyranoside (11). Sodium methoxide (catalytic amount of 1M solution in methanol) was added to a solution of compound **7** (162 mg, 0.16 mmol) in methanol (25 mL). The mixture was stirred for 2 h, then neutralized with Dowex 50 (H^+) resin, concentrated and dried in a vacuum. The residue and 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl bromide (247 mg, 0.471 mmol) was coupled and worked up as described for **9** except for flash chromatography (toluene-ethyl acetate 13:1) to give **11** (177 mg, 0.12 mmol, 78 %), $[\alpha]_D$ -14° (*c* 1.4, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -4.8, -4.4 (CH_3-Si), 18.2 (C *t*-butyl), 25.7 (CH_3 *t*-butyl), 35.5 (O-CH_2-$\underline{C}H_2$-Ar), 55.3 (CH_3-O), 59.6, 64.0, 64.7, 67.2, 67.4, 68.1, 68.4, 68.6, 68.8, 70.1, 72.2, 73.2, 76.1, 78.2, 78.3, 79.3 (C-ring, O-$\underline{C}H_2$-Ar, O-$\underline{C}H_2$-CH_2-Ar), 96.5, 97.1, 100.8, 101.6, 102.0 (C-1,1′,1′′, 2 x $\underline{C}$H-Ar), 113.7, 121.0-137.5, 159.1 (Aromatic C), 164.6, 165.3, 165.4 (C=O benzoyl).

Anal. Calcd for $C_{76}H_{80}NO_{20}SiF_3$: C, 64.6; H, 5.7; N, 1.0. Found: C, 64.8; H, 5.6; N, 0.8.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(α-D-Mannopyranosyl)-(1→3)-[*O*-(β-D-xylopyranosyl)-(1→2)]-α-D-mannopyranoside (12). 11 (95 mg, 0.067 mmol) was dissolved in acetonitrile (0.5 mL) and then added to warm (65 °C) aqueous acetic acid (20 mL, 70%). The mixture was left overnight, whereafter concentration and flash chromatography (chloroform-methanol 35:1) gave material (40 mg, 0.036 mmol, 53%)having NMR data ($CDCl_3$): ^{13}C, δ 35.6 (O-CH_2-$\underline{C}H_2$-Ar), 56.3 (CH_3-O), 62.1-77.5 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 97.5, 99.4, 100.8 (C-1,1′,1′′), 112.0 121.3, 128.4-137.2 (aromatic C), 165.6 (C=O). The above residue (40 mg, 0.036 mmol) was dissolved in dry methanol (5 mL) and methanolic sodium methoxide (0.5 ml, 0.5 M) was added. After 3 h the mixture was neutralized with Dowex (H^+) ion exchange resins, filtered and concentrated. The residue was dissolved in water, washed with diethyl ether and freeze-dried to give the *p*-methoxybenzylated intermediate (14 mg, 0.017 mmol, 48%). NMR data (D_2O): ^{13}C, δ 35.5 (O-CH_2-$\underline{C}H_2$-Ar), 57.0 (CH_3-O), 60.7, 61.6, 65.8, 66.5, 67.6, 69.0, 70.0, 71.0, 73.3, 73.5, 73.8, 76.3, 77.0,

78.2, 78.7 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 98.6, 100.8, 104.0 (C-1,1′,1′′), 113.4, 123.0 130.3-134.2, 138.8 (aromatic C). The above compound (14 mg, 0.017mmol) in water was hydrogenolyzed over Pd/C (10%, 25 mg) in a Parr aparatus for 6 h, whereafter the mixture was filtered trough Celite and freeze-dried. After gel filtration on a Bio-gel P2-column eluted with pyridinium acetate buffer (pH 5.4), **12** was collected (10 mg, 0.15 mmol, 86%), $[\alpha]_D$ +34° (*c* 1.3, water). NMR data (D_2O): ^{13}C, δ 35.5 (O-CH_2-$\underline{C}H_2$-Ar), 60.6, 61.7, 65.8, 66.7, 67.2, 69.1, 70.0, 70.8, 71.1, 73.3, 73.5, 73.9, 76.3, 76.6, 78.9 (C-2-6,2′-6′,2′′-5′′, O-$\underline{C}H_2$-CH_2-Ar), 98.6, 103.2, 104.0 (C-1,1′,1′′), 123.0, 130.7, 133.9, 138.9 (Aromatic C), ^1H (70 °C), δ 4.20 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 7.3 Hz), 4.80 (H-1), 5.08 (H-1).

Anal. Calcd for $C_{27}H_{38}NO_{16}F_3 \bullet H_2O$: C, 45.8; H, 5.7; N, 2.0. Found: C, 45.9; H, 5.4; N, 2.1.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(4,6-*O*-Benzylidene-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside (13). Sodium methoxide in methanol (0.2 mL, 1M) was added to a solution of **7** (137 mg, 0.14 mmol) in methanol (25 mL). After 2 h at room temperature, when TLC showed only deacylated product, the mixture was neutralized with Dowex 50 (H^+) resin, filtered and concentrated. The residue was then dissolved in dichloromethane (25 mL) saturated with water and containing DDQ (45 mg, 0.20 mmol). The reaction mixture was left overnight, then washed with aqueous sodium hydrogencarbonate (three times) and water (twice), dried and concentrated. After flash chromatography (toluene-ethyl acetate 6:1) **13** (89 mg, 0.11 mmol, 79%) was collected. HPLC was used to purify an analytical sample for optical rotation and elementary analysis. $[\alpha]_D$ +40° (*c* 0.8, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -5.0, -4.3 (CH_3-Si), 18.1 (C *t*-butyl), 25.7, 35.4 (O-CH_2-$\underline{C}H_2$-Ar), 63.4, 63.8, 68.2, 68.7, 69.6, 71.2, 71.6, 73.1, 78.5, 78.9,(C-ring, O-$\underline{C}H_2$-CH_2-Ar) 99.7 ($J_{C,H}$ 170 Hz) , 100.8 ($J_{C,H}$ 174 Hz), 101.5 ($J_{C,H}$ 165 Hz), 101.9 ($J_{C,H}$ 165 Hz) (C-1,1′, 2 x $\underline{C}$H-Ar), 121.0, 125.8-137.2 (aromatic C).

Anal. Calcd for $C_{42}H_{52}O_{12}NSiF_3$: C, 59.5; H, 6.2; N, 1.6. Found: C, 58.8; H, 6.1; N, 1.5.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(2,3,4-Tri-O-benzoyl-β-D-xylopyranosyl)-(1→2)-*O*-(4,6-*O*-benzylidene-3-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl)-(1→3)-[*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1→2)]-4,6-*O*-benzylidene-α-D-mannopyranoside (14). **13** (50 mg, 0.059 mmol) and 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl bromide (126 mg, 0.24 mmol) were coupled as described for **9** (silver triflate 80 mg, 0.31 mmol, 2.6-di-*tert*butyl-pyridine

40 μL, 0.18 mmol). Flash chromatography (toluene-ethyl acetate 13:1-10:1) yielded **14** (73 mg, 0.042 mmol, 71%). HPLC was used to purify an analytical sample for optical rotation and elementary analysis. $[\alpha]_D$ -35° (*c* 0.9, chloroform). NMR data ($CDCl_3$): ^{13}C, δ -4.5,-4.4 (CH_3-Si), 18.4 (C *t*-butyl), 25.8 (CH_3 *t*-butyl), 35.5 (O-CH_2-$\underline{C}H_2$-Ar), 59.4, 59.6, 64.1, 64.7, 67.3, 67.5, 68.0, 68.1, 68.3, 68.4, 68.7, 68.9, 73.5, 75.9, 76.2, 78.1, 78.7 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 95.7, 96.7, 97.3, 99.4, 101.2, 101.9 (C-1,1′,1″,1‴, 2 x $\underline{C}$H-Ar),121.0, 125.7-137.3 (aromatic C), 164.7-165.6 (C=O benzoyl)

Anal. Calcd for $C_{94}H_{92}O_{25}NSiF_3$: C, 65.0; H, 5.3; N, 0.8. Found: C, 65.4; H, 5.4; N, 0.7.

2-(*p*-Trifluoroacetamidophenyl)ethyl *O*-(β-D-Xylopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[β-D-xylopyranosyl-(1→2)]-*O*-α-D-mannopyranoside (15). **14** (95 mg, 0.055 mmol) was deprotected as described for **10** except that a small amount of acetonitrile was added to the reaction mixture to improve solubility. After deprotection in 70% acetic acid and flash chromatography (chloroform-methanol 35:1) a compound (60 mg, 0.041 mmol, 76%) was collected. NMR data ($CDCl_3$): ^{13}C, δ 35.6 (O-CH_2-$\underline{C}H_2$-Ar), 62.1-78.8 (C-ring, O-$\underline{C}H_2$-CH_2-Ar), 97.3, 98.6, 100.5, 101.1 (C-1,1′,1″,1‴), 121.1-137.0 (aromatic C), 165.4, 165.6 (C=O bensoyl). After Zemplén deacylation and purification on a Bio-Gel P-2 column **15** (20 mg, 0.024 mmol, 58%) was obtained. NMR data (D_2O): ^{13}C, δ 35.5 (O-CH_2-$\underline{C}H_2$-Ar), 60.7, 61.1, 65.9, 66.0, 66.8, 67.4, 69.1, 70.0, 70.2, 73.3, 73.5, 73.8, 76.3, 76.9, 78.2, 78.9 (C-2-6, 2′-5′,2″-6″,2‴-5‴, O-$\underline{C}H_2$-CH_2-Ar), 98.6 ($J_{C\text{-}1,H\text{-}1}$ 169 Hz), 101.2 ($J_{C\text{-}1,H\text{-}1}$ 172 Hz) (C-1,1″), 103.3 ($J_{C\text{-}1,H\text{-}1}$ 163 Hz), 103.9 ($J_{C\text{-}1,H\text{-}1}$ 161 Hz) (C-1′,1‴), 122.9, 130.7, 133.9, 138.9 (aromatic-C), ^1H (70 °C), δ 4.20 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 7.7 Hz), 4.40 (H-1, d, $J_{H\text{-}1,H\text{-}2}$ 7.3 Hz), 4.80 (H-1), 5.18 (H-1).

Anal. Calcd for $C_{27}H_{38}NO_{16}F_3 \cdot 3\ H_2O$: C, 43.6; H, 5.9; N, 1.9. Found: C, 43.7; H, 5.6; N, 2.1.

Methylation analysis of **15** showed the presence of 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylmannitol and 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methyl-mannitol.

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REFERENCES

1. R. Cherniak in *Current Topics in Medical Mycology*, Vol. 1, M. R. McGuiness, Ed.; Springer, New York, 1985, p 1.
2. M. A. Skelton, H. vanHalbeck, and R. Cherniak, *Carbohydr. Res.*, **221**, 259 (1991).
3. R. Cherniak, L. C. Morris, and S. H. Turner, *Carbohydr. Res.*, **223**, 263 (1992).
4. R. Cherniak, L. C. Morris, and S. A. Meyer, *Carbohydr. Res.*, **225**, 331 (1992).
5. P.J. Garegg, I. Kvarnström, A. Niklasson, G. Niklasson and S. C. T. Svensson, submitted to *J. Carbohydr. Chem.*.
6. F.-I. Auzanneau and D. R. Bundle, *Carbohydr. Res.*, **212**, 13 (1991).
7. P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, **149**, c9 (1986).
8. D. H. Buss and I. J. Goldstein, *J. Chem. Soc. C*, 1457 (1968).
9. R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4069 (1975).
10. J. O. Kihlberg, D. A. Leigh and D. R. Bundle *J. Org. Chem.*, **55**, 2860 (1990).
11. Y. Oikawa, T. Tanaka, K. Horito, T. Yoshioka and O. Yonemitsu, *Tetrahedron Lett.*, **25**, 5393 (1984).
12. R. Johansson and B. Samuelsson, *J. Chem. Soc. Perkin Trans.* I, 2371 (1984).
13. S. S. Jones and C. B. Reese, *J. Chem. Soc. Perkin Trans.* I, 2762 (1979).
14. H. G. Fletcher, Jr. and C. H. Hudson, *J. Am. Chem. Soc.*, **69**, 921 (1947).
15. S. Hannessian and J. Banoub, *Carbohydr. Res.*, **53**, c13 (1977).
16. P. J. Garegg and T. Norberg, *Acta Chem. Scand. Ser. B*, **33**, 116.(1979).