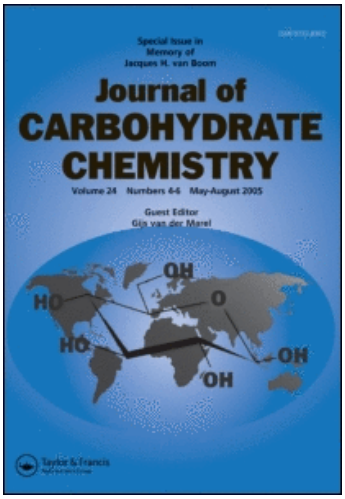

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Synthesis of *Cryptococcus neoformans* Capsular Polysaccharide Structures. Part V: Construction of Glucuronic Acid-Containing Thioglycoside Donor Blocks

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ABSTRACT

Glucuronic acid-containing di- and trisaccharide thioglycoside building blocks, ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside, ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(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)-[(benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(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 synthesized. The blocks contain an orthogonal allyl group in the 3-position of the mannose residue to allow formation of the (1 → 3)-linked mannan backbone of the CPS and benzyl ethers as persistent protecting groups to facilitate access to acetylated target structures. The glucuronic acid moiety was introduced using an acetylated trichloroacetimidate donor and the xylose residue employing the benzoylated bromo sugar to ensure β-selectivity in the couplings. Exchange to benzyl protecting groups was then performed at the di- or trisaccharide level. Assembly of suitable blocks employing DMTST as promoter in diethyl ether then afforded, in high yield and with stereoselectivity, a protected pentasaccharide corresponding to a *C. neoformans* serotype D CPS structure.

Key Words: Oligosaccharide synthesis; Convergent synthesis; Glycoconjugate vaccines.

INTRODUCTION

Cryptococcus neoformans is an opportunistic species causing severe diseases and death, especially in immunodeficient patients.[1,2] The fungi is surrounded by a thick layer of capsular polysaccharides, which is important for its virulence.[3–5] The major CPS is built up by an α-(1 → 3)-mannan backbone substituted with β-xylose residues in the 2- and 4-positions and β-glucuronic acid residues in the 2-positions (Fig. 1).[6,7] *C. neoformans* is divided into four serotypes, A–D, and the different serotypes are defined by the amount and position of the xylose residues. The CPS also contains acetyl groups at the 6-positions of the mannan backbone, but, although the acetylation

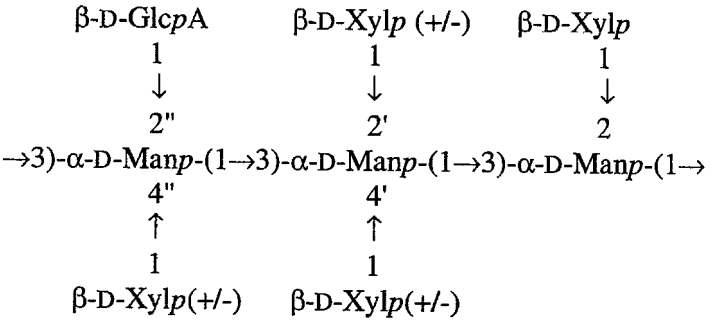


Figure 1. Schematic structure of deacetylated *Cryptococcus neoformans* CPS.

pattern is believed to be immunologically important, little is known about the distribution of the acetyl groups and its influence on the immune response.[7]

To investigate in more detail the immunological determinants of the *C. neoformans* CPS and to construct glycoconjugate vaccine candidates, the development of an efficient route to well-defined synthetic oligosaccharide structures corresponding to fragments of the native CPS would be of great value.

Due to the repetitive and similar structure of the various *C. neoformans* CPS, a block synthesis approach looks most attractive. With only six building blocks, four disaccharides (**I**, **II**, **IV**, and **V**) and two trisaccharides (**III** and **VI**) (Fig. 2), it should be possible to construct any structure wanted. In an earlier publication, we described the syntheses of three xylose-containing thioglycoside building blocks (**I**–**III**) and their efficient assembly to form penta- and hexasaccharide part structures from serotype A, B, and C CPSs.[8] Herein, we report the construction of three glucuronic acid-containing blocks (**IV**–**VI**) and an example of the use of block **IV** as a donor and subsequent acceptor to afford a fully protected pentasaccharide as a representative intermediate to deacetylated targets of serotype D structures.

RESULTS AND DISCUSSION

A strategy similar to that of the earlier synthesized blocks was applied, that is, the use of thioglycoside donors with benzyl ethers as persistent protecting groups and allyl ethers as temporary protecting groups. However, the formation of the uronic acid-containing building blocks turned out to be quite complicated, in large part due to the (possible) presence of acetate groups in the target compounds. Not only does this more or less disqualify ester-protecting groups, but it also means that the carboxyl moiety must be protected as an orthogonal ester. Furthermore, it is known that benzylation of uronic acid derivatives is troublesome.[9] Taking all this into consideration, we decided to start with a perbenzylated uronic acid derivative as donor, in spite of the fact that such precursor may not be that easily available and of the obvious problems associated with the necessity for β-selectivity in the coupling reactions. The mannose derivative **1**, earlier used in the formation of the xylose-containing building blocks,[8] was chosen as acceptor. However, all attempts to construct the desired β-linked disaccharide in an acceptable yield failed. The thioglycoside donor at best (promotor NIS/TfOH) gave a 3 : 1 α/β-ratio in 80% yield, whereas the trichloroacetimidate donor gave exclusively the α-linked disaccharide.[10] Because of this, the use of a glucose donor and introduction of the carboxyl group at the disaccharide level was investigated, but severe problems were encountered in the oxidation step, partly due to concomitant oxidation of the thioglycoside.[11]

Therefore, a third approach was tried using glucuronic acid donors with participating protecting groups. The trichloroacetimidate donor **2**,[12] easily available from glucuronic acid 3,6-lactone in four efficient steps, was coupled to acceptor **1**[8] to afford the β-linked disaccharide **3** in 58% yield (Sch. 1). Our earlier attempts to benzylate (NaH, BnBr, DMF) glucuronic acid derivatives have resulted in no product at all, even when starting from the carboxylic acid salt.[9] However, a change of solvent to DMSO, as described by Koto et al.,[13] has proven to give reasonable yields of products. Removal of the acetates in compound **3** with sodium methoxide gave the methyl ester triol, which was benzylated

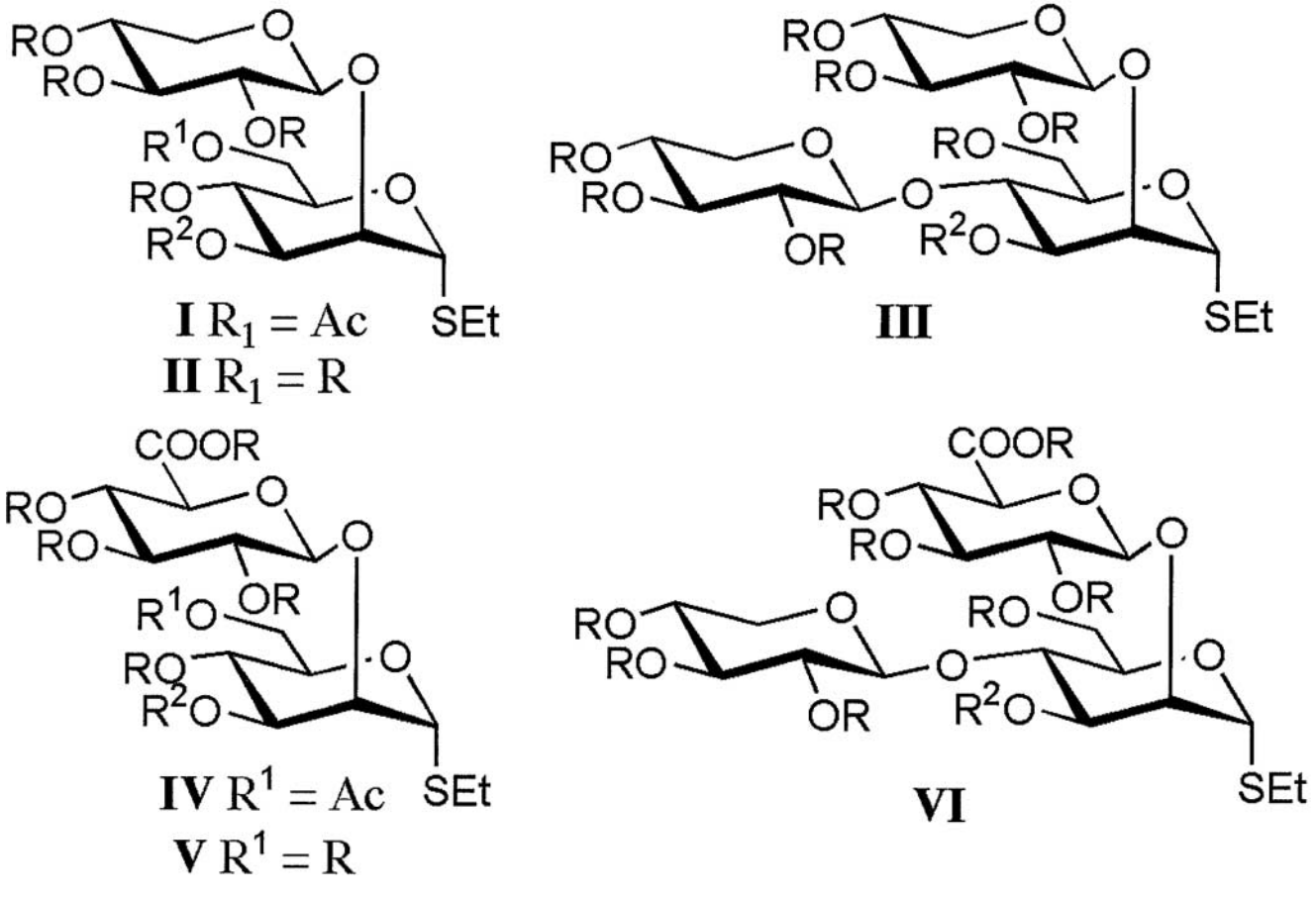
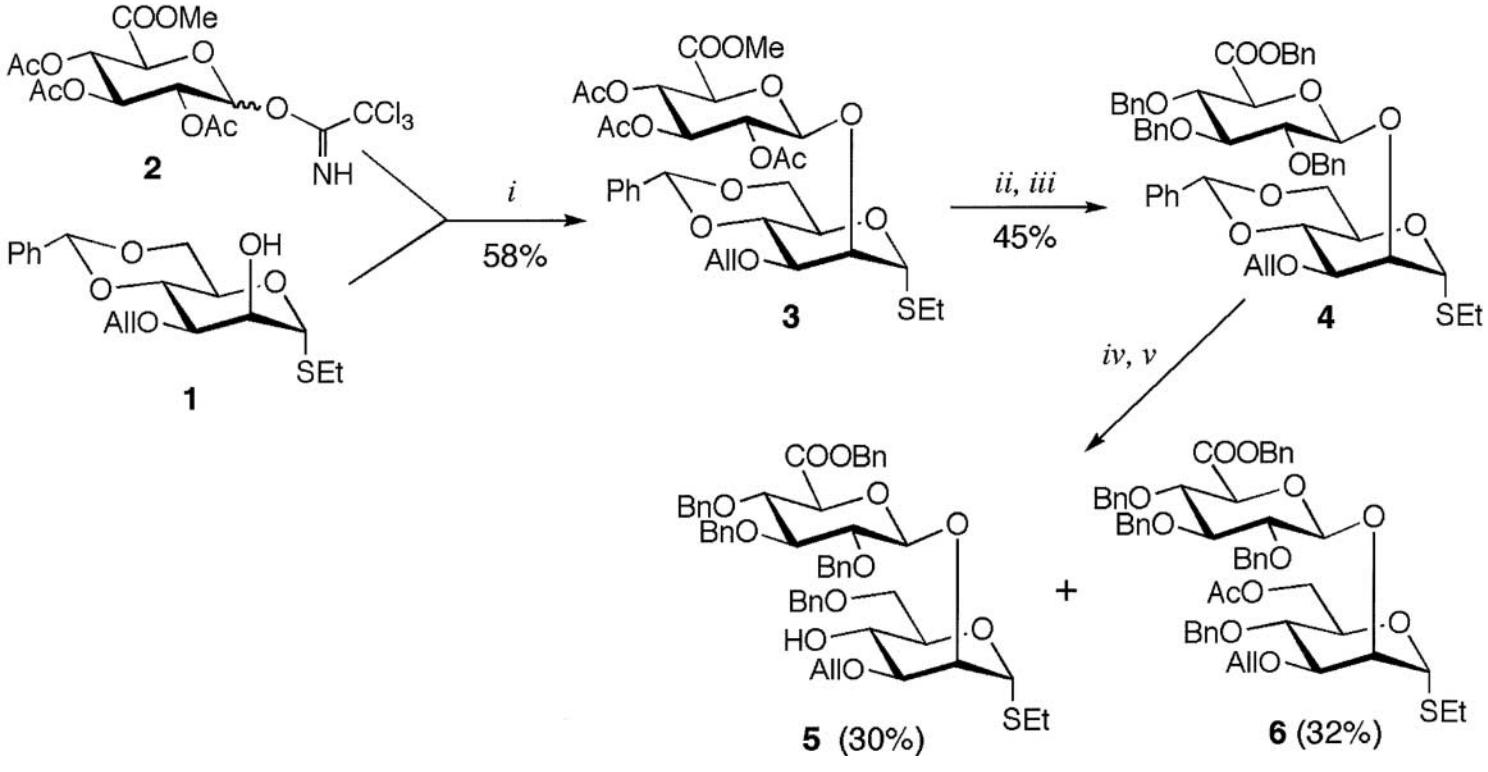


Figure 2. Desired thioglycoside building blocks. R = Persistent protecting group, removable in the presence of acetyl groups; R^2 = Temporary protecting group, removable in the presence of R and Ac groups.



Scheme 1. *i*: TMSOTf, CH_2Cl_2, −30°C; *ii*: a. NaOMe, MeOH, b. H_2O; *iii*: BnBr, NaH, DMSO; *iv*: BH_3-Me_3N, $AlCl_3$, CH_2Cl_2/Et_2O, 0°C; *v*: AcCl, collidine, CH_2Cl_2, −70°C.

(NaH, BnBr, DMSO). During this reaction only partial transesterification was observed giving a mixture of the benzyl ester **4** and the corresponding methyl ester as product. Hence, the methyl ester was saponified prior to benzylation, which afforded the pure benzyl ester **4** (45% overall yield from **3**). Reductive opening of the benzylidene acetal in compound **4**, using BH_3-$Me_3N/AlCl_3$,[14] gave an inseparable 1 : 1 mixture of the 4-OH and 6-OH derivatives. Regioselective acetylation[19] of the obtained mixture afforded an easily separable mixture of compound **5** (30%) and the first building block **6** (compare **IV** in Fig. 2) (32%).

From compound **5**, the two other building blocks **V** and **VI** are obtainable through benzylation or introduction of a xylose residue at the 4-position, respectively. However, both these blocks were more efficiently synthesized starting from derivative **3** (Sch. 2). Regioselective reductive opening ($NaCNBH_3/HCl$) of the benzylidene group in **3** gave the 6-*O*-benzyl derivative **7** (71%). Methoxide treatment of **7** afforded the methyl ester tetraol. This time no saponification step was needed because it was found that the methyl ester was completely hydrolyzed during purification by silica gel chromatography. Subsequent benzylation gave the second building block **8** (compare **V** in Fig. 2) in 50% overall yield.

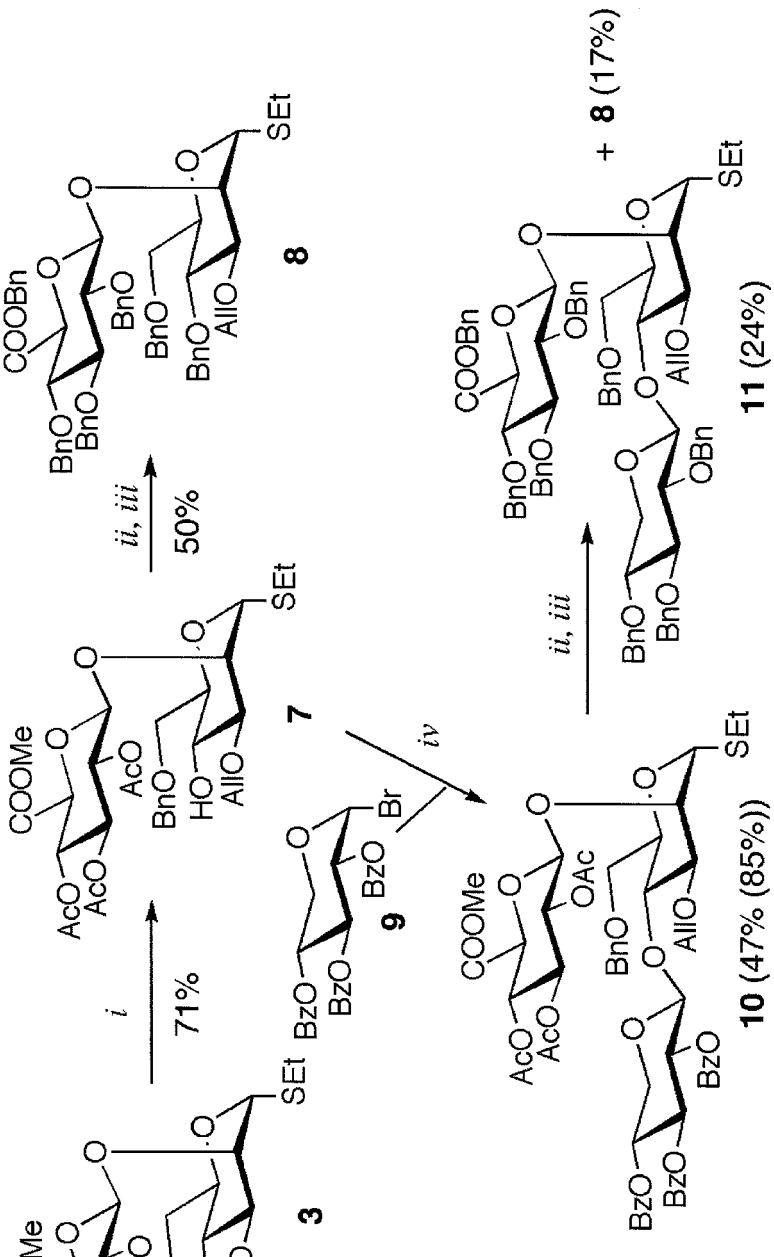
The third building block (**VI**) was also obtained from **7** by a silver triflate-promoted coupling with benzobromoxylose[15] (**9**) to obtain trisaccharide **10** (47%). In spite of a two-fold (or larger) excess of donor used, 35% of the acceptor was recovered after the coupling reaction. In the following deacylation step, the methyl ester was once more lost during silica gel chromatography. Subsequent benzylation afforded the trisaccharide building block **11** (24% from **10**) together with building block **8** (17%), due to an unexpected loss of the xylose moiety under the benzylation conditions.

In spite of the rather low yields in the benzylation steps, this approach is so far the shortest and most convenient one as long as there are no methods available to introduce the perbenzylated glucuronic acid residue with good β-selectivity. Intermediate **3** is easily obtained on a large scale, and the three different building blocks may be prepared in only three or four synthetic steps from this precursor.

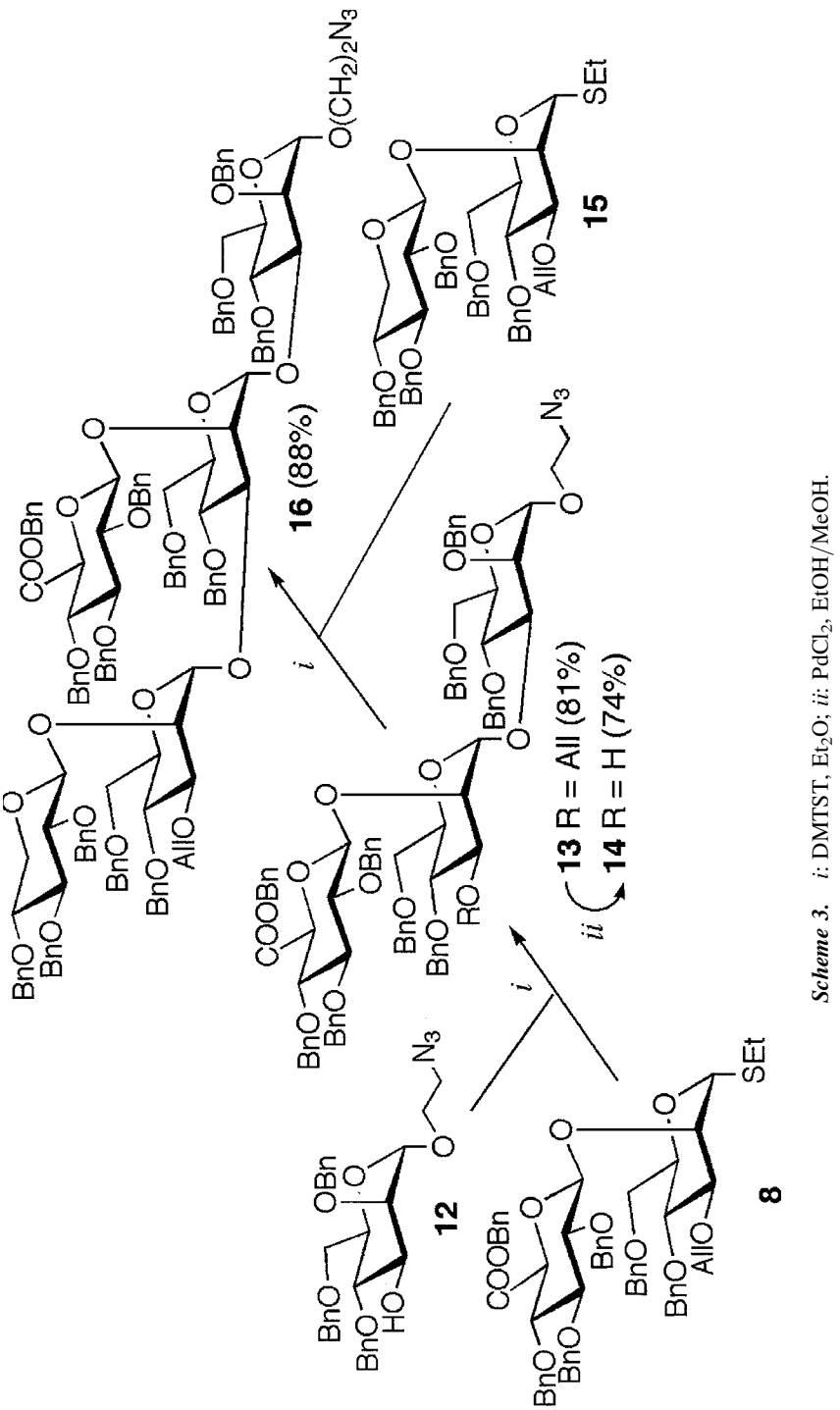
Having all the three desired glucuronic acid-containing building blocks in hand, their donor and (following deallylation) acceptor qualities could next be tested. Our assumption was that the 4,6-*O*-benzylidene acetal in the mannose confers low donor reactivity,[16] which could explain some earlier failures.[17] To evaluate this hypothesis the couplings of donors **4** and **8** to acceptor **12**[8] were compared using dimethyl(methylthio)sulfonium triflate (DMTST) as promoter. As expected, donor **8** reacted smoothly to give the trisaccharide **13** in 81% yield (Sch. 3), whereas donor **4** did not react at all. However, using the more efficient promoter system NIS/AgOTf, **4** could be activated to give a trisaccharide in good yield (58% including deallylation).[10]

Deallylation of **13**, using conditions ($PdCl_2$) worked out for the xylose-containing oligosaccharides,[8] gave compound **14** (74%), which was shown to be an excellent acceptor in a coupling with donor **15**[8] to give the protected repeating unit of *C. neoformans* type D CPS (**16**) in 88% yield. This structure has earlier been synthesized by others,[18] but their pathway does not include the possibility of elongation or the presence of acetyl groups or conjugation of the unprotected target structures. However, the agreement between ^{13}C-data is excellent, proving the structure of compound **16**.

In conclusion, we now have access to the necessary building blocks for the construction of all possible structures from *C. neoformans* serotypes A–D. The efficacy



Scheme 2. *i*: $NaCNBH_3$, HCl/Et_2O, THF; *ii*: NaOMe, MeOH; *iii*: BnBr, NaH, DMSO; *iv*: AgOTf, DTBP, CH_2Cl_2, −30°C.



Scheme 3. *i*: DMTST, Et_2O; *ii*: $PdCl_2$, EtOH/MeOH.

as donors and their efficient transformation into and subsequent use as acceptors was shown in the assembly of the protected repeating unit of deacetylated type D CPS. The latter was obtained through two subsequent stereoselective and high-yielding couplings intercepted by a deallylation step to yield the target pentasaccharide in an overall 53% yield from the available building blocks. The quest for all the repeating units (with different end groups) of the various serotypes as well as larger structures can now be launched to give an array of well-defined oligosaccharides to be used in biological experiments to try to understand the immunology of the *Cryptococcus neoformans* capsule.

EXPERIMENTAL

General methods. TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and/or 8% 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, $\delta = 0.00$) 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.

Ethyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (3). A solution of **2**[12] (623 mg, 1.30 mmol) and **1**[8] (353 mg, 1.00 mmol) in dry CH_2Cl_2 containing 4 Å molecular sieves (0.6 g) was stirred at rt for 30 min and then cooled to −20°C. Trimethylsilyl trifluoromethanesulfonate (0.45 mL of a 1 M solution in CH_2Cl_2) was added. After 2 h, the solution was neutralized by addition of *N,N*-diisopropyl-*N*-ethylamine, filtered through Celite, and concentrated. Purification by silica gel chromatography (toluene-EtOAc 5 : 1) gave **3** (387 mg, 58%); $[\alpha]_D$ +26 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.2 (CH_3CH_2S), 20.8, 20.9, 21.0 ($CH_3CO \times 3$), 26.0 (CH_3CH_2S), 53.1 (CH_3O), 64.9, 68.7, 69.6, 70.8, 71.4, 71.9, 72.9, 74.4, 78.3, 78.6 (C-2-6, C-2′-5′, CH_2CHCH_2O), 83.2 (C-1), 99.7, 102.0 (C-1′, PhCH), 117.6 (CH_2CHCH_2O), 126.4–129.3 (aromatic C), 134.9 (CH_2CHCH_2O), 137.7 (*ipso*-Bn), 167.2, 169.4, 169.7, 170.5 ($CH_3CO \times 3$, $COOCH_3$); ^1H, δ 4.62 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1′); MALDI-TOF MS: m/z calcd for $C_{31}H_{40}NaO_{14}S$ ($[M + Na]^+$): 691.2. Found: 691.2.

Ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (4). Compound **3** (200 mg, 0.30 mmol) was dissolved in MeOH and the pH was adjusted to 12 by treatment with 1 M methanolic NaOMe. The mixture was stirred at rt for 30 min when 1 mL H_2O was added. After an additional 30 min the reaction mixture was concentrated, co-evaporated twice with toluene, and purified on a short silica gel column ($CHCl_3$-MeOH 3 : 1). This residue was then dissolved in dry DMSO (6 mL) containing benzyl bromide (356 μL, 3.0 mmol) and stirred at 15°C for 10 min before NaH (60% dispersion in oil, 120 mg, 3.0 mmol) was added. The reaction was stirred for 40 min at rt and then quenched by the addition of toluene (165 mL) and water (55 mL). The organic layer was separated, washed with HOAc (5% aqueous) and brine, dried, and concentrated. Silica gel chromatography (toluene-EtOAc 9 : 1) gave **4** (120 mg, 0.14 mmol, 45%); $[\alpha]_D$ +22 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 14.9 (CH_3CH_2S), 25.6 (CH_3CH_2S), 64.4, 67.2, 68.6, 70.2, 74.0, 74.7,

75.0, 75.2, 75.7, 78.5, 78.8, 81.2, 83.0, 83.8 (C-1, C-2-6, C-2′-5′, CH_2CHCH_2O, $PhCH_2 \times 4$), 101.6, 102.5 (C-1′, PhCH), 117.1 (CH_2CHCH_2O), 125.2–138.3 (aromatic C, CH_2CHCH_2O), 167.6 (*C*OOBn); MALDI-TOF MS: m/z calcd for $C_{52}H_{56}NaO_{11}S$ ($[M + Na]^+$): 911.3. Found: 911.0.

Anal. Calcd for $C_{52}H_{56}O_{11}S$: C, 70.25; H, 6.35. Found: C, 70.37; H, 6.42.

Ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (5) and ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-6-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-1-thio-α-D-mannopyranoside (6). A solution of $AlCl_3$ (33 mg, 0.25 mmol) in diethyl ether (3 mL) was added dropwise during 30 min to a stirred mixture of **4** (56 mg, 0.06 mmol), BH_3-trimethylamine complex (177 mg, 2.5 mmol) and 4 Å molecular sieves in CH_2Cl_2 : diethyl ether (5 : 1, 7 mL) at 0°C. After 1 h, the mixture was filtered through Celite and 1 M 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 $NaHCO_3$ (aqueous sat.) and water, dried, and concentrated. Silica gel chromatography (toluene-EtOAc 3 : 1) gave a mixture of **5** and ethyl (benzyl (2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-4-*O*-benzyl-1-thio-α-D-mannopyranoside, which was submitted to a selective acetylation of the primary hydroxyl before further purification. The crude disaccharide mixture and *sym*-collidine (14 μL, 0.11 mmol) were dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to −70°C. Acetyl chloride (8 μL, 0.11 mmol) was added and the reaction was stirred for 10 min at −70°C, then quenched with MeOH and allowed to reach rt. Concentration of the mixture followed by silica gel chromatography (toluene EtOAc 9 : 1) afforded **6** (19 mg, 20 μmol, 32% over two steps) together with **5** (15 mg, 17 μmol, 30%). **6**: $[\alpha]_D$ +22 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.0 (CH_3CH_2S), 20.5 (CH_3CO), 25.8 (CH_3CH_2S), 63.3, 67.4, 69.8, 70.0, 73.9, 74.7, 75.1, 75.1, 75.2, 75.8, 76.4, 78.3, 78.7, 81.1, 82.3, 84.0 (C-1-6, C-2′-5′, CH_2CHCH_2O, $PhCH_2 \times 5$), 102.8 (C-1′), 118.0 (CH_2CHCH_2O), 127.6–129.1 (aromatic C), 134.5 (CH_2CHCH_2O), 137.8–138.4 (*ipso*-Bn), 167.8, 170.6 (CH_3CO, *C*OOBn); MALDI-TOF MS: m/z calcd for $C_{54}H_{60}NaO_{12}S$ ($[M + Na]^+$): 955.4. Found 955.4. **5**: $[\alpha]_D$ +17 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 14.9 (CH_3CH_2S), 25.6 (CH_3CH_2S), 67.3, 67.4, 69.6, 70.2, 71.8, 73.4, 74.7, 75.0, 75.1, 75.5, 75.8, 78.8, 81.0, 82.1, 83.9 (C-1-6, C-2′-5′, CH_2CHCH_2O, $PhCH_2 \times 5$), 102.8 (C-1′), 118.4 (CH_2CHCH_2O), 127.3–129.0 (aromatic C), 134.4 (CH_2CHCH_2O), 138.2–138.5 (*ipso*-Bn), 167.8 (*C*OOBn); MALDI-TOF MS: m/z calcd for $C_{52}H_{58}NaO_{11}S$ ($[M + Na]^+$): 913.4. Found: 913.4.

Anal. Calcd for $C_{52}H_{58}O_{11}S$: C, 70.09; H, 6.56. Found: C, 69.85; H, 6.73.

Ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (8). A mixture of **3** (448 mg, 0.67 mmol), $NaCNBH_3$ (421 mg, 6.7 mmol) and 3 Å molecular sieves in distilled THF (30 mL) was stirred at rt under an argon atmosphere for 30 min. A saturated solution of HCl in diethyl ether was carefully added until the evolution of gas was no longer detected. After 1 h, the reaction mixture was filtered through a layer of Celite, concentrated, and purified on a silica gel column (toluene-EtOAc 4 : 1) to give ethyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1 → 2)-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**7**, 321 mg, 0.48 mmol, 71%). NMR data: ^{13}C, δ 14.8 (CH_3CH_2S), 20.5, 20.5, 20.6 ($CH_3CO \times 3$), 25.4 (CH_3CH_2S), 52.7 (CH_3O), 67.3, 69.2, 69.9, 70.3, 70.4, 71.5, 71.8, 72.5, 73.2, 75.2, 77.6, 81.4 (C-1-6, C-2′-5′, CH_2CHCH_2O, $PhCH_2$), 99.0 (C-1′), 118.0

(CH_2CHCH_2O), 127.4–128.3 (aromatic C), 134.5 (CH_2CHCH_2O), 138.2 (*ipso*-Bn), 166.7, 169.1, 169.3, 170.2 ($CH_3CO \times 3$, $COOCH_3$). MALDI-TOF MS: m/z calcd for $C_{31}H_{42}NaO_{14}S$ ($[M + Na]^+$): 693.2. Found 692.5. A solution of **7** (233 mg, 0.35 mmol) in MeOH (5 mL) was treated with a catalytic amount of 1 M methanolic NaOMe at rt for 30 min. Dowex 50 (H^+) ion exchange resin was added and the mixture was then filtered, concentrated, and purified on a silica gel column ($CHCl_3$-MeOH 9 : 1). On the column, the methyl ester was hydrolyzed to the corresponding acid, which was directly benzylated. A mixture of this residue and benzyl bromide (356 μL, 3.0 mmol) in dry DMSO (6 mL) was stirred at 15°C. NaH (60% dispersion in oil, 120 mg, 3.0 mmol) was added. After 40 min more BnBr (356 μL, 3.0 mmol) and NaH (120 mg, 3.0 mmol) were added and the reaction was stirred for 2 h at rt. The reaction was quenched by the addition of toluene (165 mL) and water (55 mL). The organic layer was separated, washed with HOAc (5% aqueous) and brine, dried, and concentrated. Silica gel chromatography (toluene-EtOAc 9 : 1) gave **8** (170 mg, 0.17 mmol, 50% over two steps); $[\alpha]_D$ +26 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 14.9 (CH_3CH_2S), 25.5 (CH_3CH_2S), 67.3, 69.4, 70.0, 71.7, 72.1, 73.1, 74.4, 74.7, 75.0, 75.2, 75.7, 76.5, 78.2, 78.7, 80.9, 82.0, 84.0 (C-1-6, C-2′-5′, CH_2CHCH_2O, $PhCH_2 \times 6$), 102.8 (C-1′), 117.8 (CH_2CHCH_2O), 127.3–129.1 (aromatic C), 134.7 (CH_2CHCH_2O), 137.8–138.7 (*ipso*-Bn), 167.8 (*C*OOBn); MALDI-TOF MS: m/z calcd for $C_{59}H_{64}NaO_{11}S$ ($[M + Na]^+$): 1003.4. Found: 1002.5.

Anal. Calcd for $C_{59}H_{64}O_{11}S$: C, 72.22; H, 6.57. Found: C, 72.00; H, 6.71.

Ethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-[(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-(1 → 4)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (11). Silver triflate (153 mg, 0.60 mmol) dissolved in dry toluene was added at −40°C to a stirred solution of **7** (160 mg, 0.24 mmol), **9**[15] (313 mg, 0.60 mmol) and 2,6-di-*tert*-butylpyridine (107 μL, 0.48 mmol) in distilled CH_2Cl_2 (15 mL) containing crushed molecular sieves (4 Å). After 2 h, Et_3N (1 mL) was added and the stirring was continued for 15 min. The mixture was diluted with CH_2Cl_2, filtered through Celite, concentrated, and purified by silica gel chromatography (toluene-EtOAc 5 : 1) to give ethyl (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1 → 2)-[(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-(1 → 4)]-3-*O*-allyl-6-*O*-benzyl-1-thio-α-D-mannopyranoside (**10**, 125 mg, 0.11 mmol, 47% (85% based on consumed acceptor)) together with unreacted **7** (72 mg). NMR data: ^{13}C, δ 14.7 (CH_3CH_2S), 20.4, 20.4, 20.6, 25.2 (CH_3CH_2S), 52.7 (CH_3O), 61.5 (C-5′), 69.1, 69.2, 69.2, 70.5, 70.9, 70.9, 71.2, 71.9, 72.5, 72.7, 75.3, 76.2, 76.8, 81.1 (C-1-6, C-2′-4′, C-2″-5″, CH_2CHCH_2O, $PhCH_2$), 99.3, 100.3 (C-1′,1″), 117.6 (CH_2CHCH_2O), 125.2–133.3 (aromatic C), 134.9 (CH_2CHCH_2O), 138.2 (*ipso*-Bn), 165.1, 165.2, 165.5, 166.7, 169.0, 169.2, 170.1 ($PhCO \times 3$, $CH_3CO \times 3$, $COOCH_3$). MALDI-TOF MS: m/z calcd for $C_{57}H_{62}NaO_{21}S$ ($[M + Na]^+$): 1137.3. Found 1136.5. A solution of **10** (125 mg, 0.11 mmol) in MeOH (5 mL) was treated with a catalytic amount of 1M methanolic NaOMe at rt for 30 min. Dowex 50 (H^+) ion exchange resin was added and the mixture was then filtered, concentrated, and purified on a silica gel column ($CHCl_3$-MeOH 9 : 1 → 2 : 1). The methyl ester (MALDI-TOF MS: m/z calcd for $C_{30}H_{44}NaO_{15}S$ ($[M + Na]^+$): 699.2. Found 698.6) was hydrolyzed on the column to the corresponding acid (MALDI-TOF MS: Calcd for $C_{29}H_{42}NaO_{15}S$ ($[M + Na]^+$): 685.21, found 686.38). A mixture of this residue and benzyl bromide (200 μL, 1.7 mmol) in dry DMSO (5 mL) was stirred at 15°C. NaH (60% dispersion in oil, 67 mg, 1.7 mmol) was added. After 30 min more BnBr (200 μL, 1.7 mmol)

and NaH (1 mg, 3.0 mmol) were added and the reaction was stirred for 1 h at rt. The reaction was quenched by the addition of toluene (165 mL) and water (55 mL). The organic layer was separated, washed with HOAc (5% aqueous) and brine, dried, and concentrated. Silica gel chromatography (toluene-EtOAc 9 : 1) gave **11** (35 mg, 24% in two steps) together with **8** (19 mg, 17%). **11**: $[\alpha]_D$ +27 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 15.0 (CH_3CH_2S), 25.5 (CH_3CH_2S), 62.2–84.2 (C-1-6, C-2′-5′, C-2″-5″, CH_2CHCH_2O, $PhCH_2 \times 8$), 102.6, 103.3 (C-1′,1″), 116.9 (CH_2CHCH_2O), 127.4–129.1 (aromatic C), 135.2 (CH_2CHCH_2O), 137.9–138.7 (*ipso*-Bn), 167.9 (*C*OOBn); MALDI-TOF MS: m/z calcd for $C_{78}H_{84}NaO_{15}S$ ($[M + Na]^+$): 1315.5. Found: 1315.5.

Anal. Calcd for $C_{78}H_{84}O_{15}S$: C, 72.42; H, 6.57. Found: C, 72.19; H, 6.70.

2-Azidoethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14). A solution of **8** (26 mg, 26 μmol) and **12** (13 mg, 25 μmol) in dry diethyl ether (5 mL) containing powdered 4 Å molecular sieves was stirred at rt in an argon atmosphere for 30 min. DMTST (26 mg, 0.10 mmol) was added to the mixture and the stirring was continued for 30 min. After neutralization with NEt_3, the mixture was filtered through Celite and concentrated. Purification by silica gel chromatography (toluene-EtOAc 9 : 1) gave 2-azidoethyl (benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)-(3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**13**, 29 mg, 20 μmol, 81%). NMR data: ^{13}C, δ 50.3 ($N_3CH_2CH_2O$), 66.6–83.5 (C-2-6, C-2′-5′, C-2″-5″, CH_2CHCH_2O, $PhCH_2 \times 9$, $N_3CH_2CH_2O$), 97.9, 99.6 (C-1,1′), 103.2 (C-1″), 117.2 (CH_2CHCH_2O), 126.5–129.0 (aromatic C), 135.0 (CH_2CHCH_2O), 138.1–139.1 (*ipso*-Bn), 167.8 (*C*OOBn). MALDI-TOF MS: m/z calcd for $C_{86}H_{91}NaO_{17}$ ($[M + Na]^+$): 1460.6. Found 1459.7. $PdCl_2$ (60%, 6 mg, 20 μmol) was added to a solution of **13** (29 mg, 20 μmol) in EtOH : MeOH (1 : 1, 4 mL). After 30 min more $PdCl_2$ (10 mg, 34 μmol) was added. The mixture was stirred for 1 h, then filtered through Celite, concentrated, and purified on a silica gel column (toluene-EtOAc 10 : 1) to give **14** (21 mg, 15 μmol, 74%); $[\alpha]_D$ +17 (*c* 1.0, $CHCl_3$); NMR data: ^{13}C, δ 50.3 ($N_3CH_2CH_2O$), 66.6–83.0 (C-2-6, C-2′-5′, C-2″-5″, $PhCH_2 \times 9$, $N_3CH_2CH_2O$), 98.0, 100.3 (C-1,1′), 103.4 (C-1″), 126.6–128.8 (aromatic C), 137.8–139.0 (*ipso*-Bn), 168.0 (*C*OOBn); MALDI-TOF MS: m/z calcd for $C_{83}H_{87}NaO_{17}$ ($[M + Na]^+$): 1420.6. Found 1419.6.

Anal. Calcd for $C_{83}H_{87}N_3O_{17}$: C, 71.28; H, 6.27. Found: C, 71.02; H, 6.50.

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)-[(benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranosyluronate)-(1 → 2)]-(4,6-di-*O*-benzyl-α-D-mannopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16). A solution of **15**[8] (19 mg, 23 μmol) and **14** (16 mg, 11 μmol) in dry diethyl ether (4 mL) containing powdered 4 Å molecular sieves was stirred at rt in an argon atmosphere for 30 min. DMTST (12 mg, 46 μmol) was added to the mixture and the stirring was continued for 2 h. After neutralization with NEt_3, the mixture was filtered through Celite and concentrated. Purification by silica gel chromatography (toluene-EtOAc 9 : 1) gave **16** (22 mg, 10 μmol, 88%); NMR data: ^{13}C, δ 50.4 ($N_3CH_2CH_2O$), 63.7–83.4 (C-2-6, C-2′-5′, C-2″-5″, C-2‴-5‴, C-2⁗-5⁗, CH_2CHCH_2O, $PhCH_2 \times 14$, $N_3CH_2CH_2O$), 97.9, 99.9, 100.6 (C-1,1′,1‴), 102.6, 103.5 (C-1″,1⁗), 116.6 (CH_2CHCH_2O), 126.5–129.0 (aromatic C), 135.3 (CH_2CHCH_2O), 138.0–139.0 (*ipso*-Bn), 167.7 (*C*OOBn). MALDI-TOF MS: m/z calcd for $C_{132}H_{139}N_3NaO_{26}$ ($[M + Na]^+$): 2204.9. Found: 2203.6.

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