Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 2605–2609
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Synthesis of tetra- and pentasaccharides corresponding to the capsular polysaccharide of *Streptococcus pneumoniae* type 9A&L, 9N and 9A

Mia Alpe, Stefan Oscarson*

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

Received 19 March 2003; received in revised form 19 May 2003; accepted 21 May 2003

Abstract

Two tetrasaccharides, α-D-GlcA*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p* and α-D-GlcA*p*-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p* (protected form), and a pentasaccharide, α-D-Glc*p*-(1→4)-α-D-GlcA*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p* have been synthesised from 2-aminoethyl glycoside trisaccharide acceptors in a linear approach *via* consecutive α-glycosylations. Ethyl thioglycosides were used as glycosyl donors and DMTST in Et_2O or NIS/TfOH in CH_2Cl_2 were employed as promoters.

Keywords: Thioglycosides; Oligosaccharide synthesis; Glycoconjugate vaccines

1. Introduction

The bacterium *Streptococcus pneumoniae* is a major human pathogen.[1] It is divided into serotypes, each serotype corresponding to a unique structure of the capsular polysaccharide (CPS) surrounding the bacteria.[2] Serotype 9 is one of the most abundant serotypes comprising several variations: 9A, 9L, 9N, and 9V (9A with partial acetylation of the GlcA, ManNAc, and α-Glc residues). These vary slightly in their CPS structure (Fig. 1).[3–6]

Both polysaccharide and glycoconjugate vaccines against *S. pneumoniae* are now commercial. The polysaccharide vaccine consists of 23 serotypes whereas the conjugate vaccine contains seven serotypes, both, however, including serotype 9. Regarding polysaccharide vaccines, there is a consensus that large structures are needed, but with glycoconjugate vaccines there is a debate on the saccharide size needed to give protection.[7,8] To further investigate this we have earlier synthesised trisaccharide parts (residues III–I in Fig. 1) of the pentasaccharide repeating units of serotypes 9A, L and 9N.[9] The trisaccharides were synthesised as spacer glycosides, to allow conjugation to a carrier protein, and the protection pattern was designed to permit synthesis of larger structures. We now report the continued synthesis, from these trisaccharide acceptors, of tetra- and pentasaccharide structures, the latter comprises the complete repeating unit of *S. pneumoniae* type 9A.

2. Results and discussion

Since both tetra- and pentasaccharide structures were in demand, a linear synthetic approach was applied. Furthermore, we decided to use glucuronic acid donors in the first coupling in spite of the their well-known low reactivity both as donors and acceptors,[10,11] since we considered oxidation in high yield at the tetra- or pentasaccharide level an even larger problem. Both elongations, the introduction of an α-glucuronic acid moiety as well as the subsequent introduction of an α-glucose residue, were met with severe difficulties, especially considering the stereochemical outcome.

* Corresponding author. Tel.: +46-8-162480; fax: +46-8-154908.
E-mail address: s.oscarson@organ.su.se (S. Oscarson).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00271-4

→4)–α-D-Glc*p*-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9A**

→4)–α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9L**

→4)–α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9N**

V IV III II I

Fig. 1. Repeating units of *S. pneumoniae* type 9 CPS.

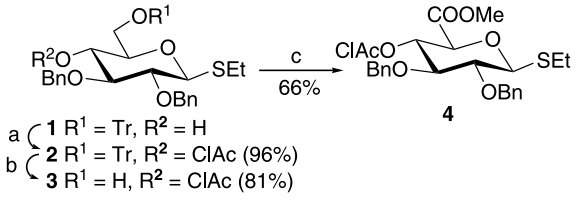
Monochloroacetylation of the known ethyl thioglycoside **1**[12] afforded **2** (Scheme 1). Removal of the trityl group followed by a two-step oxidation, a Pfitzner–Moffatt oxidation[13] to the aldehyde and a subsequent pyridine dichromate (PDC) oxidation in the presence of methanol,[14] afforded the methyl glucuronate donor **4** in 66% yield.

Our earlier experiences with thioglycoside donors in α-glucuronosylations had shown that dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) in diethyl ether gave preferentially the α-glycoside, whereas *N*-iodosuccinimide (NIS)/TfOH in CH_2Cl_2 gave mainly the β-linked glucuronate in spite of the absence of participating groups.[12] Consequently, when we started to couple donor **4** with acceptor **5**[9] to give a 9A,L type tetrasaccharide, DMTST was tried as promoter. However, this gave surprisingly mainly the β-linked tetrasaccharide. Several other conditions were tried and finally it was found that instead NIS/TfOH in CH_2Cl_2 gave the best results considering stereoselectivity and yield, 81% of a 2:1 α/β-mixture (Scheme 2). The stereoisomers were easily separated to give the α-linked tetrasaccharide **7** in 57% yield. The same conditions were then tried for the coupling of donor **4** to the glucosyl trisaccharide acceptor **6**[9] for formation of type 9N structures, which resulted in the exclusive formation of the β-linked tetrasaccharide. A return to the original 'α-coupling' conditions, i.e., DMTST in diethyl ether, with this acceptor gave the expected outcome, i.e., mainly α-glucuronosylation, albeit with a low selectivity, 70% of a 3:2 α/β-mixture. Once more, separation of the isomers was uncomplicated to yield the α-linked tetrasaccharide **8** in 43% yield.

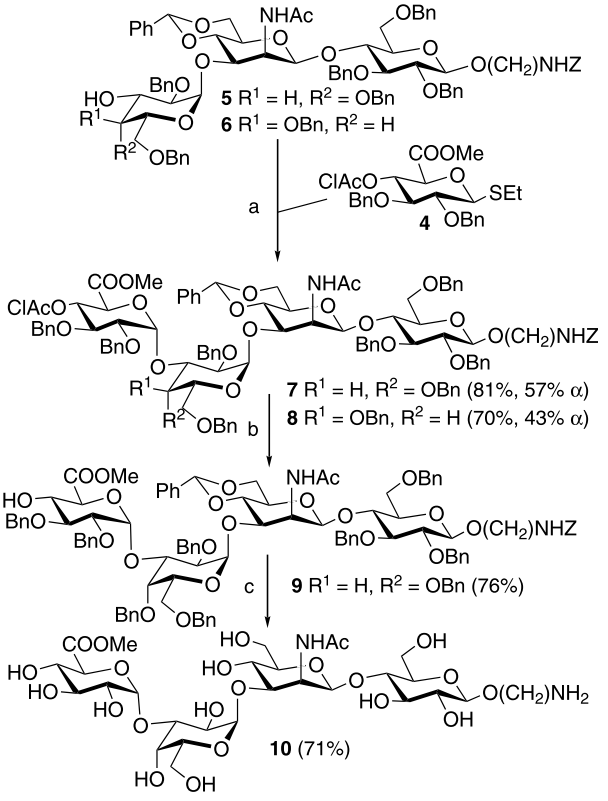
Subsequent removal of the chloroacetyl group in **7** gave the acceptor **9** (76%), hydrogenolysis of which gave the deprotected type 9A,L methyl ester tetrasaccharide **10** with a free spacer amino group ready for conjugation.

Glycosidation of acceptor **9** with glucosyl donor **11**,[15] to afford α-linked pentasaccharides corresponding to the type 9A repeating unit, was not straightforward either (Scheme 3). As found also by others,[11] the stereoselectivity in α-glycosylations of this position is low. Halide-assisted glycosylation conditions[16] could not be used due to the low reactivity of the 4-OH. The best conditions were once again DMTST in diethyl ether, which gave the pentasaccharide in 69% yield (2:1 α/β) and after chromatography the α-linked derivative **12** in 45% yield.

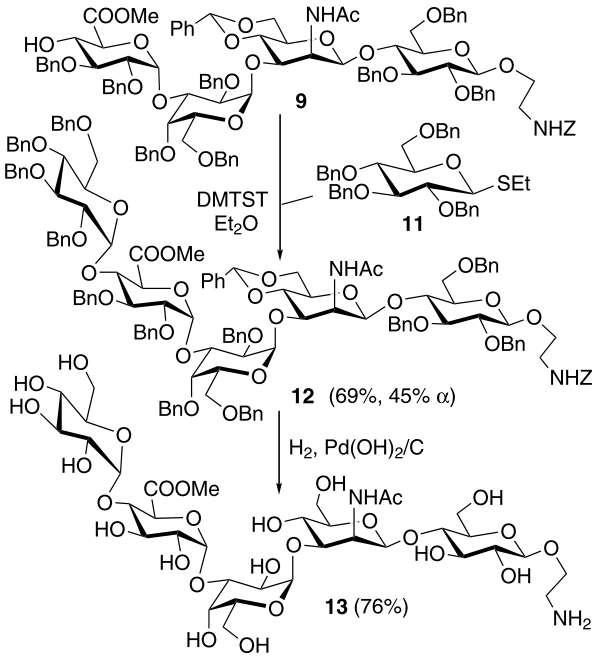
One-step deprotection of **12** through hydrogenolysis then gave the pentasaccharide repeating unit of *S. pneumoniae* type 9A **13** as its methyl ester and equipped with an aminospacer for conjugation. ^{1}H proton signals



Scheme 1. (a) ClAcCl, pyridine–CH_2Cl_2; (b) *p*TsOH, $CHCl_3$–MeOH; (c) 1. TFA, DIC, Me_2SO, pyridine, 2. PDC, MeOH.



Scheme 2. (a) NIS–TfOH, CH_2Cl_2 for **4**+**5**; DMTST, Et_2O for **4**+**6**; (b) hydrazine acetate, MeOH; (c) H_2, $Pd(OH)_2$/C.



Scheme 3.

at 5.39 (d, J 3.6 Hz, H-1^{V}), 5.31 (d, J 3.4 Hz, H-1^{III}), and 5.14 (d, J 3.2 Hz, H-1^{IV}) ppm clearly proves the α-configurations of the newly synthesised linkages. The corresponding protons in the native polysaccharide resonance at 5.42 (Glc), 5.30 (Gal), and 5.09 (GlcA) ppm.[17]

3. Experimental

3.1. General methods

Thin-layer chromatography (TLC) was carried out on E. Merck precoated 60 F_{254} plates using UV-light and/or 8% H_2SO_4 for visualization. Column chromatography was performed on silica gel (0.040–0.063 mm, Amicon) in the flash mode, if not otherwise stated. NMR spectra were recorded in $CDCl_3$ at 25 °C (internal Me_4Si, $\delta = 0.00$ ppm) or in D_2O at 70 °C (internal acetone ^{13}C $\delta = 31.0$ ppm, ^{1}H $\delta = 2.21$ ppm) on a Varian 300 or 400 MHz instrument. MALDI–TOF mass 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 concentration, which was performed under diminished pressure.

3.2. Methyl (ethyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-1-thio-β-D-glucopyranosid)uronate (4)

Chloroacetyl chloride (1.8 mL, 23 mmol) was added to a solution of **1**[12] (7.3 g, 11 mmol) in 15:1 CH_2Cl_2–Py at 0 °C. The reaction mixture was stirred for 2 h at room temperature (rt), then diluted with toluene, concentrated, and purified by silica gel chromatography (15:1 toluene–EtOAc) to give ethyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-6-*O*-triphenylmethyl-1-thio-β-D-glucopyranoside (**2**, 7.9 g, 97%); ^{13}C NMR ($CDCl_3$): δ 15.6 (SCH_2CH_3), 25.0 (SCH_2CH_3), 40.6 (CH_2Cl), 62.8, 72.0, 75.6, 75.9, 77.7, 82.1, 83.9, 85.0, 86.9 (C-1-6, $PhCH_2O$, $C(Ph)_3$), 127.3–143.8 (aromatic C), 165.8 (C=O). Compound **2** (7.9 g, 11 mmol) was dissolved in 2:1 $CHCl_3$–MeOH and the pH was adjusted to 1 (pH-indicator paper) by addition of *p*-toluenesulfonic acid. After 2 h, the mixture was washed successively with water and satd aq $NaHCO_3$, dried and concentrated. Silica gel chromatography (4:1 toluene–EtOAc) gave ethyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-1-thio-β-D-glucopyranoside (**3**, 4.3 g, 81%); ^{13}C NMR ($CDCl_3$): δ 15.1 (SCH_2CH_3), 25.1 (SCH_2CH_3), 40.4 (CH_2Cl), 61.8, 71.9, 75.5, 75.7, 77.9, 81.5, 83.4, 85.2 (C-1-6, $PhCH_2O$), 127.9–138.2 (aromatic C), 166.6 (C=O). Compound **3** (1.3 g, 2.7 mmol) was dissolved in Me_2SO (30 mL) at rt. To the solution was added Py (220 μL, 2.7 mmol), trifluoroacetic acid (102 μL, 1.4 mmol), and diisopropylcarbodiimide (DIC) (1.50 mL, 9.6 mmol), and the reaction was stirred overnight. The mixture was diluted with toluene and washed with satd aq $NaHCO_3$. After drying and concentration of the organic phase the obtained crude aldehyde was dried under diminished pressure and used for the next oxidation without further purification. The crude aldehyde was dissolved in DMF (25 mL) containing MeOH (660 μL) and cooled on ice for 30 min in a vessel covered to exclude light. PDC (6.0 g, 16.0 mmol) was added in one portion. The mixture was allowed to attain rt and react overnight and was then diluted with toluene, washed with water, dried and concentrated. Silica gel chromatography (9:1 toluene–EtOAc) gave **4** (917 mg, 66%) as a white solid; $[\alpha]_D$ −38° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 15.0 (SCH_2CH_3), 25.1 (SCH_2CH_3), 40.4 (CH_2Cl), 52.9 (CH_3O), 72.4, 75.5, 75.7, 75.9, 80.9, 82.8, 85.5 (C-1-5, $PhCH_2O$), 127.9–138.0 (aromatic C), 166.1, 167.4 (C=O). Anal. Calcd for $C_{25}H_{29}ClO_7S$: C, 59.0; H, 5.7. Found: C, 59.1; H, 5.9.

3.3. 2-(*N*-Benzyloxycarbonyl)-aminoethyl (methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8)

A solution of **4** (60 mg, 118 μmol) and **6**[9] (80 mg, 59 μmol) in dry Et_2O (5 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an Ar atmosphere for 1 h. To the mixture was added DMTST (122 mg, 472 μmol) and the stirring was continued overnight. After neutralization with Et_3N, the mixture

was filtered through Celite and concentrated. Silica gel chromatography (1:1 toluene–EtOAc) of the residue yielded **8** as an α/β-mixture (74 mg, 41.1 μmol, 70%). Repeated chromatography (2 columns, not flash-mode; 1:1 toluene–EtOAc) afforded the pure α anomer (46 mg, 25.6 μmol, 43%). $[\alpha]_D$ +18° (*c* 1.0, $CHCl_3$); Selected ^{13}C NMR data ($CDCl_3$): δ 52.6 (C-2^{II}, CH_3O), 66.5–83.5 (C-2^{I}–6^{I}, 3^{II}–6^{II}, 2^{III}–6^{III}, 2^{IV}–5^{IV}, O*C*H_2CH_2N, Ph*C*H_2O), 96.4, 97.4 (C-1^{III}, 1^{IV}), 100.0, 102.7, 104.1 (C-1^{I}, 1^{II}, benzylidene), 126.5–139.1 (aromatic C), 156.7, 166.3, 166.5, 170.6 (C=O).; β anomer: δ 96.9 (C-1^{III}), 99.9, 102.1, 102.4, 104.1 (C-1^{I}, 1^{II}, 1^{IV}, benzylidene). MALDI–TOFMS: Calcd for $C_{102}H_{109}ClN_2NaO_{25}$ ($[M+Na]^+$): 1819.7. Found 1820.2.

3.4. 2-Aminoethyl (methyl α-D-glucopyranosyluronate)-(1→3)-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside (10)

A mixture of **4** (64 mg, 0.13 mmol) and **5**[9] (100 mg, 74 μmol) in dry CH_2Cl_2 containing powdered molecular sieves (4 Å) was stirred under Ar at rt for 1 h. The solution was cooled to −30 °C, NIS (28 mg, 0.13 mmol) and TfOH (3 μL, 37 μmol) were added and the mixture was stirred for 5 h at −30 °C. The mixture was neutralized with Et_3N and filtered through Celite. The filtrate was washed with $Na_2S_2O_3$ (10% aq), $NaHCO_3$ (aq satd) and water, dried and concentrated. Purification by silica gel chromatography (1:1 toluene–EtOAc) gave 2-(*N*-benzyloxycarbonyl)-aminoethyl (methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside as an α/β-mixture (107 mg, 60 μmol, 81%). Repeated chromatography (2 columns, not flash-mode; 1:1 toluene–EtOAc) afforded the pure α anomer (**7**, 76 mg, 57%); $[\alpha]_D$ +18° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 23.3 (*C*H_3CON), 40.6, 41.6 (CH_2Cl, *C*H_2NHCOOBn), 52.5 (C-2^{II}, CH_3O), 66.7, 67.0, 68.4, 68.9, 69.4, 69.8, 72.4, 73.2, 73.4, 73.5, 73.7, 74.3, 74.6, 75.1, 75.2, 75.5, 75.9, 76.1, 78.5, 79.3, 80.1, 82.0, 83.3 (C-2^{I}–6^{I}, 3^{II}–6^{II}, 2^{III}–6^{III}, 2^{IV}–5^{IV}, O*C*H_2CH_2N, Ph*C*H_2O), 96.6, 96.8 (C-1^{III}, 1^{IV}), 99.7, 102.2, 104.0 (C-1, 1′, benzylidene), 126.4–139.3 (aromatic C), 156.7 (NH*C*OOBn), 166.4, 169.1 and 170.9 (C=O); β anomer (selected data): δ 97.1 (C-1^{III}), 99.4, 102.1, 103.7, 103.8 (C-1^{I}, 1^{II}, 1^{IV}, benzylidene). MALDI–TOFMS: Calcd for $C_{102}H_{109}ClN_2NaO_{25}$ ($[M+Na]^+$): 1819.7. Found: 1820.4. Hydrazine acetate (51 mg, 0.6 mmol) was added to a solution of **7** (51 mg, 0.3 mmol) in MeOH (5 mL) and the mixture was stirred overnight. Additional hydrazine acetate (25 mg, 14 μmol) was added and after an additional 2 h the mixture was concentrated. Silica gel chromatography (1:1 toluene–EtOAc) of the residue yielded 2-(*N*-benzyloxycarbonyl)-aminoethyl (methyl 2,3-di-*O*-benzyl-α-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**9**, 37 mg, 21.6 μmol, 76%); ^{13}C NMR ($CDCl_3$): δ 23.1 (*C*H_3CON), 41.3(*C*H_2NHCOOBn), 52.0, 52.3 (CH_3O, C-2^{II}), 66.5, 66.7, 68.2, 68.6, 69.6, 69.8, 70.9, 72.0, 72.6, 73.2, 73.5, 73.6, 73.9, 74.4, 74.9, 75.0, 75.1, 75.3, 75.8, 77.6, 78.8, 79.8, 80.6, 81.8, 83.1 (C-2^{I}–6^{I}, 3^{II}–6^{II}, 2^{III}–6^{III}, 2^{IV}–5^{IV}, O*C*H_2CH_2N, Ph*C*H_2O), 96.5, 97.2 (C-1^{III}, 1^{IV}), 99.5, 101.9, 103.8 (C-1^{I}, 1^{II}, benzylidene), 125.3–139.1 (aromatic C), 156.4 (NH*C*OOBn), 170.6, 171.2 (C=O). MALDI–TOFMS: Calcd for $C_{100}H_{109}N_2NaO_{24}$ ($[M+Na]^+$): 1743.7. Found: 1743.1. To a solution of **9** (25 mg, 14.5 μmol) dissolved in 3:2 EtOH–AcOH (5 mL) was added palladium hydroxide on activated charcoal and the mixture was hydrogenolyzed at 0.69 MPa for 72 h. Additional catalyst was added after 24 and 48 h. The mixture was centrifuged and the pellets were washed once with EtOH. The supernatants were combined and concentrated. Purification of the residue on a Bio-Gel P-2 column gave, after freeze drying, **10** (8 mg, 10.3 μmol, 71%); $[\alpha]_D$ +39° (*c* 0.8, water); Selected NMR data (D_2O): ^{13}C, δ 95.9 (C-1^{IV}), 100.3 (C-1^{II}), 101.2 (C-1^{III}), 103.0 (C-1^{I}); ^{1}H, δ 4.52 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{I}), 4.90 (s, 1 H, H-1^{II}), 5.17 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^{IV}), 5.32 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1^{III}). MALDI–TOFMS: Calcd for $C_{29}H_{50}N_2NaO_{22}$ ($[M+Na]^+$): 801.3. Found: 801.1.

3.5. 2-Aminoethyl α-D-glucopyranosyl-(1→4)-(methyl α-D-glucopyranosyluronate)-(1→3)-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside (13)

A mixture of **11**[15] (13 mg, 22.1 μmol) and **9** (19 mg, 11.0 μmol) in dry Et_2O (5 mL) containing powdered molecular sieves (4 Å) was stirred at rt in an Ar atmosphere for 1 h. To the mixture was added DMTST (11 mg, 44.1 μmol) and the stirring was continued for 6 h. After neutralization with Et_3N, the mixture was filtered through Celite and concentrated. The residue was purified on a silica gel column (2:1 toluene–EtOAc) to yield 2-(*N*-benzyloxycarbonyl)-aminoethyl (2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-glucopyranosyluronate)-(1→3)-(2,4,6-tri-,*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside as an α/β-mixture (17 mg, 7.6 μmol, 69%). Repeated chromatography (2 columns, not flash-mode; 2:1 toluene–EtOAc) afforded the pure α anomer (**12**, 11 mg, 4.9 μmol, 45%); Selected NMR data: ^{1}H, δ 5.06 (d, 1 H, 3.5 Hz), 5.42 (d, 1 H, 3.5 Hz), 5.53 (d, 1 H, 3.3 Hz). MALDI–TOFMS: Calcd for $C_{134}H_{142}N_2NaO_{29}$ ($[M+Na]^+$): 2266.0.

Found: 2266.6. To a solution of **12** (11 mg, 4.9 μmol) dissolved in 3:2 EtOH–AcOH (5 mL) was added palladium hydroxide on activated charcoal and the mixture was hydrogenolyzed at 0.69 MPa for 72 h. Additional catalyst was added after 48 h. The mixture was centrifuged and the pellets were washed once with EtOH. The supernatants were combined and concentrated. Purification of the residue on a Bio-Gel P-2 column gave, after freeze-drying, **13** (4 mg, 3.72 μmol, 76%). $[\alpha]_D$ +73° (*c* 0.5, water). Selected NMR data (D_2O): ^1H, δ 4.49 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^I), 4.88 (s, 1 H, H-1^{II}), 5.14 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1^{IV}), 5.31 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1^{III}), 5.39 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^V). MALDI–TOFMS: Calcd for $C_{35}H_{60}N_2NaO_{27}$ ($[M+Na]^+$): 963.3. Found: 963.3.

Acknowledgements

Financial support from EU (Contract Number BIO 4 CT 960158), from the Swedish Foundation for Strategic Research (the 'Glycoconjugates in Biological Systems' programme) and from the Swedish Natural Science Research Council are gratefully acknowledged.

References

1. *Streptococcus pneumoniae*; Thomasz, A. (Ed.); Molecular Biology & Mechanisms of Disease, Mary Ann Liebert, Inc., New York, 2000.
2. Lindberg, B.; Kenne, L. In *The Polysaccharides*; Aspinall, G. O., Ed.; Vol. 2; Academic Press: New York, 1995; pp 287–363.
3. Kamerling, J. P. *Pneumococcal polysaccharides: A chemical view*; In Ref. 1 pp. 81–114.
4. Jones, C.; Mulloy, B.; Wilson, A.; Dell, A.; Oates, J. E. *J. Chem. Soc., Perkin Trans. 1* **1985**, 1665–1673.
5. Richards, J. C.; Perry, M. B.; Kniskern, P. J. *Can. J. Biochem.* **1984**, *62*, 1309–1320.
6. Perry, M. B.; Daoust, V.; Carlo, D. J. *Can. J. Biochem*. **1981**, *59*, 524–533.
7. Laferrière, C. A.; Sood, R. K.; deMuys, J.-M.; Michon, F.; Jennings, H. J. *Vaccine* **1997**, *15*, 179–186.
8. Benaissa-Trouw, B.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Kraaijeveld, K.; Snippe, H. *Infect. Immun.* **2001**, *69*, 4698–4701.
9. Alpe, M.; Oscarson, S. *Carbohydr. Res.* **2002**, *337*, 1715–1722.
10. Stachulski, A. V.; Jenkins, G. N. *Natural Prod. Rep.* **1998**, *15*, 173–186.
11. Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Seeberger, P. *Angew. Chem. Int. Ed.* **2002**, *41*, 2128–2131.
12. Oscarson, S.; Svahnberg, P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 873–879.
13. Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1963**, *85*, 3027–3028.
14. O'Connor, B.; Just, B. *Tetrahedron Lett.* **1987**, *28*, 3235–3236.
15. Andersson, F.; Fügedi, P.; Garegg, P. J.; Nashed, M. *Tetrahedron Lett.* **1986**, *27*, 3919–3922.
16. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
17. Rutherford, T. J.; Jones, C.; Davies, D. B.; Elliott, A. C. *Carbohydr. Res.* **1994**, *265*, 97–111.