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SYNTHESIS OF A DISACCHARIDE COMPONENT OF THE CAPSULAR POLYSACCHARIDE ANTIGEN OF *Streptococcus pneumoniae* TYPE 1

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ABSTRACT

Methyl 2-acetamido-4-amino-2,4,6-trideoxy-3-*O*-(α-D-galactopyranosyl-uronic acid)-α-D-galactopyranoside has been synthesised. The parent disaccharide is a structural element of the capsular polysaccharide antigen of *Streptococcus pneumoniae* type 1.

INTRODUCTION

The capsular polysaccharide of *Streptococcus pneumoniae* type 1 is composed of trisaccharide repeating-units →3)-α-D-Gal*p*A-(1→3)-α-Sug*p*-(1→4)-α-D-Gal*p*A(1→, in which Sug denotes 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose[1]. In the course of immunochemical studies of this antigen, oligosaccharides that are part of this structure were needed. We now report the synthesis of the methyl glycoside (**12**) of the disaccharide α-D-Gal*p*A-(1→3)-α-Sug*p*.

RESULTS AND DISCUSSION

For the synthesis of **12**, the α-D-galactouronopyranosyl donor **9** and the diaminotrideoxy sugar precursor **6** were prepared.

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[2] (**1**) was treated with *p*-methoxybenzyl bromide and sodium hydride in *N,N*-dimethylformamide to give crystalline **2** (69%). The benzylidene group was then removed by treatment with aqueous acetic acid, and the resulting crude 4,6-diol was treated with methanesulfonyl chloride in pyridine to give crystalline **3** (73%). Selective reduction at the primary position of **3** with sodium borohydride in methyl sulfoxide[3] followed by treatment of the resulting 4-*O*-methanesulfonyl derivative **4** with sodium azide in 1,3-dimethyl-3,4,5,6-tetra-hydro-2(1*H*)-pyrimidone yielded the crystalline 4-azido-D-*galacto* derivative **5** (76%). This solvent is less harmful than

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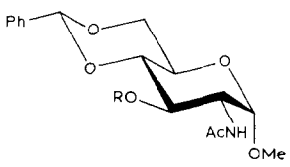
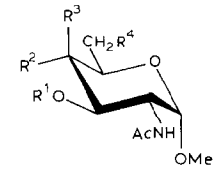


1 R = H
2 R = *p*-MeOBzl



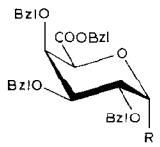
	R^1	R^2	R^3	R^4
3	*p*-MeOBzl	OMs	H	OMs
4	*p*-MeOBzl	OMs	H	H
5	*p*-MeOBzl	H	N_3	H
6	H	H	N_3	H

hexamethylphosphoramide, which has proved most efficient in similar conversions[4,5].

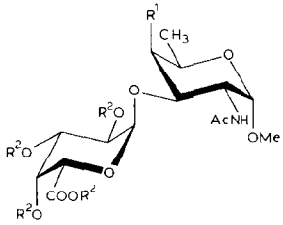
The D-*galacto* configuration of **5** was evident from its ^{13}C-n.m.r. spectrum, *e.g.*, the upfield shift of 4.3 p.p.m. of the signal of C-2 relative to that for **4**. The *p*-methoxybenzyl group in **5** was removed by oxidation[6] with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in chloroform–methanol to give, after chromatography, **6** (72%).

Methyl (methyl α-D-galactopyranosid)uronate[7] was saponified, treated first with an acidic ion-exchange resin, and then with silver oxide–benzyl bromide, to give the tetra-*O*-benzyl derivative **7** (57%). Acetolysis of **7** gave syrupy **8** (67%), treatment of which with hydrogen bromide gave the glycosyl bromide **9** (82%).

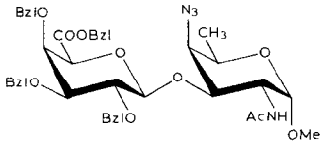
For the condensation of **9** and **6**, mercuric bromide and molecular sieves or silver triflate–collidine[8], promoters known to give high yields of *cis*-1,2-glycosides in the *gluco* and *galacto* series, proved to be unsatisfactory. Thus, in both instances, αβ-ratios of 1:3 and total yields around 55% were obtained. With halide ion-catalysis[9], the α-glycoside preponderated (αβ-ratio ~8:1), but the yield was low (35%). However, with zinc bromide in the presence of molecules sieves[10], the desired α-linked disaccharide derivative was obtained in 63% yield. A smaller amount (10%) of the β-linked disaccharide derivative was also isolated; unexpectedly, the α-linked compound had the lower positive optical rotation.



7 R = OMe
8 R = OAc
9 R = Br



10 R^1 = N_3, R^2 = Bzl
12 R^1 = NH_2, R^2 = H



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Finally, the protecting groups in **10** were removed and the amino function was generated by catalytic hydrogenation, and 73% of amorphous **12** was isolated.

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at <40° (bath). Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a JEOL FX-100 or GX-400 instrument. Chemical shifts are given in p.p.m. relative to internal Me_4Si (^{13}C, chloroform and methyl sulfoxide), external Me_4Si (^{13}C, D_2O), and HOD (^1H, D_2O). For t.l.c., Merck silica gel 60 F-254 was used. Compounds were located by quenching of u.v. fluorescence or by charring with sulfuric acid. For column chromatography, Merck silica gel 60 (0.040–0.063 mm) was used. Elemental analysis was performed by Analytische Laboratorien, Elbach, West Germany. Satisfactory elemental analyses were not obtained for syrupy products, but they were shown to be pure by chromatography and n.m.r. spectroscopy.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(p-methoxybenzyl)-α-D-glucopyranoside (**2**). — A solution of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**, 7 g) and *p*-methoxybenzyl bromide (5.2 g) in *N,N*-dimethylformamide (75 mL) was added dropwise to sodium hydride (0.78 g) at 0°. After 1 h, methanol (10 mL) was added, and the solution was diluted with chloroform (250 mL), washed twice with water, and concentrated. Distillation of xylene from the residue and recrystallisation from isobutyl methyl ketone gave **2** (6.67 g, 69%), m.p. 230–232°, $[\alpha]_{578}^{22}$ +96° (*c* 0.96, chloroform). ^{13}C-N.m.r. data (25.05 MHz, $CDCl_3$): δ 23.3 (NHAc), 52.8 (C-2), 55.2, 55.3 (2 OMe), 62.9 (C-5), 69.2 (C-6), 73.5 (PhCH_2), 75.5 (C-3), 83.0 (C-4), 99.4 (C-1), 101.5 (PhCH), 114.0, 129.7, 131.0, 159.4 (*p*-MeOBzl), 126.2, 128.2, 129.0, 137.7 (benzylidene), and 169.2 (C=O) (Found: C, 65.0; H, 6.5; N, 3.2. $C_{24}H_{27}NO_7$ calc.: C, 65.3; H, 6.2; N, 3.2%).

Methyl 2-acetamido-2-deoxy-3-O-(p-methoxybenzyl)-4,6-di-O-methylsulfonyl-α-D-glucopyranoside (**3**). — A solution of **2** (9.0 g) in aqueous 90% acetic acid (100 mL) was stirred for 10 min at 85° and then concentrated, and toluene (2 × 50 mL) was distilled from the residue. To a solution of the residue in pyridine (70 mL) was added methanesulfonyl chloride (18 mL) dropwise at 0°. The solution was allowed to attain room temperature during 2 h and then poured into a stirred mixture of ice–water (700 mL) and light petroleum (100 mL). After 2 h, the precipitate was collected, and recrystallised from methanol–water to give **3** (8.47 g, 73%), m.p. 188°, $[\alpha]_{578}^{22}$ +88° (*c* 0.76, chloroform). ^{13}C-N.m.r. data (Me_2SO-d_6): δ 22.4 (NHAc), 37.0, 39.5 (2 Ms), 52.5 (C-2), 55.0 (2 OMe), 67.3, 67.9 (C-5,6), 73.2 (PhCH_2), 76.7, 77.1 (C-3,4), 98.2 (C-1), 113.6, 129.4, 129.9, 158.9 (aromatic), and 169.4 (C=O) (Found: C, 44.5; H, 5.7; N, 2.6; S, 12.7. $C_{19}H_{29}NO_{11}S_2$ calc.: C, 44.6; H, 5.7; N, 2.7; S, 12.5%).

Methyl 2-acetamido-2,6-dideoxy-3-O-(p-methoxybenzyl)-4-O-methylsulfo-

*nyl-α-*D*-glucopyranoside* (**4**). — A mixture of **3** (5.5 g), sodium borohydride (2.3 g), and methyl sulfoxide (40 mL) was stirred at 85° for 2 h, and then added portionwise to aqueous 5% acetic acid (1 L) and kept overnight at 5°. The crystalline product was collected, and crystallised from chloroform–light petroleum to give **4** (2.87 g, 65%), m.p. 200–201° (from chloroform–light petroleum), $[\alpha]_{578}^{22}$ +92° (*c* 0.8, chloroform). ^{13}C-N.m.r. data (Me_2SO-d_6): δ 17.4 (C-6), 22.4 (NHAc), 38.4 (Ms), 52.8 (C-2), 54.6, 54.9 (2 OMe), 64.9 (C-5), 73.0 (PhCH), 76.6 (C-3), 82.9 (C-4), 98.0 (C-1), 113.5, 129.2, 130.0, 158.8 (aromatic), and 169.1 (C=O) (Found: C, 51.6; H, 6.4; N, 3.3; S, 7.8. $C_{18}H_{27}NO_8S$ calc.: C, 51.8; H, 6.5; N, 3.4; S, 7.7%).

*Methyl 2-acetamido-4-azido-2,4,6-trideoxy-3-*O*-(*p*-methoxybenzyl)-α-*D*-galactopyranoside* (**5**). — A mixture of **4** (8.0 g) and sodium azide (2.0 g) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone (40 mL) was stirred at 125° for 20 h, cooled, diluted with water (1 L), and stirred overnight. The precipitate was collected, and crystallised from methanol–water to give **5** (5.38 g, 76%), m.p. 220° (from methanol), $[\alpha]_{578}^{22}$ +102° (*c* 0.76, chloroform). ^{13}C-N.m.r. data (Me_2SO-d_6): δ 17.0 (C-6), 22.4 (NHAc), 48.5 (C-2), 54.5, 54.9 (2 OMe), 62.9, 63.9 (C-4,5), 70.8 ($PhCH_2$), 75.5 (C-3), 98.4 (C-1), 113.5, 129.0, 130.2, 158.8 (aromatic), and 169.2 (C=O) (Found: C, 55.6; H, 6.6; N, 14.8. $C_{17}H_{24}N_4O_5$ calc.: C, 56.0; H, 6.6; N, 15.4%).

*Methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-*D*-galactopyranoside* (**6**). — A solution of **5** (140 mg) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (120 mg) in chloroform–methanol (10:1, 5 mL) was stirred at 50° for 1 h, cooled, and partitioned between chloroform and saturated aqueous sodium hydrogencarbonate. The chloroform phase was concentrated, and the residue was purified on silica gel (chloroform–acetone,1:1), to yield **6** (71 mg, 72%), m.p. 220–222° (from ethyl acetate–light petroleum), $[\alpha]_{578}^{22}$ +18° (*c* 0.96 chloroform). ^{13}C-N.m.r. data (Me_2SO-d_6): δ 17.1 (C-6), 22.5 (NHAc), 49.8 (C-2), 54.6 (OMe), 63.9, 66.5, 67.6 (C-3,4,5), 98.2 (C-1), and 169.8 (C=O). A correct elemental analysis could not be obtained for this compound, but its purity was demonstrated by chromatography and n.m.r. spectroscopy.

*Benzyl (methyl 2,3,4-tri-O-*benzyl-*α-*D*-galactopyranosid)uronate* (**7**). — A solution of sodium hydroxide (0.99 g) in water (3 mL) was added to a solution of methyl (methyl α-D-galactopyranosid)uronate (5.33 g) in water (25 mL), and the mixture was kept at 0°. After 0.5 h, the mixture was added to a column (4 × 8 cm) of Dowex 50 (H^+) resin. Elution with water yielded carbohydrate-containing fractions that were combined and freeze-dried; to a solution of the residue in *N,N*-dimethylformamide (150 mL) were added silver oxide (35 g) and ground molecular sieves (3 Å, 15 g), and the mixture was stirred at 0° in the dark for 1 h. Benzyl bromide (30 mL) was then added dropwise during 1 h. The mixture was allowed to attain room temperature overnight, diluted with toluene (500 mL), filtered (Celite), washed with aqueous sodium thiosulfate and water, concentrated, and purified on silica gel (iso-octane–ethyl acetate, 2:1) to give **7** as a syrup (7.80 g, 57%), $[\alpha]_{578}^{22}$ +11° (*c* 2.5, chloroform), R_F 0.32. ^{13}C-N.m.r. data ($CDCl_3$): δ 55.9

(OMe), 66.9 (C-5), 70.6, 73.4, 73.6, 74.7, 75.9, 76.8, 78.3 (C-2,3,4, 4 PhCH$_2$), 99.2 (C-1), 126.7, 129.6, 135.3, 138.4, 138.5, 138.6 (aromatic), and 168.4 (C-6).

*Benzyl 1-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-*D*-galactopyranuronate* (**8**). — Conc. sulfuric acid (0.70 mL) was slowly added to a solution of **7** (3.5 g) in acetic acid–acetic anhydride (1:1, 30 mL) kept at 0°. After 2 h, sodium acetate (7.5 g) was added, the mixture was concentrated to dryness, the residue was partitioned between ethyl acetate and water, the organic phase was concentrated, and the residue was purified on silica gel (iso-octane–ethyl acetate, 2:1) to give **7** as a syrup (2.8 g, 67%), $[\alpha]_{578}^{22}$ +53° (*c* 1.2, chloroform), R_F 0.45. N.m.r. data: ^{1}H (99.6 MHz, $CHCl_3$), δ 6.45 (2 Hz, H-1); ^{13}C (25.05 MHz, $CDCl_3$), δ 20.9 (OAc), 67.1 (C-5), 72.8, 73.3 (2 C), 74.8 (2 C), 76.2, 77.9 (C-2,3,4, 4 PhCH$_2$), 90.4 (C-1, $J_{C\text{-}1,H\text{-}1}$ 176 Hz), 127.4–128.6, 135.0, 137.8, 138.2, 138.3 (aromatic), 167.3 (C-6), and 168.8 (OAc).

*Benzyl (2,3,4-tri-*O*-benzyl-α-*D*-galactopyranosyl bromide)uronate* (**9**). — A solution of **8** (580 mg) in dichloromethane (10 mL) was saturated with hydrogen bromide and kept at 0° for 15 min. Excess of hydrogen bromide was removed by codistillation with dichloromethane and the mixture was then concentrated. To a solution of the residue in toluene (30 mL) was added silica gel (0.25 g), and the mixture was stirred for 5 min at room temperature, filtered, and concentrated, to give **9** as a chromatographically pure, colorless oil (490 mg, 82%), $[\alpha]_{578}^{22}$ +131° (*c* 1.5, dichloromethane), R_F 0.67 (t.l.c.; toluene–ethyl acetate, 6:1). N.m.r. data: ^{1}H (99.60 MHz, $CDCl_3$), δ 6.53 (2.4 Hz, H-1); ^{13}C (25.05 MHz, $CDCl_3$), δ 67.1 (C-5), 72.6, 73.3, 74.6, 74.9, 75.4, 75.5, 78.5, (C-2,3,4, 4 PhCH$_2$), 92.3 (C-1, $J_{C\text{-}1,H\text{-}1}$ 182 Hz), 127.3–128.8, 134.8, 137.5, 137.9, 138.1 (aromatic), and 166.7 (C-6).

*Methyl 2-acetamido-4-azido-3-*O*-(benzyl 3,4,6-tri-*O*-benzyl-α-*D*-galactopyranosyluronate)-2,4,6-trideoxy-α-*D*-galactopyranoside* (**10**). — A solution of **9** (520 mg) in dichloromethane (6 mL) was added to a mixture of **6** (125 mg), zinc bromide (800 mg), and molecular sieves (4Å, 2 g) in dichloromethane (10 mL). The mixture was stirred overnight, filtered (Celite), diluted with toluene (50 mL), and stirred over aqueous sodium hydrogencarbonate for 0.5 h. The organic layer was separated, washed with water, and concentrated, and the residue was purified on silica gel (toluene–chloroform–acetone, 1:2:1) to give syrupy **10** as the main product (227 mg, 63%), $[\alpha]_{578}^{22}$ +29° (*c* 1.1, chloroform), R_F 0.50. ^{13}C-N.m.r. data ($CDCl_3$): δ 17.2 (C-6), 23.0 (NHAc), 48.5 (C-2), 55.2 (OMe), 64.2, 64.7, 66.9, 71.9, 73.3, 74.3 74.6, 76.0, 76.8, 78.3, 82.2 (C-3,4,5, C-2′,3′,4′,5′, 4 PhCH$_2$), 98.8 (C-1, $J_{C\text{-}1,H\text{-}1}$ 170 Hz), 102.7 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 170 Hz), 127.4–128.7, 135.0, 138.5 (3 C, aromatic), 168.5 (C-6′), and 170.3 (NHAc).

From subsequent fractions, **11** was obtained as a syrup (37 mg, 10%), $[\alpha]_{578}^{22}$ +67° (*c* 1.8, chloroform), R_F 0.41. ^{13}C-N.m.r. data ($CDCl_3$): δ 17.2 (C-6), 23.10 (NHAc), 48.6 (C-2), 55.1 (OMe), 64.5, 65.8, 67.3, 73.1, 74.1, 74.4, 75.0, 75.5, 77.8, 78.7, 81.3 (C-3,4,5, C-2′,3′,4′,5′, 4 PhCH$_2$), 98.9 (C-1, $J_{C\text{-}1,H\text{-}1}$ 170 Hz), 105.1 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 162 Hz), 127.1–128.6, 135.2, 138.2, 138.7, 138.9 (aromatic), 167.8 (C-6′), and 169.9 (NHAc).

Methyl 2-acetamido-4-amino-2,4,6-trideoxy-3-O-(α-D-galactopyranosyluronic acid)-α-D-galactopyranoside (**12**). — A solution of **10** (175 mg) in aqueous 90% acetic acid (25 mL) was hydrogenated at 400 kPa over 10% Pd/C (100 mg) overnight and then filtered. Gel filtration of the product on a column (2.5 × 85 cm) of Biogel P-2 gave **12** as an amorphous powder (54 mg, 73%), $[\alpha]_{578}^{22}$ +164° (*c* 0.4, water), R_F 0.24 (t.l.c.; ethyl acetate–methanol–acetic acid–water, 4:3:3:2). N.m.r. data: ^{1}H (399.78 MHz, D_2O, 50°), δ 1.33 (d, 3 H, 6.8 Hz, H-6), 1.99 (s, 3 H, NHAc), 3.40 (s, 3 H, OMe), 3.8 (dd, 1 H, 1.7 Hz, 4.3 Hz, H-4), 4.73 and 5.07 (2 d, 2 H, 3.0 Hz, H-1,1′); ^{13}C (25.05 MHz, D_2O), δ 16.7 (C-6), 23.0 (NHAc), 48.8 (C-2), 53.7, 56.6 (OMe, C-4), 63.5 (C-5), 68.4, 70.6, 71.7, 73.6 (C-2′,3′,4′,5′), 73.6 (C-3), 99.0, 99.2 (C-1,1′), 175.6 and 176.1 (C-6′, NHAc).

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