Synthesis of the Trisaccharide Component of the Repeating Unit of the Capsular Polysaccharide of *Streptococcus pneumoniae* Type 19F

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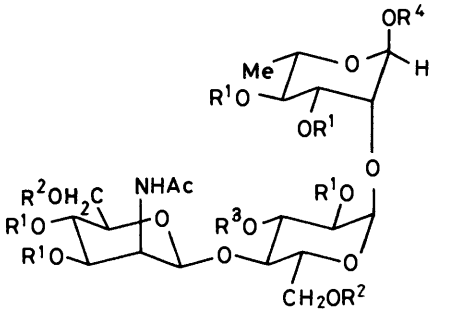
Acetolysis of methyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**3**) followed by treatment with hydrogen bromide–acetic acid afforded 4-*O*-(2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-6-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide (**5**). Compound (**5**) by $Hg(CN)_2$–$HgBr_2$ promoted condensation with benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**6**) provided the glycosylation product (22% yield) exclusively in the α-configuration.

Hydrogenolysis of the benzyl protecting groups of the obtained trisaccharide and *O*-deacetylation of the 6′ and 6″ positions eventually furnished *O*-(2-acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-α,β-L-rhamnopyranose (**1**), the trisaccharide component of the repeating unit of *Streptococcus pneumoniae* type 19F.

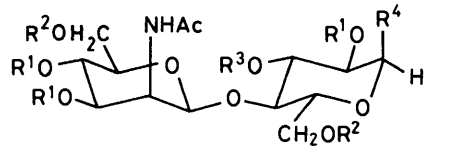
In the last decade a renewed interest in pneumococcal capsular polysaccharides for their use as vaccines[1,2] has arisen. At present against pneumococcal infections is used a multivalent vaccine, one constituent of which is the capsular polysaccharide of S.p. type 19F. The structure of this antigenic polysaccharide has been elucidated[3] and consists of repeating units composed of (→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*(1-PO_4^-)→ residues.

In a preliminary communication[4] we recently described the synthesis of the methyl glycoside of 4-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosyl)-α-D-glucopyranose (**2**), a disaccharide unit frequently encountered in the chains of pneumococcal polysaccharides.

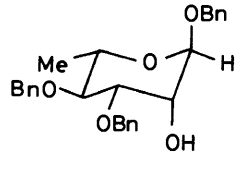
Here we report, starting from suitably blocked, anomerically activated (**2**), the synthesis of the trisaccharide *O*-(2-acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-α-D-glucopyranosyl-(1→2)-α,β-L-rhamnopyranose (**1**), the constituent of the repeating unit of the capsular polysaccharide from S.p. type 19F, which had been requested in the course of immunological studies on carbohydrate-proteine type synthetic antigens.



(**1**) $R^1=R^2=R^3=H, R^4=H$ (α+β anomer)
(**7**) $R^1=R^4=Bn, R^2=Ac, R^3=H$
(**8**) $R^1=R^4=Bn, R^2=R^3=Ac$
(**9**) $R^1=R^3=H, R^2=Ac, R^4=H$ (α + β anomer)



(**2**) $R^1=R^2=R^3=H, R^4=OH$
(**3**) $R^1=R^2=R^3=Bn, R^4=OMe$
(**4**) $R^1=R^3=Bn, R^2=Ac, R^4=OAc$ (α + β anomer)
(**5**) $R^1=Bn, R^2=Ac, R^3=H, R^4=Br$



(**6**)

Results and Discussion

For the construction of the target structure (**1**), methyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (**3**)[4] was first submitted to acetolysis with acetic anhydride–trifluoroacetic acid[5] (4:1) at room temperature to afford 4-*O*-(2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-1,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α,β-D-glucopyranose (**4**). The ^1H n.m.r. spectrum [δ 6.30 (0.7 H, *J* 3.5 Hz, 1α-H) and 5.60 (0.3 H, *J* 8 Hz, 1β-H)] and ^{13}C n.m.r. spectrum [89.3 (C-1α) and 93.6 (C-1β) p.p.m.)] of (**4**) indicated the presence of an anomeric α,β-mixture rich in the α-anomer in which the 6- and 6′-benzyl groups had been substituted by two acetyl groups.

Treatment of compound (**4**) in dichloromethane with hydrogen bromide–acetic acid at 0 °C afforded predominantly 4-*O*-(2-acetamido-6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-6-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide (**5**), in which one of the benzyl groups of (**4**) had been removed by the acidic conditions of the bromination reaction. The acid-labile benzyl group[6] proved to be the 3-*O*-benzyl of the glucosyl moiety; moreover, the bromide (**5**) was to some extent (*ca.* 15%) contaminated, probably by its 3-acetylated derivative (see the ^1H n.m.r. spectrum in the Experimental section).

The coupling reaction using benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside[7] (**6**) as glycosyl acceptor was achieved in

dichloromethane by using mercuric cyanide–mercuric bromide (3:1) as the catalyst to afford selectively the α-glycosidation product (**7**)* together with 15% of its 3′-acetylated compound (**8**) (22% overall yield).

The presence of only one anomer at the new glycosydic linkage was indicated by the ^{13}C n.m.r. spectrum of (**7**), which exhibited only three resonances in the anomeric region.

The resonance at 100.89 p.p.m. was assigned to the *N*-acetylmannosamine residue,† which carries a β-substituent, while the two resonances at 96.81 and 96.54 p.p.m. typical of anomeric carbon atoms carrying an α-substituent,‡ indicated that the newly formed glycosidic bond has the α-configuration.This was confirmed by the ^1H n.m.r. data: in the ^1H spectrum of compound (**7**) 1′-H was assigned to the doublet at δ 4.84 with an equatorial-axial coupling constant of 3.5 Hz that collapsed to a singlet on irradiation of the dd (*J* 3.5 and 10 Hz) at δ 3.40, assigned to the hydrogen on the carbon carrying the 2′-equatorial benzyl group. Moreover, when the trisaccharide (**7**) was submitted to acetylation under standard conditions, an acetylated compound, identical with (**8**), was obtained. The acetyl group introduced was located at C-3′: in fact by selective decoupling of the resonance at δ 3.47, corresponding to 2′-H, the anomeric 1′-H doublet (*J* 3.5 Hz) at δ 4.85 collapsed to a singlet, whereas a triplet (*J* 9.5 Hz) at δ 5.42 collapsed to a doublet, and thence could be attributed to 3′-H, confirming the removal of the 3-*O*-benzyl group of the glucosyl moiety in the bromination reaction.

Catalytic hydrogenolysis effected on (**7**) removed all the remaining benzyl groups, to afford the 6′,6″-acetylated trisaccharide (**9**) as a mixture of α,β-anomers at the reducing terminus. The ^1H chemical shifts values (two doublets, one at δ 4.95 for 1′-H in the β-anomer, and one at δ 4.86 for 1′-H in the α-anomer) and the coupling constant (*J* 4 Hz) once more indicate the α-configuration of the glc*p*-(1→2)-rha*p* glycosydic linkage.

O-Deacetylation with methanolic sodium methoxide through the Zémplen procedure[8] eventually afforded the expected trisaccharide (**1**). The synthetic trisaccharide (**1**) is able to inhibit *in vitro* the antigen-antibody reaction between the homologous 19F streptococcal polysaccharide and a specific antiserum; the immunological studies on (**1**) will be reported elsewhere.

Experimental

General Methods.—^1H N.m.r. spectra were recorded with a Varian XL-200 spectrometer and ^{13}C n.m.r. spectra were recorded with a Varian XL-100 spectrometer in deuteriochloroform solutions containing Me_4Si as an internal standard, unless otherwise stated. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Analytical thin layer chromatography (t.l.c.) was performed on Merck 60 F_{254} silica gel plates (0.25 mm thickness) and the spots were detected either by a u.v. lamp or by spraying with 50% aqueous H_2SO_4 and heating at 110 °C for 5 min. Column chromatography was performed on Merck 60 silica gel (70—230 mesh). Evaporation under reduced pressure was always effected with the bath temperature kept below 40 °C.

4-O-(2-*Acetamido*-6-O-*acetyl*-3,4-*di*-O-*benzyl*-2-*deoxy*-β-D-*mannopyranosyl*)-1,6-*di*-O-*acetyl*-2,3-*di*-O-*benzyl*-α,β-D-*glucopyranose* (**4**).—A solution of methyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[4] (**3**) (600 mg) in acetic anhydride (19.2 ml) was treated at 0 °C with trifluoroacetic acid (4.8 ml) and stirred for 18 h at room temperature. Solid sodium acetate was added to the mixture, which was then poured into iced water and stirred for 30 min. The mixture was extracted with dichloromethane, and the extract washed to neutrality, dried, and evaporated under reduced pressure to afford a crude residue (615 mg) which was chromatographed on silica gel [toluene–acetone, 7:1 (v/v)] to yield pure syrupy (**4**) (467 mg, 84%) (Found: C, 66.45; H, 6.4; N, 1.5. $C_{48}H_{55}NO_{14}$ requires C, 66.27; H, 6.37; N, 1.61%); $[\alpha]_D$ +11.3° (*c* 1.7, chloroform), R_F 0.38 (β anomer) and 0.30 (α anomer) (benzene–ethyl acetate, 1:1); δ 1.90—2.20 (cluster of singlets, 12 H, 3 OAc and 1 NAc), 3.30—5.0 (m, 21 H, 2-H, 3-H, 4-H, 5-H, 6-H_2, 1′-H, 2′-H, 3′-H, 4′-H, 5′-H, 6′-H_2, and 4 CH_2Ph), 5.62 (d, $J_{2',NH}$ 9 Hz, 1 H, NH), 5.60 (d, $J_{1,2}$ 8 Hz, 0.3 H, 1β-H), 6.30 (d, $J_{1,2}$ 3.5 Hz, 0.7 H, 1α-H), and 7.10—7.40 (m, 20 H, 4 Ph).

4-O-(2-*Acetamido*-6-O-*acetyl*-3,4-*di*-O-*benzyl*-2-*deoxy*-β-D-*mannopyranosyl*)-6-O-*acetyl*-2-O-*benzyl*-α-D-*glucopyranosyl Bromide* (**5**).—A solution of 33% hydrogen bromide in acetic acid (2.2 ml) was added dropwise to a cooled (0 °C) and stirred solution of the acetate (**4**) (450 mg) in dichloromethane (30 ml). The mixture was kept at 0 °C for 2.5 h after which it was diluted with dichloromethane, washed with cold water to neutrality, dried, and evaporated to afford a syrup (400 mg) (Found: C, 58.4; H, 5.6; N, 1.7. 85% $C_{39}H_{46}BrNO_{12}$ + 15% $C_{41}H_{48}BrNO_{13}$ requires C, 58.49; H, 5.78; N, 1.74%); $[\alpha]_D$ +31° (*c* 2.2, $CHCl_3$), R_F 0.38 (benzene–ethyl acetate, 1:1); δ 2.0—2.2 (cluster of singlets, 9.6 H, 2.4 OAc and 1 NAc), 3.3—5.1 (m, 18.8 H, 2-H, 3-H, 4-H, 5-H, 6-H_2, 1′-H, 2′-H, 3′-H, 4′-H, 5′-H, 6′-H_2, and 3 CH_2Ph), 5.44 (t, *J* 9.5 Hz, 0.15 H, 3-H acetylated), 5.63 (d, $J_{2',NH}$ 10 Hz, 1 H, NH), 6.29 (d, $J_{1,2}$ 4 Hz, 0.85 H, 1-H), 6.32 (d, $J_{1,2}$ 3.5 Hz, 0.15 H, 1-H corresponding to 3-OAc), and 7.10—7.40 (m, 15 H, 3 Ph).

Benzyl O-(2-*Acetamido*-6-O-*acetyl*-3,4-*di*-O-*benzyl*-2-*deoxy*-β-D-*mannopyranosyl*)-(1→4)-O-(6-O-*acetyl*-2-O-*benzyl*-α-D-*glucopyranosyl*)-(1→2)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (**7**).—A mixture of benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside[7] (**6**) (192 mg), powdered 3A molecular sieves, $Hg(CN)_2$ (152 mg), and $HgBr_2$ (72 mg) in dry dichloromethane (20 ml) was stirred in the dark under argon at room temperature for 1 h. A solution of the bromide (**5**) (376 mg) in dry dichloromethane (20 ml) was added dropwise at —20 °C. The stirred mixture was kept at room temperature for 3 days after which it was filtered through Celite and evaporated under reduced pressure. The crude mixture obtained (445 mg) was chromatographed on a SiO_2 column with benzene–ethyl acetate (7:3, v/v). Unchanged (**6**) (128 mg) was eluted first, then the α-glycosylation products (**7**) (103 mg) and (**8**) (15 mg, 22% overall yield), and a complex mixture of more polar compounds (176 mg) which was no further investigated were eluted in the order: (**7**) a syrup (Found: C, 68.75; H, 6.6; N, 1.3. $C_{66}H_{75}NO_{17}$ requires C, 68.68; H, 6.55; N, 1.21%); $[\alpha]_D$ +10° (*c* 0.8, $CHCl_3$), R_F 0.68 (benzene–ethyl acetate, 1:1); δ 1.34 (d, *J* 6 Hz, 3 H, CH_3), 2.02, 2.04, and 2.09 (3 s, 9 H, 2 OAc and 1 NAc), 3.40 (dd, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 10 Hz, 1 H, 2′-H), 3.3—4.3 (m, 14 H, 2-H, 3-H, 4-H, 5-H, 3′-H, 4′-H, 5′-H, 6′-H_2, 3″-H, 4″-H, 5″-H, and 6″-H_2), 4.53 (d, $J_{1'',2''}$ 1.5 Hz, 1 H, 1″-H), 4.84 (d, $J_{1',2'}$ 3.5 Hz, 1 H, 1′-H), 4.85 (d, $J_{1,2}$ 1.5 Hz, 1 H, 1-H), 4.35—4.95 (6 AB systems, 12 H, 6 CH_2Ph), 4.99 (ddd, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 4.0 Hz, $J_{2'',NH}$ 10 Hz, 1 H, 2″-H), 5.64 (d, $J_{2'',NH}$ 10 Hz, 1 H, NH), and 7.10—7.40 (m, 30 H, 6 Ph).

* Unfortunately the yield obtained was rather low and could not be improved even using different catalysts (such as silver triflate) or different proportions of $Hg(CN)_2$–$HgBr_2$.

† The ^{13}C chemical shifts for the anomeric carbons in the β-*N*-acetylmannosaminylglucoside (**3**) were 99.93 (C-1′) and 98.08 (C-1) p.p.m.

‡ The model disaccharide benzyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside was synthesized (unpublished results). In its ^{13}C n.m.r. spectrum the C-1 and C-1′ resonances were at 95.92 and 96.26 p.p.m., in agreement with the chemical shifts obtained for C-1 and C-1′ in the trisaccharide (**7**).

Benzyl O-(*2-Acetamido*-6-O-*acetyl*-3,4-*di*-O-*benzyl*-2-*deoxy*-β-D-*mannopyranosyl*)-(1→4)-O-(3,6-*di*-O-*acetyl*-2-O-*benzyl*-α-D-*glucopyranosyl*)-(1→2)-3,4-*di*-O-*benzyl*-α-L-*rhamnopyranoside* (**8**).—The trisaccharide (**7**) (15 mg), dissolved into dry pyridine (0.5 ml), was treated with acetic anhydride (0.25 ml). After 18 h at room temperature the solution was repeatedly evaporated under reduced pressure with toluene to yield the acetate (**8**) (14 mg) (Found: C, 68.35; H, 6.55; N, 1.25. $C_{68}H_{77}NO_{18}$ requires C, 68.27; H, 6.49; N, 1.17%); $[\alpha]_D$ +23° (*c* 1, $CHCl_3$), R_F 0.56 (benzene–ethyl acetate, 1:1); δ 1.32 (d, *J* 6 H, 3 H, CH_3), 2.01, 2.01, 2.03, and 2.13 (3 s, 12 H, 3 OAc and 1 NAc), 3.47 (dd, $J_{1',2'}$ 3.5 Hz, $J_{2',3'}$ 9.5 Hz, 1 H, 2'-H), 3.3—4.3 (m, 13 H, 2-H, 3-H, 4-H, 5-H, 4'-H, 5'-H, 6'-H_2, 3″-H, 4″-H, 5″-H, and 6″-H_2), 4.53 (d, $J_{1'',2''}$ 1.5 Hz, 1 H, 1″-H), 4.81 (d, $J_{1,2}$ 1.5 Hz, 1 H, 1-H), 4.85 (d, $J_{1',2'}$ 3.5 Hz, 1 H, 1'-H), 4.35—5.05 (m, 13 H, 6 CH_2Ph and 2″-H), 5.42 (t, *J* 9.5 Hz, 1 H, 3'-H), 5.62 (d, $J_{2'',NH}$ 10 Hz, 1 H, NH), and 7.10—7.40 (m, 30 H, 6 Ph).

O-(2-*Acetamido*-6-O-*acetyl*-2-*deoxy*-β-D-*mannopyranosyl*)-(1→4)-O-(6-O-*acetyl*-α-D-*glucopyranosyl*)-(1→2)-α,β-L-*rhamnopyranose* (**9**).—The trisaccharide (**7**) (75 mg) was dissolved into methanol (10 ml), treated with Pd–C (10%, 20 mg), and shaken under hydrogen atmosphere for 18 h. The catalyst was filtered off and the solvent evaporated under reduced pressure to yield the title compound (**9**) (36 mg) as an anomeric α,β-mixture (Found: C, 46.75; H, 6.3; N, 2.2. $C_{24}H_{39}NO_{17}$ requires C, 46.98; H, 6.41; N, 2.28%); $[\alpha]_D$ +28° (*c* 0.5, MeOH), R_F 0.59 (ethyl acetate–isopropyl alcohol–water, 3:3:1); δ (CD_3OD, one drop D_2O) 1.27 and 1.29 (2 d, *J* 7 and 6 Hz, 3 H, CH_3), 2.04 and 2.05 (2 s, 3 H, NAc), 2.08, 2.10, and 2.12 (3 s, 6 H, 2 OAc), 3.2—4.5 (m, 15 H, 2-H, 3-H, 4-H, 5-H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H_2, 3″-H, 4″-H, 5″-H, and 6″-H_2), 4.59 (dd, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 4.5 Hz, 1 H, 2″-H), 4.72 and 4.73 (2 d, $J_{1'',2''}$ 1.5 Hz, 1 H, 1″-H), 4.77 (d, $J_{1,2}$ 1 Hz, 0.3 H, 1α-H), 4.86 and 4.95 (2 d, $J_{1',2'}$ 4 Hz, 1 H, 1'-H), and 5.12 (d, $J_{1,2}$ 1.5 Hz, 0.7 Hz, 1β-H).

O-(2-*Acetamido*-2-*deoxy*-β-D-*mannopyranosyl*)-(1→4)-O-(α-D-*glucopyranosyl*)-(1→2)-α,β-L-*rhamnopyranose* (**1**).—The anomeric mixture of compound (**9**) (32 mg) was dissolved into methanol (10 ml) and a 1% sodium methoxide solution (0.1 ml) was added. After being stirred for 18 h the solution was neutralized with Dowex 50 (H^+). The resin was filtered off and the solvent was evaporated under reduced pressure to give solid amorphous (**1**) (25 mg) (Found: C, 45.0; H, 6.8; N, 2.5. $C_{20}H_{35}NO_{15}$ requires C, 45.37; H, 6.66; N, 2.65%); $[\alpha]_D$ +15.9° (*c* 0.3, H_2O), R_F 0.28 (ethyl acetate–isopropyl alcohol–water, 3:3.1); δ (D_2O) 1.11 and 1.12 (2 d, *J* 6.5 and 5.5 Hz, 3 H, CH_3), 1.90 (s, 3 H, NAc), 3.2—4.0 (m, 15 H, 2-H, 3-H, 4-H, 5-H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H_2, 3″-H, 4″-H, 5″-H, and 6″-H_2), 4.38 (dd, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 4.5 Hz, 1 H, 2″-H), 4.72 (d, $J_{1'',2''}$ 1.5 Hz, 1 H, 1″-H), 4.77 (d, $J_{1,2}$ 0.5 Hz, 0.35 H, 1α-H), 4.82 and 4.91 (2 d, $J_{1',2'}$ 3.5 Hz, 1 H, 1'-H), and 5.05 (d, $J_{1,2}$ 1 Hz, 0.65 H, 1β-H).

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