Carbohydrate Research, 180 (1988) 242–245
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Streptococcus pneumoniae type 19A polysaccharide. Synthesis of the trisaccharide component of the repeating unit

LUIGI PANZA, FIAMMA RONCHETTI*, AND LUCIO TOMA

Centro di Studio per le Sostanze Organiche Naturali del C.N.R. and Dipartimento di Chimica Organica e Industriale dell'Università, Via Venezian 21, 20133 Milano (Italy)

(Received May 22nd, 1987; accepted for publication, September 17th, 1987)

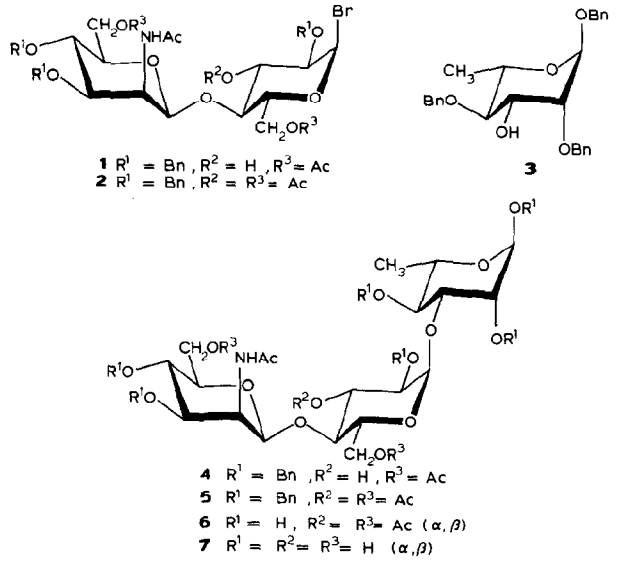
Recently[1] the structure of the type 19A capsular polysaccharide has been reinvestigated; the polysaccharide is linear and can be represented by the repeating unit, →4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1-PO_4^-→. We describe herein the synthesis of the trisaccharide component of the repeating unit of *Streptococcus pneumoniae* type 19A starting from 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-glucopyranosylbromide (**1**), which we have recently employed[2] for the synthesis of the trisaccharide repeating unit of *Streptococcus pneumoniae* type 19F. Condensation of the α-bromide **1**, contaminated by 15% of its 3-*O*-acetyl derivative[2] **2**, with benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside[3] (**3**) in the presence of the promoters 3:1 mercury(II) cyanide–mercury(II) bromide in dichloromethane yielded 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→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**4**), in mixture with its 3′-acetate **5**. This mixture was isolated in ~31% yield. As unreacted **3** was recovered in 42% yield, the yield of the condensation reaction was ~54%.

The α-D configuration of the glucopyranosyl residue bond was ascertained by ^{1}H-n.m.r. spectroscopy. The spectrum of the glycosylation mixture exhibited two doublets, one at δ 5.10 ($J_{1',2'}$ 3.5 Hz) for H-1′ of the 3′-OH free derivative **4**, and one at δ 5.14 ($J_{1',2'}$ 3.5 Hz) for the same H-1′ of the 3′-OAc compound **5**, in a 4:1 ratio; the coupling constants were indicative of an equatorial–axial coupling. The β-D anomer could not be detected either by chromatography or ^{1}H-n.m.r. spectroscopy, showing that the condensation reaction had proceeded with complete α stereoselectivity.

The glycosylation mixture was acetylated to yield benzyl *O*-(2-acetamido-6-

*To whom correspondence should be addressed at: Dipartimento di Chimica e Biochimica medica, Via Saldini 50, 20133 Milano (Italy).

0008-6215/88/$ 03.50



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→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**5**). In this case, the ^{1}H-n.m.r. spectrum showed only one doublet at δ 5.14 (*J* 3.5 Hz) for H-1′. The protecting benzyl groups were cleaved off by hydrogenolysis on palladium–charcoal to afford the 3′,6,6′-triacetylated trisaccharide **6** as an α,β anomeric mixture. Again ^{1}H-n.m.r. spectroscopy indicated an α-D configuration for the glucopyranosyl residue; in the ^{1}H-n.m.r. spectrum of **6**, irradiation of the triplet at δ 5.31 (*J* 9.5 Hz) corresponding to H-3′, caused a collapse to a doublet (*J* 4 Hz) of the double doublet at δ 3.63 (*J* 4 and 9.5 Hz) which, therefore, could be attributed to H-2′. On subsequent irradiation of this last resonance, the doublets at δ 4.98 and 5.03 having equatorial–axial coupling constants of 4 Hz collapsed to two singlets and so could be assigned to H-1′. The triacetate **6** was finally deacetylated by Zemplén's procedure[4] to give the desired trisaccharide **7**.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter. ^{1}H-n.m.r. spectra were recorded with a Varian XL-200 spectrometer for solutions in (^{2}H)chloroform containing Me_4Si as an internal standard, unless otherwise stated. Analytical t.l.c. was performed on Merck 60 F_{254} Silica gel plates (0.25 mm thickness), and the spots were detected either by u.v. irradiation or by spraying with 50% aqueous H_2SO_4 and heating at 110° 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 <40°.

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→3)-2,4-di-*O*-benzyl-α-*L*-rhamnopyranoside* (**5**). — A mixture of benzyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside[4] (**3**, 103 mg), powdered molecular sieves 4A, $Hg(CN)_2$ (60 mg), and $HgBr_2$ (29 mg) in dry dichloromethane (10 mL) was stirred in the dark under Ar for 1 h at room temperature. A solution of the bromide **1** (190 mg) in dry dichloromethane (10 mL) was added dropwise at −40°. The mixture was kept at room temperature under stirring for 2 days, and then was filtered through Celite and evaporated *in vacuo*. The crude mixture (240 mg) was chromatographed on a Silica gel column with 4:1 (v/v) benzene–ethyl acetate. Unreacted **3** (43 mg) was eluted first, and then the α-glycosylation products **4** and **5** (85 mg, 31% yield), followed by a complex mixture of more polar compounds (96 mg), in which a 1-OH-free tetrasaccharide originating by self condensation was probably present; this mixture was not further investigated; R_F (3:2 benzene–ethyl acetate), 0.61 (**4**), 0.52 (**5**); ^{1}H-n.m.r. (**4**): δ 1.17 (d, 3 H, *J* 6 Hz, CH_3), 1.90, 1.99, and 2.08 (3 s, 9 H, 2 OAc and 1 NAc), 3.47 (dd, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$ 10 Hz, H-2'), 3.3–4.3 (m, 14 H, H-2,3,4,5,3',4',5',6',6',3'',4'',5'',6'',6''), 4.54 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1''), 4.80 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.35–4.95 (m, 12 H, 6 CH_2Ph), 4.98 (ddd, 1 H, $J_{1'',2''}$ 1.5, $J_{2'',3''}$ 4.0, $J_{2'',NH}$ 9.5 Hz, H-2''), 5.10 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 5.57 (d, 1 H, $J_{2'',NH}$ 9.5 Hz, NH), 7.10–7.40 (m, 30 H, 6 Ph).

The mixture of **4** and **5** (75 mg) was dissolved into dry pyridine (0.5 mL) and treated with acetic anhydride (0.25 mL). After 18 h at room temperature, the solution was repeatedly evaporated *in vacuo* with toluene to yield 73 mg of the triacetate **5**, $[\alpha]_D^{20}$ −3° (*c* 2.6, chloroform); ^{1}H-n.m.r.: δ 1.28 (d, 3 H, *J* 6 Hz, CH_3), 1.92, 1.99, 2.00, 2.02 (4 s, 12 H, 3 OAc and 1 NAc), 3.3–4.3 (m, 14 H, H-2,3,4,5,2',4',5',6',6',3'',4'',5'',6'',6''), 4.54 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1''), 4.85 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.35–5.05 (m, 13 H, 6 CH_2Ph and H-2''), 5.14 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 5.53 (t, 1 H, *J* 9.5 Hz, H-3'), 5.56 (d, 1 H, $J_{2'',NH}$ 9.5 Hz, NH), 7.10–7.40 (m, 30 H, 6 Ph).

Anal. Calc. for $C_{68}H_{77}NO_{18}$: C, 68.27; H, 6.49; N, 1.17. Found: C, 68.12; H, 6.54; N, 1.29.

*O-(2-Acetamido-6-*O*-acetyl-2-deoxy-β-*D*-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-α-*D*-glucopyranosyl)-(1→3)-α,β-*L*-rhamnopyranose* (**6**). — The trisaccharide **5** (60 mg) was dissolved into methanol (10 mL), treated with Pd–C (10%, 20 mg), and shaken under an H_2 atmosphere for 18 h. The catalyst was filtered off and the solvent evaporated *in vacuo* to yield **6** (29 mg) as an anomeric α,β-mixture, $[\alpha]_D^{20}$ +44° (*c* 1.5, methanol), R_F 0.52 (6:2:1 ethyl acetate–2-propanol–water); ^{1}H-n.m.r. (CD_3OD, one drop D_2O): δ 1.27 (d, 2.55 H, *J* 6 Hz, CH_3-α), 1.31 (d, 0.45 H, CH_3-β), 2.03 (s, 3 H, NAc), 2.09, 2.12, and 2.13 (3 s, 9 H, 3 OAc), 3.63 (dd, 1 H, $J_{1',2'}$ 3.5, $J_{2',3'}$ 9.5 Hz, H-2'), 3.2–4.4 (m, 13 H, H-2,3,4,5,4',5',6',6',3'',4'',5'',6'',6''), 4.42 (dd, 1 H, $J_{1'',2''}$ 1.5, $J_{2'',3''}$ 4.5 Hz, H-2''), 4.63 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1''), 4.71 (br. s, 0.15 H, H-1-β), 4.98 (d, 0.85 H, $J_{1',2'}$ 4 Hz, H-1'-α), 5.03 (d, 0.15 H, $J_{1',2'}$ 4 Hz, H-1'-β), 5.04 (d, 0.85 H, $J_{1,2}$ 1.5 Hz, H-1-α), 5.31 (t, 1 H, *J* 9.5 Hz, H-3').

Anal. Calc. for $C_{26}H_{41}NO_{18}$: C, 47.63; H, 6.30; N, 2.14. Found: C, 47.32; H, 6.44; N, 2.11.

O-*(2-Acetamido-2-deoxy-β-*D*-mannopyranosyl)-(1→4)*-O-*α*-D*-glucopyranosyl-(1→3)-α,β-*L*-rhamnopyranose* (**7**). — The anomeric mixture of **6** (29 mg) was dissolved into methanol (10 mL) and a 1% sodium methoxide solution (0.1 mL) was added. After 18 h under stirring, the solution was neutralized with Dowex 50 (H^+) cation-exchange resin. The resin was filtered off and the solvent was evaporated *in vacuo* to give amorphous **7** (23 mg), $[\alpha]_D^{20}$ +31° (*c* 0.75, water), R_F 0.29 (3:3:1 ethyl acetate–2-propanol–water); ^{1}H-n.m.r. (D_2O): δ 1.11 (d, 1.95 H, *J* 6.5 Hz, CH_3), 1.12 (d, 1.05 H, *J* 5.5 Hz, CH_3), 1.90 (s, 3 H, NAc), 3.2–4.0 (m, 15 H, H-2,3,4,5,2′,3′,4′,5′,6′,6′,3″,4″,5″,6″,6″), 4.38 (dd, 1 H, $J_{1'',2''}$ 1, $J_{2'',3''}$ 4 Hz, H-2″), 4.66 (br. s, 0.35 H, H-1-β), 4.73 (d, 1 H, $J_{1'',2''}$ 1 Hz, H-1″), 4.89 (d, 0.65 H, $J_{1',2'}$ 4 Hz, H-1′-α), 4.92 (d, 0.35 H, $J_{1',2'}$ 4 Hz, H-1′-β), 4.98 (d, 0.65 H, $J_{1,2}$ 1.5 Hz, H-1-α).

Anal. Calc. for $C_{20}H_{35}NO_{15}$: C, 45.37; H, 6.66; N, 2.65. Found: C, 45.08; H, 6.80; N, 2.49.

ACKNOWLEDGMENT

The authors thank Professor G. Russo for helpful discussions.

REFERENCES

1 E. Katzenellenbogen and H. J. Jennings, *Carbohydr. Res.*, 124 (1983) 235–245.
2 L. Panza, F. Ronchetti, G. Russo, and L. Toma, *J. Chem. Soc., Perkin Trans. 1*, (1987) 2745–2747.
3 A. Lipták, P. Fügedi, and P. Nánási, *Carbohydr. Res.*, 65 (1978) 209–217.
4 G. Zemplén, *Ber. Dtsch. Chem. Ges.*, 59 (1926) 1254–1266.