Carbohydrate Research, 229 (1992) 155–160
Elsevier Science Publishers B.V., Amsterdam

Note

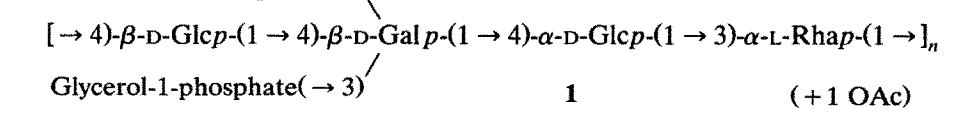
Synthesis of a repeating pentasaccharide fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 18C

Augusta M.P. van Steijn, Jos G.M. van der Ven, Paul van Seeventer, Johannis P. Kamerling, and Johannes F.G. Vliegenthart

Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht (Netherlands)

(Received August 28th, 1991; accepted October 11th, 1991)

In the framework of our investigations on the development of synthetic vaccines[1], based on oligosaccharide conjugates, against infections by *Streptococcus pneumoniae* serotypes, attention has been focused on the preparation of structural elements of the capsular polysaccharide of serotype 18C[2] (**1**), being one of the constituents of Pneumovax© 23. Recently, we have carried out[3] the synthesis of the non-glycerol-phosphorylated elements β-D-Gal*p*-(1 → 4)-α-D-Glc*p*-(1 → 3)-α-L-Rha*p*-OMe and α-D-Glc*p*-(1 → 2)-[β-D-Glc*p*-(1 → 4)]-β-D-Gal*p*-OMe, and the glycerol-phosphorylated elements *sn*-Glycerol-(3-P → 3)-β-D-Gal*p*-(1 → 4)-α-D-Glc*p*-(1 → 3)-α-L-Rha*p*-OMe and α-D-Glc*p*-(1 → 2)-[*sn*-Glycerol-(3-P → 3)]-[β-D-Glc*p*-(1 → 4)]-β-D-Gal*p*-OMe.



1 (+1 OAc)

For the synthesis of the non-glycerol-phosphorylated pentasaccharide repeating unit, α-D-Glc*p*-(1 → 2)-[β-D-Glc*p*-(1 → 4)]-β-D-Gal*p*-(1 → 4)-α-D-Glc*p*-(1 → 3)-L-Rha*p* (**2**αβ), of the capsular polysaccharide of *S. pneumoniae* type 18C, the trisaccharide derivative benzyl 2,4-di-*O*-benzyl-3-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside[3] (**3**) was used as a key intermediate. This intermediate offers also the possibility to prepare the glycerol-phosphate-containing analogue of **2**.

Deacetylation of **3** (→ **4**), followed by benzylidenation afforded **5** (77% from **3**). Coupling of **5** with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[4] in ether

Correspondence to: Dr. J.P. Kamerling, Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands.

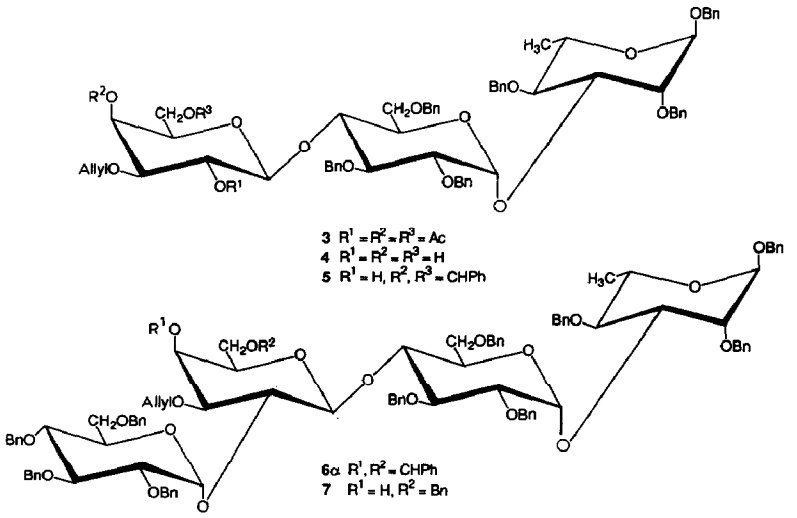
TABLE I

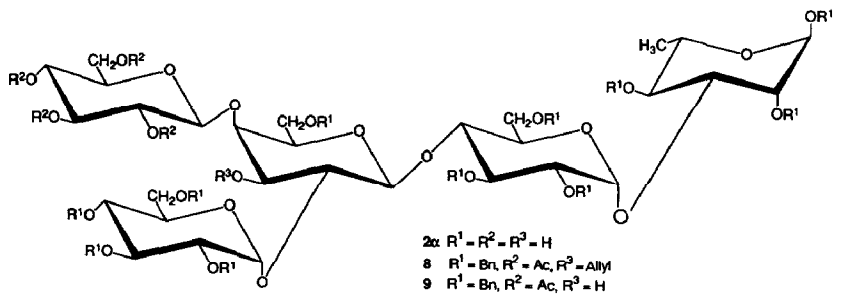
500-MHz ^{1}H-NMR data [a] for pentasaccharide **2**

Proton/J	δ(ppm)/J (Hz)						
	α-Rha	β-Rha	α-Glc′(α) [b]	α-Glc′(β) [b]	β-Gal″	α-Glc‴	β-Glc⁗
H-1	5.153	4.863	5.081	5.107	4.655/2 [c]	5.338	4.672
H-2	4.126	4.147	3.625	3.625	3.758	3.519	3.355
H-3	3.861	3.683	3.939	3.941	3.864	3.733	3.507
H-4	3.546	3.476	3.811	3.803	4.189	3.443	3.423
H-5	3.906 [d]	3.453 [d]	4.128	4.110	n.d.	4.083	3.42
CH_3	1.292	1.309	–	–	–	–	–
$J_{1,2}$	1.8	~0	3.8	3.8	8.1	3.9	8.1

[a] Chemical shifts are relative to the signal of internal acetone (δ 2.225 ppm in D_2O). [b] Doubling of the α-Glc′ series is due to the anomers of Rha. [c] Two chemical shift values due to the anomers of Rha. [d] Assignment of the anomeric configuration is also based on the δ values of Rha H-5 (ref. 11).

with methyl triflate[5] as the promoter yielded the tetrasaccharide derivatives **6α** (50%) and **6β** (26%), having the additional glucosyl unit in α- and β-(1 → 2)-linkages, respectively. Selective opening of the 4,6-*O*-benzylidene ring of **6α**, using sodium cyanoborohydride–hydrochloric acid in tetrahydrofuran[6], gave the corresponding 6-benzyl ether (**7**, 51%). Condensation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in dichloromethane–toluene, using silver triflate as the catalyst at −25°, gave **8** (77%). Deallylation[7] of **8** (→ **9**, 61%), then deacetylation, and debenzylation afforded the target compound **2αβ** (91%). The ^{1}H-NMR data of **2**, obtained by 2D COSY and 2D HOHAHA measurements, are presented in Table I.





EXPERIMENTAL

General methods.—^{1}H-NMR spectra (360 and 500 MHz) were recorded at 25° with a Bruker HX 360 or AM 500 spectrometer (Bijvoet Center, Utrecht University). 2D Double-quantum-filtered ^{1}H–^{1}H correlation spectra (2D DQF ^{1}H–^{1}H COSY) were recorded in the phase-sensitive mode[8], and 2D homonuclear Hartmann–Hahn spectra (2D HOHAHA) with a MLEV-17 mixing sequence of 120 ms (ref. 9). ^{13}C-NMR spectra (APT, 50 MHz) were recorded at 25° with a Bruker WP 200 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^{1}H, and to the signal for internal Me_4Si ($CDCl_3$; indirectly to $CDCl_3$, δ 76.9) or external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C.

Column chromatography was performed on Kieselgel 60 (Merck, < 230 mesh) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by charring with H_2SO_4 after examination under UV light. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. In the workup procedures, washings were carried out three times with appropriate quantities of H_2O or aq 5% $NaHCO_3$ unless indicated otherwise. Solvents were evaporated under reduced pressure at 40° (bath). All solvents were distilled from appropriate drying agents.

Because of hygroscopicity, satisfactory elemental analyses of **2αβ**, **8**, and **9** could not be obtained.

*Benzyl 3-*O*-[4-*O*-(3-*O*-allyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-2,3,6-tri-*O*-benzyl-α-*D*-glucopyranosyl]-2,4-di-*O*-benzyl-α-*L*-rhamnopyranoside* (**5**).—To a solution of benzyl 2,4-di-*O*-benzyl-3-*O*-[2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-galactopyranosyl)-α-D-glucopyranosyl]-α-L-rhamnopyranoside[3] (**3**; 349 mg, 0.33 mmol) in MeOH (10 mL) was added sodium methoxide to pH 10. After stirring for 48 h, the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (75 : 25 CH_2Cl_2–EtOAc) of the residue gave **4**, isolated as a syrup. To a solution of **4** in *N*,*N*-dimethylformamide (1 mL) and α,α-dimethoxytoluene (3 mL) was added *p*-toluenesulfonic acid (25 mg). The

solution was stirred overnight (TLC, 95:5 CH_2Cl_2–EtOAc; **5** R_F 0.30), diluted with CH_2Cl_2 (400 mL), washed with aq 5% $NaHCO_3$ and H_2O, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue afforded **5**, isolated as a glass (289 mg, 77%), $[\alpha]_D$ +17° (*c* 1, $CHCl_3$). NMR data ($CDCl_3$): ^{13}C, δ 139.1–137.3 and 128.6–126.3 ($C_6H_5CH_2O$ and C_6H_5CH), 135.0 ($H_2C=CHCH_2O$), 117.1 ($H_2C=CHCH_2O$), 103.6, 100.9 (C-1″ and Ph*C*H), 97.2, 95.0 (C-1,1′), 17.8 (C-6); ^1H, δ 7.412–7.166 (m, 35 H, 7 Ph), 5.459 (m, 1 H, $H_2C=CHCH_2O$), 5.456 (s, 1 H, PhC*H*), 5.309 and 5.197 (2 m, each 1 H, $H_2C=CHCH_2O$), 5.131 (d, 1 H, H-1′), 1.358 (d, 3 H, H-6,6,6), $J_{1',2'}$ 3.5, $J_{5,6}$ 6.1 Hz.

Anal. Calcd for $C_{70}H_{76}O_{15}$: C, 72.64; H, 6.62. Found: C, 72.28; H, 6.62.

Benzyl 3-O-{4-O-[3-O-allyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-O-benzyl-α,β-D-glucopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-α-D-glucopyranosyl}-2,4-di-O-benzyl-α-L-rhamnopyranoside (**6αβ**).—To a solution of **5** (537 mg, 0.46 mmol) and ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside[4] (542 mg, 0.93 mmol) in ether (20 mL), containing powdered 4A molecular sieves (1.5 g), was added methyl triflate[5] (0.5 mL, 4.56 mmol), and the mixture was stirred for 16 h. Triethylamine (2 mL) was added and, after 5 min, the mixture was diluted with CH_2Cl_2 (250 mL), filtered through Celite, washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. Column chromatography [7:3 light petroleum (bp 40–60°)–EtOAc] of the residue gave **6α**, isolated as a white glass (391 mg, 50%), $[\alpha]_D$ +52° (*c* 1, $CHCl_3$), R_F 0.41; and **6β**, isolated as a white glass (205 mg, 26%), $[\alpha]_D$ +21° (*c* 1, $CHCl_3$), R_F 0.64. NMR data ($CDCl_3$): **6α** ^{13}C, δ 139.0–137.2 and 128.9–126.4 ($C_6H_5CH_2O$ and C_6H_5CH), 134.9 ($H_2C=CHCH_2O$), 117.1 ($H_2C=CHCH_2O$), 102.1, 100.9 (C-1″ and Ph*C*H), 97.2, 96.0, and 95.1 (C-1,1′,1‴), 18.0 (C-6); ^1H, δ 7.443–7.055 (m, 55 H, 11 Ph), 5.956 (m, 1 H, $H_2C=CHCH_2O$), 5.471 (s, 1 H, PhC*H*), 5.312 (d, 1 H, H-1‴), 5.144 (d, 1 H, H-1′), 5.255 and 5.076 (2 m, each 1 H, $H_2C=CHCH_2O$), 1.228 (d, 3 H, H-6,6,6), $J_{5,6}$ 6.2, $J_{1',2'}$ 3.8, $J_{1''',2'''}$ 3.4 Hz; **6β** ^{13}C, δ 139.4–137.3 and 129.5–126.5 ($C_6H_5CH_2O$ and C_6H_5CH), 134.9 ($H_2C=CHCH_2O$), 117.0 ($H_2C=CHCH_2O$), 102.4, 102.0, and 101.3 (C-1″,1‴ and Ph*C*H), 97.3, 96.1 (C-1,1′), 17.8 (C-6).

Anal. Calcd for $C_{104}H_{110}O_{20}$: C, 74.35; H, 6.60. Found **6α**: C, 74.13; H, 6.88. Found **6β**: C, 74.19; H, 6.97.

Benzyl 3-O-{4-O-[3-O-allyl-6-O-benzyl-2-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-α-D-glucopyranosyl}-2,4-di-O-benzyl-α-L-rhamnopyranoside (**7**).—To a solution of **6α** (360 mg, 0.21 mmol) and sodium cyanoborohydride[6] (168 mg, 2.7 mmol) in freshly distilled tetrahydrofuran (10 mL), containing 3A molecular sieves (1.5 g), was added satd HCl in ether until the evolution of gas ceased. After 1 h, when TLC [7:3 light petroleum (bp 40–60°)–EtOAc] indicated the reaction to be complete, the mixture was diluted with CH_2Cl_2 (250 mL) and H_2O (50 mL), filtered through Celite, washed with aq 5% $NaHCO_3$ and H_2O, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue gave **7**, isolated as a syrup (183 mg, 51%), $[\alpha]_D$ +45° (*c* 1, $CHCl_3$), R_F 0.58. ^{13}C-NMR data ($CDCl_3$): δ 138.9–137.2 and 128.7–

127.2 ($C_6H_5CH_2O$), 134.0 ($H_2C=CHCH_2O$), 118.0 ($H_2C=CHCH_2O$), 101.7 (C-1″), 97.3, 96.1, and 95.0 (C-1,1′,1‴), 18.0 (C-6).

Anal. Calcd for $C_{104}H_{112}O_{20}$: C, 74.26; H, 6.71. Found: C, 74.59; H, 6.72.

Benzyl 3-O-{4-O-[3-O-allyl-6-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranosyl]2,3,6-tri-O-benzyl-α-D-glucopyranosyl}-2,4-di-O-benzyl-α-L-rhamnopyranoside (**8**).—A solution of silver triflate (160 mg, 0.6 mmol) in toluene (2.5 mL) was added dropwise during 30 min in the dark to a stirred mixture of **7** (132 mg, 78 μmol), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (117 mg, 0.29 mmol), and powdered 4A molecular sieves (500 mg) in CH_2Cl_2 (2 mL) and toluene (2 mL) at −40°. The stirring was continued for 2.5 h at −25°, until TLC (6:1 toluene–acetone) showed the absence of **7** (R_F 0.74). Pyridine was added, and the mixture was diluted with CH_2Cl_2 (150 mL), filtered through Celite, washed with aq 10% sodium thiosulfate (2 × 25 mL) and H_2O, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue afforded **8**, isolated as a glass (122 mg, 77%), $[\alpha]_D$ +32° (*c* 1, $CHCl_3$), R_F 0.59. NMR data ($CDCl_3$): ^{13}C, δ 170.5, 170.2, 169.3, and 168.8 (4 $COCH_3$), 138.9–137.2 and 128.9–127.3 ($C_6H_5CH_2O$), 134.1 ($H_2C=CHCH_2O$), 117.5 ($H_2C=CHCH_2O$), 102.2, 99.2, 97.4, 97.0, and 95.5 (C-1,1′,1″,1‴,1⁗), 20.5–20.4 ($COCH_3$), 18.0 (C-6); ^1H, δ 7.330–7.121 (m, 55 H, 11 Ph), 5.929 (m, 1 H, $H_2C=CHCH_2O$), 2.028, 1.963, and 1.893 (3 s, 6, 3, 3 H, 4 Ac), 1.281 (d, 3 H, H-6,6,6), $J_{5,6}$ 6.2 Hz.

Benzyl 2,4-di-O-benzyl-3-O-{4-O-[6-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-O-benzyl-α-D-glucopyranosyl}-α-L-rhamnopyranoside (**9**).—A solution of **8** (25 mg, 12.4 μmol), tris(triphenylphosphine)rhodium(I) chloride (15 mg, 16 μmol), and 1,4-diazobicyclo[2.2.2]octane (5 mg, 45 μmol) in 7:3:1 EtOH–benzene–H_2O (3 mL) was boiled under reflux[7] for 16 h, then concentrated. The residue was dissolved in 95:5 acetone–H_2O (4 mL) containing mercury(II) oxide (5 mg, 23 μmol) and mercury(II) chloride (20 mg, 74 μmol), and the solution was stirred for 1.5 h at room temperature. When TLC [6:4 light petroleum (bp 40–60°)–EtOAc] indicated complete conversion into **9** (R_F 0.58), the mixture was concentrated and then dissolved in CH_2Cl_2 (100 mL). The solution was washed with aq 30% KBr (3 × 15 mL) and H_2O, dried (Na_2SO_4), filtered, and concentrated. Column chromatography of the residue afforded **9**, isolated as a syrup (15 mg, 61%), $[\alpha]_D$ +57° (*c* 1, $CHCl_3$). ^1H-NMR data ($CDCl_3$): δ 7.377–7.085 (m, 55 H, 11 Ph), 2.072, 2.024, 1.973, and 1.760 (4 s, each 1 H, 4 Ac), 1.196 (d, 3 H, H-6,6,6).

3-O-[4-O-(2-O-α-D-Glucopyranosyl-4-O-β-D-glucopyranosyl-β-D-galactopyranosyl)-α-D-glucopyranosyl]-α,β-L-rhamnopyranose (**2αβ**).—To a solution of **9** (25 mg, 13 μmol) in MeOH (5 mL) was added sodium methoxide to pH 10. After 16 h, the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. Column chromatography (R_F 0.46, 85:10:5 CH_2Cl_2–acetone–MeOH) of the residue afforded deacetylated **9**, isolated as a syrup. To a solution of the residue in

3:2 MeOH–2-propanol (5 mL) containing a few drops of acetic acid was added 10% Pd–C (15 mg), and the mixture was hydrogenolysed at 4 kg/cm^2 for 16 h, filtered, and concentrated to give **2**$\alpha\beta$, isolated as a white powder (9.3 mg, 91%), $[\alpha]_D$ +77° (*c* 1, H_2O). ^{13}C-NMR data (D_2O): δ 105.3 (161 Hz) and 102.9 (163 Hz) (C-1″,1‴), 99.1 (172 Hz) (C-1‴), 96.3 (172 Hz) (C-1′, α-Rha anomer), 96.1 (170 Hz) (C-1′, β-Rha anomer), 94.9 (169 Hz) (C-1α)[10], 94.8 (156 Hz) (C-1β)[10], 62.3, 62.0, 61.5, and 61.0 (C-6′,6″,6‴,6⁗), 18.2 (C-6). For the ^{1}H-NMR data, see Table I.

ACKNOWLEDGMENTS

This investigation was supported by the Netherlands Foundation for Chemical Research (SON/NWO), the Institute of Molecular Biology and Medical Biotechnology (IMB, Utrecht University), and the Netherlands Innovation Directed Programme for Biotechnology (IOP-b). We thank Dr. B.R. Leeflang for recording the 2D ^{1}H-NMR spectra.

REFERENCES

1 J.E.G. van Dam, A. Fleer, and H. Snippe, *Antonie van Leeuwenhoek; J. Microbiol. Serol.*, 58 (1990) 1–47.
2 C. Lugowski and H.J. Jennings, *Carbohydr. Res.*, 131 (1984) 119–129.
3 A.M.P. van Steijn, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*,211 (1991) 261–277.
4 F. Weygand and H. Zeimann, *Justus Liebigs Ann. Chem.*, (1962) 179–198.
5 H. Lönn, *J. Carbohydr. Chem.*, 6 (1987) 301–306.
6 P.J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
7 E.J. Corey and W.J. Suggs, *J. Org. Chem.*, 38 (1973) 3224.
8 D. Marion and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 113 (1983) 967–974.
9 A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
10 K. Bock, C. Pedersen, and H. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 193–225.
11 C. Laffite, A.M. Nguygen Phuoc Du, F. Winternitz, R. Wylde, and F. Pratviel-Sosa, *Carbohydr. Res.*, 67 (1978) 91–103.