Carbohydrate Research, 150 (1986) 285–289
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of *p*-aminophenyl β-D-ribofuranoside 3-(D-ribit-5-yl phosphate)*

PER J. GAREGG†, ROLF JOHANSSON**, INGVAR LINDH, AND BERTIL SAMUELSSON***

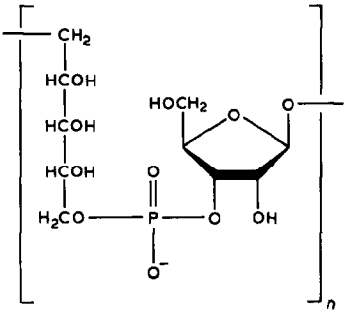
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

(Received June 6th, 1985; accepted for publication, August 30th, 1985)

The bacterium *Haemophilus influenzae* type b, which, *inter alia,* causes meningitis in children, produces a capsular antigen having the structure[1,2] **1**. We have reported the synthesis of 1-*O*-β-D-ribofuranosyl-D-ribitol[3] and its 5-phosphate[4], of which the latter was inactive in inhibition studies. Since the naturally occurring antigen is a diphosphate, we have now synthesised the title compound, which contains a *p*-aminophenyl unit suitable for coupling to a protein[5]. The biological evaluation of the conjugate as a possible artificial antigen will be described elsewhere.

Tritylation of *p*-nitrophenyl β-D-ribofuranoside[6,7] afforded 85% of the 5-trityl ether **2**, monobenzylation of which, using a phase-transfer technique[8], gave 59% of the 2-*O*-benzyl derivative **3**. The constitution of **3** was shown by n.m.r. spectroscopy (see Experimental) and by methylation analysis[9,10].

5-*O*-Trityl-D-ribose[11] was reduced by sodium borohydride, and the product



1

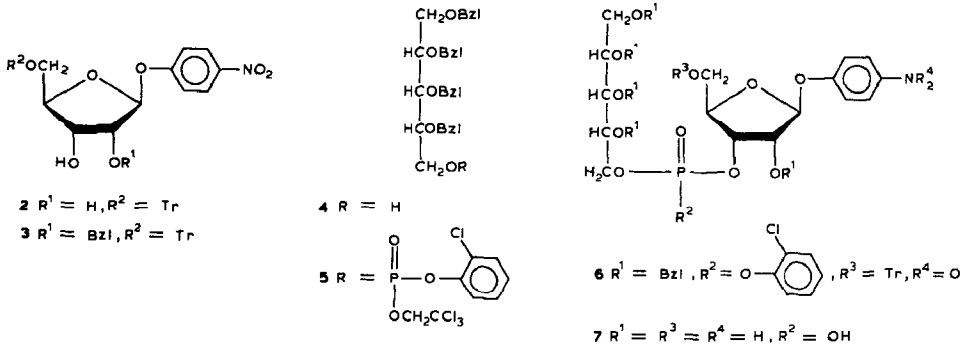
*Dedicated to Professor N. K. Kochetkov.

†Present address: Department of Organic Pharmaceutical Chemistry, Uppsala Biomedical Center, Box 574, S-751 23 Uppsala, Sweden.

**Present address: KabiVitrum AB, S-112 87 Stockholm, Sweden.

***Present address: Astra Pharmaceutical Production, S-151 85 Södertälje, Sweden.

0008-6215/86/$ 03.50



was fully benzylated[12]. Detritylation then gave 49% of 1,2,3,4-tetra-*O*-benzyl-D-ribitol (**4**), which was 5-phosphorylated using 2,2,2-trichloroethyl 2-chlorophenyl phosphorochloridate to yield 98% of the triphosphate **5** (diastereomeric mixture). After removal of the trichloroethyl group from **5** by treatment with zinc powder, the resulting diphosphate was activated with 3-nitro-1-(2,4,6-tri-isopropylbenzenesulfonyl)-1,2,4-triazole and coupled[13] to **3** to give 88% of **6** (diastereomeric mixture). Deblocking of **6** and purification of the product gave 36% of **7** as a zwitterion. Considerable difficulties were experienced in the deblocking of **6**, and the conditions described reflect extensive experimentation. The constitution of **7** follows from its preparation from **6** and from n.m.r. data.

EXPERIMENTAL

General methods. — These were the same as those previously reported[14]. ^{13}C-N.m.r. assignments are based on literature data[15,16]. N.m.r. data accorded with the structures postulated and only relevant data are given below.

p-*Nitrophenyl 5-O-triphenylmethyl-β-D-ribofuranoside* (**2**). — Trityl chloride (2.7 g, 9.6 mmol) was added at room temperature to a stirred solution of *p*-nitrophenyl β-D-ribofuranoside[6,7] (2.0 g, 7.4 mmol) in pyridine (40 mL). After storage for 20 h at room temperature and then 1 h at 50°, the solution was concentrated and the product was eluted from a column of silica gel with toluene–ethyl acetate (1:1) to give **2** (3.2 g, 85%), $[\alpha]_D$ −110° (*c* 1, chloroform). ^{13}C-N.m.r. data [25 MHz, $(CD_3)_2SO$]: δ 63.3 (C-5), 69.9 (C-3), 74.9 (C-2), 82.8 (C-4), 85.7 (Ph_3C), 105.1 (C-1).

p-*Nitrophenyl 2-O-benzyl-5-O-triphenylmethyl-β-D-ribofuranoside* (**3**). — A mixture of **2** (1.9 g, 3.7 mmol), tetrabutylammonium hydrogensulfate (0.25 g, 0.74 mmol), and benzyl bromide (0.75 mL, 6.3 mmol) in dichloromethane (120 mL) and aqueous 5% sodium hydroxide was boiled under reflux for 20 h, and then cooled to room temperature. The dichloromethane layer was washed with water and concentrated, and the residue was eluted from a column of silica gel with toluene–ethyl acetate (7:1) to give **3** (1.3 g, 59%), $[\alpha]_D$ −65° (*c* 1, chloroform). N.m.r. data [$(CD_3)_2SO$]: ^{13}C, δ 63.1 (C-5), 69.8 (C-3), 71.9 ($PhCH_2$, t, off-resonance), 81.8

(C-2), 85.8 (Ph_3C, s, off-resonance), 83.0 (C-4), 102.7 (C-1); ^1H (100 MHz, $CDCl_3$), δ 2.55 (d, J 6.8 Hz, H-3), 3.10 (dd, $J_{4,5}$ 4.5 Hz, $J_{5,5'}$ 10 Hz, H-5), 3.40 (dd, $J_{4,5'}$ 4.5 Hz, $J_{5,5'}$ 10 Hz, H-5'), 4.21 (m, H-4), 4.36 (s, OH), 4.76 (3 H, H-2, OCH_2Ph), 5.76 (d, J 1.0 Hz, H-1).

Addition of trichloroacetyl isocyanate[17] to a solution of **3** in $CDCl_3$ converted the hydroxyl group into a carbamate and resulted in a major down-field shift in the signal for H-3 to δ 5.38 (1 H, dd, $J_{2,3}$ 4.8, $J_{3,4}$ 4.4 Hz). In homonuclear decoupling experiments, couplings of H-3 to H-2 and H-4, of H-4 to H-3 and H-5, and of H-2 to H-1 were demonstrated.

Anal. Calc. for $C_{37}H_{33}NO_2$: C, 73.6; H, 5.5; N, 2.3. Found: C, 73.8; H, 5.7; N, 2.2.

Methylation analysis[9,10] (methylation, hydrolysis, borohydride reduction, acetylation, and g.l.c.–m.s.)[9,10] of **3** gave 1,2,4,5-tetra-*O*-acetyl-3-*O*-methylribitol.

The presumed 3-*O*-benzyl regioisomer of **3** was also isolated (18%).

*1,2,3,4-Tetra-*O*-benzyl-*D*-ribitol* (**4**). — Sodium borohydride (0.50 g, 13.2 mmol) was added at room temperature to a stirred solution of 5-*O*-triphenylmethyl-D-ribofuranose[11] (4.0 g, 10.2 mmol) in dichloromethane–methanol (4:1, 100 mL). After 4 h, acetic acid was added to pH 6, and the solution was diluted with dichloromethane (200 mL) and washed with water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. To a solution of the residue in *N,N*-dimethylformamide (100 mL) was added sodium hydride (1.96 g, 81.6 mmol) and then benzyl bromide (8.38 g, 49.0 mmol) dropwise at 0°. After 18 h at room temperature, methanol (excess) was added, and the solution was diluted with ether (400 mL), washed with water, dried (Na_2SO_4), filtered, and concentrated. A solution of the residue in aqueous 80% acetic acid (150 mL) was kept at 60° overnight and then concentrated to dryness. Column chromatography (4:1 toluene–ethyl acetate) of the residue gave **4** (2.5 g, 49%), $[\alpha]_D$ +13° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 61.4 (t, off-resonance, C-5), 69.8 (t, off-resonance, C-1), 71.8, 72.4, 73.3, 73.9 (4 CH_2Ph), 78.3, 78.9, 79.1 (C-2,3,4).

*1,2,3,4-Tetra-*O*-benzyl-*D*-ribitol 5-(2,2,2-trichloroethyl 2-chlorophenyl phosphate)* (**5**). — 2,2,2-Trichloroethyl 2-chlorophenyl phosphorochloridate (1.2 g, 3.2 mmol) was added to a stirred solution of **4** (0.97 g, 1.9 mmol) in chloroform–pyridine (2:1, 10 mL). After 2 h, the solution was diluted with chloroform (20 mL), washed sequentially with 0.5M sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (10:1 toluene–ethyl acetate) of the residue gave diastereomeric **5** (1.5 g, 98%), $[\alpha]_D$ −7° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 68.5 and 68.8 ($J_{C,P}$ 6.1 Hz, C-5), 69.1 (C-1), 72.3, 73.3, 73.6 (4 CH_2Ph), 77.1, 77.3, 77.8 (C-2,3,4), 94.6 (d, $J_{C,P}$ 12.2 Hz, CCl_3).

p-*Nitrophenyl 2-*O*-benzyl-5-*O*-triphenylmethyl-β-*D*-ribofuranoside 3-(2-chlorophenyl 1,2,3,4-tetra-*O*-benzyl-*D*-ribit-5-yl phosphate)* (**6**). — Zinc powder (654 mg, 10 mmol) and acetic acid (60 mg, 1 mmol) were added to a stirred solution of **5** (835 mg, 1 mmol) in pyridine (5 mL). The temperature rose to 35°.

More zinc powder (327 mg, 5 mmol) was added. After 20 min, insoluble material was collected on Celite and washed with chloroform (200 mL). T.l.c. (85:10:5 ethyl acetate–methanol–water) of the filtrate showed a single spot, R_F 0.3. The solution was extracted with M triethylammonium hydrogencarbonate (25 mL), 0.1M triethylammonium hydrogencarbonate (25 mL), and finally aqueous disodium ethylenedinitrilotetra-acetate. The organic layer was concentrated to a syrup, and *p*-nitrophenyl 2-*O*-benzyl-5-*O*-triphenylmethyl-β-D-ribofuranoside (573 mg, 0.95 mmol) was added. The mixture was dried by three co-concentrations with anhydrous pyridine (30 mL). 3-Nitro-1-(2,4,6-tri-isopropylbenzenesulfonyl)-1,2,4-triazole (495 mg, 1.3 mmol) was added, and then just enough anhydrous pyridine to allow stirring. After 10 h at room temperature, the mixture was diluted with chloroform (50 mL), washed with aqueous 5% sodium hydrogencarbonate (20 mL), and water (25 mL), dried (Na_2SO_4), filtered and concentrated. Column chromatography (9:1 toluene–ethyl acetate) of the residue gave diastereomeric **6** (1.13 g, 88%), $[\alpha]_D$ −38° (*c* 1, chloroform). ^{13}C-N.m.r. data [$(CD_3)_2CO$)]: ribosyl residue, δ 62.7 (C-5), 81.6 (C-2), 82.7 and 82.9 ($J_{C,P}$ 6.1 Hz, C-4), 103.5 (C-1).

Anal. Calc. for $C_{74}H_{71}ClNO_{14}P$: C, 70.8; H, 5.7; N, 1.1; P, 2.4. Found: C, 70.9; H, 5.6; N, 1.1; P, 2.3.

p-*Aminophenyl* β-D-*ribofuranoside 3-(*D-*ribit-5-yl phosphate)* (**7**). — A solution of **6** (1.0 g, 0.81 mmol) in pyridine (2 mL) containing a few drops of water was treated with pyridine-2-aldoxime (0.28 g, 2.3 mmol) and *N,N,N,N*-tetramethylguanidine (0.27 g, 2.3 mmol) at room temperature for 6 h. T.l.c. (85:10:5 ethyl acetate–methanol–water) then showed complete disappearance of **6** and the presence of a slower-moving compound. The mixture was diluted with chloroform (100 mL), washed with water, dried (Na_2SO_4), filtered, and concentrated. The residue was subjected to short-column chromatography (95:5 dichloromethane–methanol). The appropriate fractions were concentrated, and a solution of the residue in 2% trifluoroacetic acid in dichloromethane (10 mL) was stored at 20° for 5 h, then washed with aqueous sodium hydrogencarbonate, dried, filtered, and concentrated. Short-column chromatography of the residue, as described, yielded a syrupy product, a solution of which in ethyl acetate (50 mL) was hydrogenated at atmospheric pressure over platinum oxide (100 mg), prehydrogenated in ethyl acetate (25 mL). The hydrogen uptake corresponded to the conversion of a nitro into an amino group. The product was then hydrogenated at 400 kPa over 10% Pd/C (350 mg) in aqueous 95% ethanol (50 mL) containing aqueous 37% hydrochloric acid (60 mg) for 24 h. The product was purified by passage through a column of Bio-Gel P-2 (elution with water) to give **7** (127 mg, 36%), $[\alpha]_D$ −51° (*c* 1, water). N.m.r. data: ^{13}C (100 MHz, D_2O, 1,4-dioxane, δ 67.4000), δ 63.37 and 63.44 (C-5 ribose, C-1 ribitol), 67.85 ($J_{C,P}$ 5.5 Hz, C-4 ribitol), 72.04 ($J_{C,P}$ 7.9 Hz, C-5 ribitol), 72.78, 73.18 (C-2,3 ribitol), 75.03 (d, $J_{C,P}$ 3.7 Hz, C-3 ribose), 75.30 (d, $J_{C,P}$ 5.49 Hz, C-2 ribose), 83.79 (d, $J_{C,P}$ 6.1 Hz, C-4 ribose), 106.56 (C-1 ribose), 119.4, 120.7 (aromatic C), aromatic C-N^+H_3 and C–O not recorded; ^{31}P (162 MHz, D_2O, H_3PO_4 0.00 p.p.m.), δ 0.29.

ACKNOWLEDGMENTS

We thank Professors B. Lindberg and J. Stawiński for valuable discussions, and the Swedish Natural Science Research Council and the National Swedish Board for Technical Development for financial support.

REFERENCES

1 R. M. Crisel, R. S. Baker, and D. E. Dorman, *J. Biol. Chem.*, 250 (1975) 4926–4930.
2 P. Branefors-Helander, C. Erbing, L. Kenne, and B. Lindberg, *Acta Chem. Scand., Ser. B*, 30 (1976) 276–277.
3 P. J. Garegg, B. Lindberg, and B. Samuelsson, *Carbohydr. Res.*, 58 (1977) 219–221.
4 P. J. Garegg and B. Samuelsson, *Carbohydr. Res.*, 86 (1980) 293–296.
5 D. H. Buss and I. J. Goldstein, *J. Chem. Soc., C*, (1968) 1457–1461.
6 E. F. Recond and H. Rinderknecht, *Helv. Chim. Acta*, 42 (1959) 1171–1173.
7 K. Honma, K. Nakazima, T. Uematsu, and A. Hamada, *Chem. Pharm. Bull.*, 24 (1976) 394–399.
8 P. J. Garegg, T. Iversen, and S. Oscarson, *Carbohydr. Res.*, 50 (1976) c12–c14.
9 H. Björndal, C.-G. Hellerquist, B. Lindberg, and S. Svensson, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
10 P.-E. Jansson, L. Kenne, H. Liedgren, and J. Lönngren, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–75.
11 B. L. Kahn and N. J. Oppenheimer, *Carbohydr. Res.*, 69 (1979) 308–310.
12 J. S. Brimacombe and O. A. Ching, *Carbohydr. Res.*, 8 (1968) 82–88.
13 J. F. M. de Rooij, G. Willie-Hazeleger, P. H. van Deursen, J. Serdijn, and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 98 (1979) 537–548.
14 P. J. Garegg, H. Hultberg, and S. Oscarson, *J. Chem. Soc., Perkin Trans. 1*, (1982) 2395–2397.
15 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
16 G. Zon and J. D. Robbins, *Carbohydr. Res.*, 114 (1983) 103–121.
17 V. W. Goodlett, *Anal. Chem.*, 37 (1965) 431–432.