Carbohydrate Research, 195 (1990) C1–C4
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

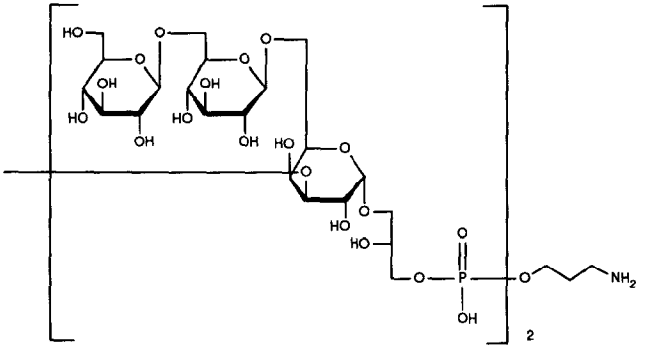
Solid-phase synthesis of a cell-wall component of *Haemophilus (Actinobacillus) pleuropneumoniae* serotype 2

GERRIT H. VEENEMAN, HUMPHREY F. BRUGGHE, HANS VAN DEN ELST, AND JACQUES H. VAN BOOM*
Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden (The Netherlands)

(Received May 30th, 1989; accepted for publication, November 27th, 1989)

Haemophilus (Actinobacillus) pleuropneumoniae (HPP) is a worldwide causative agent of porcine infections[1,2] that are highly pathogenic when inoculated by the respiratory route. Ten serotypes based on HPP capsular polysaccharides (CPS) have been reported[3].

Perry *et al.*[4] showed that the CPS from HPP serotype 2 (HPP-2) comprises tetrasaccharide units linked by phosphodiester bonds, but the presence of either *sn*-glycerol 1- or 3-phosphate moieties was not determined. The synthesis of the CPS-HPP-2 fragment **1**† covalently linked to a spacer molecule, and with two *sn*-glycerol 3-phosphate linkages, has been published[5].



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We now report a solid-phase synthesis[6] of the HPP-2 fragment **2** that contains a covalently linked spacer suitable for conjugation with macromolecular carriers.

The first stage involved preparation of the glycerol unit **8** attached to a solid

* Author for correspondence.

† The glycerol stereochemistry of the structures **7**, **8**, **10–12**, **16**, **17**, **19**, and **20–26** in the previous publication[5] has the glycerol configuration wrongly drawn in every unit. Thus, structure **26** is now correctly presented as **1**.

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support. Tritylation of 1-*O*-allyl-*sn*-glycerol (**3**), obtained by allylation[7] of 2,3-*O*-isopropylidene-*sn*-glycerol[8] and subsequent acid hydolysis, with 4,4′-dimethoxytrityl chloride (DMTr-Cl) gave **4**, $[\alpha]_D$ +3.8° (*c* 2)*, R_F 0.34 (1:1 ether–hexane). Benzylation of **4** furnished **5** (72%), R_F 0.85 in 1:1 ether–hexane. Deallylation of **5** by the two-step method of Oltvoort *et al.*[9] afforded **6** (89%), $[\alpha]_D$ +40° (*c* 2), R_F 0.58 (97:3 dichloromethane–acetone). Acylation of **6** with succinic anhydride and a catalytic amount of 4-dimethylaminopyridine (DMAP) in pyridine gave **7** (82%), $[\alpha]_D$ +22° (*c* 2), R_F 0.35 (95:5 dichloromethane–acetone). Immobilization of **7** on aminopropyl-functionalized controlled porous glass (CPG)[10] was achieved with di-isopropylcarbodi-imide and 1-hydroxybenzotriazole to yield, after acetylation with 0.25M DMAP in acetic anhydride–collidine–acetonitrile (1:1:8), immobilized **8** with a loading capacity of 51 μmol.g^{-1} of resin, as gauged by spectrophotometry[10] of the dimethoxytrityl cation released by acidolysis (λ_{max} 489 nm).

In the second stage, selective dimethoxytritylation at the primary position of **10** (ref. 5) afforded **11**, $[\alpha]_D$ +16° (*c* 2), R_F 0.62 (95:5 dichloromethane–acetone), which was treated with the reagent **12** (ref. 11) to give the donor unit **13** (95%) as a mixture of diastereoisomers (R_F 0.70; 95:5 dichloromethane–acetone). ^{31}P-N.m.r. data (CD_3CN): δ 149 and 151.

In the final stage, elongation of **8** with **13** was achieved by the DNA solid-phase synthesis protocol[12], using an automated DNA Synthesizer. Each elongation cycle (Table I) involved four chemical steps, each of which was preceded by washing the solid support for 2 min with anhydrous acetonitrile. The preparation of **14** involved four consecutive cycles. The coupling step 2 had to be repeated in order to ensure an acceptable coupling efficiency (85%). Acidolysis (step 1) of **14** and subsequent reaction of **15** with the reagent **16** (ref. 13) in the presence of 1*H*-tetrazole, followed by oxidation (step 4), gave immobilized **17**. Desuccinylation, deacetylation, and elimination of the P(V)-protecting groups of **17** by ammonolysis for 17 h at 50° furnished partially deblocked **18**, which, in turn, was purified by chromatography on Sephadex LH-20 and

TABLE I

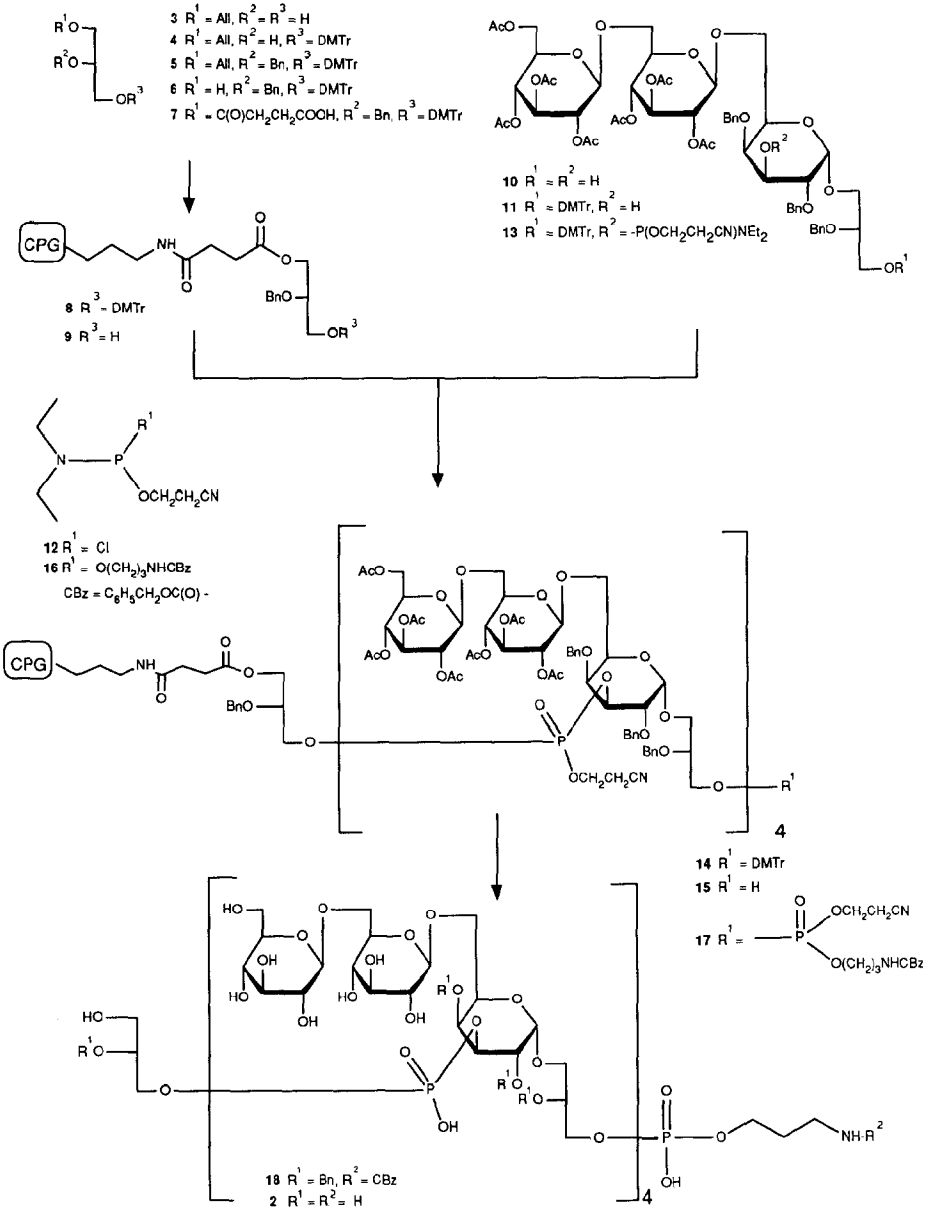
Chemical steps involved in each elongation cycle

Step	*Manipulation*	*Solvents and reagents*[a]	*Time (min)*
1	Detritylation	2% Trichloroacetic acid in CH_2Cl_2	3
2	Coupling	**13**[b], 1-*H*-tetrazole[c], MeCN	30[d]
3	Capping	0.25M 4-Dimethylaminopyridine in Ac_2O–collidine–MeCN (1:1:8)	3
4	Oxidation	0.02M I_2 in MeCN–collidine–H_2O (10:1:5)	3

[a] Reactions were performed on 200 mg (10 μmol) of resin **8**. [b] 0.1M **13** (0.3 mL) in MeCN. [c] 0.5M 1-*H*-Tetrazole (0.6 mL) in MeCN. [d] Step 2 was executed twice.

* Values of $[\alpha]_D$ were measured for solutions in chloroform at 25°, unless noted otherwise. Compounds for which $[\alpha]_D$ values are recorded gave satisfactory elemental analyses.

converted into the corresponding Na^+ salt by using a cation-exchange resin. Hydrogenolysis of the Na^+ salt of **17** in the presence of 10% Pd–C in aqueous methanol afforded the target HPP-2 fragment **2** (17%), $[\alpha]_D$ +13.5° (*c* 0.2, water). The ^{31}P-n.m.r. spectrum of **2** contained signals at δ 1.34 and 0.75 in the ratio of 1:4, and the ^{1}H-n.m.r. spectrum (D_2O, 20°) contained signals for anomeric protons at δ 4.98 (m, 4 H) for the four α-D-galactopyranosyl residues, and 4.48 (m, 8 H) for the eight β-D-glucopyranosyl



residues, in addition to signals at δ 4.30 (m, 4 H, Gal H-3), 4.20 (m, 4 H, Gal H-4), and 3.13 (t, 2 H, aminopropyl H-3), in good agreement with the data for natural[4] and synthetic[5] samples of similar structures.

The above solid-phase approach for synthesis of **2** and other [6-14] structurally related teichoic acid-type capsular polysaccharides may also facilitate the synthesis of other naturally occurring teichoic acids, the repeating units of which are linked by phosphodiester bonds. Moreover, the spacer-containing polymer **2** may open the way to the construction of a synthetic vaccine against HPP-2.

ACKNOWLEDGMENTS

We thank Dr. C. Erkelens for recording the n.m.r. spectra, and the staff of the Microanalytical Laboratory, University College, Dublin for the elemental analyses.

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