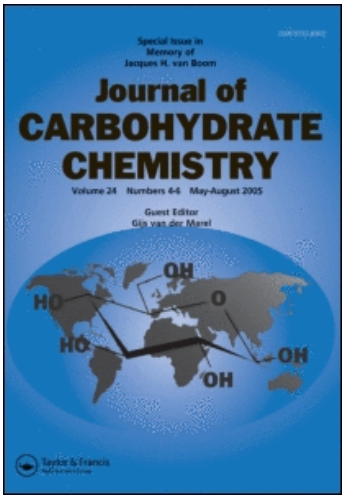


## Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

# Solid-Phase Synthesis of an Analog of Haemophilus Influenzae Type B Capsular Polysaccharide

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**To cite this Article** Nilsson, Magnus and Norberg, Thomas(1998) 'Solid-Phase Synthesis of an Analog of Haemophilus Influenzae Type B Capsular Polysaccharide', Journal of Carbohydrate Chemistry, 17: 2, 305 — 316
**To link to this Article: DOI:** 10.1080/07328309808002330
**URL:** http://dx.doi.org/10.1080/07328309808002330

J. CARBOHYDRATE CHEMISTRY, 17(2), 305-316 (1998)

# SOLID-PHASE SYNTHESIS OF AN ANALOG OF HAEMOPHILUS INFLUENZAE TYPE B CAPSULAR POLYSACCHARIDE

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*Received March 18, 1997 - Final Form November 12, 1997*



## ABSTRACT

A pentameric spacer-containing glyceryl-ribitol phosphate structure **2** was synthesized using a solid-phase protocol. The *H*-phosphonate **16**, synthesized from a D-ribitol derivative and (*S*)-1,2-*O*-isopropylideneglycerol, was used as monomer. Compound **2** is a simplified fragment of *Haemophilus influenzae* capsular polysaccharide, where the glyceryl part replaces the original ribosyl moiety.

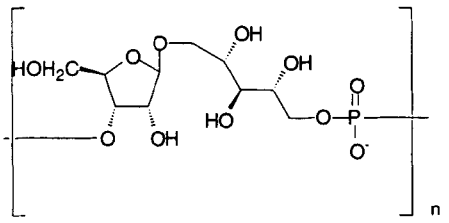
## INTRODUCTION

Modern vaccines for prevention of Haemophilus influenzae type b (Hib) infections consist of PRP (polyribosylribitol phosphate, **1**) conjugated to a carrier protein. Several such vaccines have recently been licenced in the USA and elsewhere for pediatric use. The PRP used in these vaccines is isolated from bacterial cell culture supernatants, and therefore hetereogeneous in molecular size. To investigate a corresponding chemical method of preparation (that would give a PRP of more defined size) we previously synthesized[2,3] oligomers 5 or 10 monomers in length. These oligomers were terminated by a chemical group

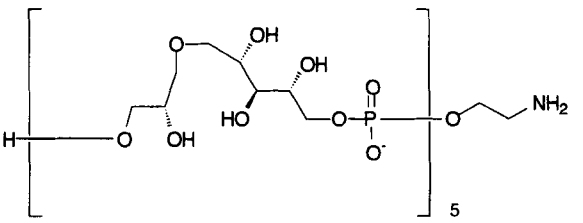
suitable for protein conjugation. Conjugates prepared from these oligomers gave[4] high titers in monkeys against PRP in preliminary immunization experiments. These results are in accordance with earlier observations,[5-7] that it is possible to induce anti-PRP antibodies with conjugates of small, oligomeric structures, as well as with larger PRP conjugates. Thus, it appears that the requirements on molecular size are not very strict for PRP antibody induction.

We have now started an investigation of the corresponding requirements on PRP backbone structure. It is known,[8,9] that there is antigenic and immunological cross-reactivity between HiB PRP and the structurally similar E. coli capsular polysaccharide, which is identical in composition, but different in linkage (1-5 and 1-2 phosphodiester, respectively). Therefore, it appears that considerable structural variation can be tolerated, provided that the phosphodiester structure is intact. To verify this, we have synthesized a simplified pentameric PRP analog (**2**), where a glyceryl group replaces the original ribosyl unit, and thus the acid-labile glycosidic linkage is replaced by a more stable ether linkage. The phosphodiester linkage is left unchanged. The ability of this analog to bind to or elicit anti-PRP antibodies will be investigated and compared to that of the original structure.





**1**



**2**

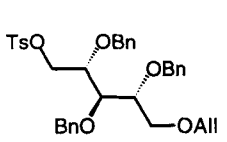
## RESULTS AND DISCUSSION

### Monomer synthesis:

The monomers **15** and **16**, used in the solid-phase synthesis of **2**, were synthesized as follows:

Treatment of 5-*O* -allyl-2,3,4-tri-*O*-benzyl-D-ribitol[2] with *p*-toluenesulphonyl chloride in pyridine gave the corresponding tosylate **3**, the tosyl group of which was displaced with (*S*)-1,2-*O*-isopropylidene-glycerol **4** (sodiumhydride / *N,N*-dimethylformamide), to give compound **5** in a yield of 66 %. This compound was then subjected to the reaction sequence deisopropylidenation (aq. 80 % acetic acid), tritylation (trityl chloride, pyridine),

benzylation (benzyl bromide, sodium hydride) and detritylation (aq. 80 % acetic acid) to give **9** in 74 % yield. Benzoylation (benzoyl chloride, pyridine) of **9** followed by deallylation (first tris(triphenylphosphine)rhodium(I) chloride, then aq. 80 % acetic acid) gave **11** in 82 % yield. Monomethoxytritylation (monomethoxytrityl chloride in pyridine) followed by debenzoylation (sodium methoxide) gave compound **13** in 82 % yield. Treatment of **13** with successively, succinic anhydride and 4-nitrophenol/*N,N'*-diisopropylcarbodiimide gave **15**

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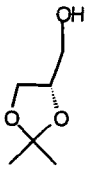


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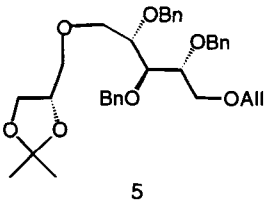


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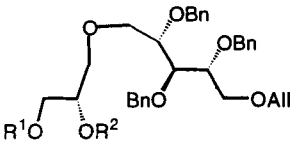
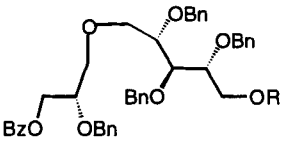
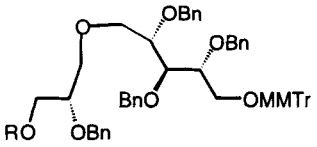


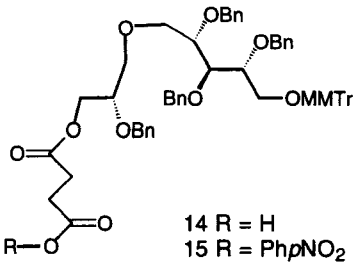

6 $R^1 = R^2 = H$
7 $R^1$ = Tr, $R^2 = H$
8 $R^1$ = Tr, $R^2$ = Bn
9 $R^1 = H$, $R^2$ = Bn



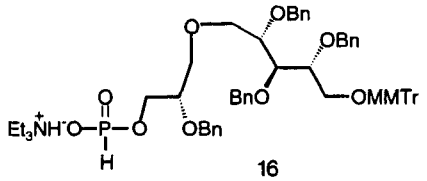

10 R = All
11 R = H



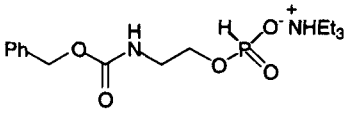

12 R = Bz
13 R = H




14 R = H
15 R = Ph*p*$NO_2$




16




17

(89 % yield), suitable as chain initiation monomer in the solid-phase synthesis. Treatment of compound **13** with phosphorous acid and 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane in pyridine gave the *H*-phosphonate **16** (96 % yield), suitable for use as chain propagation monomer in the solid-phase synthesis. The terminating compound **17** was prepared in 46 % yield from CBZ-ethanolamine[10] by treatment with an excess of phosphorous trichloride and imidazole in acetonitrile, followed by hydrolysis.

**Solid-phase synthesis**

With the monomers **15, 16** and **17** in hand, the solid-phase synthesis and subsequent deprotection were carried out, essentially as previously described.[2] The coupling yields (estimated by spectrophotometric determination of released monomethoxytrityl cation in the next cycle) were in the 90 - 98 % range. Some additional observations were made: 1) A high concentration of the monomer **16** during the coupling step was important. Concentrations below those given in the experimental part resulted in lower coupling yields, which could not be improved by double-coupling. This again[2] indicates, that reaction of the resin OH-groups with some reactive species other than the *H*-phosphonate is causing a reduction in coupling yields ("capping"). 2). The first deprotection attempt gave considerable amounts of what appeared (MALDI-TOF MS, NMR) to be monomethyl phosphotriester derivatives of **2**. Their occurrence was most probably due to side-reactions during the oxidation and cleavage steps. A cleaner product was obtained in subsequent attempts, where the water content in the pyridine-water-iodine oxidation mixture as well as the reaction time were increased.

The final product **2** was obtained, after gel filtration on a Bio-gel P2 column, in 70 % yield, slightly contaminated (according to NMR and MALDI-TOF MS) by shorter homologs and monomethyl phosphotriesters.

## EXPERIMENTAL

**General Methods.** Concentrations were performed at reduced pressure (<40 °C bath temperature). Optical rotations were measured at 23 °C (*c* 1.0, chloroform) unless otherwise stated, using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded for solutions in $CDCl_3$ (internal $Me_4Si$, $\delta = 0.00$) or $D_2O$ ($^1H$ and $^{13}C$, internal acetone, $\delta$ = 2.225 and 30.7, respectively, $^{31}P$, external 85% $H_3PO_4$ in $D_2O$, $\delta = 0.00$) at 303 °K with Varian VXR-400, Bruker DRX 400, DRX 500a or DRX 600 spectrometers. Only selected NMR data is reported.

Assignments were corroborated by appropriate 2-D experiments. Pairwise assignments marked with an asterisk* could be reversed. High-resolution mass spectra were recorded with a JEOL JMS-SX/SX-102A mass spectrometer, using the FAB ion source and glycerol as matrix. For MALDI-TOF mass spectrometry, a LDI-1700XS instrument was used (negative ion mode). The MALDI MS sample was prepared by treating a solution (2 mg /mL $H_2O$) of compound **2** with ion exchange resin ($NH_4^+$ form), then 4 μL of the supernantant was mixed with 20 μL of a matrix solution (2,4,6-trihydroxyacetophenone, 0.5 M in methanol) containing 5 μL aq. 0.1 M ammonium citrate and the mixture was then applied onto the spectrometer probe. TLC was performed on Silica Gel $F_{254}$ (Merck, Darmstadt, Germany) with detection by UV light and by charring with 5% sulfuric acid in ethanol or 1 % ninhydrin in 1-butanol. Column chromatography was performed on silica gel (0.035-0.070 mm, Matrex LC 60A, Grace, Helsingborg, Sweden). Silanizations were performed by treating the glassware for 1 min with a solution of 5 % dichlorodimethylsilane in pentane, and then drying. Molecular sieves (4Å, rods) for solvents, were freshly dried at 300 °C/0.5 torr overnight. Dichloromethane was distilled from $P_2O_5$ when necessary. Pyridine was of pro analysi quality, and when appropriate, was first distilled from ninhydrin and then fractionally distilled from anhydrous barium oxide. *N,N*-dimethylformamide was of pro analysi qualiy and dried with 4Å molecular sieves when appropriate. Pivaloyl chloride (99 %) was redistilled before use. Bio-gel P2 columns were packed and eluted with degassed aq. 5% 1-butanol. (*S*)-1,2-*O*-isopropylideneglycerol was purchased from Sigma-Aldrich Chemical Co (St. Louis, USA). Aminomethyl resin was from Bachem Feinchemikalien AG (Switzerland).

**5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-[(*S*)-1'-deoxy-2',3'-*O*-isopropylidene-glyceryl]-D-ribitol (5).** A mixture of 5-*O*-allyl-2,3,4-tri-*O*-benzyl-D-ribitol[2] (4.63 g, 10.0 mmol) and pyridine (84 mL) was stirred and cooled (0° C) while *p*-toluenesulphonyl chloride (3.83 g, 20.1 mmol) was added. When addition was complete, the mixture was further stirred at rt overnight. Water (2 mL) was then added to destroy excess reagent, and the mixture was diluted with dichloromethane, washed with aq. 2 M sulphuric acid, aq. 1 M sodium hydrogen carbonate, water, dried ($MgSO_4$), filtered and concentrated. A solution of the residue, containing compound **3**, and (*S*)-1,2-*O*-isopropylideneglycerol (9.17 g, 69 mmol) in *N,N*-dimethylformamide (70 mL) was added dropwise to a cooled mixture (0° C) of sodium hydride (80 % in mineral oil, 2.94 g, 98 mmol) in *N,N*-dimethylformamide (75 mL). After complete addition, the reaction mixture

formed a brown solid which was converted to a slurry through addition of more *N,N*-dimethylformamide (100 mL). The reaction mixture was stirred overnight at rt, then methanol was added to destroy excess sodium hydride. The solution was partitioned between toluene and water, and the aqueous layer was washed with toluene. The combined toluene layers were washed with water, dried ($MgSO_4$), filtered and concentrated. Column chromatography with ethyl acetate in toluene (0-20 %, stepwise gradient elution) of the residue gave **5** (3.84 g, 6.6 mmol, 66 %) as a colorless syrup, $[\alpha]_{578}$ +1°. NMR data ($CDCl_3$): $^{13}C$, δ 25.5, 26.8 ($C(\boldsymbol{C}H_3)_2$), 67.0 (C-3'), 70.1, 71.9, 72.2, 72.4, 72.4, 72.5, (C-1, 5, $O\boldsymbol{C}H_2Bn$, $OCH_2\boldsymbol{C}H{=}CH_2$), 73.8 (C-1') 74.7 (C-2'), 78.4, 78.6, 78.8 (C-2, 3, 4), 109.3 ($\boldsymbol{C}(CH_3)_2$), 116.7 ($OCH_2CH{=}\boldsymbol{C}H_2$), 134.9 ($OCH_2\boldsymbol{C}H{=}CH_2$); $^1H$, δ 1.35, 1.40 (2 s, $C(C\boldsymbol{H}_3)_2$), 3.40 (dd, $J_{1a',2'}$ = 5.8 Hz, $J_{1a',1b'}$ = 9.8 Hz, H-1a'), 3.50 (dd, $J_{1b',2'}$ = 5.8 Hz, H-1b'), 3.71 (m, H-3a'), 4.00 (dd, $J_{2',3b'}$ = 6.5 Hz, $J_{3a',3b'}$ = 8.3 Hz, H-3b'), 4.22 (p, H-2'), 5.14, 5.16, 5.23, 5.27 (4 m, $CH{=}C\boldsymbol{H}_2$) 5.89 (m, $C\boldsymbol{H}{=}CH_2$). HRMS: Calcd for $C_{35}H_{45}O_7$: 577.3165. Found 577.3227 ($M+H^+$).

**5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-[(*R*)-2'-*O*-benzyl-1'-deoxyglyceryl]-D-ribitol (9)**. Compound **5** (2.85 g, 4.9 mmol) in aq. 80 % acetic acid (50 mL) was stirred for 46 h at 50 °C and the reaction mixture was concentrated and co-concentrated with toluene. The residue, containing compound **6**, was dissolved in pyridine (40 mL) and trityl chloride (2.33 g, 8.3 mmol) was added. The mixture was stirred at 50 °C overnight, and the reaction mixture was then diluted with toluene and concentrated. The residue was partitioned between 2:1 dichloromethane-water and the organic layer was washed with aq. 2 M sulphuric acid, aq. 1 M sodium hydrogen carbonate, water, dried ($Na_2SO_4$), filtered and concentrated. The residue, containing compound **7**, and benzyl bromide (1.75 mL, 14.7 mmol) in *N,N*-dimethylformamide (40 mL) was added dropwise to a stirred slurry of sodium hydride (80 % in mineral oil, 0.485 g, 16.2 mmol) in *N,N*-dimethylformamide (50 mL). After complete addition, the reaction mixture was stirred for 36 h at rt, then methanol was added to destroy excess sodium hydride. The mixture was partitioned between toluene and water, and the aqueous layer was extracted with toluene. The combined toluene layers were washed with water, dried ($Na_2SO_4$), filtered and concentrated. The residue, containing compound **8**, was dissolved in aq. 80 % acetic acid (50 mL) and the solution was stirred overnight at 50 °C, after which the reaction mixture was concentrated and co-concentrated with toluene. Column chromatography with ethyl acetate in toluene (stepwise gradient elution, 0-20 %) of the crude product gave **9** (2.31 g, 3.6 mmol, 74 %) as a syrup, $[\alpha]_{578}$ +3°. NMR data ($CDCl_3$): $^{13}C$, δ 62.8 (C-3'), 70.0,

71.5, 71.7, 72.1, 72.2, 72.4, 72.4, 73.8 (C-1, 5, 1', O$CH_2$Bn, O$CH_2$CH=$CH_2$), 77.9, 78.4, 78.4, 78.7 (C-2, 3, 4, 2'), 116.8 (OCH$_2$CH=$CH_2$), 134.9 (OCH$_2$$CH$=CH$_2$). HRMS: Calcd for $C_{39}H_{47}O_7$: 627.3322. Found 627.3345 (M+H$^+$).

**1-*O*-[(*S*)-3'-*O*-Benzoyl-2'-*O*-benzyl-1'-deoxyglyceryl]-2,3,4-tri-*O*-benzyl-D-ribitol (11).** Benzoyl chloride (0.83 mL, 7.15 mmol) was added dropwise to a stirred, cooled (0 °C) solution of **9** (2.29 g, 3.6 mmol) in pyridine (40 mL). After complete addition, the reaction mixture was stirred another 150 min at rt. Water was added to destroy excess benzoyl chloride, and the mixture was diluted with dichloromethane, washed with aq. 2 M sulphuric acid, aq. 1 M sodium hydrogen carbonate, water, dried ($MgSO_4$), filtered and concentrated. The residue, containing compound **10**, was dissolved in ethanol-toluene-water 40:16:5 (61 mL), tris(triphenylphosphine)-rhodium(I)chloride (0.165 g, 0.18 mmol) was added, and the mixture was refluxed overnight, then diluted with water and extracted with ethyl ether. The combined ether layers were washed with aq. saturated potassium chloride, dried ($Na_2SO_4$), filtered and concentrated. The residue was dissolved in aq. 80 % acetic acid (50 mL) and the solution was stirred at rt overnight, then the mixture was concentrated, and coconcentrated with toluene. The residue was purified by column chromatography with ethyl acetate in toluene (stepwise gradient elution, 0-25 %) to give **11** (2.03 g, 2.93 mmol, 82 %) as a syrup, $[\alpha]_{578}$ +11°. NMR data ($CDCl_3$): $^{13}$C, δ 61.4 (C-5), 64.4 (C-3'), 71.2, 71.5, 72.0, 72.2, 72.6, 74.0 (C-1, 1', O$CH_2$Bn), 75.9, 78.3, 78.9, 79.1 (C-2, 3, 4, 2'), 166.3 (C=O). HRMS: Calcd for $C_{43}H_{47}O_8$: 691.3271. Found 691.3358 (M+H$^+$).

**2,3,4-Tri-*O*-benzyl-1-*O*-[(*R*)-2'-*O*-benzyl-1'-deoxyglyceryl]-5-*O*-monomethoxytrityl-D-ribitol (13).** Monomethoxytrityl chloride (0.73 g, 2.4 mmol) was added to a solution of **11** (1.25 g, 1.8 mmol), dry pyridine (0.44 mL, 5.4 mmol) and DMAP (0.11 g, 0.90 mmol) in dry dichloromethane (40 mL) at room temperature. After stirring for 3.5 h, the solution was partitioned between aq. 1M sodium bicarbonate and dichloromethane. The aqueous layer was washed with dichloromethane. The combined organic layers were washed with brine, dried ($Na_2SO_4$), filtered and concentrated to obtain crude **12**. The crude **12** was stirred with 0.1 M methanolic sodium methoxide (40 mL) overnight at room temperaure, then Dowex 50 (H$^+$) resin was added until neutral, and the solution was filtered and concentrated. Column chromatography with ethyl acetate - petroleum ether (20-40 %, stepwise gradient elution) gave **13** (1.28 g, 1.5 mmol, 82 %) as a colorless syrup, $[\alpha]_{578}$ -11°. NMR data ($CDCl_3$): $^{13}$C, δ 55.2 (O$CH_3$), 62.9 (C-3'*), 63.7 (C-5), 71.5, 71.9, 72.1, 72.5, 72.6, 73.6 (C-1, 1'*, O$CH_2$Bn), 77.9, 78.7, 78.8, 78.9 (C-2, 3, 4, 2'), 86.4 ($C$(Ar)$_3$); $^1$H, δ 3.35 (dd, $J_{4,5a}$ = 5.7 Hz, $J_{5a,5b}$ = 10.3 Hz, H-5a), 3.43 (dd,

$J_{4,5b}$ = 2.7 Hz, H-5b), 3.48 (dd, $J_{1a',2'}$ = 5.3 Hz, $J_{1a',1b'}$ = 10.1 Hz, H-1a'*), 3.54 (dd, $J_{1b',2'}$ = 4.9 Hz, H-1b'*), 3.61 (m, 2H, H-2', H-3a'*), 3.66 (m, H-3), 3.70 (dd, $J_{2',3b'}$ = 6.6 Hz, $J_{3a',3b'}$ = 14.1 Hz, H-3b'*), 3.75 (s, $OCH_3$), 3.87 (m, H-4). HRMS: Calcd for $C_{56}H_{58}O_8Na$: 881.4029. Found 881.4097 ($M+Na^+$).

**2,3,4-Tri *O*-benzyl-1-*O*-[(*S*)-2'-*O*-benzyl-3'-*O*-{3"-(*p*-nitrophenyloxycarbonyl)propionyl}-1'-deoxyglyceryl]-5-*O*-monomethoxytrityl-D-ribitol (15).** Succinic anhydride (0.22 g, 2.2 mmol) was added to a solution of **13** (0.66 g, 0.77 mmol), DMAP (0.045 g, 0.37 mmol) in pyridine (10 mL) at room temperature. After stirring overnight, the reaction mixture was concentrated and co-concentrated with toluene. The residue was dissolved in chloroform and washed with brine and water. The organic layer was dried ($Na_2SO_4$), and concentrated to dryness to obtain crude **14** as a white foam (0.74 g). Crude **14** (0.50 g, 0.52 mmol) in pyridine (5 mL) was mixed with 4-nitrophenol (0.145 g, 1.04 mmol), the mixture was stirred for 10 min, then *N,N'*-diisopropylcarbodiimide (0.16 mL, 1.0 mmol) was added, and the mixture was stirred overnight at rt. The mixture was then partitioned between 1:1 dichloromethane-water, the organic layer was dried ($Na_2SO_4$), filtered and concentrated. Column chromatography (toluene-ethyl acetate-pyridine 90:10:0.5) of the residue gave **15** (0.50 g, 0.46 mmol, 89 %) as a colorless syrup, $[\alpha]_{578}$ -41° (*c* 0.1). NMR data ($CDCl_3$): $^{13}C$, δ 29.3, 29.7 ($O=CCH_2CH_2C=O$), 55.2 ($OCH_3$), 63.7, 64.5, 70.8, 72.1, 72.1, 72.5, 72.6, 73.6 (C-1, 5, 1', 3', $OCH_2Bn$), 75.7, 78.3, 78.8, 79.0 (C-2, 3, 4, 2'), 144.7 (aromatic C, $PhpNO_2$), 170.0, 171.7 (C=O). HRMS: Calcd for $C_{66}H_{65}O_{13}NNa$: 1102.4354. Found 1102.4495 ($M+Na^+$).

**2,3,4-Tri-*O*-benzyl-1-*O*-[(*S*)-2'-*O*-benzyl-1'-deoxyglyceryl]-5-*O*-monomethoxytrityl-D-ribitol 3'-*H*-phosphonate triethylammonium salt (16).** A solution of **13** (1.86 g, 2.2 mmol) in pyridine (7.8 mL) was mixed with a 2 M solution of phosphorous acid in pyridine (10.9 mL), then 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane (2.26 g, 12.3 mmol) was added and the mixture was stirred for 19 h at rt. An aq. solution of triethylammonium bicarbonate (1 M, 8.4 mL) was added to destroy excess reagent, then the mixture was diluted with dichloromethane. The organic layer was washed with aq. 1 M triethylammonium bicarbonate and concentrated, the residue was purified by column chromatography using a stepwise gradient of methanol (0-100 %) in dichloromethane-pyridine (99:1). Appropriate fractions were pooled and concentrated, and the residue was partitioned between dichloromethane and 0.5 M aq. triethylammonium bicarbonate. Finally, the organic layer was concentrated and co-concentrated with dichloromethane several times (to remove traces of

water) to give **16** (2.12 g, 2.1 mmol, 96 %) as an amorphous compound, $[\alpha]_{578}$ -16°. NMR data ($CDCl_3$): $^{13}C$, δ 8.4 ($NCH_2\mathit{C}H_3$), 45.3 ($N\mathit{C}H_2CH_3$), 55.2 ($OCH_3$), 63.2 ($J_{C,P}$ = 4.0 Hz, C-3'), 63.8 (C-5), 71.9, 72.0, 72.0, 72.5, 72.6, 73.6 (C-1, 1', $O\mathit{C}H_2Bn$), 77.7, 77.7, 78.8, 79.2 (C-2, 3, 4, 2') 86.4 ($\mathit{C}(Ar)_3$); $^1H$, δ 1.23 (t, $NCH_2C\mathit{H}_3$), 2.94 (q, $NC\mathit{H}_2CH_3$), 3.34 (dd, $J_{4,5a}$ = 5.4 Hz, $J_{5a,5b}$ = 10.2 Hz, H-5a), 3.41 (dd, $J_{4,5b}$ = 2.0 Hz, H-5b), 3.53 (m, 2H, H-1'a,b), 3.64 (m, H-3), 3.74 (s, $OC\mathit{H}_3$), 3.75 (m, H-2'), 3.87 (m, H-4), 3.96 (m, 2H, H-3a',b') 6.86 (d, $J_{H,P}$=622 Hz, PH). HRMS: Calcd for $C_{56}H_{57}O_{10}P$: 921.3848. Found 921.3829 ($M+H^+$).

***N*-Carbobenzyloxyethanolamine H-phosphonate triethylammonium salt (17).** Phosphorus trichloride (2.62 mL) was added to a stirred and cooled (ice) solution of imidazole (6.13 g) in dry acetonitrile (100 mL). A precipitate formed, eventually stirring by swirling was necessary. Then triethylamine (13.9 mL) was added, followed by dropwise addition of a solution of CBZ-ethanolamine[10] (1.95 g) in dry acetonitrile (25 mL). After 1 h stirring with cooling in ice, water (5 mL) was added, then, after 15 min, the mixture was partitioned between water (300 mL) and dichloromethane (300 mL). The aqueous layer was washed with dichloromethane (2 x 250 mL), then extracted with 4:1 dichloromethane-1-butanol (4 x 250 mL). The dichloromethane-butanol extracts were concentrated, then purified by chromatography on a silica gel column (120 g), packed in dichloromethane-methanol (9:1). The column was eluted with 200 mL of the same solvent, then with 100 mL portions of : 8:2, 6:4, 4:6, 2:8 dichloromethane-methanol, and finally with methanol. Fractions containing material were pooled and concentrated. The residue was taken up in water (20 mL) and the solution was slowly passed through a column of Dowex-50 x 4 (triethylammonium form, 200-400 mesh, 7 x 2 cm) by eluting with 80 mL of water. The fractions containing material were pooled and evaporated, then lyophilized. The residue (1.77 g) was coevaporated several times from dry dichloromethane and then pumped with a vacuum pump, residue: 1.64 g (46 %). NMR data ($D_2O$): $^1H$, δ 1.27 (t, 9H, $NCH_2C\mathit{H}_3$), 3.17 (q, 6H, $NC\mathit{H}_2CH_3$), 3.35 (t, 2H, $NC\mathit{H}_2CH_2O$), 3.89 (m, 2H, $NCH_2C\mathit{H}_2O$), 5.13 (s, 2H, $PhC\mathit{H}_2$), 6.67 (d, $J_{P,H}$ = 637 Hz, P-H), 7.43 (s, 5H, Ar-H); $^{13}C$, δ 8.9 ($NCH_2\mathit{C}H_3$), 42.0 ($N\mathit{C}H_2CH_2O$), 47.4 ($NCH_2\mathit{C}H_3$), 63.4 (d, $NCH_2CH_2O$), 67.7 ($PhCH_2$). HRMS: Calcd for $C_{10}H_{13}NO_5P$: 258.0531. Found 258.0594 ($M+H^+$).

**Functionalization of the solid support with 16.** Aminomethyl resin (80-200 mesh, 0.9 mmol/g, 0.602 g, 0.54 mmol) was mixed with *N,N*-dimethylformamide (20 mL) in a silanized round-bottom flask, after 10 min the

supernantant liquid was removed, and a solution of compound **15** (0.417 g, 0.39 mmol) in *N,N*-dimethylformamide (3 mL) was added followed by triethylamine (0.18 mL). The flask was rotated on a rotavapor at room temperature for 24 h, then the material was filtered off on a silanized glass filter. The solid was washed with *N,N*-dimethylformamide (5 x 10 mL) and pyridine (5 x 10 mL), transferred to a silanized round-bottom flask and rotated with pyridine/acetic anhydride 2:1 (9 mL) 24 h (to acetylate unreacted amino groups). The reagents were filtered off and the resin was washed with pyridine (5 x 10 mL), dichloromethane (6 x 8 mL), diethyl ether (3 x 10 mL). Drying in vacuum gave the monomer-functionalized resin (0.906 g, 0.41 mmol/ g resin, by weight increase). The degree of loading was also determined by treating an aliquot of the dry resin with trifluoroacetic acid in dichloromethane (0.5 % by volume), measuring the absorbance at 478 nm (due to released monomethoxytrityl cation) and comparing the absorbance with a standard curve. This gave a value of 0.44 mmol/ g resin. The standard curve was prepared by treatment of known amounts of 5-*O*-monomethoxytrityl-2,3,4-tri-*O*-benzyl-1-*O*-(2,5-di-*O*-benzyl-ß-D-ribofuranosyl)-D-ribitol 3-*H*-phosphonate triethylammonium salt[2] with trifluoroacetic acid in dichloromethane (0.5 % by volume), and measurement of the absorbance at 478 nm.

**General procedure for solid-phase synthesis.** The solid-phase synthesis was carried out manually in an apparatus[11] of the stationary, fritted glass filter bottom type, where nitrogen is pushed up through the filter to provide agitation. Reagents and solvents were added directly into the vessel with Pasteur pipettes. During filtrations, extra pressure was obtained with the aid of a syringe on top of the closed vessel. All glassware was dried overnight at 120 °C, and allowed to cool in a desiccator. Molecular sieves (4Å) were added to the pyridine 24 h before use and all solvent mixtures were prepared freshly. Mixtures of **16** and molecular sieves (4Å) in dichloromethane-pyridine 4:1 were prepared 1 h before coupling. These mixtures were activated immediately before use by addition of pivaloyl chloride, mixing for a few seconds then transferring to the resin in the solid-phase synthesis apparatus. To estimate coupling yields, acid washings were collected, diluted to 50 mL with trifluoroacetic acid in dichloromethane (0.5 % by volume), and the absorbance (at 535 nm, to give a reading < 1.0) was compared to the absorbance of the acid filtrate from the previous cycle.

**Solid-phase synthesis of the protected linker-containing pentamer 18.** The dry and silanized solid-phase apparatus was charged with dry, functionalized resin (0.202 g, 0.083 mmol monomer). Dichloromethane (10 mL)

was added and the mixture was agitated for 10 min, filtered and then subjected to the following cycle:

| | | | |
|---|---|---|---|
| 1 | Trifluoroacetic acid - dichloromethane (0.5% by vol) | 6 x 7 mL | 6 x 1 min |
| 2 | Dichloromethane | 6 x 7 mL | 6 x 1 min |
| 3 | Dichloromethane - pyridine (4:1 by vol) | 7 mL | 1 min |
| 4 | Pivaloyl chloride (50 μL, 0.41 mmol) was added to a vial containing **16** (0.42 g, 0.41 mmol) and molecular sieves (4 Å) in dichloromethane-pyridine 4:1 (3.65 mL). This mixture and a dichloromethane vial-rinse solution (0.9 mL) was added to the resin. | | 10 min |
| 5 | Dichloromethane-pyridine (4:1 by vol) | 7 mL | 1 min |
| 6 | Dichloromethane | 6 x 7 mL | 6 x 1 min |

The cycle (1-6) was repeated four times with compound **16**, and finally once with the terminating spacer **17** (0.18 g, 0.50 mmol) using pivaloyl chloride (60 μL, 0.50 mmol). Coupling yields, according to absorbance readings for the first four cycles, were 90-98%. The recovery of compound **16** from the filtrates of step 4, was, after column chromatography, 65 % of the theoretical.

**Oxidation, removal of oligomer and deprotection.** Oxidation and removal from the resin was performed in the apparatus according to the following scheme:

| | | | |
|---|---|---|---|
| 7 | Wash: pyridine | 1 x 7 mL | 1 x 1 min |
| 8 | Wash: pyridine-water 95:5 | 2 x 7 mL | 2 x 1 min |
| 9 | Oxidation: 1% $I_2$ (w/ v) in pyridine-water 95:5 | 7 mL | 60 min |
| 10 | Wash: pyridine | 6 x 7 mL | 6 x 1 min |
| 11 | Wash: dichloromethane | 6 x 7 mL | 6 x 1 min |
| 12 | Removal from resin: sodium methoxide (1.2 mL, 0.5 M) in methanol-dioxane 1:1 (12 mL) | | 16 h |
| 13 | Rinsing of resin: dioxane-methanol 1:1 | 3 x 7 mL | 3 x 1 min |
| 14 | Rinsing of resin: methanol | 3 x 5 mL | 3 x 5 min |

The combined solutions from step 12-14 were treated with acetic acid (35 μL) and concentrated at 30 °C. The residue was dissolved in ethyl acetate-ethanol-water 1:2:2 (12.5 mL) containing acetic acid (30 μL), and hydrogenated over Pd/ C (0.12 g, 10%) at atmospheric preassure and rt for 16 h. More water (2.5 mL) and

Pd/ C (0.12 g, 10 %) was then added and the hydrogenation was continued for another 16 h. The mixture was then filtered, concentrated and lyophilized. The residue was purified by gel filtration to give the final product **2** (87 mg, 70 %) as a white powder, slightly contaminated with shorter homologs (< 5 %, by MALDI-TOF MS) and pentameric monomethyl phosphotriesters (8% on a molar basis, by NMR, $^{31}P$, δ 1.03, MALDI-TOF MS: *m/z* 1516), probably generated during the oxidation-deprotection treatment. NMR data ($D_2O$): $^{1}H$, δ 3.29 (t, $J_{H,H}$ = 5.1 Hz, $OCH_2CH_2$-$NH_2$), 3.58 (dd, $J_{H1'a,H2'}$ = 6.3 Hz, $J_{H1'a,H1'b}$ = 11.8 Hz, H-1'a) 3.61-3.79 (m, H-2, H-5a,b) 3.66 (dd, $J_{H1'b,H2'}$ = 4.2 Hz, $J_{H1'a,H1'b}$ = 11.8 Hz, H-1'b), 3.87-4.09 (m, H-3'a,b), 3.92 (m, H-4), 3.98-4.03 (m, H-3), 4.03-4.08 (m, H-2'), 4.12 (q, 2H, $J_{H,P}$ = 6.1 Hz, $OCH_2CH_2NH_2$), $^{13}C$, δ 40.5 ($OCH_2CH_2NH_2$, $^{3}J_{C,P}$ = 8.1 Hz), 62.3 ($OCH_2CH_2NH_2$, $J_{C,P}$ = 5.5 Hz) 63.1 (C-1'), 67.0 (C-3', $J_{C,P}$ = 5.2 Hz), 69.7 (C-2', $^{3}J_{C,P}$ = 8.1 Hz), 70.9 (C-3, $^{4}J_{C,P}$ = 2.2 Hz), 71.3 (C-4, $^{3}J_{C,P}$ = 8.1 Hz), 72.1 (C-2), 72.2 (C-5, $J_{C,P}$ = 7.0 Hz), 72.4 (C-1), $^{31}P$, δ 0.90 (end P), 1.36 (backbone P). MALDI-TOF MS: Calcd for $C_{42}H_{91}NO_{46}P_5$: 1501. Found: 1501 $[M-H]^-$.



## ACKNOWLEDGEMENTS

We thank SBL Vaccin AB, Stockholm, Sweden for financial support and Dr. Corine Sandström at our department for expert help with NMR assignments.

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