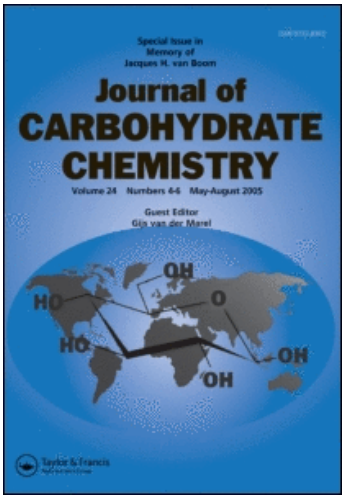


Journal of Carbohydrate Chemistry

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

Solid-Phase Synthesis of a Fragment of the Capsular Polysaccharide of *Haemophilus Influenzae* Type B Using *H*-Phosphonate Intermediates

Stinabritt Nilsson[a]; Marita Bengtsson[a]; Thomas Norberg[a]
[a] Organic Synthesis Department BioCarb Technology AB, Lund, Sweden

To cite this Article Nilsson, Stinabritt , Bengtsson, Marita and Norberg, Thomas(1992) 'Solid-Phase Synthesis of a Fragment of the Capsular Polysaccharide of *Haemophilus Influenzae* Type B Using *H*-Phosphonate Intermediates', Journal of Carbohydrate Chemistry, 11: 3, 265 — 285
To link to this Article: DOI: 10.1080/07328309208017993
URL: http://dx.doi.org/10.1080/07328309208017993

J. CARBOHYDRATE CHEMISTRY, 11(3), 265-285 (1992)

SOLID-PHASE SYNTHESIS OF A FRAGMENT OF THE CAPSULAR POLYSACCHARIDE OF *HAEMOPHILUS INFLUENZAE* TYPE B USING *H*-PHOSPHONATE INTERMEDIATES

Stinabritt Nilsson, Marita Bengtsson and Thomas Norberg

Organic Synthesis Department,
BioCarb Technology AB, S-223 70 Lund, Sweden

Received September 23, 1991 - Final form December 30, 1991

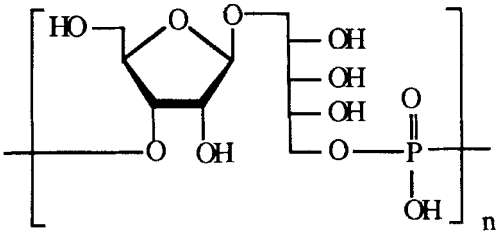
ABSTRACT

A pentameric fragment of the capsular polysaccharide of *Haemophilus influenzae* type b, containing a linker group, was synthesized by a simple solid-phase procedure. Cross-linked polystyrene was used as a solid phase, and a benzyl-protected ribosylribitol *H*-phosphonate was used as monomer. A non-promoted glycosidation reaction was used for preparation of the ribosylribitol monomer.

INTRODUCTION

Haemophilus influenzae type b (Hib) is the most common causative agent of meningitis in children. Antibodies which bind to the capsular polysaccharide polyribosylribitol phosphate[1,2] (PRP) of the bacterium protect

against disease, and successful vaccination is therefore possible if such antibodies can be induced. Several vaccine formulations have been evaluated for this purpose. The best results have been obtained with conjugate vaccines,[3,4,5] consisting of PRP fragments conjugated to a carrier protein. The PRP is isolated by multi-stage processes from bacterial cell culture supernatants.



Polyribosylribitol phosphate (PRP)

An alternative source of PRP structures for vaccine evaluation and production is chemical synthesis. Several laboratories have reported succesful syntheses of PRP fragments on a small scale, using either solution[6-14] or solid-phase[15] techniques. However, to date no report has appeared describing a protocol simple enough to be considered for production of the larger amounts (0.1-1.0 g) needed for vaccine production and evaluation.

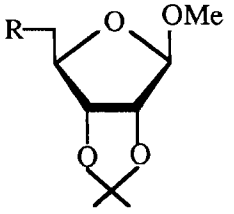
We now report an alternative synthesis of the monomer **11**[13,14] and its subsequent oligomerization, using simple and efficient *H*-phosphonate chemistry[16,17,18] and highly substituted polystyrene beads as solid phase. We also report synthesis of the linker monomer **20**, with which the oligomer chain was terminated. After deprotection, the pentameric PRP fragment **21**, suitable for linking to a carrier protein, was obtained.

RESULTS AND DISCUSSION

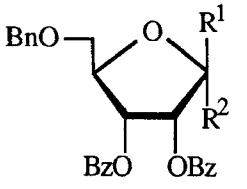
Monomer Synthesis

Among the various monomers used in previously reported syntheses of PRP fragments, compound **11** was chosen for this work since it contains only benzyl protecting groups which can be removed in one step at the end of the synthesis. Compound **11** was synthesized by a route similar to the one reported,[13,14] but with some improvements. Briefly, methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**1**, prepared in one step[19] from D-ribose) was

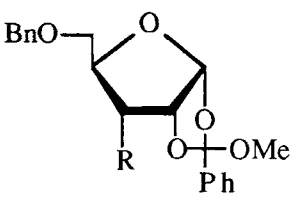
benzylated (benzyl chloride/sodium hydroxide in *N,N*-dimethylformamide) to give **2**[13,14] (94% yield), which was converted to the crystalline methyl glycoside **3** by the sequence partial hydrolysis followed by benzoylation (51% yield). The major byproduct was unreacted **2**. Treatment of **3** with hydrogen bromide in dichloromethane gave the corresponding bromide **4**, which was reacted in situ with methanol and collidine to give the orthoester **5**. Debenzoylation (sodium methoxide in methanol) followed by allylation (allyl bromide/sodium hydroxide in *N,N*-dimethylformamide) gave the allylated orthoester **6** (59% yield from **3**). Brief treatment of **6** with trimethylsilyl chloride gave the chloride **7**, which was used directly in the subsequent glycosylation reaction. During investigation of the silver triflate promoted glycosylation with **7**, it was realized, that this furanosyl halide is reactive enough to form glycosides with alcohols without a promoter. Thus, the alcohol **8**[9,10,13,14] was reacted with **7** at room temperature for 16 h in dichloromethane solution in the presence of molecular sieves as acid scavenger. The obtained product **9** (84% yield) was subjected to the reaction sequence debenzoylation (sodium methoxide), benzylation (benzyl chloride /sodium hydroxide), and deallylation [tris(triphenylphosphine)rhodium(I) chloride/aqueous acetic acid] giving the diol **10**[13,14] in 74% yield (from **9**). Monomethoxytritylation of **10**, with monomethoxytrityl chloride in pyridine as described,[14] gave the key compound **11** in 88% yield. Treatment of **11** with successively, succinic anhydride and *p*-nitrophenol/*N,N'*-diisopropylcarbodiimide[20] gave **12** (87% yield), suitable for use as chain initiation monomer in the solid-phase synthesis. Treatment of **11** with phosphorous acid and 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane in pyridine[21] gave the *H*-phosphonate **13** (84% yield), suitable for use as chain propagation monomer in the solid-phase synthesis.



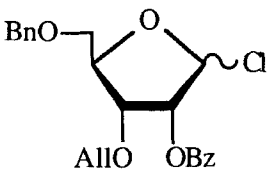
1 R = OH
2 R = OBn



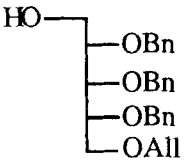
3 R^1 = OMe, R^2 = H
4 R^1, R^2 = H, Br



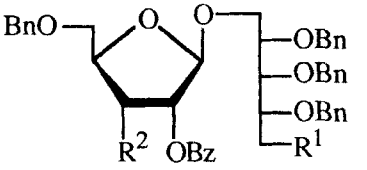
5 R = OBz
6 R = OAll



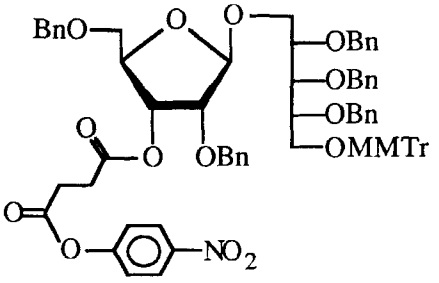
7



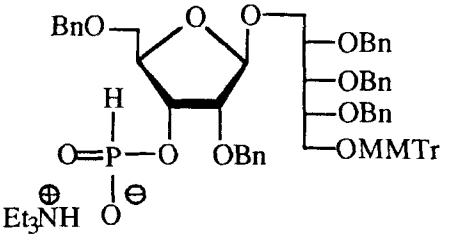
8



9 $R^1 = R^2 = OAll$
10 $R^1 = R^2 = OH$
11 $R^1 = OMMTr$, $R^2 = OH$

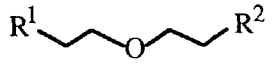


12

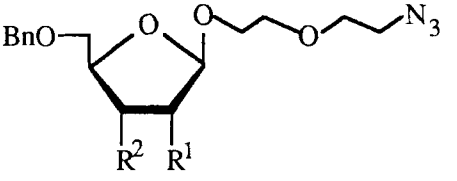


13

For synthesis of the chain terminating linker monomer **20**, ribosyl donor **7** was reacted with 2-(2-azidoethoxy)ethanol (**17**, prepared in four steps from diethylene glycol, via compounds **14-16**) and molecular sieves in dichloromethane to give riboside **18** (92% yield). Subsequent protecting group manipulations (debenzoylation, benzylation and deallylation) gave **19** (46% yield). The deallylation could not be effected with either tris(triphenylphosphine)rhodium(I) chloride, Pd/C in aqueous acetic acid or potassium *tert*-butoxide/dimethyl sulfoxide. However, selenium dioxide[22] gave acceptable conversion, although the yield was moderate. Conversion into the *H*-phosphonate **20** (89%) was done as for compound **13**.



14 $R^1 = OTr$, $R^2 = OH$
15 $R^1 = OTr$, $R^2 = OTs$
16 $R^1 = OTr$, $R^2 = N_3$
17 $R^1 = OH$, $R^2 = N_3$



18 $R^1 = OBz$, $R^2 = OAll$
19 $R^1 = OBn$, $R^2 = OH$
20 $R^1 = OBn$, $R^2 = OPH(O)O^{\ominus}Et_3NH^{\oplus}$

The monomers **12**, **13** and **20** were used in the solid-phase synthesis described below.

Solid-phase Synthesis

In previous work aimed at solid-phase synthesis of PRP oligomers or related capsular polysaccharides, the solid phase chosen was controlled pore glass[15,23] or NH_2-monobeads.[24] Controlled pore glass or silica gel has, with few recent exceptions,[25] also been the material of choice for solid-phase synthesis of RNA or DNA fragments.[20] In the present work, however, a high loading on the solid phase was desired, to make possible preparation of large amounts of material using a minimum amount of solvents and solid phase. Therefore, we used crosslinked polystyrene as solid phase, giving loadings in the range of 0.5 millimoles/gram, as compared to 0.05 millimoles/g as a typical loading for controlled pore glass or silica gel. The swelling properties of polystyrene resin, however, renders it less suitable for use in the usual column-based DNA synthesis machines. Instead, we used a simple reaction vessel stirred by nitrogen gas, bubbled into the vessel through the bottom, made of fritted glass. The synthesis was carried out manually, but can easily be automated.

In previous work, the phosphotriester,[8,9,11] or phosphoramidite chemistries[12,13,14,15] were used for creation of the phosphodiester linkages of Hib capsular polysaccharide fragments. However, in our opinion, the *H*-phosphonate procedure should be the most suitable for larger scale synthesis because of its simplicity (few operations per cycle) and economy (no use of expensive reagents, and simple recovery of excess *H*-phosphonate). Therefore, this procedure was used in the present work.

H-phosphonate monoesters have been activated for coupling to alcohols with sterically hindered acid chlorides, such as pivaloyl chloride[18,26,27] or adamantanecarbonyl chloride.[28] Usually, the activating agent is added to a mixture of the *H*-phosphonate monoester, pyridine and the alcohol ("regular" coupling). In the solid-phase synthesis of oligoribonucleotides and oligodeoxynucleotides the used ratio of acyl chloride to *H*-phosphonate monoester has been 4:1 or 5:1 for rapid activation of the *H*-phosphonate monoester, and minimal competitive acylation of the alcohol group on the solid phase.[18,28,29] Coupling efficiencies have been in the range of 91-100% using 2-40 equivalents of monomer.[26-28,30,31] Premixing the *H*-phosponate with excess acyl chloride (coupling with "preactivation"), in solvent systems containing pyridine, before addition to the alcohol, produces bisacyl phosphites,[32] leading to lower coupling efficiencies. However, by using

acetonitrile/quinoline as solvent, the rate of bisacyl phosphite formation was made significantly slower leading to higher coupling efficiencies, thus eliminating the negative effect of "preactivation".[33]

We found that "regular" coupling in dichloromethane/pyridine with 10 equivalents of pivaloyl chloride and 5 equivalents of monomer **13** gave a coupling efficiency of approximately 81%. By using 5 equivalents of pivaloyl chloride and 5 equivalents of monomer **13**, the coupling efficiency of the first coupling step was approximately 95%, but then gradually diminished to approximately 86% for the fourth coupling step. Even the use of $4 \times 0.98^{(n-1)}$ equivalents (where n = 1 to 4) of pivaloyl chloride in a pentamer synthesis showed, with an average coupling efficiency of 95%, a tendency towards diminishing coupling efficiencies in the coupling steps. The reason for the low coupling efficiencies is probably competitive acylation by pivaloyl chloride of the alcohol function on the resin. Since this has not been a major problem in nucleoside chemistry, this means that the *H*-phosphonate **13** is less reactive than the corresponding nucleoside *H*-phosphonates and/or that the alcohol function on the solid phase in this case is more reactive than the alcohol function in the corresponding nucleoside case.

Premixing the monomer **13** and pivaloyl chloride, before addition to the solid phase, should reduce the amount of capping of the alcohol, provided that pivaloyl chloride is not added in excess. (A low amount of pivaloyl chloride should also minimize bisacyl phosphite formation, even if "preactivation" is performed in the presence of pyridine.[29,32]) We found that the "preactivation" of 5 equivalents of monomer **13** with 5 equivalents of pivaloyl chloride in dichloromethane/pyridine 5.25/1 for 2 min, followed by coupling to the resin-bound alcohol for 10 min, gave an average coupling efficiency of 96% in the synthesis of a pentamer, and the coupling efficiency seemed to be constant throughout the coupling steps. The use of excess pivaloyl chloride gave a lower coupling efficiency.

With these considerations in mind, the linker-containing pentamer **21** was prepared carrying out the following operations using **12**, **13** and **20**:

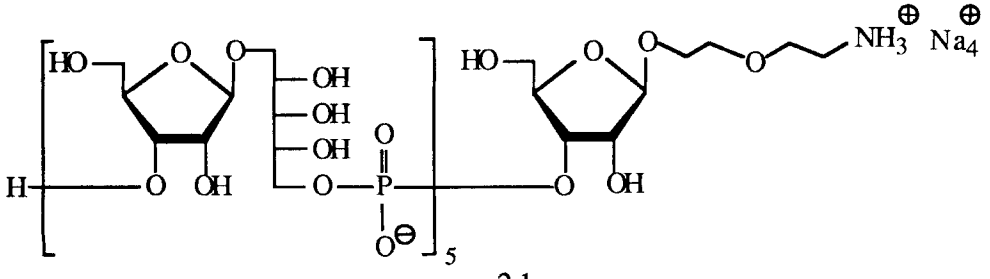
1) Chain initiation was done by reacting **12** with crosslinked amino-polystyrene, followed by capping with acetic anhydride/pyridine. The amount of monomethoxytrityl cation released on treatment with 0.5% trifluoroacetic acid in dichloromethane indicated a functionalization of approximately 0.5 mmol/g dry resin.

2) Deprotection was done by treatment with 0.5% trifluoroacetic acid in dichloromethane.

3) Pivaloyl chloride (5 equivalents) was added to a solution of **13** (5 equivalents) in dichloromethane/pyridine. After stirring for 2 min the "preactivation" mixture was added to the resin and allowed to react for 10 min. The coupling efficiency was estimated by comparing the absorbance at 478 nm of the deprotection fluid with that from the previous cycle. The excess *H*-phosponate could be recovered from the filtrate in 81% of the theoretical amount.

After repeating steps 2 and 3 the desired amount of times, the terminating linker monomer **20** was coupled using the same conditions as for coupling of **13.**

4) Oxidation was performed on the resin with iodine in 98/2 pyridine/water,[34,35] then the oligomer was removed from the resin by treatment with methanolic sodium methoxide in dioxane/methanol. Since no simultaneous *N*-deacylation of a nucleotide base derivative was desired, as in the analogous detachment reaction (concd ammonia, heat) in oligonucleotide chemistry,[20] these milder, more typical *O*-deacylation conditions could be used. The obtained crude material was hydrogenated with palladium on charcoal, and then purified by gel filtration or preparative HPLC. Purification by gel filtration gave no baseline separation between the spacer containing pentamer and the lower oligomeres, although the sample was recirculated on the gel filtration column. Fractions containing the desired compound in >90% purity were pooled. The material was transformed into the sodium form by ion exchange giving **21** as a white powder in 40% yield. Purification of the crude material from the hydrogenation by preparative HPLC, followed by transformation of the material into the sodium form by ion exchange gave **21** in 41% yield and a high degree of purity (>99%). NMR and FAB-MS analysis of the material verified the structure. That the ribitol and linker ribofuranoside linkages were β were indicated by comparison of the ^{13}C NMR-shifts with those published for methyl ribofuranosides.[36] The small H-1,H-2 coupling constants for compounds **9** and **18** also indicate β-coupling products.[37]



21

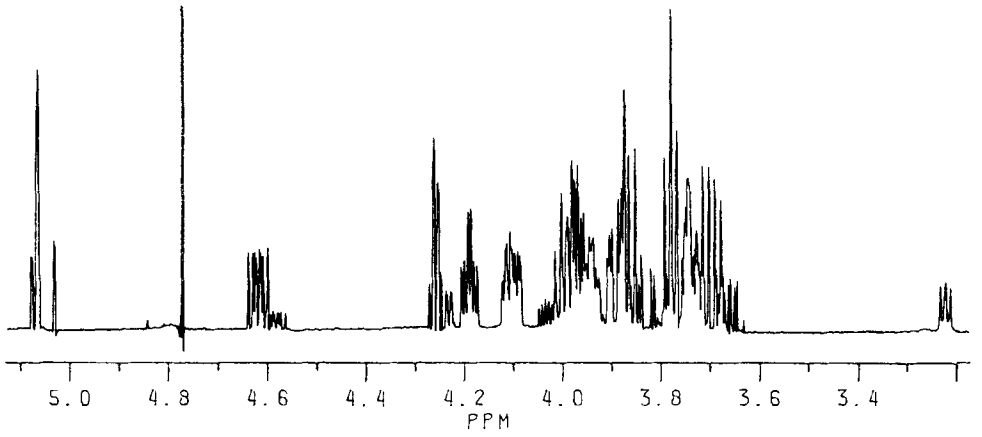


Fig. 1. ^1H NMR spectrum of compound **21** in D_2O.

Thus, efficient and comparatively simple synthesis of a Hib capsular polysaccharide fragment was performed. Work towards automatization, yield optimization of the solid-phase synthesis procedure, and further simplification of the monomer synthesis is in progress and will be reported in a separate paper.

EXPERIMENTAL

General Methods. Melting points are corrected. Concentrations were performed under reduced pressure at <40 °C bath temperature. Optical rotations were measured at 25 °C (*c* 0.5, chloroform) unless otherwise stated, using a Perkin-Elmer 241 Polarimeter. NMR spectra were recorded at 300 K with a Bruker AM 500 instrument. The following reference signals were used: Me_4Si, δ 0.0 (^1H in $CDCl_3$); $CDCl_3$, δ 77.0 (^{13}C in $CDCl_3$); Me_2CO, δ 2.225 (^1H in D_2O) and external dioxan, δ 67.4 (^{13}C in D_2O). Only selected NMR data are reported. Assignments were based on 2 D COSY, J resolved, decoupling, DEPT, proton-carbon and proton-phosphorus correlation experiments. In the NMR shift tables signals from ribitol atoms are marked with '. The FAB-MS spectrum was recorded with a VG ZAB-SE mass spectrometer. The primary beam consisted of xenon atoms with a maximum energy of 8 keV. The

samples were dissolved in thioglycerol and the positive ions were extracted and accelerated over a potential of 10 kV. TLC was performed on Silica Gel F_{254} (Merck, Darmstadt, Germany) with detection by UV light and/or by charring with 5% aqueous sulfuric acid. Column chromatography was performed on silica gel (60Å, 40-63 μm; Merck, Darmstadt, Germany). Elemental analyses were not obtained for amorphous compounds. These were purified by column chromatography and the purity was ascertained by TLC, in at least two different solvent systems, and by NMR spectroscopy. Organic solutions were dried over magnesium sulfate. Powdered molecular sieves (4Å; Fluka, Buchs, Switzerland) were heated to 300 °C under vaccum overnight. Dichloromethane, *N,N*-dimethylformamide and pyridine were bought puriss.; absolute, over molecular sieve (Fluka, Buchs, Switzerland). Methanol was dried over 3Å molecular sieves, and dioxane over 5Å molecular sieves. Pivaloyl chloride was redistilled before use. Bio-Beads S-X1 beads were bought from Bio-Rad Laboratories, Richmond, California, United States.

Methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (2). This compound was prepared by a modification of the published[14] procedure. Benzyl chloride (295 mL, 2.54 mol) was added dropwise to a stirred mixture of compound **1**[19] (374 g, 1.83 mmol), powdered sodium hydroxide (380 g, 9.5 mmol) and *N,N*-dimethylformamide (1750 mL). The temperature was kept between room temperature and 45 °C. After 2 h, methanol (300 mL) was added to destroy excess benzyl chloride. After stirring for 1 h the mixture was partitioned between water and toluene. The aqueous layer was washed with toluene and the combined organic layers were washed with water, dried and concentrated. Distillation of the residue in the presence of barium carbonate gave **2** (507 g, 94%), bp 125-135 °C (0.3 mbar). Physical constants were as reported.[14]

Methyl 5-*O*-benzyl-2,3-di-*O*-benzoyl-β-D-ribofuranoside (3). Aqueous formic acid (200 mL, 95% by vol) was mixed, at 21 °C, with **2** (31 g, 0.11 mol). After stirring for 30 min at 21 °C, the solution was cooled in ice to slow down the hydrolysis reaction. It was then slowly added to a vigorously stirred mixture of sodium hydroxide (240 g), water (1.0 L), ice (1.0 kg) and dichloromethane (1.0 L). The organic layer was separated and the water phase was extracted with more dichloromethane (4x500 mL). The combined extracts were concentrated, and the residue was co-concentrated with pyridine (100 mL).

The residue was dissolved in pyridine (200 ml) and benzoyl chloride (34 mL, 0.29 mol) was added dropwise while stirring and cooling in ice. The ice bath was removed and the mixture was stirred for 16 h at room temperature. Water (2 mL) was added to destroy excess benzoyl chloride and after 15 min the mixture was partitioned between water and dichloromethane. The organic layer was washed with water, 1 M sulfuric acid and saturated sodium hydrogen carbonate, dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 9/1 by vol). Fractions containing **3** were pooled and taken up in methanol. On cooling (4 °C), crystals of **3** were obtained (25 g, 51%), mp 68-69 °C, $[\alpha]_D$ +51°. NMR data ($CDCl_3$): ^1H, δ 3.44 (s, OCH_3), 3.71 (dd, $J_{4,5a}$=6.5 Hz, $J_{5a,5b}$=10.4 Hz, H-5a), 3.79 (dd, $J_{4,5b}$=3.9 Hz, H-5b), 4.57 (m, $J_{3,4}$=6.5 Hz, H-4), 4.64 (m, CH_2Ph), 5.15 (d, $J_{1,2}$=1.0 Hz, H-1), 5.61 (dd, $J_{2,3}$=5.0 Hz, H-2), 5.68 (dd, H-3).

Anal. Calcd for $C_{27}H_{26}O_7$: C, 70.1; H, 5.7. Found: C, 70.0; H, 5.7.

3-*O*-Allyl-5-*O*-benzyl-1,2-*O*-methoxybenzylidene-α-D-ribofuranose (6). A solution of hydrogen bromide in dichloromethane was prepared by dropwise addition of acetyl bromide (30 mL, 0.40 mol) to a mechanical stirred solution of methanol (15 mL, 0.37 mol) in dichloromethane (800 mL). Compound **3** (50 g, 0.11 mol) was added, and the mixture was stirred at room temperature for 50 min. The solution, containing mainly the bromide **4**, was stirred and cooled in ice while 2,4,6-trimethylpyridine (270 mL) was added dropwise, followed by addition of methanol (100 mL). The mixture was stirred for 1.5 h at room temperature, washed with water, dried, concentrated and co-concentrated with methanol.

The residue, containing crude orthoester **5**, was dissolved in methanolic sodium methoxide (350 mL, 0.1 M). After 3 h, CO_2(s) was added until the pH of the solution was 8.0, then the mixture was concentrated and co-concentrated twice with toluene. The residue was dissolved in *N,N*-dimethylformamide (500 mL), and powdered sodium hydroxide (33 g, 0.82 mol) was added. The mixture was stirred while allyl bromide (33 ml, 0.38 mol) was added dropwise. After 1 h the mixture was partitioned between toluene and water. The organic layer was washed with water, dried and concentrated. Purification of the residue on a silica gel column (toluene/ethyl acetate/pyridine, 90/10/1 by vol) gave syrupy **6** (25 g, 59%), $[\alpha]_D$ +115°. NMR data ($CDCl_3$): ^1H, δ 3.21 (s, OCH_3), 3.48 (dd, $J_{4,5a}$=4.0 Hz, $J_{5a,5b}$=11.4 Hz, H-5a), 3.63 (dd, $J_{4,5b}$=2.0 Hz, H-5b), 3.75 (m, $J_{3,4}$=9.2 Hz, H-4), 3.82 (dd, $J_{2,3}$=4.7 Hz, H-3), 4.02, 4.17 (2 m, CH_2-CH=CH_2), 4.48 (m, CH_2Ph), 4.80 (t, $J_{1,2}$=4.2 Hz, H-2), 5.18, 5.28 (2 m, CH=CH_2), 5.90 (m, CH=CH_2), 6.03 (d, H-1).

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(3-*O*-allyl-2-*O*-benzoyl-5-*O*-benzyl-β-D-ribofuranosyl)-D-ribitol (9). A mixture of **6** (19 g, 48 mmol) and trimethylsilyl chloride (100 mL) was stirred at room temperature for 20 minutes, then concentrated and co-concentrated with dichloromethane. The residue was dissolved in dichloromethane (50 mL) and added to a mixture of compound **8**[10,14] (24 g, 52 mmol), 4Å molecular sieves (24 g) and dichloromethane (100 mL). The mixture was stirred at room temperature overnight. Pyridine (6 mL) was added and the mixture was filtered. The filtrate was washed with 1 M sulfuric acid, saturated sodium hydrogen carbonate, dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 15/1 by vol) to give syrupy **9** (33 g, 84%), $[\alpha]_D$ +6°. NMR data ($CDCl_3$): ^{13}C, δ 67.1, 69.6, 71.4, 71.6, 71.9, 72.0, 72.1, 73.0, 73.6 (C-5,1',5', OCH_2Ph, OCH_2-CH=CH_2), 74.3, 77.8, 77.8, 78.0, 78.4, 80.4 (C-2,3,4,2',3',4'), 104.9 (C-1), 116.5, 117.3 (CH=CH_2), 165.2 (C=O); ^1H, δ 3.57 (dd, $J_{4,5a}$=6.3 Hz, $J_{5a,5b}$=10.5 Hz, H-5a), 3.64 (dd, $J_{4,5b}$=3.6 Hz, H-5b), 4.12 (dd, $J_{2,3}$=4.4 Hz, $J_{3,4}$=7.8 Hz, H-3), 4.29 (m, H-4), 5.04 (s, H-1), 5.07, 5.15, 5.17, 5.25 (4 m, CH=CH_2), 5.41 (d, H-2), 5.75, 5.89 (2 m, CH=CH_2).

2,3,4-Tri-*O*-benzyl-1-*O*-(2,5-di-*O*-benzyl-β-D-ribofuranosyl)-D-ribitol (10). A solution of **9** (23 g, 28 mmol) in dichloromethane (20 mL) was mixed with methanolic sodium methoxide (100 mL, 0.1 M). The solution was kept at 40 °C for 3 h, then neutralized with Dowex 50 (H^+) resin, concentrated and co-concentrated with toluene.

The residue was dissolved in *N*,*N*-dimethylformamide (200 mL). Powdered sodium hydroxide (11 g, 0.28 mol) was added, followed by dropwise addition of benzyl chloride (9.8 mL, 85 mmol). After stirring overnight, methanol (20 mL) was added to destroy excess benzyl chloride. After 1 h, the mixture was partitioned between toluene and water. The organic layer was washed with water, dried and concentrated.

The residue was dissolved in ethanol/toluene/water (120 mL, 30/10/5 by vol) and tris(triphenylphosphine)rhodium(I) chloride (667 mg, 0.72 mmol) was added, followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (0.20 mL, 1.3 mmol). The mixture was refluxed for 2 h, then concentrated and taken up in acetic acid/water (130 mL, 9/1 by vol). The solution was heated at 80 °C for 1 h, cooled and concentrated. The concentrate was partitioned between diethyl ether and water. The organic layer was washed with water, dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate, 8/2 by vol) to give syrupy **10** (15 g, 74%). The physical constants were as reported.[14]

5-*O*-Monomethoxytrityl-2,3,4-tri-*O*-benzyl-1-*O*-(2,5-di-*O*-benzyl-β-D-ribofuranosyl)-D-ribitol (11). This compound was prepared as described[14] from **10** and monomethoxytrityl chloride. With toluene/ethyl acetate/pyridine, 95/5/1 by vol, as eluent in the chromatographic purification, the yield was 88%.

5-*O*-Monomethoxytrityl-2,3,4-tri-*O*-benzyl-1-*O*-(2,5-di-*O*-benzyl-3-*O*-[3-(*p*-nitrophenyloxycarbonyl)propionyl]-β-D-ribofuranosyl)-D-ribitol (12). Succinic anhydride (0.65 g, 6.5 mmol) was added to a solution of compound **11** (2.01 g, 2.00 mmol) in pyridine (10 mL) containing 4-(*N*,*N*-dimethylamino)pyridine (61 mg, 0.50 mmol). After 16 h, water (0.5 mL) was added to destroy excess anhydride. After 3 h, the mixture was partitioned between toluene/ethyl acetate (1/1 by vol) and aqueous phosphate buffer (0.1 M, pH 6.5). The organic layer was washed with the phosphate buffer, concentrated and co-concentrated with toluene. The residue was dissolved in pyridine (5 mL) containing *p*-nitrophenol (0.42 g, mmol, 3.0 mmol), then *N*,*N*'-diisopropylcarbodiimide (0.47 ml, 3.0 mmol) was added. After 16 h, the mixture was partitioned between dichloromethane and water. The organic layer was dried and concentrated. The residue was purified by column chromatography (toluene/ethyl acetate/pyridine, 95/5/1 by vol). Syrupy **12** (2.13 g, 87%) was obtained, $[\alpha]_D$ -13°. NMR data ($CDCl_3$): ^1H, δ 2.75, 2.82 (2 m, $O{=}CCH_2CH_2C{=}O$), 3.35 ($J_{4',5a'}$=5.6 Hz, $J_{5a',5b'}$=10.3 Hz, H-5a'), 3.42 (dd, $J_{4',5b'}$=2.7 Hz, H-5b'), 3.51 (m, H-5a,5b), 3.74 (s, $PhOCH_3$), 4.08 (dd, $J_{1,2}$=2.0 Hz, $J_{2,3}$=5.1 Hz, H-2), 4.31 (q, $J_{3,4}$=5.3 Hz, H-4), 4.99 (d, H-1), 5.18 (t, H-3).

5-*O*-Monomethoxytrityl-2,3,4-tri-*O*-benzyl-1-*O*-(2,5-di-*O*-benzyl-β-D-ribofuranosyl)-D-ribitol 3-*H*-phosphonate triethylammonium salt (13). A solution of phosphorous acid in dry pyridine (35 mL, 2 M) was added to a solution of **11** (7.0 g, 6.9 mmol) in dry pyridine (25 mL), followed by 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane (7.3 g, 40 mmol). After 16 h, aqueous triethylammonium bicarbonate (14 mL, 1 M) was added and the mixture was stirred for 30 min, then partitioned between dichloromethane and aqueous triethylammonium bicarbonate (0.5 M). The organic layer was washed with water, dried and concentrated. The residue was dissolved in dichloromethane/pyridine (100/1 by vol) and applied to a silica gel column, packed in the same solvent. Stepwise gradient elution with an increasing concentration (0-15% v/v) of methanol/pyridine (100/1 by vol) in dichloromethane/pyridine (100/1 by vol) gave, after concentration of appropriate fractions, a residue, which was partitioned between dichloro-

methane and aqueous triethylammonium bicarbonate (0.1 M). The organic layer was washed with water, dried and concentrated to give **12** (6.8 g, 84%), $[\alpha]_D$ -22°. NMR data ($CDCl_3$): ^{13}C, δ 8.6 (NCH_2CH_3), 45.3 (NCH_2CH_3), 63.8, 67.8, 72.0, 72.2, 72.3, 72.6, 73.2, 73.6 (C-5,1',5', CH_2Ph), 73.2, 78.4, 78.8, 79.0, 81.4 (C-2,4,2',3',4'), 81.8 (d, $J_{C,P}$ 5.7 Hz, C-3), 86.3 (CPh_3), 105.9 (C-1); ^1H, δ 1.15 (t, NCH_2CH_3), 2.85 (q, NCH_2CH_3), 3.35 (m, H-5a'), 3.40 (m, H-5b'), 3.52 (dd, $J_{4,5a}$=6.8 Hz, $J_{5a,5b}$=10.6 Hz, H-5a), 3.68 (dd, $J_{1a',1b'}$=10.8 Hz, $J_{1a',2'}$=2.8 Hz, H-1a'), 3.68 (dd, $J_{4,5b}$=3,1 Hz, H-5b), 3.74 (s, $PhOCH_3$), 3.82 (m, $J_{1b',2'}$=5.9 Hz, H-2'), 3.92 (dd, H-1b'), 4.01 (dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=4.8 Hz, H-2), 4.32 (m, $J_{3,4}$=6.2 Hz, H-4), 4.69 (m, $J_{3,P}$=10.5 Hz, H-3), 4.98 (d, H-1), 6.93 (d, $J_{H,P}$=621 Hz, P-H).

2-[(2-Trityloxy)ethoxy]ethanol (14). To a solution of diethylene glycol (78 mL, 0.82 mol) in pyridine (400 mL) was added trityl chloride (100 g, 359 mmol) at room temperature. After 5 h the mixture was partitioned between dichloromethane and water. The organic layer was washed with cold 2 M sulfuric acid, water and saturated sodium hydrogen carbonate, dried and concentrated. The residue was crystallized from dichloromethane/light petroleum to give **14** (64 g, 51%), mp 114-116 °C. NMR data ($CDCl_3$): ^{13}C, δ 61.9, 63.4, 70.6, 72.3 (CH_2), 86.7 (CPh_3), 127.0, 127.8, 128.7, 144.0 (aromatic C).

Anal. Calcd for $C_{23}H_{24}O_3$: C, 79.3; H, 6.9. Found: C, 79.2; H, 7.1.

2-[(2-Trityloxy)ethoxy]ethyl *p*-toluenesulfonate (15). *p*-Toluenesulfonyl chloride (49 g, 0.26 mol) was added in portions to a stirred solution of **14** (52 g, 0.15 mol) in pyridine (300 mL) at 0 °C. After 3 h at 0 °C the mixture was poured onto water containing sodium hydrogen carbonate. After stirring for 15 min the mixture was extracted with dichloromethane. The organic layer was washed with water, 2 M sulfuric acid and saturated sodium hydrogen carbonate, dried and concentrated. The residue was crystallized from ethanol to give **15** (63 g, 83%), mp 96-97 °C. NMR data ($CDCl_3$): ^{13}C, δ 21.6 (CH_3), 63.3, 68.7, 69.3, 70.8 (CH_2), 86.6 (CPh_3), 126.9, 127.7, 127.9, 128.6, 129.7, 133.0, 143.9, 144.6 (aromatic C).

Anal. Calcd for $C_{30}H_{30}O_5S$: C, 71.7; H, 6.0; S, 6.4. Found: C, 71.7; H, 6.0; S, 6.2.

2-(2-Azidoethoxy)-*O*-tritylethanol (16). A mixture of **15** (60 g, 0.12 mmol) and sodium azide (24 g, 0.37 mol) in *N,N*-dimethylformamide (120 mL) was stirred at 100 °C for 1 h. After cooling the mixture was partitioned between diethyl ether/ethyl acetate (1/2 by vol) and water. The organic layer was washed with water, dried and evaporated. The residue was crystallized from ethanol to give **16** (40 g, 88%), mp 95-97 °C. NMR data ($CDCl_3$): ^{13}C, δ 50.9, 63.4, 70.1, 70.8 (CH_2), 86.6 (CPh_3), 126.9, 127.7, 128.7, 144.0 (aromatic C).

Anal. Calcd for $C_{23}H_{23}N_3O_2$: C, 74.0; H, 6.2; N, 11.3. Found: C, 73.9; H, 6.3; N, 10.8.

2-(2-Azidoethoxy)ethanol (17). A solution of compound **16** (29 g, 78 mmol) in acetic acid/water (170 mL, 7/3 by vol) was stirred at 70 °C for 1 h. The mixture was cooled, and the precipitated material was filtered and washed with acetic acid/water (170 mL, 7/3 by vol). The filtrate was concentrated and co-concentrated with toluene. Distillation of the residue gave, at 118-122 °C and 27 mbar, compound **17** contaminated with a small amount (about 5%) of its acetylated derivative. Treatment of the distillate with methanolic sodium methoxide (2.5 mL, 1 M) in methanol (60 mL) overnight, neutralization with Dowex 50 (H^+) resin, filtration, concentration and co-concentration with successively toluene and dichloromethane gave pure **17** (8.0 g, 79%). NMR data ($CDCl_3$): ^{13}C, δ 50.7, 61.7, 70.0, 72.4.

2-(2-Azidoethoxy)ethyl 3-*O*-allyl-2-*O*-benzoyl-5-*O*-benzyl-β-D-ribofuranoside (18). The orthoester **6** (2.06 g, 5.17 mmol) was treated with trimethylsilyl chloride (19 mL) for 15 min, then concentrated and co-evaporated once with toluene. The residue was dissolved in dichloromethane (12 mL) and added to a mixture of **17** (1.13 g, 8.62 mmol) and 4Å molecular sieves (2.9 g) in dichloromethane (12 mL). After stirring at room temperature overnight the mixture was filtered through a layer of Celite. The filtrate was concentrated and purified by column chromatography (light petroleum/ethyl acetate, 3/1 by vol) to give **18** as a syrup (2.38 g, 92%), $[\alpha]_D$ +23°. NMR data ($CDCl_3$): ^{13}C, δ 50.5 (CH_2N_3), 66.7, 69.9, 70.1, 71.3, 71.8, 73.2 (C-5, $OCH_2CH{=}CH_2$, OCH_2CH_2-OCH_2), 74.3, 77.7, 80.5 (C-2,3,4), 105.3 (C-1), 117.5 ($CH{=}CH_2$), 165.4 (C=O); ^1H, δ 3.34 (m, CH_2N_3), 3.62 (dd, $J_{4,5a}$=6.4 Hz, $J_{5a,5b}$=10.2 Hz, H-5a), 3.74 (dd, $J_{4,5b}$=3.5 Hz, H-5b), 4.22 (dd, $J_{2,3}$=4.5 Hz, $J_{3,4}$=7.9 Hz, H-3), 4.32 (m, H-4), 5.16 (s, H-1), 5.48 (d, H-2), 5.77 (m, $CH{=}CH_2$).

2-(2-Azidoethoxy)ethyl 2,5-di-*O*-benzyl-β-D-ribofuranoside (19). A solution of **18** (2.38 g, 4.78 mmol) in methanol (20 mL) was treated with sodium methoxide (0.80 mL, 1 M) overnight, then neutralized with Dowex 50 (H^+) resin, filtered and concentrated.

The residue was dissolved together with benzyl chloride (0.72 mL, 6.2 mmol) in *N,N*-dimethylformamide (20 mL) and added dropwise to sodium hydride (0.23 g, 9.6 mmol) at 0 °C under nitrogen. The cooling bath was removed after 15 min and after 2 h at room temperature methanol (2 mL) was added to destroy excess sodium hydride. The mixture was partitioned between toluene and water. The organic phase was washed with water, dried and concentrated to a yellow syrup.

A solution of this material and acetic acid (0.40 mL) in dioxane (14 mL) was treated with selenium dioxide (0.57 mg, 5.1 mmol) at reflux for 40 min. The mixture was cooled and filtered through a layer of Celite. The filtrate was concentrated and purified by column chromatography (toluene/dichloromethane, 3/1 by vol) to give **19** (0.97 g, 46%), $[\alpha]_D$ +5°. NMR data ($CDCl_3$): ^{13}C, δ 50.6 (CH_2N_3), 66.8, 69.9, 70.2, 71.5, 72.7, 73.3 (CH_2Ph, $OCH_2CH_2OCH_2$), 71.7, 81.9, 83.2 (C-2,3,4), 104.7 (C-1); ^1H, δ 3.32 (m, CH_2N_3), 3.67 (dd, $J_{4,5b}$=3.7 Hz, $J_{5a,5b}$=10.4 Hz, H-5b), 3.92 (broad d, $J_{2,3}$=5.2 Hz, H-2), 4.09 (m, $J_{3,4}$=6.4 Hz, $J_{4,5a}$=6.4 Hz, H-4), 4.20 (m, $J_{3,OH}$=9.2 Hz, H-3), 5.05 (broad s, H-1).

2-(2-Azidoethoxy)ethyl 2,5-di-*O*-benzyl-β-D-ribofuranoside 3-*H*-phosphonate triethylammonium salt (20). To a solution of **19** (0.71 g, 1.6 mmol) in 2 M phosphorous acid in pyridine (8 mL) was added 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinane (1.5 g, 8.1 mmol). After stirring overnight 0.5 M aqueous triethylammonium hydrogen carbonate (4 mL) was added carefully to destroy excess reagent. The mixture was partitioned between dichloromethane and 0.5 M aqueous triethylammonium hydrogen carbonate. The organic layer was washed with water, dried and concentrated. The residue was purified by column chromatography with a stepwise gradient of methanol in dichloromethane (0/100 to 20/80 by vol). Fractions containing the desired compound were concentrated, and partitioned between dichloromethane and aqueous triethylammonium bicarbonate (0.1 M). The organic layer was washed with water, dried and concentrated to give **20** (0.87 g, 89%), $[\alpha]_D$ -5°. NMR data ($CDCl_3$): ^{13}C, δ 8.3 (NCH_2CH_3), 45.3 (NCH_2CH_3), 50.6 (CH_2N_3), 67.0, 69.9, 70.2, 71.2, 72.4, 73.2 (C-5, CH_2Ph, $OCH_2CH_2OCH_2$), 73.3, 81.3 (C-2,4), 81.4 (d, $J_{C,P}$ 5.7 Hz, C-3), 105.7 (C-1); ^1H, δ 1.17 (t, NCH_2CH_3), 2.88 (m, NCH_2CH_3), 3.29 (m, CH_2N_3), 3.72 (dd, $J_{4,5b}$=3.3 Hz, $J_{5a,5b}$=10.7 Hz, H-5b), 4.03 (dd, $J_{1,2}$=1.8 Hz, $J_{2,3}$=4.6 Hz, H-2), 4.33 (m, $J_{3,4}$=6.1 Hz, $J_{4,5a}$=6.1 Hz, H-4), 4.76 (m, $J_{3,P}$=10.1 Hz, H-3), 5.01 (d, H-1), 6.87 (d, $J_{H,P}$=645 Hz, P-H).

Functionalization of the solid support. Bio-Beads S-X1, chloromethylated, 200-400 mesh, 1.25 meq/g (4.48 g, 5.6 mmol Cl), was reacted with di-*tert*-butyl iminodicarboxylate potassium salt[38] (2.71 g, 10.6 mmol) as described.[39] The *tert*-butyloxycarbonyl groups were removed by shaking the resin at room temperature with dichloromethane/trifluoroacetic acid/anisole (50 mL, 50/45/5 by vol) for 2 min. The mixture was filtered and the resin was treated once more with new reagent (50 mL) for 20 min. The solid was filtered and thoroughly washed in turn with dichloromethane (2x50 mL), dichloromethane/*N*,*N*-dimethylformamide (2x50 mL, 1/1 by vol), *N*,*N*-dimethyl-

formamide (2x50 mL), dichloromethane/*N,N*-dimethylformamide (2x50 mL, 1/1 by vol) and finally with dichloromethane (3x50 mL). Drying in vacuum gave the aminomethylated resin (4.83 g).

A mixture of this resin (760 mg), compound **12** (954 mg, 0.777 mmol) and triethylamine (0.30 mL) in *N,N*-dimethylformamide (6 mL) was rotated in a silanized round-bottomed flask overnight. The resin was washed with *N,N*-dimethylformamide (5x5 mL) and pyridine (4x5 mL). Unreacted amino-groups were acetylated with acetic anhydride/pyridine (12 mL, 1/2 by vol) overnight. Washing with successively pyridine (3x5 mL), *N,N*-dimethyl-formamide (3x5 mL) and dichloromethane (3x5 mL) gave the monomer functionalized resin (1.33 g, 0.46 mmol monomer/g). The extent of loading of the ribosylribitol monomer on the resin was determined, by liberating the monomethoxytrityl cation from a few milligrams of the resin, with trifluoroacetic acid in dichloromethane (0.5% by vol), measurement of the absorbance at 478 nm and comparison with a standard curve. This was prepared by treatment of known amounts of 5'-*O*-monomethoxytrityl-thymidine with trifluoroacetic acid in dichloromethane (0.5% by vol), and measurement of the UV absorbance at 478 nm.

Solid-phase synthesis of the protected linker-containing pentamer. The silanized solid-phase apparatus was charged with functionalized resin (200 mg, 0.092 mmol monomer). The resin was stirred in dichloromethane (7 mL) by nitrogen gas for 5 min, filtered and then exposed to the following cycle:

1	Trifluoroacetic acid in dichloromethane (0.5% by vol)	6x7 mL	6x1 min
2	Dichloromethane	6x7 mL	6x1 min
3	Dichloromethane/pyridine (4/1 by vol)	7 mL	1 min
4	Pivaloyl chloride (56 μL, 0.46 mmol) in dichloro-methane (1.0 mL) was added to compound **13** (539 mg, 0.46 mmol) or compound **20** (280 mg, 0.46 mmol) in dichoromethane/pyridine (4.0 mL, 4/1 by vol). After stirring for 2 min the mixture was added to the solid phase.		10 min
5	Dichloromethane/pyridine (4/1 by vol)	7 mL	1 min
6	Dichloromethane	6x7 mL	6x1 min

The filtrates from step 1 were collected to determine the amount of released monomethoxytrityl cation, by absorption measurements at 478 nm. The filtrates from steps 4 and 5 were collected, stirred with water (2 mL) for 1

h, concentrated and then purified by extraction and column chromatography as described for compound **13**, to give an 81% recovery of the theoretical amount of *H*-phosphonate **13**.

The cycle was performed four times with the ribosylribitol *H*-phosphonate **13**, and finally once with the terminating linker **20**. The resin was then treated, in the same apparatus, with a freshly prepared solution of iodine (1% w/v) in pyridine/water (10 mL, 98/2 by vol) for 30 min. The resin was washed with pyridine (6x7 mL) and dichloromethane (6x7 mL).

Deprotection of the oligomer. A mixture of the resin from above, sodium methoxide (0.60 mL, 1 M) and dioxane/methanol (12 mL, 1/1 by vol) was rotated at room temperature overnight. Acetic acid (35 μL) was added, the mixture was filtered, and the filtrate was concentrated.

A solution of this crude material and acetic acid (30 μL) in ethyl acetate/ethanol/water (12.5 mL, 1/2/2 by vol) was hydrogenated over Pd/C (120 mg, 10%) at 60 °C and atmospheric pressure overnight. The mixture was filtered and concentrated. The residue was purified by gel filtration or preparative HPLC.

Purification by gel filtration: The crude material from the hydrogenation was applied to a Fractogel TSK HW-50 (S) column (2.6 x 90 cm) and eluted with water. Fractions were checked by HPLC (C-18, Nucleosil 5 μm, 1 x 30 cm; 10 mM triethylamminium acetate and 2.5% acetonitrile in water, pH 5.3). Fractions with more than 95% of the linker-containing pentamer were pooled separately from fractions containing less of the desired substance. The impure pool was applied to the Fractogel TSK HW-50 (S) column in 10 mM ammonium bicarbonate pH 6.2, and purified by recirculation. The obtained fractions containing more than 90% of the linker-containing pentamer were pooled, concentrated and desalted. The combined pools of material were passed through a column (2 x 3 cm) of Dowex 50W x 2 (Na^+-form) with water. The eluate was concentrated. Lyophilization gave compound **21** (76 mg, 40%).

Purification by preparative HPLC: The crude material from the hydrogenation could also be purified by preparative HPLC (C-18, Nucleosil 5 μm, 1 x 30 cm) using 0.10 M aqueous triethylammonium acetate with 2.5% acetonitrile, pH 5.3, as eluent. Fractions containing the desired substance were pooled, desalted on a gel filtration column and converted into the sodium form by ion exchange as described above. Compound **21** (77 mg, 41%) was obtained in a high degree of purity (>99%), $[\alpha]_D$ -30° (*c* 0.5, H_2O). NMR data (D_2O), ribose and ribitol residues are named A to F and A' to E', respectively,

where A is the non-phosphorylated ribose residue, and F is the ribose residue of the linker: ^{13}C, δ 40.0 ($CH_2CH_2NH_2$), 63.1, 63.2 (C-5 B,C,D,E,F), 63.4 (C-5 A), 67.5 (broad d, C-5' A',B',C',D',E'), 68.0, 70.4 (OCH_2CH_2O), 69.3 ($CH_2CH_2NH_2$), 69.4 (C-1' A',B',C',D',E'), 70.9, 72.2 (C-2',3' A',B',C',D',E'), 71.4 (C-3 A), 71.7-71.8 (C-4' A',B',C',D',E'), 74.5 (C-2 F), 74.6 (C-2 B,C,D,E), 75.0 (C-3 F), 75.1, 75.1 (C-2 A, C-3 B,C,D,E), 82.7-82.8 (C-4 B,C,D,E,F), 83.5 (C-4 A), 107.5 (C-1 B,C,D,E,F), 107.7 (C-1 A); ^1H δ, 3.22 (broad t, $CH_2CH_2NH_2$), 3.66 (dd, $J_{4,5a}$=6.3 Hz, $J_{5a,5b}$=12.3 Hz, H-5a F), 3.66 (dd, $J_{4,5a}$=6.1 Hz, $J_{5a,5b}$=12.2 Hz, H-5a A), 3.69 (dd, $J_{4,5a}$=6.1 Hz, $J_{5a,5b}$=12.2 Hz, H-5a B,C,D,E), 3.74 (OCH_2CH_2O), 3.78 ($CH_2CH_2NH_2$), 3.83 (dd, $J_{4,5b}$=3.0 Hz, H-5b A), 3.87 (H-1'b A',B',C',D',E'), 3.89 (H-5b B,C,D,E,F), 3.94 (H-4' A',B',C',D',E'), 3.99 (H-5'a A',B',C',D',E'), 4.03 (m, H-4 A), 4.10 (m, H-2 A, H-5'b A',B',C',D',E'), 4.19 (m, $J_{3,4}$=6.5 Hz, $J_{4,5b}$=3.1 Hz, H-4 B,C,D,E,F), 4.23 (dd, $J_{1,2}$=1.5 Hz, $J_{2,3}$=4.6 Hz, H-2 F), 4.26 (m, H-3 A, H-2 B,C,D,E), 4.58 (m, $J_{3,P}$=8.2 Hz, H-3 F), 4.62 (m, $J_{2,3}$=4.6 Hz, $J_{3,P}$=8.2 Hz, H-3 B,C,D,E), 5.03 (broad s, H-1 A), 5.06 (broad s, H-1 B,C,D,E), 5.07 (d, H-1 F). The ^1H NMR spectrum is shown in Fig. 1.

FAB-MS of compound **21** showed a pseudo molecular ion $[M+NH_4]^+$ of m/z 1986, which is consistent with the mass of the linker-containing pentamer, where all phosphate groups are protonated. $[M+NH_4]^+$ is frequently found when thioglycerol is used as matrix. The ammonium ion probably originates from a contamination.

ACKNOWLEDGEMENTS

We thank Mr. Gunnar Grönberg (Analytical Department, BioCarb AB) for recording the NMR spectra, Mr. Stefan Strömberg (Analytical Department, BioCarb AB) for recording the FAB-MS spectrum, and Mr. Åke Moberg and Ms. Karin Erlansson (Isolation and Separation Department, BioCarb AB) for technical assistance. We also thank Prof. Jacek Stawinski and Dr. Roger Strömberg (Department of Organic Chemistry, University of Stockholm) for valuable discussions.

REFERENCES AND FOOTNOTES

1. R. M. Crisel, R. S. Baker, and D. E. Dorman, *J. Biol. Chem.*, **250**, 4926 (1975).

2. P. Branefors-Helander, C. Erbing, L. Kenne, and B. Lindberg, *Acta Chem. Scand.*, **B30**, 276 (1976).

3. P. W. Anderson, Jr., E. C. Stein, and R. A. Insel in *Conjugate Vaccines;* J. M. Cruse, R. E. Lewis, Jr., Eds.; Karger AG: Basel, 1989, p 115.

4. E. D. Shapiro, *Pediatr. Clin. North Am.*, **37**, 567 (1990).

5. D. A. Clements, and G. L. Gilbert, *Aust. N. Z. J. Med.*, **20**, 828 (1990).

6. P. J. Garegg, B. Lindberg, and B. Samuelsson, *Carbohydr. Res.*, **58**, 219 (1977).

7. P. J. Garegg, and B. Samuelsson, *Carbohydr. Res.*, **86**, 293 (1980).

8. P. J. Garegg, R. Johansson, I. Lindh, and B. Samuelsson, *Carbohydr. Res.*, **150**, 285 (1986).

9. P. Hoogerhout, D. Evenberg, C. A. A. van Boeckel, J. T. Poolman, E. C. Beuvery, G. A. van der Marel, and J. H. van Boom, *Tetrahedron Lett.*, **28**, 1553 (1987).

10. J. P. G. Hermans, L. Poot, M. Kloosterman, G. A. van der Marel, C. A. A. van Boeckel, D. Evenberg, J. T. Poolman, P. Hoogerhout, and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **106**, 498 (1987).

11. P. Hoogerhout, C. W. Funke, J. -R. Mellema, G. N. Wagenaars, C. A. A. van Boeckel, D. Evenberg, J. T. Poolman, A. W. M. Lefeber, G. A. van den Marel, and J. H. van Boom, *J. Carbohydr. Chem.*, **7**, 399 (1988).

12. Z. Y. Wang, and G. Just, *Tetrahedron Lett.*, **29**, 1525 (1988).

13. L. Chan, and G. Just, *Tetrahedron Lett.*, **29**, 4049 (1988).

14. L. Chan, and G. Just, *Tetrahedron*, **46**, 151 (1990).

15. C. J. J. Elie, H. J. Muntendam, H. van den Elst, G. A. van den Marel, P. Hoogerhout, and J. H. van Boom, *Rec. Trav. Chim. Pays-Bas*, **108**, 219 (1989).

16. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Chemica Scripta*, **25**, 280 (1985).

17. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Chemica Scripta*, **26**, 59 (1986).

18. B. C. Froehler, and M. D. Matteucci, *Tetrahedron Lett.*, **27**, 469 (1986).

19. N. J. Leonard, and K. L. Carraway, *J. Heterocycl. Chem.*, **3**, 485 (1966).

20. *Oligonucleotide Synthesis - a Practical Approach*, M. J. Gait, Ed.; IRL Press: Oxford, 1984.

21. J. Stawinski, and M. Thelin, *Nucleosides Nucleotides*, **9**, 129 (1990).

22. K. Kariyone, and H. Yazawa, *Tetrahedron Lett.*, **33**, 2885 (1970).

23. G. H. Veeneman, H. F. Brugghe, H. van den Elst, and J. H. van Boom, *Carbohydr. Res.*, **195**, C1 (1990).

24. P. Westerduin, G. H. Veeneman, Y. Pennings, G. A. van der Marel, and J. H. van Boom, *Tetrahedron Lett.*, **28**, 1557 (1987).

25. F. Bardella, E. Giralt, and E. Pedroso, *Tetrahedron Lett.*, **31**, 6231 (1990).

26 P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, and R. Strömberg, *Tetrahedron Lett.*, **27**, 4051 (1986).

27. P. J. Garegg, I. Lindh, T. Regberg, J. Stawinski, and R. Strömberg, *Tetrahedron Lett.*, **27**, 4055 (1986).

28. A. Andrus, J. W. Efcavitch, L. J. McBride, and B. Giusti, *Tetrahedron Lett.*, **29**, 861 (1988).

29. R. Strömberg, *Chem. Commun. Univ. Stockholm*, **1**, 1 (1987).

30. H. Takaku, S. Yamakage, O. Sakatsume, and M. Ohtsuki, *Chem. Lett.*, 1675 (1988).

31. B. L. Gaffney, and R. A. Jones, *Tetrahedron Lett.*, **29**, 2619 (1988).

32. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *Nucleosides Nucleotides*, **6**, 655 (1987).

33. V. A. Efimov, and I. Y. Dubei, *Sov. J. Bioorg. Chem. Engl. Transl.*, **16**, 125-131 (1990).

34. K. K. Ogilvie, and M. J. Nemer, *Tetrahedron Lett.*, **21**, 4145 (1980).

35. P. J. Garegg, T. Regberg, J. Stawinski, and R. Strömberg, *J. Chem. Soc., Perkin Trans. I*, 1269 (1987).

36. P. A. J. Gorin, and M. Mazurek, *Can. J. Chem.*, **53**, 1212 (1975).

37. J. D. Stevens, and H. G. Fletcher Jr., *J. Org. Chem.*, **33**, 1799 (1968).

38. The salt was prepared by mixing di-*tert*-butyl iminodicarbonate[39] (4.0 g, 18 mmol), in ethanol/water (28 mL, 10/4 by vol), with aqueous potassium hydroxide (8.7 mL, 2.0 M), concentration and drying in vaccum.

39. L. Grehn, and U. Ragnarsson, *Synthesis*, 275 (1987).