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Synthesis of octa- and dodecamers of D-ribitol-1-phosphate and their protein conjugates

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This paper is dedicated to Professor András Lipták on the occasion of his 70th birthday

Abstract—The bacterial cell-wall-associated teichoic acids contain predominantly D-ribitol residues interconnected by phosphodiester linkages. Because of their location, these antigens may be vaccine candidates as part of conjugate vaccines. Here, we describe the synthesis of extended oligomers of D-ribitol-1-phosphate linked to a spacer having an amino group at its terminus. The synthesis utilized a fully protected D-ribitol-phosphoramidite that was oligomerized in a stepwise fashion followed by deprotection. The free oligomers were connected to bovine serum albumin using oxime chemistry. Thus, the ribitol phosphate oligomers were converted into keto derivatives, and the albumin counterpart was decorated with aminooxy groups. Reaction of the functionalized saccharide and protein moieties afforded conjugates having up to 20 ribitol phosphate chains.

Keywords: Glycoconjugate; Phosphodiester; Ribitol phosphate; Synthesis; Teichoic acid; Vaccine

1. Introduction

Polymers of D-ribitol phosphate (teichoic acids) are major cell-wall components of many Gram-positive bacteria. These anionic structures form wall-associated carbohydrate-containing linear chains, where the repeating units are connected through phosphodiester linkages, covalently linked to cell-wall muramic acid residues in the peptidoglycan. The physiological roles of teichoic acids include ion exchange and control of the activity of autolytic enzymes that are important for growth and division of bacterial cells.[1] There are several types of teichoic acids, and their biosynthesis, function and compositions have been described.[2–4] Typically, poly(D-ribitol phosphates) are substituted with different sugars including amino sugars or a D-alanine ester.[1] The size and degree of substitution varies and depends on the age of the cells and the composition of the growth media.[5] The size of teichoic acids isolated from *Bacillus subtilis* and *Staphylococcus aureus* was reported to be around 25 kDa accounting for an average range of 45–60 repeating units.[6,7] Common are 1,5-poly(D-ribitol phosphates) found in staphylococci, bacilli and 3,5-poly(ribitol phosphates) in *Nocardiopsis* species.[8] Pathogenic Gram-negative bacteria like *Haemophilus influenzae* types a and b also contain ribitol phosphate moieties in their capsular polysaccharides (CPS's). Antibodies elicited by *H. influenzae* type b CPS, composed of D-ribosyl-D-ribitol phosphate repeating subunit, were shown to precipitate poly(D-ribitol phosphates) of *S. aureus*, *Bacillus pumilus* and *Lactobacillus plantarum*.[9] We have shown recently that antibodies induced by a protein conjugate of an unseparable mixture of

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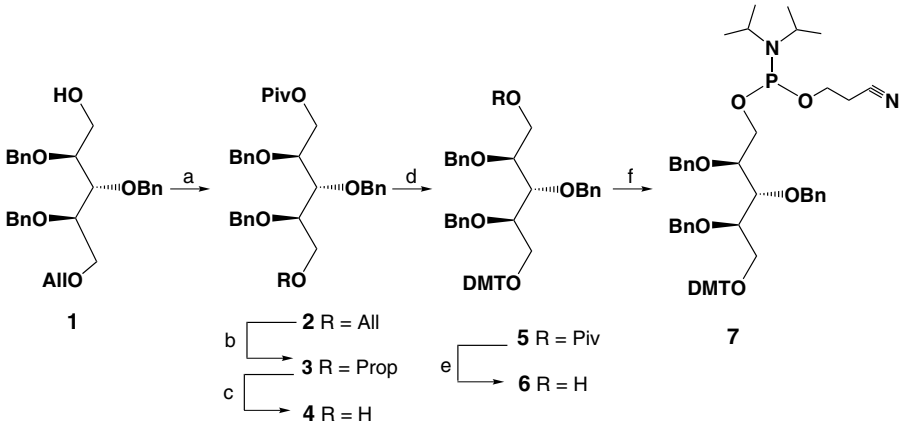
poly(D-ribitol phosphate) and poly(glycerol phosphate) of *B. pumilus* Sh 18 recognized CPS's of *H. influenzae* types a and b, as well as those of *S. aureus* and *S. epidermidis* teichoic acids.[10] So far we have not been able to isolate a pure chain of poly(D-ribitol phosphate) from any Gram-positive bacteria. We note that related structures, including oligomers of ribosyl-ribitol phosphate, have been prepared by chemical synthesis either in a stepwise approach[11] or by controlled polymerization.[12] However, the synthesis of polymers of ribitol phosphate has not yet been described. According to our experience, the size of an oligosaccharide to induce polysaccharide-specific antibody response when covalently linked to an immunogenic protein should preferably be in the decamer range.[13,14] Therefore, we decided to synthesize an octamer and a dodecamer of ribitol phosphate to investigate the role of the chain length in the immunological properties of ribitol phosphate oligomers.

Here, we report chemical synthesis of compounds **34** and **35**, that is, octa- and dodecamers of D-ribitol phosphate carrying a reactive amino group that enables their conjugation to proteins. We also describe the conjugation of the target compounds to bovine serum albumin using a method recently reported by Kubler-Kielb and Pozsgay,[15] using thioether[16] and oxime chemistries.[17–19] Such conjugates will be used for the development of a single-component experimental vaccine likely capable of acting against several pathogens carrying D-ribitol phosphate moieties in their surface antigens.

2. Results and discussion

For the preparation of the target compounds **34** and **35**, the phosphoramidite method[20] which has been developed for solid-phase oligonucleotide synthesis[21] and adopted by van Boom and co-workers for the synthesis of a broad range of phosphodiester-linked oligomers of carbohydrates, was chosen.[22]

In applying van Boom's phosphoramidite protocol, we have chosen a stepwise sequential approach using D-ribitol derivative **7** as the key building block. In compound **7**, the phosphityl moiety serves as the precursor for the phosphodiester part of the final products, the 4,4′-dimethoxytrityl (DMT) group is a temporary protecting group at the site of chain extension, whereas the three benzyl groups provide permanent protection until the last stages of the synthesis. Preparation of phosphoramidite **7** started with D-ribitol derivative **1** that was synthesized from D-ribonolactone as described by van Boom and co-workers.[23,24] Compound **1** was treated with trimethylacetyl chloride in pyridine to give the pivaloyl derivative **2** (Scheme 1). The pivaloyl group was chosen over the conventional *O*-acetyl protection because of the higher stability of pivaloyl esters during the tritylation step (see below). Next, the allyl group at O-5 was removed in a two-step procedure involving isomerization into the propenyl derivative using (1,5-cyclooctadiene)-bis(methyldiphenylphosphine)iridium hexafluorophosphate[25,26] (→**3**) followed by mercury(II) chloride-assisted hydrolysis to afford **4** in a 92% combined yield over three steps. The ribitol alcohol **4** so obtained was converted to the trityl derivative **5** using 4,4′-dimethoxytrityl chloride in the presence of Hünig's base to afford compound **5** (85%). Next, the trimethylacetyl protecting group was removed under alkaline conditions to give the hydroxy derivative **6** in 90% yield. That this compound was the optically pure D-isomer was shown by its conversion to either (−)-(*R*)- or (+)-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl derivative using the corresponding acid chlorides, to afford the so-called Mosher esters.[27] The observation of only one resonance in the ^{19}F NMR spectrum provides a proof of **6** being a single stereoisomer; any isomerization during the preceding steps would have led to an equilib-

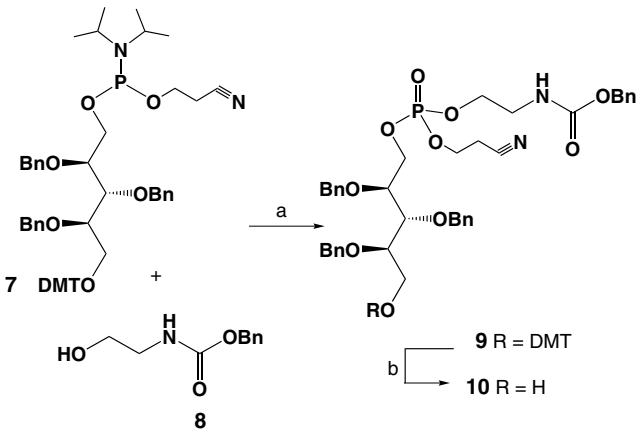


Scheme 1. Reagents and conditions: (a) 1.05 equiv of pivaloyl chloride, C_5H_5N, 0 °C, 1.5 h; (b) (1,5-cyclooctadiene)-bis(methyldiphenylphosphine)iridium hexafluorophosphate (cat.), THF, 23 °C, 4 h; (c) 1.06 equiv of HgO and 1.05 equiv of $HgCl_2$, acetone–H_2O, 23 °C, 0.5 h; (d) 1.25 equiv of EtN(*i*-Pr)$_2$, 1 equiv of DMTCl, CH_3CN, 23 °C, 1.5 h; (e) 2 equiv of NaOMe, dioxane and MeOH, 23 °C, 72 h; (f) 5 equiv of EtN(*i*-Pr)$_2$, 2.45 equiv of chloro-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite, 1,2-dichloroethane, 0–23 °C, 2 h.

rium mixture exhibiting more than one line in the ^{19}F spectrum. On the other hand, when the same experiment was performed on the Mosher esters of the corresponding D,L ribitol derivative (not described in the experimental), the ^{19}F NMR spectrum showed two signals of equal intensity, indicating two stereoisomers. Subsequently, ribitol **6** was phosphitylated using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in a mixture of Hünig's base and dichloromethane to afford phosphoramidite **7** in 61% yield.

In our original strategy, we planned to oligomerize compound **7** in an automatic solid-phase oligonucleotide synthesizer,[24] using standard phosphoramidite chemistry[20] consisting of the following steps: (1) 1*H*-tetrazole-assisted attachment to the glass-attached spacer, (2) oxidation of the phosphite triester into phosphate triester using iodine, (3) capping of unreacted 'acceptor' and (4) cleavage of the DMT group using trichloroacetic acid. After 6 cycles, the linkage between the solid support and the spacer was cleaved by the action of ammonia (not described in the experimental). Surprisingly, an intractable product mixture was obtained that did not appear to contain the targeted hexamer. This failure prompted us to reinvestigate the oligomerization using solution-phase chemistry.

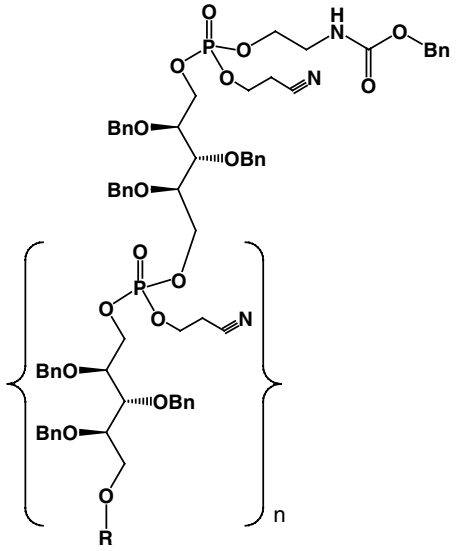
Thus, phosphoramidite **7** was combined with the spacer **8** by treatment with 1*H*-tetrazole in MeCN, followed by oxidation with iodine to afford phosphotriester **9** in 77% yield (Scheme 2). Attempted removal of the DMT group from compound **9** by trichloroacetic acid simultaneously also cleaved the phosphotriester moiety. This observation explained our failure to use standard phosphoramidite chemistry in our attempted solid-phase synthesis as described above. Next, we investigated less aggressive conditions, and eventually we found that an 85:10:5 mixture of acetic acid, CH_2Cl_2 and water selectively cleaved the DMT group in compound **9** and



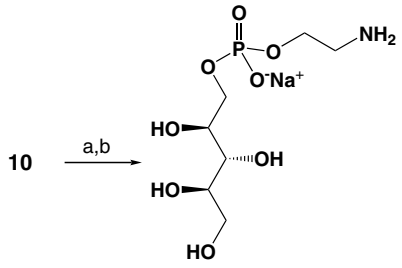
Scheme 2. Reagents and conditions: (a) 10 equiv of a 0.45 M solution of tetrazole in CH_3CN, CH_3CN, 23 °C, 1 h, then 0.5 M solution of iodine in 2:1 THF–water (excess); (b) AcOH–H_2O, 23 °C, 2 h.

afforded **10** in 88% yield without compromising the integrity of the phosphotriester moiety. However, the reaction time increased up to 4 h, compared with just a few minutes for such a cleavage in the standard oligonucleotide synthesis. We hypothesized that the use of aqueous acetic acid instead of trichloroacetic acid for the removal of the DMT group would require even longer reaction times in solid-phase approach, thus abolishing one of its main attractions, that is, increased speed over solution-phase chemistry. Therefore, we decided to use solution-phase chemistry throughout this project.

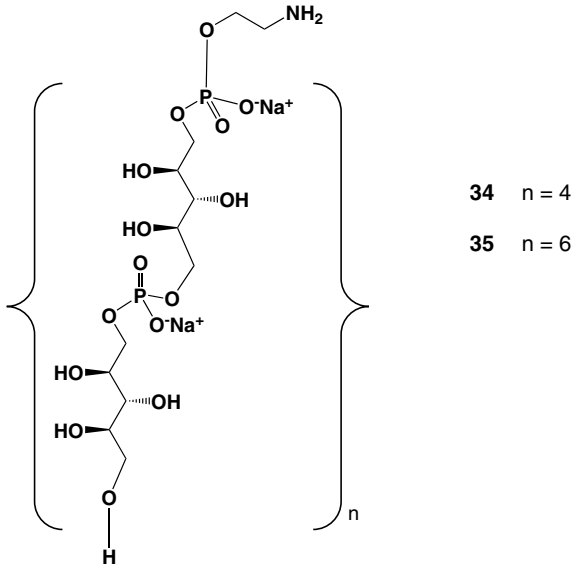
Next, alcohol **10** was coupled with phosphoramidite **7** as described for the preparation of **9** to afford the dimer **11** from which the 4,4′-dimethoxytrityl group was removed by acetic acid to afford the 'acceptor' **12**. Subsequent iterative chain extensions, presented in detail in Section 4, using the reaction sequence as described above, afforded the higher membered oligomers of ribitol phosphate up to the dodecamer **32**.



11	n = 1	R = DMT	**22**	n = 6	R = H
12	n = 1	R = H	**23**	n = 7	R = DMT
13	n = 2	R = DMT	**24**	n = 7	R = H
14	n = 2	R = H	**25**	n = 8	R = DMT
15	n = 3	R = DMT	**26**	n = 8	R = H
16	n = 3	R = H	**27**	n = 9	R = DMT
17	n = 4	R = DMT	**28**	n = 9	R = H
18	n = 4	R = H	**29**	n = 10	R = DMT
19	n = 5	R = DMT	**30**	n = 10	R = H
20	n = 5	R = H	**31**	n = 11	R = DMT
21	n = 6	R = DMT	**32**	n = 11	R = H

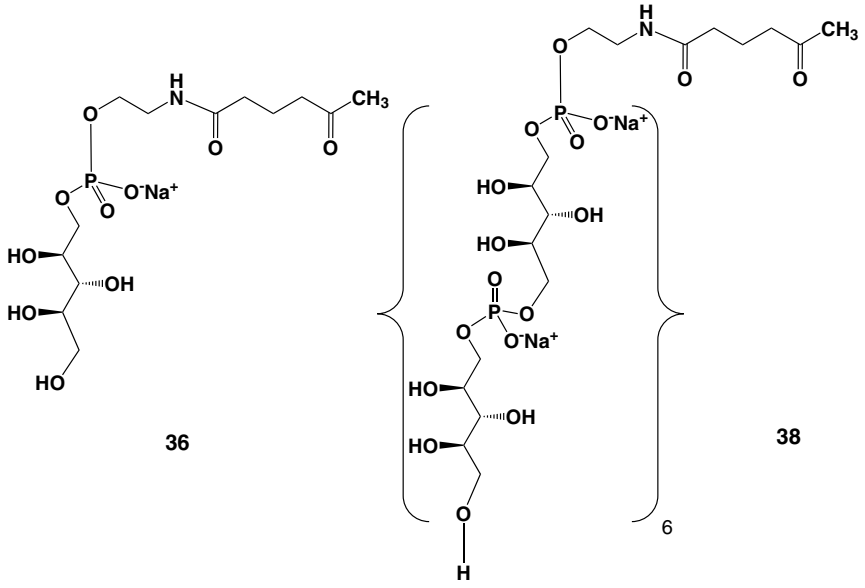


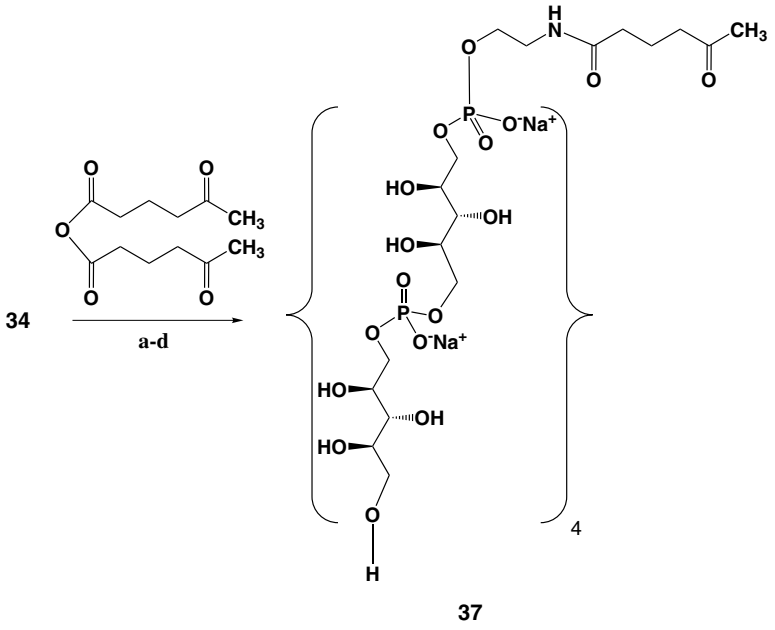
Scheme 3. Reagents and conditions: (a) MeOH, concd NH_4OH, 50 °C, 8 h, Dowex 50 × 8–100(Na^+); (b) H_2, 10% Pd/C, 2:1 *t*-BuOH–water, 23 °C, 2 days.



Having assembled extended oligomers of D-ribitol phosphate, we started the removal of the protecting groups. Hydrolysis of the cyanoethyl group from the monomeric phosphotriester **10** was uneventful as expected to provide a phosphodiester that was subjected to catalytic hydrogenation/hydrogenolysis. Surprisingly, when the hydrogenolysis was carried out in ethanol, the only product that was isolated was the *N*-ethyl derivative of the targeted ribitol phosphate. While the formation of the *N*-ethyl derivative was unexpected, N-alkylation during hydrogenolysis in primary or secondary alcohols is not unprecedented and is explained by the formation of an aldehyde from the solvent by its catalytic oxidation, presumably by oxygen adsorbed on the catalyst, followed by Schiff-base formation and subsequent reduction.[28–30] Subsequently, we found that the formation of the *N*-ethyl derivative could be completely suppressed when the hydrogenolysis was carried out in *t*-BuOH, affording the free ribitol phosphate **33** as the only product (Scheme 3). Removal of the protecting groups from the higher membered oligomers **24** and **32** was performed similarly and the unprotected octa- (**34**) and dodecamer (**35**) were isolated in 66% and 80% yields, respectively. The structures of compounds **34** and **35** were verified by NMR and mass-spectrometric methods (see Section 4). Particular proof was provided by the ^{31}P NMR spectra of **34** and **35**, which exhibited two sets of characteristic resonances in the ratio of 7:1 and 11:1, respectively, indicating a different environment of just one of the phosphodiester linkages, as expected.

Covalent attachment of **33**, **34** and **35** to bovine serum albumin (BSA) was performed according to the procedure of Kubler-Kielb and Pozsgay.[15] The method is based on the well-documented oxime formation between an aldehyde or a ketone and an aminooxy compound





Scheme 4. Reagents and conditions: (a) 5-ketohexanoic anhydride (excess), MeOH–H_2O, triethylamine, 23 °C, 20 min; (b) Bio-Gel P2 chromatography in 0.02 M C_5H_5N–AcOH; (c) freeze drying; (d) Dowex 50 × 8–100(Na^+), H_2O.

Table 1. Conjugates of ribitol phosphates **36**, **37** and **38** with bovine serum albumin

Ribitol phosphate	Amount of the aminooxy groups on BSA (μmol)	Amount of hapten (μmol)	Mass by MALDI [Da]	Hapten loading on BSA (mol/mol)	Protein/sugar ratio (mol/mol)
36	4	7.3	81,249	18	1:0.1
37	4	5.8	106,077	15	1:0.5
38	1.2	1.9	106,110	10	1:0.5

and proceeds under mild conditions. We have chosen to introduce aminooxy groups in the protein and convert the carbohydrate counterpart into a keto derivative. For example, octamer **34** was reacted with 5-ketohexanoic anhydride in aqueous MeOH in the presence of triethylamine (Scheme 4). Purification of the product by chromatography on a Bio-Gel P2 column, followed by conversion to the sodium form using Dowex 50 × 8–100 (Na^+), afforded compound **37** in a 85% yield. The monomer **33** and the dodecamer **35** were similarly acylated to afford the corresponding ketohexanoyl derivatives **36** and **38**. The keto derivatives **36**–**38** were allowed to react with aminooxylated BSA in pH 7.4 phosphate buffer as recently reported.[15] Table 1 shows the results of some typical conjugation experiments, indicating average incorporation levels of 10–18 saccharide units per mole of BSA as determined by MALDI-TOF mass spectrometry.

3. Conclusions

We constructed spacer-linked, higher membered ribitol phosphate oligomers from a suitably protected, monomeric ribitol phosphoramidite by stepwise polymerization. We have also demonstrated that the amino spacer can serve as a handle to conjugate the oligomers to proteins under mild conditions using oxime chemistry. The immunogenicity and antigenicity of the protein conjugates described here and conjugates of the octa- and dodecamers with medically useful proteins are currently being evaluated.

4. Experimental

4.1. General methods

All chemicals were of commercial grade and used without purification. Solvents for chromatography were distilled prior to use. Anhydrous solvents were obtained from Aldrich Chemical Company. Bovine serum albumin was purchased from Sigma Chemical Company. Column chromatography was performed on Silica Gel 60 (0.040–0.063 mm). The ^1H and ^{13}C NMR spectra were recorded at either 500 or 300 MHz and 125 and 75.5 MHz, respectively. Internal references: TMS (0.000 ppm for ^1H for solutions in $CDCl_3$), acetone

(2.225 ppm for ^{1}H and 31.00 ppm for ^{13}C for solutions in D_2O), MeOH (3.358 ppm for ^{1}H and 49.68 ppm for ^{13}C for solutions in D_2O) and $CDCl_3$ (77.00 ppm for ^{13}C for solutions in $CDCl_3$). Coupling constants are given in hertz. The electrospray-ionization mass spectra (ESIMS) were recorded at the Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD. For the MALDI-TOF mass spectra, the protein conjugates were dissolved in 0.1% TFA in 30% aq MeCN and applied to the target in sinapinic acid matrix. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

4.2. 5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-pivaloyl-D-ribitol (2)

To a stirred solution of 5-*O*-allyl-2,3,4-tri-*O*-benzyl-D-ribitol[23,24,31,32] (**1**) (4.63 g, 10.0 mmol) in dry pyridine (25 mL) was added dropwise pivaloyl chloride (1.30 mL, 10.5 mmol) at 0 °C within 5 min. Stirring at 0 °C was continued for 25 min, then at 23 °C for 90 min. Water (1 mL) was added, and the reaction mixture was concentrated. A solution of the residue in 1:1 Et_2O–hexane was washed successively with water and with 1 M aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in toluene, followed by concentration under reduced pressure. Compound **2** thus obtained was used in the next step without further purification. A small amount was purified by column chromatography for analytical purposes. ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 9H, CH_3), 3.58–3.72 (m, 2H), 3.82–3.92 (m, 3H), 3.94–3.98 (m, 2H), 4.16–4.24 (dd, 1H), 4.50–4.76 (m, 7H), 5.14–5.25 (m, 2H), 5.82–5.94 (m, 1H), 7.2–7.4 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 27.2, 38.8, 63.9, 69.8, 72.18, 72.20, 72.4, 73.8, 77.5, 78.2, 78.5, 116.8, 127–129, 134.8, 138.2, 138.3, 138.4, 178.3. HRESIMS: Calcd for $C_{34}H_{42}N_{12}LiO_6$, *m*/*z* 553.3145; found, *m*/*z* 553.3141. Anal. Calcd for $C_{34}H_{42}O_6$: C, 74.70; H, 7.74. Found: C, 74.84; H, 7.65.

4.3. 2,3,4-Tri-*O*-benzyl-1-*O*-pivaloyl-5-*O*-(propen-1-yl)-D-ribitol (3)

A solution of compound **2** (5.46 g, 10.0 mmol) in THF (10 mL) was alternatively degassed and placed under helium. (1,5-Cyclooctadiene)-bis(methyldiphenylphosphine)iridium hexafluorophosphate[25,26] (~5 mg) was added, followed by degassing as above. A stream of H_2 was passed through the solution for 5 min. Next, the reaction mixture was degassed, then a gentle stream of helium was passed through it for 4 h. The reaction mixture was concentrated, and compound **3** thus obtained was used in the next step without purification. A small amount was purified by column chromatography for analytical purposes. ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 9H), 1.54 (m, 3H), 3.80–3.95 (m, 5H), 4.15–4.25 (dd, 1H), 4.50–4.80 (m, 8H), 6.15–6.25 (dd, 1H), 7.2–7.4 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 12.6, 27.3, 38.9, 63.7, 68.5, 72.2, 72.5, 73.9, 77.4, 77.5, 78.3, 98.8, 127.0–129.0, 138.2–138.4, 146.6, 178.4. HRESIMS: Calcd for $C_{34}H_{42}LiO_6$, *m*/*z* 553.3145; found, *m*/*z* 553.3148. Anal. Calcd for $C_{34}H_{42}O_6$: C, 74.70; H, 7.74. Found: C, 74.61; H, 7.70.

4.4. 2,3,4-Tri-*O*-benzyl-1-*O*-pivaloyl-D-ribitol (4)

To a solution of compound **3** (5.46 g, 10.0 mmol) in acetone (115 mL) and water (8 mL), HgO (2.29 g, 10.6 mmol) and $HgCl_2$ (2.86 g, 10.5 mmol) were added.[24] The resulting suspension was stirred at room temperature for 30 min. The solids were removed by filtration. The filtrate was successively washed with 50% satd aq KI, 1% aq $NaHSO_3$ and 1 M aq $NaHCO_3$, followed by separation of the phases. The organic phase was dried (Na_2SO_4). Removal of the volatiles under reduced pressure afforded product **4** (4.65 g, 92% for three steps) as an amorphous material that was used in the next step without further purification. A small amount was purified by column chromatography for analytical purposes. ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 9H), 2.15 (t, 1H), 3.70–3.85 (m, 4H), 3.85–3.95 (m, 2H), 4.2–4.3 (dd, 1H), 4.5–4.8 (m, 6H), 7.2–7.4 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 27.3, 38.9, 61.2, 63.7, 72.1, 72.3, 74.1, 77.4, 78.7, 78.8, 127.0–129.0, 138.0, 138.1, 178.4. HRESIMS: Calcd for $C_{31}H_{38}LiO_6$, *m*/*z* 513.2796; found, *m*/*z* 513.2828. Anal. Calcd for $C_{31}H_{38}O_6$: C, 73.49; H, 7.56. Found: C, 73.19; H, 7.70.

4.5. 2,3,4-Tri-*O*-benzyl-5-*O*-(4,4′-dimethoxytrityl)-1-*O*-pivaloyl-D-ribitol (5)

Compound **4** (4.65 g, 9.18 mmol) was dissolved in dry MeCN, followed by concentration. This procedure was repeated two more times. To a solution of the residue in dry MeCN (30 mL) were added at 23 °C *N*,*N*-diisopropylethylamine (2.0 mL, 11.5 mmol) and 4,4′-dimethoxytrityl chloride (3.11 g, 9.18 mmol). After 90 min, the solution was treated with 1 M aq $NaHCO_3$ (15 mL), followed by concentration. A solution of the residue in 1:1 Et_2O–hexane was washed with 1 M aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography using 100:0→85:15 hexane–EtOAc containing 0.5% Et_3N as the eluant to afford **5** (6.30 g, 85%) as a syrup. A small amount was purified by column chromatography for analytical purposes. ^{1}H NMR ($CDCl_3$) (selected data): δ 1.18 (s, 9H), 3.3–3.4 (dd, 1H), 3.5–3.55 (dd, 1H), 3.7–3.95 (m, 10H), 4.1–4.2 (dd, 1H), 4.45–4.8 (m, 6H), 7.05–7.5 (28H). ^{13}C NMR ($CDCl_3$):

δ 27.2, 38.7, 55.1, 63.4, 64.2, 72.3, 72.6, 73.6, 77.8, 78.6, 78.8, 86.1, 110.0–160.0, 178.3. HRESIMS: Calcd for $C_{52}H_{56}LiO_8$, *m*/*z* 815.4135; found, *m*/*z* 815.4120. Anal. Calcd for $C_{52}H_{56}O_8$: C, 77.20; H, 6.98. Found: C, 77.33; H, 7.16.

4.6. 2,3,4-Tri-*O*-benzyl-5-*O*-(4,4′-dimethoxytrityl)-D-ribitol (6)

Compound **5** (6.30 g, 7.79 mmol) was dissolved in dry dioxane, and the resulting solution was concentrated under reduced pressure. This procedure was repeated two more times. To a solution of the residue in dry dioxane (10 mL), dry MeOH (15 mL) and NaOMe in MeOH (3 mL, 30% w/w, 16 mmol) were added. After 72 h the mixture was concentrated. A solution of the residue in a 1:1 mixture of Et_2O and hexane was washed with water and 1 M aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography using 9:1→7:3 hexane–EtOAc containing 0.5% Et_3N as the eluant to give **6** (5.06 g, 90%) as an amorphous material. A small amount was purified by column chromatography for analytical purposes. ^{1}H NMR ($CDCl_3$): δ 2.17 (br t, 1H), 3.38 (m, 2H), 3.70–3.78 (m, 9H), 3.8–4.0 (m, 2H), 4.44–4.74 (m, 6H), 6.70–7.50 (28H). ^{13}C NMR ($CDCl_3$): δ 55.2, 61.4, 63.3, 71.9, 72.7, 73.8, 78.6, 78.9, 79.4, 86.2, 110.0–160.0. Anal. Calcd for $C_{47}H_{48}O_7$: C, 77.88; H, 6.67. Found: C, 77.92; H, 6.75.

4.7. 2-Cyanoethyl [2,3,4-tri-*O*-benzyl-5-*O*-(4,4′-dimethoxytrityl)-1-D-ribityl] *N*,*N*-diisopropylphosphoramidite (7)

A solution of compound **6** (2.52 g, 3.48 mmol) in dry pyridine was concentrated under reduced pressure. To a solution of the residue in dry 1,2-dichloroethane (15 mL) was added *N*,*N*-diisopropylethylamine (3.0 mL, 17.4 mmol). To the solution thus obtained chloro-2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (1.9 mL, 8.52 mmol) was added dropwise at 0 °C within 5 min under stirring. Stirring at 0 °C was continued for 25 min, then at 23 °C for 90 min. The reaction mixture was concentrated. A solution of the residue in 1:1 EtOAc–hexane was washed with 1 M aq $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography using 9:1→4:1 hexane–EtOAc containing 0.5% Et_3N as the eluant to give **7** (1.96 g, 61%) as a syrupy material. ^{1}H NMR ($CDCl_3$): δ 1.08–1.18 (m), 2.30–2.50 (m), 3.76 (s). ^{13}C NMR ($CDCl_3$): δ 20.10, 20.13, 20.16, 20.20, 24.5, 42.94, 43.01, 43.06, 43.14, 55.08, 55.09, 58.18, 58.25, 58.37, 58.44, 86.0, 117.58, 117.64. ^{31}P NMR ($CDCl_3$): δ 148.8, 149.1. Anal. Calcd for $C_{56}H_{65}N_2O_8P$: C, 72.71; H, 7.08. Found: C, 72.89; H, 7.00.

4.8. [(*N*-Benzyloxycarbonyl)-2-aminoethyl] 2-cyanoethyl [2,3,4-tri-*O*-benzyl-5-*O*-(4,4′-dimethoxytrityl)-1-D-ribityl] phosphate (9)

To a stirred solution of compound **7** (2.4 g, 2.59 mmol) and benzyl *N*-(2-hydroxyethyl) carbamate **(8)** (1.52 g, 7.78 mmol) in dry MeCN (70 mL) was added a 0.45 M solution of tetrazole in MeCN (57.6 mL, 25.9 mmol) at 23 °C. After 1 h, dry pyridine (2 mL, 24.7 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction was quenched with satd aq $Na_2S_2O_3$. The organic solvent was removed under reduced pressure. A solution of the residue in CH_2Cl_2 was washed with water, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography using 9:1 CH_2Cl_2–acetone as the eluant to afford **9** (2.06 g, 77%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.09–7.5 (m, 29H), 6.7–6.78 (m, 4H), 5.18–5.26 (m, 1H), 5.05 (br s), 3.74 (br s, 6H), 3.26–3.4 (m, 4H), 2.33–2.5 (m, 2H). HRESIMS: Calcd for $C_{60}H_{63}N_2NaO_{12}P$, *m*/*z* 1057.4016; found, *m*/*z* 1057.3981.

4.9. [(*N*-Benzyloxycarbonyl)-2-aminoethyl] 2-cyanoethyl (2,3,4-tri-*O*-benzyl-1-D-ribityl) phosphate (10)

To a stirred mixture of compound **9** (2 g, 1.94 mmol) in CH_2Cl_2 (10 mL) and water (5 mL) was added glacial HOAc (85 mL). The mixture was stirred at 23 °C for 4 h followed by concentration under vacuum. Toluene was added to and evaporated from the residue. A solution of the residue in CH_2Cl_2 was washed with a satd aq $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated. Column chromatography of the residue using 9:1 CH_2Cl_2–acetone as the eluant gave **10** (1.25 g, 88%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.2–7.4 (m, 20H), 5.27–5.34 (m, 1H), 5.06 (br s, 2H), 3.65–3.78 (m, 2H) 3.28–3.4 (m, 2H), 2.42–2.57 (m, 2H). HR-ESIMS: Calcd for $C_{39}H_{46}N_2O_{10}P$, *m*/*z* 733.2890; found, *m*/*z* 733.2878.

4.10. Dimer (11)

To a solution of compound **7** (3.16 g, 3.4 mmol) and compound **10** (1.25 g, 1.7 mmol) in dry MeCN (130 mL) was added a 0.45 M solution of tetrazole in MeCN (75.7 mL, 34 mmol) at 23 °C. After stirring for 1 h, dry pyridine (4.3 mL, 53.2 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for the preparation of **9**. The residue was purified by column chromatography using 95:5→9:1 CH_2Cl_2–acetone as the eluant to afford **11** (2.3 g, 86%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.02–7.48 (m, 44H), 6.67–6.76 (m), 5.22–5.3 (m, 1H), 5.05 (br s, 2H), 3.75 (s, 6H), 3.22–3.4 (m, 4H), 2.35–2.56

(m, 2H), 2.1–2.35 (m, 2H). HRESIMS: Calcd for $C_{89}H_{95}N_3NaO_{19}P_2$, *m/z* 1594.593; found, *m/z* 1594.5876.

4.11. De(dimethoxytrityl)ation of dimer (12)

Compound **11** (2.3 g, 1.46 mmol) was processed as described for **10**. Column chromatography (9:1→4:1 CH_2Cl_2–acetone) of the residue gave **12** (1.61 g, 87%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 7.09–7.48 (m, 35H), 5.23–5.31 (m, 1H), 5.06 (br s, 2H), 3.63–3.75 (m, 2H), 3.24–3.44 (m, 2H), 2.39–2.55 (m, 2H), 2.23–2.38 (m, 2H). HRESIMS: Calcd for $C_{68}H_{77}N_3NaO_{17}P_2$, *m/z* 1292.4626; found, *m/z* 1292.4689.

4.12. Trimer (13)

To a solution of compound **7** (2.3 g, 2.5 mmol) and compound **12** (1.61 g, 1.27 mmol) in dry MeCN (80 mL) was added a 0.45 M solution of tetrazole in MeCN (55.3 mL, 25 mmol) at 23 °C. After stirring for 1 h, dry pyridine (3.2 mL, 40 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→4:1 CH_2Cl_2–acetone) to afford **13** (2.25 g, 84%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.96–7.58 (m, 59H), 6.68–6.86 (m, 4H), 5.23–5.33 (m, 1H), 5.06 (d, 2H), 3.73, 3.78 (2s), 3.23–3.42 (m, 4H), 2.37–2.53 (m, 2H), 2.11–2.35 (m). HR-ESIMS: Calcd for $C_{118}H_{127}N_4NaO_{26}P_3$, *m/z* 2131.7850; found, *m/z* 2131.7874.

4.13. De(dimethoxytrityl)ation of trimer (14)

Compound **13** (2.25 g, 1.06 mmol) was processed as described for **10**. Column chromatography (9:1→4:1 CH_2Cl_2–acetone) of the residue gave **14** (1.67 g, 87%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.96–7.6 (m, 50H), 5.23–5.33 (m, 1H), 5.06 (br s, 2H), 3.63–3.76 (m, 2H) 3.24–3.41 (m, 2H), 2.37–2.58 (m, 2H), 2.23–2.38 (m, 4H); HRESIMS: Calcd for $C_{97}H_{109}N_4NaO_{24}P_3$, *m/z* 1829.6542; found, *m/z* 1829.6611.

4.14. Tetramer (15)

To a solution of compound **7** (1.67 g, 1.82 mmol) and compound **14** (1.67 g, 0.92 mmol) in dry MeCN (80 mL) was added a 0.45 M solution of tetrazole in MeCN (40.3 mL, 18.2 mmol) at 23 °C. After stirring for 1 h, dry pyridine (2.3 mL, 28.7 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→4:1 CH_2Cl_2–acetone) to afford **15** (2.12 g, 87%) as a colorless syrup. ^{1}H NMR ($CDCl_3$): δ 6.92–7.59 (m, 74H), 6.67–6.77 (m, 4H), 5.24–5.33 (m, 1H), 5.06 (br s, 2H), 3.73 (s, 6H), 3.24–3.43 (m, 4H), 2.37–2.54 (m, 2H), 2.1–2.36 (m, 6H). HRESIMS: Calcd for $C_{147}H_{159}N_5NaO_{33}P_4$, *m/z* 2668.9766; found, *m/z* 2668.9802.

4.15. De(dimethoxytrityl)ation of tetramer (16)

Compound **15** (2.12 g, 0.80 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **16** (1.58 g, 84%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.96–7.58 (m, 65H), 5.22–5.33 (m, 1H), 5.06 (d, 2H), 3.62–3.76 (m, 2H) 3.27–3.41 (m, 2H), 2.37–2.52 (m, 2H), 2.15–2.36 (m, 6H). HRESIMS: Calcd for $C_{126}H_{141}N_5NaO_{31}P_4$, *m/z* 2366.8459; found, *m/z* 2366.8696.

4.16. Pentamer (17)

To a solution of compound **7** (1.21 g, 1.32 mmol) and compound **16** (1.58 g, 0.67 mmol) in dry MeCN (70 mL) was added a 0.45 M solution of tetrazole in MeCN (29.2 mL, 13.2 mmol) at 23 °C. After stirring for 1 h, dry pyridine (1.67 mL, 20.8 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **17** (1.9 g, 89%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.94–7.57 (m, 89H), 6.66–6.77 (m, 4H), 5.21–5.32 (m, 1H), 5.06 (d, 2H), 3.75 (s, 6H), 3.26–3.41 (m, 4H), 2.37–2.53 (m, 2H), 2.11–2.36 (m, 8H). HRESIMS: Calcd for $(C_{176}H_{191}N_6Na_2O_{40}P_5)^{2+}$, *m/z* 1614.579; found, *m/z* 1614.5890.

4.17. De(dimethoxytrityl)ation of pentamer (18)

Compound **17** (1.9 g, 0.59 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **18** (1.45 g, 85%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.93–7.56 (m, 80H), 5.22–5.32 (m, 1H), 5.06 (d, 2H), 3.61–3.75 (m, 2H) 3.26–3.41 (m, 2H), 2.38–2.53 (m, 2H), 2.14–2.36 (m, 8H). HRESIMS: Calcd for $(C_{155}H_{173}N_6O_{38}P_5H_2)^{2+}$, *m/z* 1441.532; found, *m/z* 1441.5338.

4.18. Hexamer (19)

To a solution of compound **7** (0.9 g, 0.98 mmol) and compound **18** (1.45 g, 0.50 mmol) in dry MeCN (60 mL) was added a 0.45 M solution of tetrazole in MeCN (21.7 mL, 9.8 mmol) at 23 °C. After stirring for 1 h, dry pyridine (1.24 mL, 15.4 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water

until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **19** (1.59 g, 85%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.94–7.58 (m, 104H), 6.67–6.77 (m, 4H), 5.24–5.33 (m, 1H), 5.06 (br s, 2H), 3.73 (s, 6H), 3.25–3.42 (m, 4H), 2.38–2.53 (m, 2H), 2.12–2.37 (m, 10H). HRESIMS: Calcd for $C_{205}H_{223}Li_2N_7O_{47}$, *m/z* 1867.201; found, *m/z* 1867.205.

4.19. De(dimethoxytrityl)ation of hexamer (20)

Compound **19** (1.59 g, 0.43 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **20** (1.34 g, 91%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.93–7.59 (m, 95H), 5.23–5.32 (m, 1H), 5.07 (br s, 2H), 3.62–3.76 (m, 2H) 3.26–3.43 (m, 2H), 2.38–2.53 (m, 2H), 2.15–2.37 (m, 10H). HRESIMS: Calcd for $(C_{184}H_{205}N_7O_{45}P_6H_2)^{2+}$, *m/z* 1710.128; found, *m/z* 1710.141.

4.20. Heptamer (21)

To a solution of compound **7** (0.7 g, 0.76 mmol) and compound **20** (1.34 g, 0.39 mmol) in dry MeCN (50 mL) was added a 0.45 M solution of tetrazole in MeCN (16.8 mL, 7.6 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.96 mL, 11.9 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **21** (1.48 g, 89%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.93–7.57 (m, 119H), 6.67–6.77 (m, 4H), 5.23–5.32 (m, 1H), 5.07 (br s, 2H), 3.74 (s, 6H), 3.26–3.41 (m, 4H), 2.38–2.52 (m, 2H), 2.12–2.37 (m, 12H). HRESIMS: Calcd for $(C_{234}H_{255}N_8O_{54}P_7H_2)^{2+}$, *m/z* 2152.272; found, *m/z* 2152.260.

4.21. De(dimethoxytrityl)ation of heptamer (22)

Compound **21** (1.48 g, 0.35 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **22** (1.25 g, 90%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.92–7.58 (m, 110H), 5.24–5.31 (m, 1H), 5.06 (d, 2H), 3.63–3.76 (m, 2H) 3.27–3.41 (m, 2H), 2.37–2.52 (m, 2H), 2.15–2.36 (m, 12H). HRESIMS: Calcd for $(C_{213}H_{237}N_8O_{52}P_7H_2)^{2+}$, *m/z* 1979.225; found, *m/z* 1979.236.

4.22. Octamer (23)

To a solution of compound **7** (0.57 g, 0.62 mmol) and compound **22** (1.25 g, 0.31 mmol) in dry MeCN (50 mL) was added a 0.45 M solution of tetrazole in MeCN (13.7 mL, 6.2 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.78 mL, 9.7 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **23** (1.36 g, 90%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.94–7.56 (m, 134H), 6.67–6.76 (m, 4H), 5.23–5.32 (m, 1H), 5.06 (d, 2H), 3.73 (s, 6H), 3.24–3.41 (m, 4H), 2.37–2.53 (m, 2H), 2.11–2.36 (m, 14H). HRESIMS: Calcd for $(^{13}C_2C_{261}H_{287}N_9O_{61}P_8)^{2+}$, *m/z* 2405.3957; found, *m/z* 2405.4067. Anal. Calcd for $C_{263}H_{287}N_9O_{61}P_8$: C, 65.84; H, 6.03. Found: C, 65.63; H, 6.01.

4.23. De(dimethoxytrityl)ation of octamer (24)

Compound **23** (1.36 g, 0.28 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **24** (1.1 g, 87%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.94–7.57 (m, 125H), 5.24–5.33 (m, 1H), 5.07 (d, 2H), 3.63–3.74 (m, 2H) 3.27–3.40 (m, 2H), 2.38–2.53 (m, 2H), 14H). HRESIMS: Calcd for $(^{13}C_2C_{240}H_{269}Li_2N_9O_{59}P_8)^{2+}$, *m/z* 2254.331; found, *m/z* 2254.3398. Anal. Calcd for $C_{242}H_{269}N_9O_{59}P_8$: C, 64.65; H, 6.03. Found: C, 64.51; H, 6.10.

4.24. Nonamer (25)

To a solution of compound **7** (0.288 g, 0.31 mmol) and compound **24** (0.94 g, 0.21 mmol) in dry MeCN (25 mL) was added a 0.45 M solution of tetrazole in MeCN (7 mL, 3.14 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.4 mL, 4.94 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **25** (0.95 g, 85%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.93–7.56 (m, 149H), 6.68–6.76 (m, 4H), 5.22–5.29 (m, 1H), 5.06 (d, 2H), 3.74 (s, 6H), 3.23–3.42 (m, 4H), 2.38–2.52 (m, 2H), 2.10–2.35 (m, 16H). HRESIMS: Calcd for $(C_{292}H_{319}N_{10}O_{68}PLi_2)^{2+}$, *m/z* 2674.4929; found, *m/z* 2674.5158. Anal. Calcd for $C_{292}H_{319}N_{10}O_{68}P_9$: C, 65.73; H, 6.03. Found: C, 65.46; H, 6.02.

4.25. De(dimethoxytrityl)ation of nonamer (26)

Compound **25** (0.95 g, 0.18 mmol) was processed as described for **10**. Column chromatography of the residue using 9:1→7:3 CH_2Cl_2–acetone as the eluant gave **26** (0.75 g, 83.7%) as a syrup. ^1H NMR ($CDCl_3$): δ 6.95–7.56 (m, 140H), 5.22–5.28 (m, 1H), 5.06 (d, 2H), 3.65–3.72 (m, 2H) 3.29–3.36 (m, 2H), 2.38–2.52 (m, 2H), 2.14–2.36 (m, 16H). HRESIMS: Calcd for $(C_{271}H_{301}Li_3N_{10}O_{66}P_9)^{3+}$, *m/z* 1684.6237; found, *m/z* 1684.6179.

4.26. Decamer (27)

To a solution of compound **7** (0.205 g, 0.224 mmol) and compound **26** (0.75 g, 0.15 mmol) in dry MeCN (18 mL) was added a 0.45 M solution of tetrazole in MeCN (5 mL, 2.24 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.286 mL, 3.54 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **27** (0.77 g, 88%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.93–7.45 (m, 164H), 6.65–6.77 (m, 4H), 5.20–5.29 (m, 1H), 5.06 (d, 2H), 3.72 (s, 6H), 3.26–3.40 (m, 4H), 2.38–2.52 (m, 2H), 2.10–2.35 (m, 18H). Anal. Calcd for $C_{321}H_{351}N_{11}O_{75}P_{10}$: C, 65.65; H, 6.02. Found: C, 65.63; H, 6.26.

4.27. De(dimethoxytrityl)ation of decamer (28)

Compound **27** (0.77 g, 0.13 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **28** (0.63 g, 86%) as a syrup. ^{1}H NMR ($CDCl_3$): δ6.95–7.56 (m, 155H), 5.22–5.27 (m, 1H), 5.06 (d, 2H), 3.65–3.72 (m, 2H) 3.29–3.38 (m, 2H), 2.39–2.52 (m, 2H), 2.12–2.38 (m, 18H). Anal. Calcd for $C_{300}H_{333}N_{11}O_{73}P_{10}$: C, 64.68; H, 6.03. Found: C, 64.53; H, 6.26.

4.28. Undecamer (29)

To a solution of compound **7** (0.156 g, 0.17 mmol) and compound **28** (0.63 g, 0.11 mmol) in dry MeCN (13.5 mL) was added a 0.45 M solution of tetrazole in MeCN (3.8 mL, 1.7 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.217 mL, 2.68 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **29** (0.605 g, 83.5%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.94–7.56 (m, 179H), 6.69–6.76 (m, 4H), 5.21–5.29 (m, 1H), 5.07 (d, 2H), 3.72 (s, 6H), 3.24–3.40 (m, 4H), 2.38–2.51 (m, 2H), 2.10–2.36 (m, 20H). Anal. Calcd for $C_{350}H_{383}N_{12}O_{82}P_{11}$: C, 65.58; H, 6.02. Found: C, 65.51; H, 6.14.

4.29. De(dimethoxytrityl)ation of undecamer (30)

Compound **29** (0.605 g, 0.094 mmol) was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **30** (0.495 g, 86%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.93–7.55 (m, 170H), 5.21–5.29 (m, 1H), 5.06 (d, 2H), 3.63–3.72 (m, 2H) 3.29–3.39 (m, 2H), 2.38–2.52 (m, 2H), 2.13–2.36 (m, 20H). Anal. Calcd for $C_{329}H_{365}N_{12}O_{80}P_{11}$: C, 64.69; H, 6.02. Found: C, 64.54; H, 6.17.

4.30. Dodecamer (31)

To a solution of compound **7** (0.112 g, 0.121 mmol) and compound **30** (0.495 g, 0.081 mmol) in dry MeCN (10 mL) was added a 0.45 M solution of tetrazole in MeCN (2.72 mL, 1.21 mmol) at 23 °C. After stirring for 1 h, dry pyridine (0.156 mL, 1.93 mmol) was added, followed by a 0.5 M solution of iodine in 2:1 THF–water until the brown color persisted. The reaction mixture was processed as described for **9**. The residue was purified by column chromatography (9:1→7:3 CH_2Cl_2–acetone) to afford **31** (0.466 g, 83%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.93–7.54 (m, 194H), 6.69–6.77 (m, 4H), 5.22–5.28 (m, 1H), 5.06 (d, 2H), 3.74 (s, 6H), 3.23–3.42 (m, 4H), 2.39–2.52 (m, 2H), 2.10–2.38 (m, 22H). Anal. Calcd for $C_{379}H_{415}N_{13}O_{89}P_{12}$: C, 65.52; H, 6.02. Found: C, 65.61; H, 6.24.

4.31. De(dimethoxytrityl)ation of dodecamer (32)

Compound **31** (0.466 g, 0.067 mmol) was dissolved in CH_2Cl_2 (10 mL) and water (5 mL). Glacial HOAc (50 mL) was added, and the mixture was stirred at room temperature overnight. The reaction mixture was processed as described for **10**. Column chromatography (9:1→7:3 CH_2Cl_2–acetone) of the residue gave **32** (0.373 g, 83.8%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 6.93–7.55 (m, 185H), 5.22–5.28 (m, 1H), 5.06 (d, 2H), 3.64–3.73 (m) 3.28–3.39 (m, 2H), 2.39–2.52 (m, 2H), 2.12–2.38 (m, 22H). Anal. Calcd for $C_{358}H_{397}N_{13}O_{87}P_{12}$: C, 64.70; H, 6.02. Found: C, 64.74; H, 6.16.

4.32. 2-(Aminoethyl) (1-D-ribityl) phosphate (33)

To a solution of compound **10** (210 mg, 0.286 mmol) in MeOH (4 mL) was added concd NH_4OH (2 mL). The mixture was stirred under reflux for 8 h. After concentration, the residue was purified on Sephadex LH-20 using 1:1 MeOH–CH_2Cl_2 as the eluant. A solution of the resulting syrup in MeOH was treated with Dowex 50 × 8–100 (Na^+), followed by filtration and removal of the volatiles from the filtrate under reduced pressure. A solution of residue in 1:1 *t*-BuOH–H_2O (10 mL) was stirred under hydrogen at 200 psi for 2 days in the presence of 10% Pd/C (~200 mg). The catalyst was removed by filtration through layer of Celite, and the filtrate was concentrated. A solution of the residue was freeze dried to afford amorphous **33** (68 mg, 88.0%). ^{1}H NMR (D_2O): δ 4.10–4.04 (m, 3H), 3.98 (dd, 1H, *J* 5.0, 11.5 Hz), 3.93 (m, 1H), 3.84 (m, 1H), 3.80 (dd, 1H, *J* 3.2, 11.4 Hz), 3.74 (t, 1H, *J* 5 Hz), 3.65 (dd, 1H, *J* 7.0, 11.7 Hz), 3.19 (t, 2H, *J* 4.7 Hz); ^{13}C NMR δ 72.7, 72.3, 71.5 (d, *J* 8.0 Hz), 67.3 (d, *J* 5.6 Hz), 63.8 (d, *J*

5.2 Hz), 63.0, 40.9 (d, *J* 8.9 Hz); ^{31}P NMR δ 1.77. HR-ESIMS: Calcd for $C_7H_{18}LiNNaO_8P$, *m*/*z* 305.0828; found, *m*/*z* 305.0824.

4.33. Deprotection of octamer 24 (34)

To a solution of compound **24** (380 mg, 0.084 mmol) in MeOH (4 mL) was added concd NH_4OH (2 mL). The mixture was stirred under reflux for 8 h, then was processed as described for **33** to afford amorphous **34** (110 mg, 66.2%). ^{1}H NMR (D_2O): δ 4.16–4.11 (m, 1H), 4.11–4.04 (m), 4.04–3.92 (m) 3.86 (ddd, 1H), 3.84–3.78 (m), 3.76 (t, 1H, *J* 6.0 Hz), 3.66 (dd, 1H, *J* 7.1, 12.0 Hz), 3.29 (t, 2H, *J* 4.8 Hz); ^{13}C NMR δ 72.8, 72.5, 71.95, 71.90, 71.7, 71.6, 67.4 (d, *J* 5.4 Hz), 67.3 (d, *J* 5.0 Hz), 67.3, 67.2, 63.1, 62.6 (d, *J* 4.7 Hz), 40.8 (d, *J* 8.1 Hz); ^{31}P NMR δ 2.35 (7P), 1.62 (1P). Anal. Calcd for $C_{42}H_{87}NNa_8O_{57}P_8$: C, 25.87; H, 4.50. Found: C, 25.96; H, 4.60.

4.34. Deprotection of dodecamer 32 (35)

Compound **32** (210 mg, 0.031 mmol) was dissolved in MeOH (4 mL) and concd NH_4OH (2 mL). The mixture was stirred at reflux for 8 h, then was processed as described for **33** gave **35** (72 mg, 80%). ^{1}H NMR (D_2O): δ 4.15–4.40 (m), 4.04–3.92 (m) 3.86 (ddd, 1H), 3.84–3.77 (m), 3.75 (t, 1H, *J* 6.0 Hz), 3.65 (dd, 1H, *J* 7.3, 12.2 Hz), 3.28 (t, 2H); ^{13}C NMR δ 72.8, 72.5, 71.9 (11C), 71.7 (11C), 71.6 (11C), 67.4 (d, *J* 4.5 Hz), 67.3 (11C), 67.2 (11C), 63.1, 62.9 (d, *J* 4.6 Hz), 40.9 (d, *J* 7.7 Hz); ^{31}P NMR 2.39, 2.37, 2.35, 2.33, 1.64. MALDI-TOFMS: Calcd for $C_{62}H_{127}NNa_{13}O_{85}P_{12}$, *m*/*z* 2917.19; found, *m*/*z* 2917.0. Anal. Calcd for $C_{62}H_{127}NNa_{12}O_{85}P_{12}$: C, 25.73; H, 4.42. Found: C, 25.8; H, 4.38.

4.35. Preparation of the *N*-(5-ketohexanoyl) derivatives of ribitol phosphates 33, 34 and 35

To a solution of ribitol phosphate **34** (19 mg) in H_2O (0.3 mL) was added MeOH (1.5 mL) followed by Et_3N (0.1 mL). This solution was treated with 5-ketohexanoic anhydride (~35 mg) at 23 °C. After 20 min, the volatiles were removed in a stream of air. The residue was purified on a Bio-Gel P2 column (80 × 2 cm) using 1:1 0.02 M C_5H_5N–0.02 M HOAc as the eluant. Fractions containing the product were pooled and freeze dried. To a solution of the residue in water was added Dowex 50 × 8–100 (Na^+) in excess, and the mixture was gently mixed for 30 min, followed by filtration and freeze drying of the filtrate to afford ketone **37** as a glass (17 mg, 85%). ^{1}H NMR (D_2O): ^{1}H δ 4.15–4.04 (m), 4.04–3.92 (m), 3.86–3.78 (m), 3.76 (t, 1H, *J* 6.0 Hz), 3.66 (dd, 1H, *J* 7.4 Hz, *J* 11.7 Hz), 3.44 (t, 2H, *J* 4.7 Hz), 2.62 (t, 2H, *J* 7.4 Hz), 2.28 (t, 2H, *J* 7.4 Hz), 2.21 (s, 3H), 1.84 (m, 2H); ^{31}P NMR δ 2.39 (7P), 1.97 (1P). Ribitol phosphates **33** and **35** were converted to their respective 5-ketohexanoyl derivatives (**36** and **38**) in a similar manner and were obtained in similar yields.

4.36. Conjugation of ketohexanoyl derivatives 36, 37 and 38 to aminooxylated bovine serum albumin

Bovine serum albumin was derivatized with *O*-(3-thiolpropyl)hydroxylamine as described.[15] Average incorporation of the linker was 30 mol/1 mol of protein, as assayed by MALDI-TOFMS using sinapinic acid as the matrix. To a solution of aminooxy-BSA (10 mg, 0.135 μmol containing 4 μmol of aminooxy-linker) in 0.1 M phosphate buffer containing 1 mM EDTA and 0.1% glycerol at pH 7.4 was added either the monomer **36** (3.0 mg, 7.3 μmol) or the octamer **37** (15 mg, 5.8 μmol), respectively. In another experiment, aminooxy-BSA (3 mg, 0.04 μmol containing 1.2 μmol aminooxy-linker) was reacted with dodecamer **38** (6 mg, 1.9 μmol). All reactions were carried out at pH 6.5, at 23 °C, for 18 h, in 2.5 mL of total volume. After 18 h the conjugation mixture was applied to a Sephadex G50 column made up in 0.2 M NaCl. The fractions containing the conjugates were pooled and analyzed by MALDI-TOFMS (Table 1).

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Supplementary data

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