Synthetic methods to glycerol teichoic acids

Hogendorf, W.F.J.

Chapter 3

Automated Solid Phase TA Synthesis

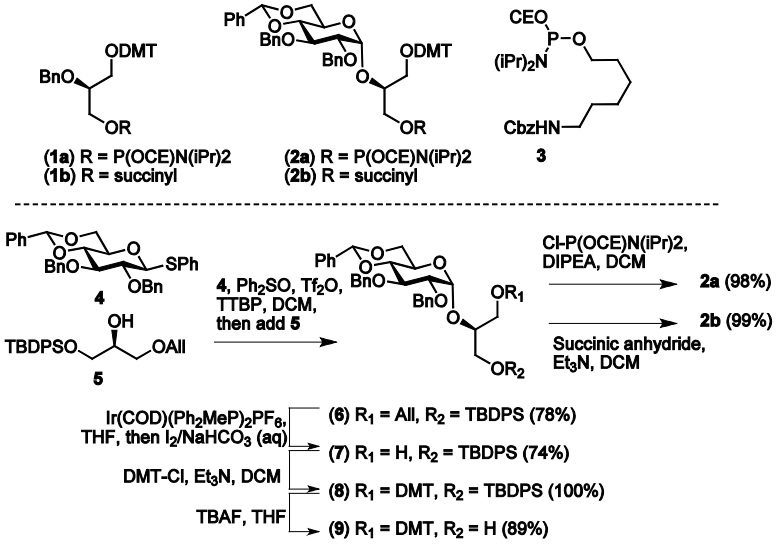
Introduction

Teichoic acids (TAs) have been implied as antigenic structures[1,2] and are therefore interesting targets for the development of (synthetic) vaccines against increasingly resistant Gram-positive species, such as *E. faecalis*[3,4] and *S. aureus*.[5,6] Because of the microheterogeneity of the TA in biological preparations, the establishment of structure activity relations has been troublesome and the biological activity of TA preparations from biological sources has been subject of debate.[7-9] Therefore, a library of synthetic TA structures would be a powerful means to find the optimal length and substitution pattern of an antigen. In **chapter two** the solution phase synthesis of a (kojibiosyl) GTA hexamer is described.[10] Although this approach can be used for a large scale synthesis of a single target compound, it is too labor intensive to generate a library of compounds.

Automated solid phase synthesis is a well established methodology in oligonucleic acid synthesis, giving rapid access to (a library of) oligomers of desired length and composition.[11-13] Because of their repetitive nature and connection through phosphodiester linkages, TAs resemble oligonucleic acids. Therefore, an automated solid phase approach, in combination with the chemistry described in **chapter two**, was deemed suitable to obtain a set of synthetic TA fragments.

This chapter describes the synthesis of GTA oligomers up to a length of a 20-mer, using a commercial DNA synthesizer. Glucosylated fragments, a substitution that is found in several species of *Staphylococcus* and *Bacillus*,[14,15] have been obtained using tailor-made glucosyl glycerol phosphoramidite. The library of synthetic TAs has been evaluated on their antigenicity in an opsonophagocytic inhibition assay (OPIA), revealing a length-activity relationship and a high inhibitory potential of two glucosylated hexaglycerol phosphates.[16]

Scheme 1. Building blocks for the automated solid phase TA synthesis.

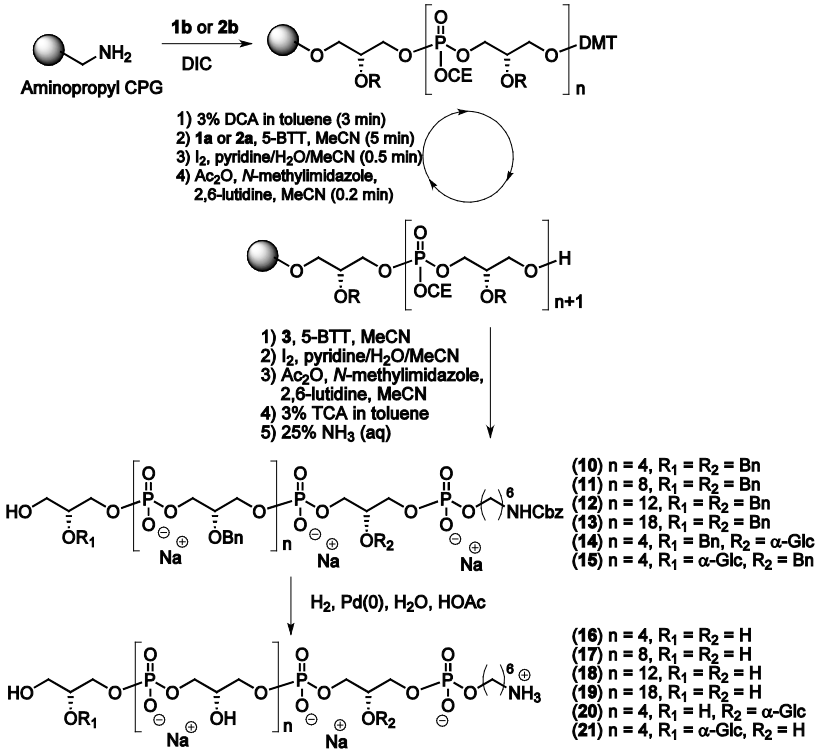


Results and Discussion

Based on the state-of-the-art in nucleic acid synthesis, and as described in **chapter two**, cyanoethyl (CE) protected glycerol phosphoramidite building blocks were employed (see scheme 1).[13] The alcohol functions of the building blocks, that were used for elongation, mediated glycosylation of glycerol alcohol **5**.[18-21] The resulting glucosylglycerol **6** was transformed into **2a/b** as follows: iridium catalyzed isomerization of the allyl ether[22] and subsequent cleavage of the resulting enol ether using iodine under basic conditions liberated the primary alcohol (**7**), which was capped with a DMT group to give compound **8**. Next the silyl group was removed to provide alcohol **9**, which was either phosphitylated to provide the phosphoramidite building block **2a**, or treated with succinic anhydride to give the glucosylglycerol linker **2b**.

With the required building blocks in hand the next goal was to explore the automated solid phase TA oligomer synthesis (Scheme 2). As a first objective the assembly of spacer containing TA-hexamer **10** was investigated. To this end aminopropyl CPG was functionalized with succinyl glycerol **1b**. Cleavage of the DMT group with 3% dichloroacetic acid (DCA) in toluene and concomitant determination of the loading led to a CPG with a loading of 100 μmol/g. At this point, the CPG-glycerol was installed into the ÄKTA™ oligopilot™ DNA/RNA synthesizer. For the coupling step the resin was treated with 5 equiv. of phosphoramidite **1a** and 5-benzylthiotetrazole[23] (5-BTT, 22.5 equiv.) as activator in acetonitrile for 5 minutes. Oxidation of the intermediate phosphites was achieved with I_2 in pyridine/H_2O (1 min.), after which a

Scheme 2. Automated solid phase TA synthesis.



capping step (*N*-methylimidazole/acetic anhydride (Ac_2O)/2,6-lutidine) was introduced to cap any unreacted alcohol functionalities. To complete the elongation cycle the DMT-groups were removed with 3% DCA in toluene. The coupling efficiency using this protocol was generally > 98% as judged from the automatic DMT-count. In the 6th cycle of the hexamer assembly, the glycerol phosphoramidite **1a** was replaced by benzyloxy-carbonyl aminohexanol phosphoramidite **3** to terminate the sequence.[24] Cleavage of the hexamer from the CPG using aqueous ammonia, and concomitant cyanoethyl removal liberated the partially protected TA-hexamer **10**. Two different protocols for the purification of the partially protected oligomers were examined: RP-HPLC purification and anion exchange chromatography followed by desalination. In the case of hexamer **10**, the former protocol provided the desired oligomer in the highest yield (18% overall from aminopropyl CPG).

There was a possibility that the phosphodiester linkages in the synthetic targets would be labile to base treatment, in analogy to the lability of RNA fragments.[13] To investigate the base lability of the partially protected TA oligomer, hexamer **10** was subjected to a treatment with 25% aqueous ammonia for a prolonged period of time at room temperature and at 40 °C. No degradation of the hexamer could be detected by LCMS analysis after 24h at 40 °C, indicating that transesterification of the

phosphodiesters is not a significant risk under the reaction conditions used. Using the conditions described above, the following TA-oligomers were assembled: unsubstituted 10-mer **11**, unsubstituted 14-mer **12**, unsubstituted 20-mer **13** and mono glucosyl substituted 6-mers **14** and **15**. TA-fragment **15** was assembled on CPG resin functionalized with glucosyl glycerol succinate **2b** (loading 100 μmol/g). The results of the syntheses are summarized in Table 1, from which it becomes clear that the syntheses of the longer and substituted fragments proceeded with even greater efficiency than the assembly of hexamer **10**. With increasing size of the oligomers, purification by ion exchange chromatography became more efficient than RP-HPLC purification. For TA 20-mer **13** and the glucosyl substituted hexamers **14** and **15** LCMS analysis of the crude reaction products showed significant peak broadening and therefore these compounds were solely purified using the ion-exchange protocol. From the excellent yields of glucosyl substituted hexamers **14** and **15** it becomes clear that the glycerol C-2 glucose substitution had no adverse effect on the coupling efficiency of the building blocks used. Notably, the benzylidene functionality on the glucosyl moiety in **15** proved to be stable to the repeated detritylation steps. Global deprotection of the partially protected TA-fragments was achieved by hydrogenolysis of the benzyl ethers, Cbz-group and benzylidene functionality over palladium black followed by desalination (Scheme 2), uneventfully leading to TA-target structures **16-21**, as summarized in Table 1. The structure of the final products was confirmed by NMR spectroscopy and high resolution mass spectrometry. In the NMR spectra the relative ratio of the integrals of the peaks belonging to the spacer CH_2 groups, the spacer CH_2-N group and the terminal glycerol CH_2-O moiety with respect to the bulk signal originating from of all other glycerol protons, provided proof for the integrity of the structures.

To establish their antigenic activity, the prepared TA fragments (**16-21**) were tested in an opsonophagocytic inhibition assay, as described by Theilacker *et al.*[3,4,16] Also, kojibiosyl GTA hexamer **22** (figure 1), of which the synthesis is described in **chapter two** was taken along in this biological evaluation. In this assay, rabbit serum raised against purified *E. faecalis* LTA is used to kill *E. faecalis* bacteria. Blocking the

Table 1. Automated solid phase synthesis and deprotection yields.

Entry	Compound	Yield (RP-HPLC	Yield (ion exchange)	Deprotection (yield)
1	**10**	18%	10%	**16** (65%)
2	**11**	29%	16%	**17** (78%)
3	**12**	8%	21%	**18** (84%)
4	**13**		24%	**19** (95%)
5	**14**		32%	**20** (68%)
6	**15**		36%	**21** (86%)

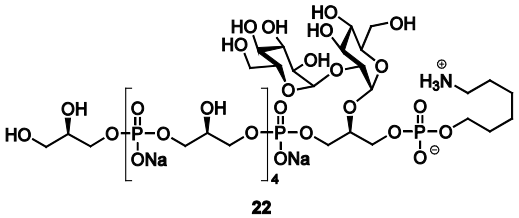


Figure 1. Hexamer **22**.

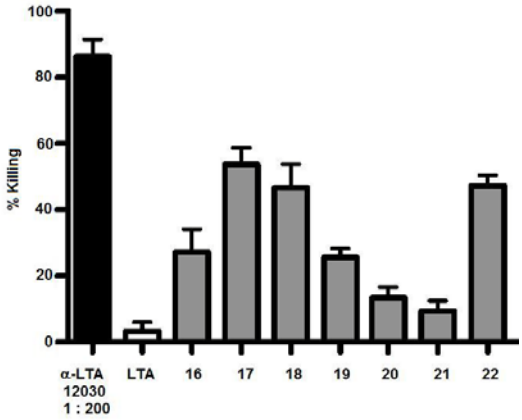


Figure 2. Results OPIA. α-LTA 12030 represents the killing of the serum at 1:200 dilution. LTA is used as a positive control. Compounds **16-22** are used in a concentration of 100 μg/ml**.**

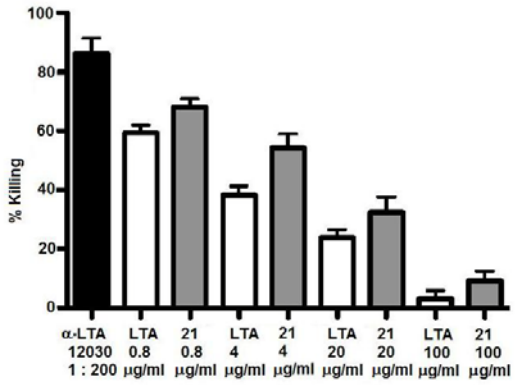


Figure 3. OPIA of LTA and compound **21** at different concentrations.

opsonic antibodies in the serum with an inhibitor will lead to reduced killing. The inhibitory potential of the synthesized TA fragments at a concentration of 100 μg/mL is displayed in Figure 2, which shows that the smallest fragment tested, hexamer **16**, is capable of inhibiting the opsonophagocytic killing. For the 10-, 14- and 20-mer a length-dependence is observed for the inhibitory potential. The longer the fragments are the better the binding is to the opsonic antibodies, resulting in reduced killing. Interestingly, the glucose substituted TA fragments **20** and **21** were found to be relatively very potent inhibitors. This is striking since the α-glucosyl substituent is known from the TA from several *Staphylococcus and Bacillus* strains, but has not been found in *E. faecalis* TA.[14,15] In contrast, hexamer **22**, bearing the kojibiosyl substitution naturally occurring in *E. faecalis* TA, showed a significantly lower inhibition. Compound **21** proved to be the most active compound of the series, showing already some inhibition at a concentration of 0.8 μg/ml. Furthermore, compound **21** showed a comparable concentration-inhibition relation as native LTA (see figure 3), which makes this a promising candidate for the future development of a vaccine comprising **21** or a close analogue as a synthetic TA-antigen.[16]

Conclusion

This chapter describes the development of automated solid phase methodology to synthesize glycerol phosphate teichoic acid fragments. Tailor made glycerol phosphoramidite building blocks were used as building blocks in combination with a commercially available synthesizer, to produce partially protected TA fragments. With a full coupling cycle, taking approximately 15 minutes, a TA 20-mer was produced in 5 hours. Functionalized glycerol phosphate building blocks could also be used in the synthesis to allow the assembly of substituted TA fragments. Employing the solid phase methodology, a small library of TA fragments was generated which was tested for activity in an opsonophagocytic inhibition assay, revealing a clear TA-length-activity relationship. The assay also revealed glucosyl substituted TA-hexamers **20** and **21** as promising lead candidates for future vaccine development. Interestingly, hexamer **22**,containing the kojibiosyl substitution as found in *E. Faecalis* LTA, did not show any opsonophagocytic inhibition. Hexamer **21** can also be synthesized in a light fluorous fashion (see **chapter five**) and conjugated to several immunogenic carrier proteins (see **chapter seven**).

Experimental section

General Procedures and Material: All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/l, in 10% aqueous H_2SO_4 followed by charring at +/- 140 ^oC. Some unsaturated compounds were visualized b y spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in H_2O. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on a Propol automated polarimeter (Sodium D-line, λ = 589 nm) with a concentration of 10 mg/ml (c = 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300 and are reported in cm^{-1}. ^{31}P, ^{1}H, and ^{13}C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 100 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 ^oC) with resolution R = 60000 at *m/z* 400 (mass range *m/z* = 150-2000) and dioctylphthalate (*m/z* = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was conducted on a JASCO system using an Alltima C18 analytical column (5 µm particle size, flow:1.0 ml/min). Absorbance was measured at 214 nm. Solvent system: A: water, B: MeCN, C:0.15 M NH_4OAc in H_2O , gradient (13.5 min): 80% A, 10% B, 10% C $\rightarrow$ 0% A, 90% B, 10% C. This system was coupled to a PE/SCIEX API165 (Perkin-Elmer) mass spectrometer.

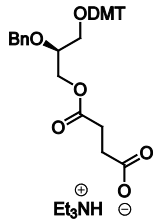
Procedure for automated solid-phase synthesis, purification and global deprotection of TA oligomers: Aminopropyl modified controlled pore glass support (CPG, Fluka) was loaded with (glucosyl)glycerol succinates **1b** or **2b** and the loading was determined (loading: 100 μmol/g CPG) using the method described by Pon.[25] The automated syntheses were performed on a synthesizer (ÄKTA™ oligopilot plus™, GE Healthcare) on a scale of 100-150 mg functionalized CPG (10-15 μmol glycerol derivative) and started off with acidolysis of the dimethoxytrityl ether using 3% dichloroacetic acid in toluene (15 ml, 3 min). After flushing with acetonitrile (5ml, 1 min), the resulting alcohol was reacted with phosphoramidites **1a** or **2a** (0.1 M in MeCN, 5 eq) and 5-benzylthiotetrazole (BTT, 0.3M in MeCN, 22.5 eq) for 5 min using a cycled flow. After flushing with MeCN (5ml, 1 min), oxidation of the intermediate phosphite was performed using I_2 (0.05 M in pyridine/H_2O 9/1, 2ml, 1 min). A flushing step with MeCN (5ml, 1 min) was followed by a capping step (1 ml of a 1/1 mixture of capping solution A (20 v/v% *N*-methylimidazole in MeCN) and capping solution B (20 v/v% Ac_2O, 15 v/v% 2,6-lutidine in MeCN for 12s). After flushing with MeCN (5ml, 1 min), a detritylation step was performed using the before mentioned cocktail and the molecule was elongated using phosphoramidites **1a** or **2a** using the same set of reactions (coupling, oxidation, capping, detritylation). The average coupling efficiency was measured by quantitative UV-detection (400 nm) of the dimethoxytrityl cation during each detritylation step. When the desired length was obtained, spacer phosphoramidite **3** (0.1 M in MeCN, 2 x 5 eq, 2 x 5 min) was coupled to the CPG-TA-oligomer using 5-BTT (0.3M in MeCN, 2 x 22.5 eq) and, subsequently treated with I_2 (0.05 M in pyridine/H_2O 9/1, 2ml, 1 min), before it was released from the solid support using 25% NH_4OH (10ml, 1h, the cyanoethyl groups are concomitantly released at this stage). The solvents were then removed *in vacuo* before the crude oligomer was purified using method A or B.

Purification method A: RP-HPLC (Gilson preparative HPLC system; column: Alltima C18, particle size: 5 μm, dimensions: 10/250 mm; eluent: (10 mM NH_4OAc in H_2O)/MeCN, 9/1 → 1/9, detection: UV (215 and 254 nm), the fractions containing product were collected and the solvents were removed under reduced pressure. Repeated lyophilization (twice) of the residue was followed by eluting the purified oligomer through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.

Purification method B: Anion-exchange chromatography (device: ÄKTA Explorer™, GE Healthcare; column: Q-sepharose HR16/10, GE Healthcare; eluent: buffer A (50 mM NaOAc, 50 mM $NaClO_4$), buffer B (50mM NaOAc, 500mM $NaClO_4$), gradient 1/0 → 0/1) followed by desalination using size-exclusion chromatography (Sephadex G10 (hexamer **10**) or Sephadex G25 (all other oligomers), GE Healthcare, dimensions: 26/60 mm, eluent: 0.15 M NH_4HCO_3). The purified oligomer was lyophilized twice before it was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.

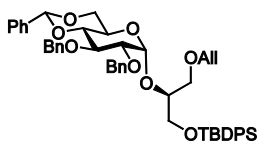
Deprotection: The oligomers (1-5 μmol) were dissolved in H_2O (3-6 ml) together with AcOH (3-6 drops) and treated for 3 days with Palladium black (20-40 mg)/H_2. Subsequently, the mixture was filtered and the solvents removed under reduced pressure before the residue was purified by size-exclusion chromatography (Sephadex HW40, Toyopearl, dimensions: 16/60 mm, eluent: 0.15 M Et_3NHOAc or 0.15 M NH_4OAc). After repeated lyophilization, the purified product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use).

Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by HRMS and NMR (^{1}H, ^{13}C, ^{31}P) analysis.



1-*O*-(Triethylammonium succinate)-2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (1b)

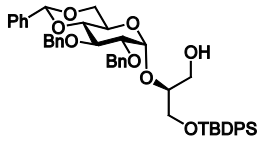
To a solution of 2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (2.50 g, 5.16 mmol) and Et_3N (7.87 ml, 56.8 mmol) in DCM (35 ml) was added succinic anhydride (2.58 g, 25.8 mmol). The mixture was stirred for 10 min at 0 °C, followed by the addition of a catalytic amount of DMAP. After stirring 2h at RT, the mixture was diluted with DCM (80 ml) and washed with 0.5 M HCl (50 ml), sat. aq. $NaHCO_3$ (40 ml) and brine (40 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by column chromatography (MeOH/DCM/Et_3N), afforded glycerol succinate **1b** (3.26 g, 4.75 mmol, 92%) as white foam. $[\alpha]_D^{20}$ (MeOH): +8.2; IR: 829, 1034, 1177, 1250, 1609, 1736, 2343, 2359; ^{1}H NMR (400 MHz, CD_3CN): δ = 1.11 (t, 9H, *J* = 7.3 Hz, 3 x CH_3 Et_3N), 2.35 - 2.45 (m, 4H, 2 x CH_2 succinyl), 2.85 (q, 6H, *J* = 7.3 Hz, 14.6 Hz, 3 x CH_2 Et_3N), 3.14 - 3.22 (m, 2H, 2 x CH*H* glycerol), 3.72 - 3.74 (m, 7H, 2 x OMe, CH glycerol), 4.15 - 4.23 (m, 2H, 2 x CH*H* glycerol), 4.57 (s, 2H, CH_2 Bn), 6.83 (d, 4H, *J* = 8.8 Hz, H_{arom}), 7.17 - 7.33 (m, 12H, H_{arom}), 7.44 (d, 2H, *J* = 7.6 Hz, H_{arom}); ^{13}C NMR (100 MHz, CD_3CN): δ = 9.2 (3 x CH_3 Et_3N), 30.9 (CH_2 succinyl), 31.7 (CH_2 succinyl), 45.9 (3 x CH_2 Et_3N), 55.8 (2 x OMe), 63.7 (CH_2 glycerol), 64.4 (CH_2 glycerol), 72.6 (CH_2 Bn), 77.2 (CH glycerol), 86.8 (C_q DMT), 113.9 (CH_{arom}), 127.6, 128.4, 128.6, 128.7, 128.8, 129.1, 130.8 (CH_{arom}), 136.9, 139.7, 146.1, 159.5 (C_q Bn, 4 x C_q DMT), 173.8, 176.9 (2 x CO succinate); HRMS (free acid): $C_{35}H_{36}O_8$ + Na^+ requires 607.2302, found 607.2298.



1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (6)

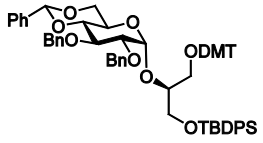
A solution of glucose donor **4** (2.70 g, 5.00 mmol), TTBP (2.86 g, 11.5 mmol) and Ph_2SO (1.11 g, 5.50 mmol) in freshly distilled DCM (100 ml), together with activated MS 3Å, was stirred under argon at RT for 30 min. The mixture was then cooled to -75 °C and stirred for another 15 min. After the addition of Tf_2O (0.93 ml, 5.5 mmol) the mixture was stirred for 45 min at -75 °C and, subsequently, glycerol acceptor **5** (2.22 g, 5.99 mmol) was added. After stirring for 2 hrs at -75 °C, the mixture was allowed to warm to room temperature overnight. The reaction was by the addition of moist Et_3N (3.4 ml, 25 mmol) and stirred for 30 min. After washing with sat. aq. $NaHCO_3$ (30 ml) and brine (30 ml), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was dissolved in pyridine (50 ml) and Ac_2O (10 ml) and stirred for 2 hrs. The solvents were removed *in vacuo* before the residue was redissolved in Et_2O (125 ml) and washed with H_2O (2 x 40 ml) and brine (40 ml). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. Column chromatography (EtOAc/PE) of the residue gave α-glucoside **6** (3.10 g, 3.87 mmol, 78%), as a colourless oil with a minor amount (< 7%, based on ^{1}H-NMR analysis) of the β-product. $[\alpha]_D^{20}$ ($CHCl_3$): +4.2; IR: 737, 1088, 1369, 1454, 1751, 2855, 2924; ^{1}H NMR (400 MHz, α-anomer): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.53 - 3.69 (m, 4H, CH glycerol, CH*H* glycerol, H-2, H-6), 3.71 - 3.81 (m, 3H, 3 x CH*H* glycerol), 3.86 - 3.92 (m, 1H, H-5), 3.99 - 4.04 (m, 5H, CH_2 allyl, H-3, H-4, H-6), 4.74 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 11.8 Hz, CH*H* Bn), 4.80 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.87 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 5.16 (dd, 1H, *J* = 1.3 Hz, 10.4 Hz, CH*H* allyl), 5.26 (dd, 1H, *J* = 1.6 Hz, 17.2 Hz, CH*H* allyl), 5.26 (d, 1H, *J* = 3.8 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 5.88 (ddd, 1H, *J* = 5.2 Hz, 10.4 Hz, 22.4 Hz, CH allyl), 7.24 - 7.46 (m, 21H, H_{arom}), 7.65 - 7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz, α-anomer): δ = 19.2 (C_q *t*-Bu, 26.8 (3 x CH_3 TBDPS), 62.4 (C-5), 63.9 (CH_2 glycerol), 68.8 (C-6), 70.8 (CH_2 glycerol), 72.3 (CH_2 allyl), 72.5 (CH_2 Bn), 75.2 (CH_2 Bn), 76.7, 78.2 (C-3, C-

4), 79.0 (CH glycerol), 82.1 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 116.9 (CH_2 allyl), 126.0 - 129.7 (CH_{arom}), 133.1, 133.2 (2 x C_q phenyl), 134.6 (CH allyl), 135.5 (CH_{arom}), 137.5, 138.3, 138.8 (2 x C_q Bn, C_q benzylidene); HRMS: $C_{49}H_{56}O_8Si$ + Na^+ requires 823.3637, found 823.3631.



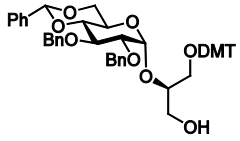
1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-*sn*-glycerol (7)

A solution of glycoside **6** (0.400 g, 0.499 mmol) in freshly distilled THF (3.0 ml) was stirred under argon for 30 min. After the addition of $Ir(COD)(Ph_2MeP)_2PF_6$ (0.042 g, 10 mol%) the solution turned red and the mixture was purged with H_2 (g) until the solution turned colourless again (~30s). After stirring under argon for 2 h, the mixture was diluted with THF (7.0 ml) and sat. aq. $NaHCO_3$ (25 ml). Upon addition of I_2 (0.190 g, 0.75 mmol), the mixture was allowed to stir for 1.5 h at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, sat. aq. NaS_2O_3 (2 x 20 ml) and brine (20 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (EtOAc/toluene/PE) afforded **7** (281 mg, 0.369 mmol, 74%) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +3.2; IR: 737, 995, 1030, 1076, 1369, 1736, 2858, 2932; ^{1}H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.17 (bs, 1H, OH), 3.53 - 3.68 (m, 5H, 2 x CH*H* glycerol, H-2, H-4, H-6), 3.72 - 3.90 (m, 4H, 2 x CH*H* glycerol, CH glycerol, H-5), 3.99 (dd, 1H, *J* = 4.9 Hz, 10.1 Hz, H-6), 4.04 (at, 1H, *J* = 9.3 Hz, H-3), 4.70 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.86 (d, 1H, *J* = 3.9 Hz, H-1), 4.88 (d, 1H, *J* = 11.7 Hz, CH*H* Bn), 4.93 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 5.50 (s, 1H, CH benzylidene), 7.25 - 7.46 (m, 21H, H_{arom}), 7.63 - 7.66 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-Bu, 26.8 (3 x CH_3 TBDPS), 62.7 (C-5), 62.9 (CH_2 glycerol), 63.9 (CH_2 glycerol), 68.8 (C-6), 74.5 (CH_2 Bn), 75.2 (CH_2 Bn), 78.9 (C-2, C-3), 81.8 (CH glycerol), 82.3 (C-4), 99.6 (C-1), 101.2 (CH benzylidene), 126.0 - 129.8 (CH_{arom}), 133.0, 133.1 (2 x C_q phenyl), 135.5 (CH_{arom}), 137.3, 137.5, 138.5 (2 x C_q Bn, C_q benzylidene); HRMS: $C_{46}H_{52}O_8Si$ + Na^+ requires 783.3324, found 783.3325.



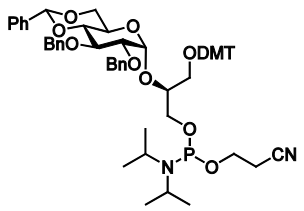
1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (8)

To a solution of alcohol **7** (4.68 g, 6.14 mmol) in DCM (50 ml) were added, respectively, DiPEA (1.61 ml, 9.22 mmol) and DMT-Cl (2.50 g, 7.37 mmol). The mixture was allowed to stir overnight after which it was quenched by the addition of MeOH (5.0 ml). After stirring 15 min the mixture was washed with H_2O (20 ml) and brine (20 ml) and the organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et_3N) yielded **8** (6.51 g, 6.12 mmol, 100%) as white foam. $[\alpha]_D^{20}$ (MeOH): +17.2; IR: 1034, 1088, 1250, 1508, 2343, 2361; ^{1}H NMR (400 MHz): δ = 0.97 (s, 9H, *t*-Bu TBDPS), 3.30 (dd, 1H, *J* = 6.2 Hz, 9.8 Hz, CH*H* glycerol), 3.46 (dd, 1H, *J* = 4.4 Hz, 10.0 Hz), 3.52 - 3.64 (m, 3H, H-2, H-4, H-6), 3.72 - 3.80 (m, 8H, 2 x CH*H* glycerol, 2 x OMe), 3.90 - 3.97 (m, 1H, H-5), 4.00 - 4.07 (m, 3H, CH glycerol, H-3, H-6), 4.59 (d, 1H, *J* = 12.4 Hz, CH*H* Bn), 4.64 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.79 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.87 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 5.21 (d, 1H, *J* = 3.6 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 6.74 - 6.76 (m, 4H, H_{arom}), 7.12 - 7.46 (m, 30H, H_{arom}), 7.58 - 7.62 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-Bu, 26.8 (3 x CH_3 TBDPS), 55.1 (2 x OMe), 62.6 (C-5), 64.1 (2 x CH_2 glycerol), 68.9 (C-6), 72.5 (CH_2 Bn), 75.2 (CH_2 Bn), 77.5, 78.3 (CH glycerol, C-3), 78.9 (C-2), 82.2 (C-4), 86.5 (C_q DMT), 97.2 (C-1), 101.3 (CH benzylidene), 113.1 (CH_{arom}), 126.1 - 130.1 (CH_{arom}), 133.2, 133.3 (2 x C_q phenyl), 135.5 (CH_{arom}), 136.0, 136.0, 137.6, 138.1, 138.8 (2 x C_q Bn, C_q benzylidene, 5 x C_q DMT); HRMS: $C_{67}H_{70}O_{10}Si$ + H^+ requires 1063.4811, found 1063.4804.



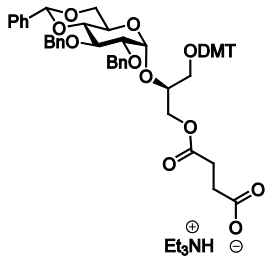
2-*O*-(2,3-di-*O*-Benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (9)

Compound **8** (6.33 g, 5.95 mmol) was dissolved in THF (60 ml) and after addition of TBAF (1M in THF, 10.7 ml) stirred overnight. After evaporation of the solvents under reduced pressure the resulting oil was purified by column chromatography (EtOAc/PE/Et_3N), giving alcohol **9** (4.37 g, 5.30 mmol, 89%) as a white foam. $[\alpha]_D^{20}$ (MeOH): +29.2; IR: 1032, 1076, 1250, 1508, 1609, 2343, 2361; ^{1}H NMR (400 MHz): δ = 2.40 (bs, 1H, OH), 3.28 (dd, 1H, *J* = 6.3 Hz, 9.8 Hz, CH_2 glycerol), 3.34 (dd, 1H, *J* = 5.4 Hz, 9.9 Hz, CH_2 glycerol), 3.54 (dd, 1H, *J* = 3.8 Hz, 9.4 Hz, H-2), 3.61 (at, 1H, *J* = 9.5 Hz, H-4), 3.66 - 3.77 (m, 9H, CH_2 glycerol, H-6, 2 x OMe), 3.87 (ddd, 1H, *J* = 3.5 Hz, 6.1 Hz, 11.8 Hz, CH glycerol), 3.98 (dd, 1H, *J* = 4.8 Hz, 10.0 Hz, H-5), 4.02 (at, 1H, *J* = 9.3 Hz, H-3), 4.25 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.59 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 11.3 Hz, CH_2 Bn), 4.90 (d, 1H, *J* = 11.3 Hz, CH_2 Bn), 4.97 (d, 1H, *J* = 3.9 Hz, H-1), 5.54 (s, 1H, CH benzylidene), 6.80 (4, 4H, *J* = 8.9 Hz, H_{arom}), 7.16 - 7.36 (m, 20H, H_{arom}), 7.43 - 7.49 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.1 (2 x OMe), 62.9 (C-5), 63.3 (CH_2 glycerol), 63.9 (CH_2 glycerol), 68.9 (C-6), 73.2 (CH_2 Bn), 75.3 (CH_2 Bn), 78.4 (C-3), 78.8 (C-2), 78.8 (CH glycerol), 82.0 (C-4), 86.6 (C_q DMT), 97.4 (C-1), 101.2 (CH benzylidene), 113.1 (CH_{arom}), 126.0 - 130.0 (CH_{arom}), 133.0 (2 x C_q phenyl), 135.8, 137.3, 137.9, 138.7, 144.6, 158.5 (2 x C_q Bn, C_q benzylidene, 5 x C_q DMT); HRMS: $C_{51}H_{52}O_{10}$ + Na^+ requires 847.3453, found 847.3455.



1-*O*-([*N*,*N*-diisopropylamino]-2-cyanoethoxy-phosphite)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (2a)

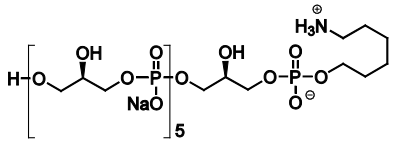
To a cooled (0 °C) solution of **9** (1.24 g, 1.50 mmol) and DiPEA (0.42 ml, 2.4 mmol) in freshly distilled DCM (30 ml) was added 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (0.462 g, 1.95 mmol). After stirring overnight, the reaction was quenched by the addition of H_2O (2.0 ml) and washed with H_2O (10 ml) and brine (10 ml), respectively. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et_3N) gave phosphoramidite **2a** (1.50 g, 1.46 mmol, 98%) as white foam. ^{31}P NMR (161.7 MHz, CD_3CN): δ = 149.0, 149.3 (diastereoisomers); ^{1}H NMR (400 MHz, CD_3CN): δ = 1.07 - 1.15 (m, 12H, 4 x CH_3 isopropylamino), 2.46 - 2.50 (m, 2H, CH_2 cyanoethyl), 3.11 - 3.29 (m, 2H, 2 x CH isopropylamino), 3.51 - 4.27 (m, 19H, CH_2 cyanoethyl, 2 x OMe, 2 x CH_2 glycerol, CH glycerol, H-2, H-3, H-4, H-5, H-6, H-6), 4.57 - 4.62 (m, 2H, CH_2 Bn), 4.79 (m, 2H, CH_2 Bn), 5.15 (m, 1H, H-1), 5.60 (s, 1H, CH benzylidene), 6.80 (d, 4H, *J* = 8.1 Hz, H_{arom}), 7.11 - 7.47 (m, 24H, H_{arom}); HRMS: $C_{60}H_{69}N_2O_{11}P$ + H^+ requires 1025.4712, found 1025.4720.



1-*O*-(Triethylammoniumsuccinate)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (2b)

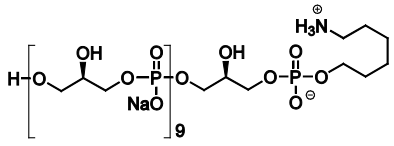
To a solution of **9** (3.21 g, 3.89 mmol) and Et_3N (5.93 ml, 42.8 mmol) in DCM (40 ml) was added succinic anhydride (1.95 g, 19.45 mmol). A catalytic amount of DMAP was added and the reaction was stirred for 100 min. The mixture was diluted with DCM (50 ml) and washed with H_2O (2 x 50 ml) after which the organic layer was concentrated under reduced pressure. The triethylammonium salt of succinyl ester **2b** (3.93 g, 3.83 mmol, 99%) was obtained as white foam. $[\alpha]_D^{20}$ (MeOH): +31.6 °; IR: 829, 1034, 1250, 1508, 1738,

2343, 2361; ^{1}H NMR (400 MHz, CD_3CN): δ = 1.11 (t, 9H, *J* = 7.3 Hz, 3 x CH_3 Et_3NH), 2.36 - 2.39 (m, 2H, CH_2 succinyl), 2.47 - 2.50 (m, 2H, CH_2 succinyl), 2.82 (q, 6H, *J* = 7.3 Hz, 14.6 Hz, 3 x CH_2 Et_3N), 3.21 (m, 2H, CH_2 glycerol), 3.56 (dd, 1H, *J* = 3.7 Hz, 9.3 Hz, H-2), 3.64 (at, 1H, *J* = 9.5 Hz, H-4), 3.69 - 3.75 (m, 7H, 2 x OMe, H-6), 3.87 (at, 1H, *J* = 9.3 Hz, H-3), 3.94 - 4.00 (m, 2H, CH glycerol, H-5), 4.15 (dd, 1H, *J* = 6.4 Hz, 11.6 Hz, CH_2 glycerol), 4.23 - 4.26 (m, 2H, CH_2 glycerol, H-6), 4.58 (s, 2H, CH_2 Bn), 4.79 (s, 2H, CH_2 Bn), 5.10 (d, 1H, *J* = 3.7 Hz, H-1), 5.61 (s, 1H, CH benzylidene), 6.84 (d, 4H, *J* = 8.9 Hz, H_{arom}), 7.14 - 7.50 (m, 24H, H_{arom}); ^{13}C NMR (100 MHz, CD_3CN): δ = 9.5 (3 x CH_3 Et_3N), 30.9 (CH_2 succinyl), 31.7 (CH_2 succinyl), 45.9 (3 x CH_2 Et_3N), 55.9 (2 x OMe), 63.7 (CH_2 glycerol), 63.7 (C-5), 65.0 (CH_2 glycerol), 69.5 (C-6), 73.4 (CH_2 Bn), 75.3 (CH_2 Bn), 75.7 (CH glycerol), 79.0 (C-3), 80.3 (C-2), 82.6 (C-4), 87.3 (C_q DMT), 114.1 (CH_{arom}), 127.2 - 131.0 (CH_{arom}), 136.7, 136.8, 138.9, 139.4, 140.1, 146.0, 159.6 (2 x C_q Bn, 4 x C_q DMT, C_q benzylidene), 174.0, 176.8 (2 x CO succinate); HRMS (as free acid): $C_{55}H_{56}O_{13}$ + Na^+ requires 947.3613, found 947.3627.



Hexaglycerolphosphate (16)

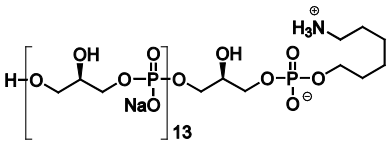
Synthesis on 10 μmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.3% (5 couplings). Purification method A gave the semiprotected hexamer (**10**) as white amorphous solid (3.4 mg, 1.8 μmol, 18%). Method B (synthesis on 15 μmol scale) gave **10** as white amorphous solid (2.7 mg, 1.5 μmol, 10%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.85 min, $C_{74}H_{99}NO_{33}P_6$ + H^+ requires 1716.5 found 1716.6.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (4P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.12 (m, 4H, 2 x CH_2 hexylspacer), 1.26 (m, 2H, CH_2 hexylspacer), 1.42 (m, 2H, CH_2 hexylspacer), 2.92 (t, 2H, *J* = 6.9 Hz, CH_2-N hexylspacer), 3.50 - 3.61 (m, 3H, CH glycerol, CH_2 glycerol), 3.68 - 3.93 (m, 29H, CH_2-O hexylspacer, 11 x CH_2 glycerol, 5 x CH glycerol), 4.41 - 4.54 (m, 12H, 6 x CH_2 Bn), 4.92 (s, 2H, CH_2 benzylcarbamate), 7.16 - 7.32 (m, 35H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 25.6, 26.5, 29.7, 30.7 (4 x CH_2 hexylspacer), 41.3 (CH_2-N hexylspacer), 61.4 (CH_2 glycerol), 65.1, 65.4, 65.5 - 65.7, 67.2, 67.6 (CH_2-O hexylspacer, 11 x CH_2 glycerol, CH_2 benzylcarbamate), 72.7, 73.0 (6 x CH_2 Bn), 78.0 - 78.1 (5 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.7 (CH_{arom}), 137.5, 138.4 - 138.5 (6 x C_q Bn, C_q benzylcarbamate), 159.2 (C_q benzylcarbamate); HRMS: $[C_{74}H_{99}NO_{33}P_6 + 2H]^{2+}$ requires 858.7335, found 858.7340; Deprotection: The semiprotected hexamer (**10**, 4.7 mg, 2.5 μmol) was deprotected using the standard procedure yielding hexamer **16** (1.9 mg, 1.6 μmol, 65%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.2 (3P), 1.3 (2P); ^{1}H-NMR (600 MHz): δ = 1.36 - 1.38 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.64 (m, 4H, 2 x CH_2 hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.54 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, CH*H* glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, CH*H* glycerol), 3.78 - 3.91 (m, 25H, CH_2-O hexylspacer, 11 x CH_2 glycerol, CH glycerol), 3.96 - 4.01 (m, 5H, 5 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.3 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 66.9 - 67.3 (CH_2-O hexylspacer, 11 x CH_2 glycerol), 70.4 - 70.6, 71.7 (6 x CH glycerol); HRMS: $C_{24}H_{57}NO_{31}P_6$ + H^+ requires 1042.1413, found 1042.1425.



10-mer (17)

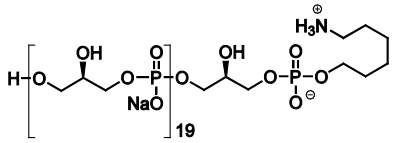
Synthesis on 10 μmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.0% (9 couplings). Purification method A gave the semiprotected decamer (**11**) as white amorphous solid (8.5 mg, 2.9 μmol, 29%). Method B (synthesis on 15 μmol scale) gave the semiprotected decamer (**11**) as white amorphous solid (7.1 mg, 2.4 μmol, 16%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 6.15 min,

$[C_{114}H_{151}NO_{53}P_{10} + 2H]^{2+}$ requires 1346.8 found 1346.8.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (8P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.03 (m, 2H, CH_2 hexylspacer), 1.08 (m, 2H, CH_2 hexylspacer), 1.20 (m, 2H, CH_2 hexylspacer), 1.38 (m, 2H, CH_2 hexylspacer), 2.86 (m, 2H, CH_2-N hexylspacer), 3.49 - 3.59 (m, 3H, CH glycerol, CH_2 glycerol), 3.64 - 3.92 (m, 49H, CH_2-O hexylspacer, 19 x CH_2 glycerol, 9 x CH glycerol), 4.33 - 4.47 (m, 20H, 10 x CH_2 Bn), 4.82 (bs, 2H, CH_2 benzylcarbamate), 7.00 - 7.22 (m, 55H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 25.7, 26.6, 29.8, 30.7 (4 x CH_2 hexylspacer), 41.2 (CH_2-N hexylspacer), 61.3 (CH_2 glycerol), 65.1, 65.5 - 65.6, 67.1, 67.5 (CH_2-O hexylspacer, 19 x CH_2 glycerol, CH_2 benzylcarbamate), 72.6, 72.8 - 72.9 (10 x CH_2 Bn), 78.0 - 78.1 (9 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.6 (CH_{arom}), 138.4 - 138.5 (10 x C_q Bn, C_q benzylcarbamate) 158.9 (C_q benzylcarbamate); HRMS: $[C_{114}H_{151}NO_{53}P_{10} + 2Na]^{2+}$ requires 1369.3173, found 1369.3181; Deprotection: The partially protected decamer (**11**, 7.10 mg, 2.44 μmol) was deprotected using the standard procedure yielding decameric GTA **17** (3.6 mg, 1.9 μmol, 78%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.3 (7P), 1.3 (2P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.39 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.65 (m, 4H, 2 x CH_2 hexylspacer), 2.95 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.55 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, CH*H* glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, CH*H* glycerol), 3.72 (m, 1H, CH*H* glycerol), 3.80 - 4.02 (m, 49H, CH_2-O hexylspacer, 18 x CH_2 glycerol, 1 x CH*H* glycerol, 10 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.5, 30.3 (4 x CH_2 hexylspacer), 40.3 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 66.9 - 67.3 (CH_2-O hexylspacer, 19 x CH_2 glycerol), 70.4 - 70.5, 71.3, 71.6, 71.7 (10 x CH glycerol); HRMS: $C_{36}H_{85}NO_{51}P_{10}$ + H^+ requires 1658.1537, found 1658.1553.



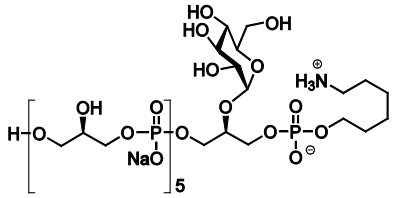
14-mer (18)

Synthesis on 10 μmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.1% (13 couplings). Purification method A gave the semiprotected 14-mer (**12**) as white amorphous solid (3.0 mg, 0.75 μmol, 8%). Method B gave the semiprotected 14-mer (**12**) as white amorphous solid (8.2 mg, 2.1 μmol, 21%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.84 min, $[C_{154}H_{203}NO_{73}P_{14} + 3H]^{3+}$ requires 1224.0 found 1224.4.; ^{31}P-NMR (162 MHz, D_2O): δ = 0.7 - 1.1 (14P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.99 (m, 2H, CH_2 hexylspacer), 1.05 (m, 2H, CH_2 hexylspacer), 1.15 (m, 2H, CH_2 hexylspacer), 1.35 (m, 2H, CH_2 hexylspacer), 2.83 (m, 2H, CH_2-N hexylspacer), 3.47 - 3.58 (m, 3H, CH glycerol, CH_2 glycerol), 3.60 - 3.92 (m, 69H, CH_2-O hexylspacer, 27 x CH_2 glycerol, 13 x CH glycerol), 4.29 - 4.44 (m, 28H, 14 x CH_2 Bn), 4.72 (bs, 2H, CH_2 benzylcarbamate), 6.91 - 7.15 (m, 75H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 24.7, 25.6, 28.9, 29.8 (4 x CH_2 hexylspacer), 40.3 (CH_2-N hexylspacer), 60.2 (CH_2 glycerol), 64.1, 64.4 - 64.6, 66.1, 66.4 (CH_2-O hexylspacer, 27 x CH_2 glycerol, CH_2 benzylcarbamate), 71.6, 71.8 (14 x CH_2 Bn), 77.0 - 77.1 (13 x CH glycerol), 78.2 (CH glycerol), 127.6 - 128.6 (CH_{arom}), 137.5 - 137.6 (14 x C_q Bn, C_q benzylcarbamate); HRMS: $[C_{154}H_{203}NO_{73}P_{14}$ + 2Na$]^{2+}$ requires 1857.4174 found 1857.4167; Deprotection: The semiprotected 14-mer (**12**, 5.5 mg, 1.4 μmol) was deprotected using the standard procedure yielding 14-mer glycerol TA **18** (3.0 mg, 1.2 μmol, 84%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.1 - 1.3 (14P); ^{1}H-NMR (600 MHz, D_2O): δ =1.37 - 1.41 (m, 4H, 2 x CH_2 hexylspacer), 1.60 - 1.67 (m, 4H, 2 x CH_2 hexylspacer), 2.97 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.57 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, CH*H* glycerol), 3.64 (dd, 1H, *J* = 4.1 Hz, 11.8 Hz, CH*H* glycerol), 3.71 - 3.76 (m, 1H, CH*H* glycerol), 3.78 - 4.04 (m, 69H, CH_2-O hexylspacer, 26 x CH_2 glycerol, CH*H* glycerol, 14 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.1 (CH_2 glycerol), 65.8 (CH_2 glycerol), 67.3 - 67.7 (CH_2-O hexylspacer, 26 x CH_2 glycerol), 70.5 - 70.6, 71.3, 71.7 (14 x CH glycerol); HRMS: $C_{48}H_{112}NO_{71}P_{14}$ + H^+ requires 2273.1584, found 2273.1562.



20-mer (19)

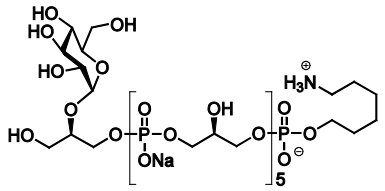
Synthesis on 15 µmol scale (150 mg glycerol-CPG). Average coupling efficiency: 98.5% (19 couplings). Purification method B gave the semiprotected 20-mer (**13**) as white amorphous solid (20.2 mg, 3.62 µmol, 24%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/9 in 13.5 min): r.t. 5.23 min, $[C_{214}H_{281}NO_{103}P_{20} + 4H]^{4+}$ requires 1284.6 found 1284.8.; ^{31}P-NMR (162 MHz, D_2O): δ = 0.8 (1P), 1.0 (18P), 1.1 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.96 (m, 2H, CH_2 hexylspacer), 1.03 (m, 2H, CH_2 hexylspacer), 1.13 (m, 2H, CH_2 hexylspacer), 1.33 (m, 2H, CH_2 hexylspacer), 2.82 (m, 2H, CH_2-N hexylspacer), 3.43 - 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.57 - 4.08 (m, 99H, CH_2-O hexylspacer, 39 x CH_2 glycerol, 19 x CH glycerol), 4.17 - 4.44 (m, 40H, 20 x CH_2 Bn), 4.69 (bs, 2H, CH_2 benzylcarbamate), 6.85 - 7.13 (m, 105H, H_{arom}), 7.68 (s, 1H, NH); ^{13}C NMR (150 MHz, D_2O): δ = 25.7, 26.6, 30.0, 30.7 (4 x CH_2 hexylspacer), 41.3 (CH_2-N hexylspacer), 61.1 (CH_2 glycerol), 64.9 - 65.6, 67.1, 67.2, 67.6 (CH_2-O hexylspacer, 39 x CH_2 glycerol, CH_2 benzylcarbamate), 72.5 - 72.8 (20 x CH_2 Bn), 77.7 - 78.1 (19 x CH glycerol), 79.1 (CH glycerol), 128.6 - 129.6 (CH_{arom}), 137.5, 138.4 - 138.7 (20 x C_q Bn, C_q benzylcarbamate), 158.4 (C_q benzylcarbamate); HRMS: $[C_{214}H_{296}N_6O_{103}P_{20} + 3H]^{3+}$ (mass + 5 x NH_3) requires 1740.7715, found 1740.7691; Deprotection: The partially protected 20-mer (**13**, 6.2 mg, 1.1 µmol) was deprotected using the standard procedure yielding 20-mer glycerol TA **19** (3.8 mg, 1.1 µmol, 95%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 - 1.4 (20P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.59 - 1.71 (m, 4H, 2 x CH_2 hexylspacer), 2.95 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.56 (dd, 1H, *J* = 6.2 Hz, 11.8 Hz, CH*H* glycerol), 3.63 (dd, 1H, *J* = 4.3 Hz, 11.9 Hz, CH*H* glycerol), 3.72 (m, 1H, CH*H* glycerol), 3.81 - 4.04 (m, 99H, CH_2-O hexylspacer, 38 x CH_2 glycerol, CH*H* glycerol, 20 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 65.7 (CH_2 glycerol), 66.9 - 67.5 (CH_2-O hexylspacer, 37 x CH_2 glycerol), 67.7 (CH_2 glycerol), 70.4 - 70.7, 71.3, 71.7 (20 x CH glycerol); HRMS: $[C_{66}H_{155}NO_{101}P_{20} + 2H]^{2+}$ requires 1599.5961, found 1599.5971.



glucosylated hexamer (20)

Synthesis on 15 µmol scale (150 mg glycerol-CPG). Average coupling efficiency: 98.2% (5 couplings). Purification method B gave the semiprotected hexamer (**14**) as white amorphous solid (10.5 mg, 4.86 µmol, 32%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.80 min, $[C_{94}H_{119}NO_{38}P_6 + 2H]^{2+}$ requires 1029.3 found 1029.2.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (1P), 1.1 (3P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.85 - 1.17 (m, 6H, 3 x CH_2 hexylspacer), 1.37 (m, 2H, CH_2 hexylspacer), 2.84 (m, 2H, CH_2-N hexylspacer), 3.34 - 4.11 (m, 38H, CH_2-O hexylspacer, 12 x CH_2 glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.29 - 4.71 (m, 16H, 7 x CH_2 Bn, CH_2 benzylcarbamate), 5.22 (bs, 1H, H-1), 5.28 (bs, 1H, CH benzylidene), 6.86 - 7.43 (m, 45H, H_{arom}); HRMS: $[C_{94}H_{119}NO_{38}P_6 + NH_4 + H]^{2+}$ requires 1037.3123, found 1037.3120; Deprotection: The partially protected hexamer (**14**, 10.5 mg, 4.86 µmol) was deprotected using the standard procedure yielding hexamer monoglucosylglycerol TA **20** (4.4 mg, 3.3 µmol, 68%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 0.9 (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P); ^{1}H-NMR (600 MHz, D_2O): δ =1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.65 (m, 4H, 2 x CH_2 hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.34 (at, 1H, *J* = 9.6 Hz, H-4), 3.46 (dd, 1H, *J* = 3.7 Hz, 9.9 Hz, H-2), 3.55 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, CH*H* glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, CH*H* glycerol), 3.69 - 3.72 (m, 3H, H-3, H-5, H-6), 3.79 - 4.00 (m, 30H, CH_2-O hexylspacer, 11 x CH_2 glycerol, 5 x CH glycerol,

H-6), 4.05 (m, 1H, CH glycerol), 5.11 (d, 1H, *J* = 3.7 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.5 (C-6), 63.0 (CH_2 glycerol), 65.2 (CH_2 glycerol), 66.1 (CH_2 glycerol) 67.0 - 67.4 (CH_2-O hexylspacer, 9 x CH_2 glycerol), 70.4 - 70.6 (4 x CH glycerol, C-4), 71.7 (CH glycerol), 72.5 (C-2), 72.8 (C-5), 73.9 (C-3), 76.3 (CH glycerol), 98.7 (C-1); HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1957.



glucosylated hexamer (21)

Synthesis on 15 μmol scale (150 mg glucosylglycerol-CPG). Average coupling efficiency: 96.9% (5 couplings). Purification method B gave the semiprotected hexamer (**15**) as white amorphous solid (11.6 mg, 5.37 μmol, 36%). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.55 min, $C_{94}H_{119}NO_{38}P_6$ + H^+ requires 2057.6 found 2058.0.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 - 1.3 (6P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.90 - 1.15 (m, 6H, 3 x CH_2 hexylspacer), 1.33 (m, 2H, CH_2 hexylspacer), 2.80 (m, 2H, CH_2-N hexylspacer), 3.22 - 4.18 (m, 38H, CH_2-O hexylspacer, 12 x CH_2 glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.30 - 4.69 (m, 16H, 7 x CH_2 Bn, CH_2 benzylcarbamate), 5.12 - 5.20 (m, 2H, H-1, CH benzylidene), 6.86 - 7.26 (m, 45H, H_{arom}); HRMS: $[C_{94}H_{119}NO_{38}P_6$ + $2H]^{2+}$ requires 1028.7991, found 1028.7996; Deprotection: The partially protected hexamer (**15**, 2.2 mg, 0.99 μmol) was deprotected using the standard procedure yielding hexamer monoglucosyl TA **21** (1.1 mg, 0.85 μmol, 86%) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.59 - 1.65 (m, 4H, 2 x CH_2 hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.36 (at, 1H, *J* = 9.7 Hz, H-4), 3.48 (dd, 1H, *J* = 3.8 Hz, 9.9 Hz, H-2), 3.68 - 3.73 (m, 4H, H-3, 2 x H-6, CH*H* glycerol), 3.77 - 4.02 (m, 32H, CH_2-O hexylspacer, 11 x CH_2 glycerol, CH*H* glycerol, 6 x CH glycerol, H-5), 5.12 (d, 1H, *J* = 3.7 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.6, 30.3 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.5 (CH_2 glycerol), 62.2 (C-6), 65.2 (CH_2 glycerol), 66.9 - 67.2 (CH_2-O hexylspacer, 11 x CH_2 glycerol), 70.4 - 70.6 (5 x CH glycerol, C-4), 72.4 (C-2), 72.9 (C-5), 73.8 (C-3), 77.8 (CH glycerol), 98.8 (C-1); HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1956.

References

1. Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686-723.
2. Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, *6*, 276-287.
3. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange,T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703–5712.
4. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839.
5. Morath, S.; Geyer, A.; Hartung, T. *J. Exp. Med.* **2001,** *193*, 393-397.
6. Morath, S.; Stadelmaier, A.; Geyer, A.; Schmidt, R. R.; Hartung, T. *J. Exp. Med.* **2002,** *195*, 1635-1640.
7. Hashimoto, M.; Tawaratsumida, K.; Kariya, H.; Kiyohara, A.; Suda, Y.; Kirikae, F.; Kirikae, T.; Gotz, F. *J. Immunol.*, **2006**, *177*, 3162-3169.
8. von Aulock, S.; Hartung, T.; Hermann, C. *J. Immunol.* **2007**, *178*, 2610-2611.
9. Hashimoto, M.; Furuyashiki, M.; Suda, Y. *J. Immunol.* **2007**, *178*, 2610-2611.
10. Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, *18*, 3668-3678.
11. Caruthers, M.H. *Science* **1985**, *230*, 281-285.
12. Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1992**, *48*, 2223-2311.
13. Reese, C.B. *Org. Biomol. Chem.* **2005**, *3*, 3851-3868.
14. Pollack, J.H.; Neuhaus, F.C. *J. Bacteriol.* **1994**, *176*, 7252-7259.
15. Endl, J.; Seidl, H.P.; Fiedler, F.; Schleifer, K.H. *Arch. Microbiol.* **1983**, *135*, 215-
16. Hogendorf, W.F.J.;Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, 47, 8961-8963.
17. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. *J. Am. Chem. Soc.* **1963**, *85*, 3821-3827.
18. Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926-4930.
19. Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480.
20. Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.
21. Codée, J.D.C.; van den Bos, L.J.; Litjens, R.E.J.N.; Overkleeft, H.S.; van Boom, J.H.; van der Marel, G.A. *Org. Lett.* **2003**, *5*, 1947-1950.
22. Oltvoort, J. J.; van Boeckel, C. A. A.; de Koning, J. H.; van Boom, J. H. *Synthesis* **1981**, 305-308.
23. Welz, R.; Müller, S. *Tetrahedron Lett.* **2002**, *43*, 795-797.
24. The use of the commercially available *N*-MMT-6-aminohexanol phosphoramidite led to the formation of *N*-cyanoethyl side products in the cleavage/deprotection step. Although this could be prevented by the use of 1,4-dithiothreitol (DTT) in the cleavage cocktail, we preferred the use of the stable benzyl carbamate **3**.
25. Pon, R.T. *Methods in Molecular Biology*; Agrawal, S., Ed.; Humana Press, Totowa, New Jersey, **1993**, Vol. 20, pp 465-496.