Synthetic methods to glycerol teichoic acids

Hogendorf, W.F.J.

Chapter 5

Light Fluorous Synthesis of Glucosylated GTAs

Introduction

The automated solid phase strategy described in **chapter three**, led to the generation of a small library of teichoic acid (TA) fragments. The antigenicity of these molecules was evaluated by means of an opsonophagocytic inhibition assay (OPIA). In this assay the inhibition of killing *Enterococcus faecalis* by rabbit antibodies raised against a biological preparation of native enterococcal LTA by the synthetic antigens was quantified. It was found that two glucosylated TA fragments showed the highest inhibitory potency (**1** and **2**, figure 1) when tested in such an assay (see figures 2 and 3, **chapter three**).[1] A next step in the immunological evaluation of **1** and **2** comprises the coupling of these molecules to carrier proteins and test the potential of the resulting conjugates as a vaccine against *Enterococcus faecalis*.[2-4] In order to achieve this, sufficient quantities of **1** and **2** are required which cannot easily be generated using an automated solid phase synthesis approach.

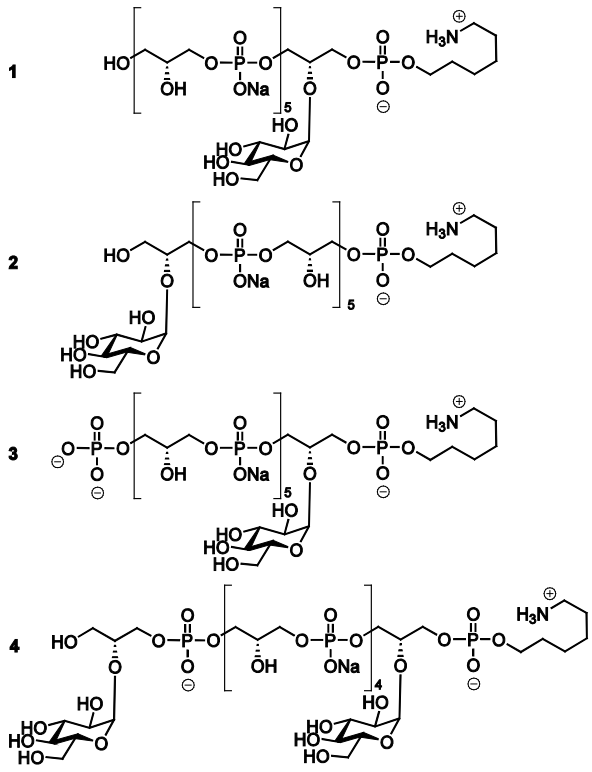


Figure 1. Glucosylated hexamers **1-4**.

In **chapter four**, a light fluorous[5-10] approach is described, which allowed the rapid production of multimilligram amounts of TAs. With this method, starting from

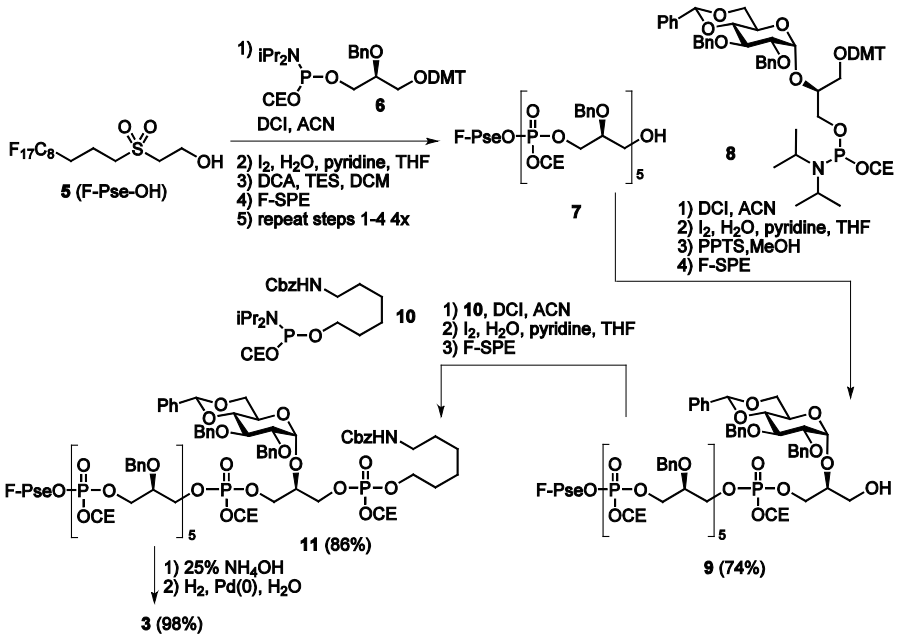
the perfluorooctylpropylsulfonyl ethanol linker (F-Pse), it was possible to perform a quick purification of the intermediates using fluorous solid phase extraction (F-SPE) after each elongation cycle. At the end of the synthesis, the fluorous linker is cleaved and the molecules undergo a final hydrogenolysis step, giving multimilligram quantities of the target TAs bearing a terminal phosphomonoester.[11]

This chapter first discusses the synthesis of TA fragment **3**, a phosphorylated version of hexamer **1**, which is attained using the earlier described light fluorous approach. To enable the light fluorous synthesis of TAs without terminal phosphate an alternative fluorous linker is required. The perfluorooctylpropyl succinyl linker proved suitable for this, as is illustrated by the synthesis of lead TA **2** and TA **4** in which a second glucosyl moiety is incorporated.[12] Finally, the antigenic properties of the prepared TAs (**2**,**3**,**4**) are probed in an OPIA, as described earlier, whereby the influence of a terminal phosphate and a second glucosyl moiety on the immunological activity could be established.

Results and Discussion

As described in **chapter four** perfluorooctylpropylsulfonylethanol (F-Pse) can be used effectively as a phosphate protecting group and concomitantly serve as a fluorous linker in the solution phase synthesis of TA fragments.[11] First the synthesis of hexamer **3**, a phosphorylated analogue of TA fragment **1**, was explored, using this

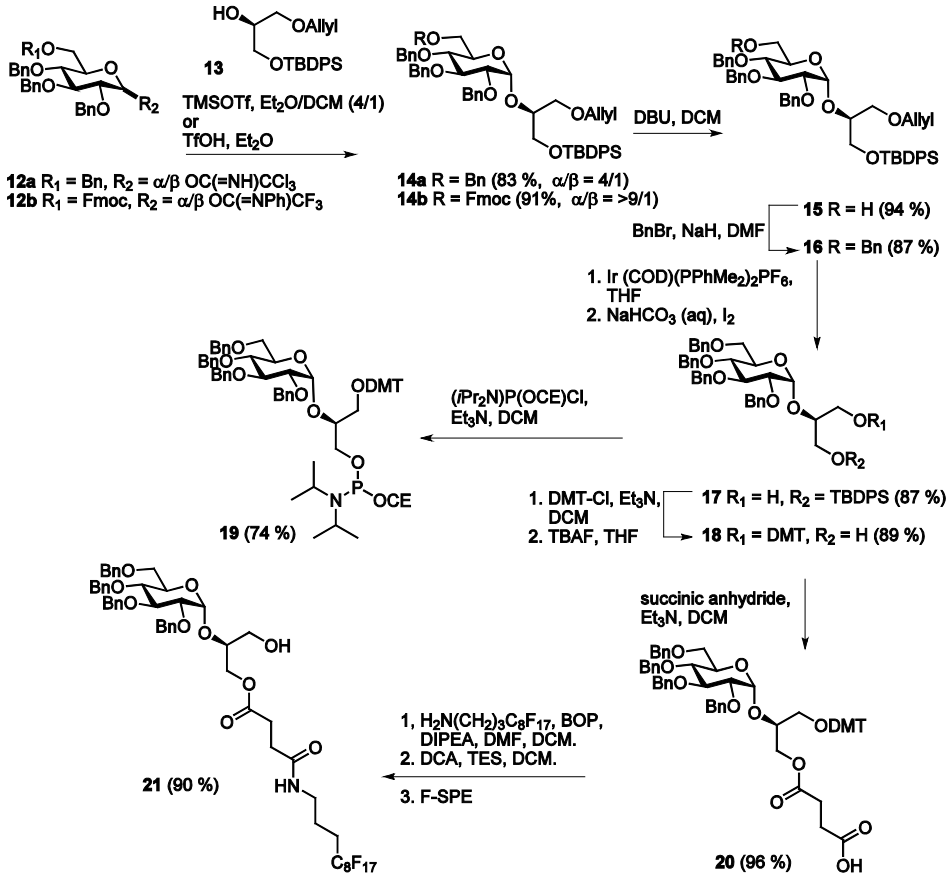
Scheme 1. Assembly of TA-fragment **3**.



linker-system. Thus, F-Pse **5**[13] was elongated in a stepwise manner (Scheme 1) with glycerol phosphoramidite **6**[14] in an elongation process, which comprises coupling, oxidation, detritylation and, finally, a F-SPE[7] purification step. Before the F-SPE purification the crude reaction mixture was partitioned between acetonitrile/water (80/20) and hexane to remove the bulk of TES and DMT-H that are released during the detritylation step in order to simplify the F-SPE purification. Repeating this process four times led to pentamer **7** which was then subjected to an adapted elongation process. Coupling with benzylidene protected glucosylglycerol phosphoramidite **8**[1] under agency of DCI and standard oxidation was followed by detritylation using the milder PPTS/MeOH cocktail as the 4,6-*O*-benzylidene moiety is unstable towards DCA/TES.[14,15] The presence of the lipophilic protected carbohydrate moiety did not influence the F-SPE purification and the target compound was obtained uneventfully in 74% yield. At this stage an aminospacer was introduced to allow the conjugation of the target structure to, for instance, a carrier protein. Condensation of hexamer **9** and phosphoramidite **10** was followed by oxidation and F-SPE to give the fully protected construct **11** in 86% yield. Deprotection of hexamer **11** started by removal of the cyanoethyl (CE) and F-Pse groups by overnight treatment with aqueous ammonia at 40 °C. The semi-protected intermediate was separated from the released fluorous linker (perfluorooctylpropylsulfonylethyl amine) using a Et_2O/H_2O extraction. Subsequently, the benzylidene acetal, benzyl ethers and benzyl carbamate were removed by means of hydrogenolysis (Pd/H_2), leading to the target hexamer **3** in 98% yield.

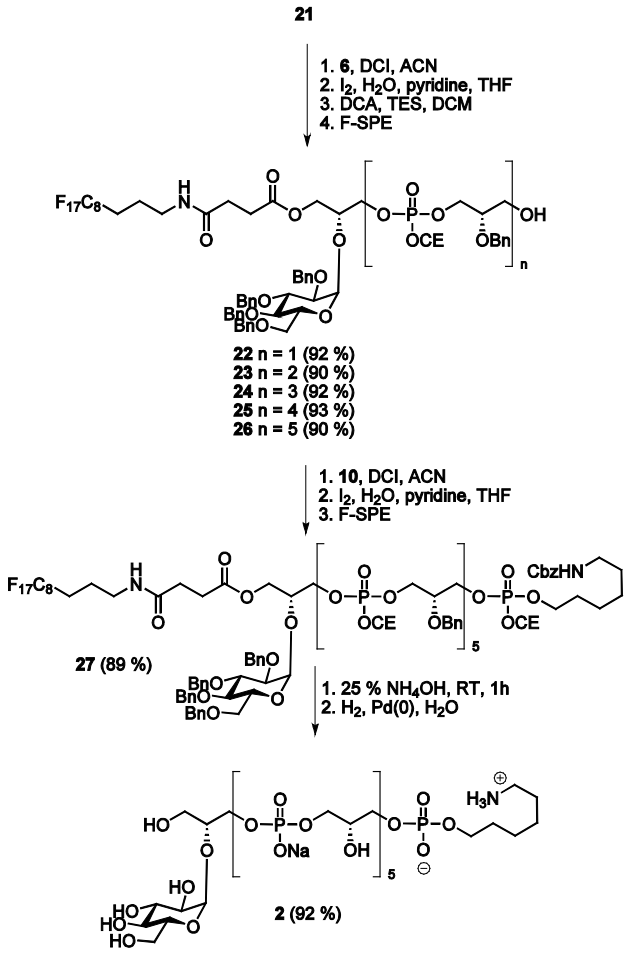
To attain the light fluorous assembly of TA fragments without a terminal phosphate moiety the next objective was to find a suitable fluorous hydroxyl protecting group. Inspired by contemporary DNA synthesis methods, a succinyl type linker was deemed suitable because of its stability towards phosphoramidite chemistry, oxidation and detritylation conditions.[16,17] The base lability of a fluorous succinyl linker allows the same deprotection strategy as employed in the synthesis of hexamer **3**. Moreover, attention was paid to the development of a more acid-stable glucosyl glycerol synthon that allows the incorporation of a glucosyl moiety at any stage of the elongation sequence. As described earlier, the benzylidene acetal does not withstand the standard detritylation conditions, necessitating the use of a carefully controlled procedure for the removal of the temporary DMT group. Therefore, the synthesis of the more acid stable tetra-*O*-benzyl glucosyl synthon **19** was undertaken (Scheme 2). A crucial step en route to synthon **19** is the stereoselective introduction of the α-glucosidic linkage. First the use of per-benzylated glucosyl imidate **12a**[18] for the construction of this linkage was explored. Condensation of this donor with glycerol acceptor **13** in DCM led to formation of product **14a** with poor selectivity (α/β = 2/1). The use of ether as co-solvent[19] improved the α/β-ratio (4/1), but the anomeric mixture proved to be inseparable. Next, a glucosyl donor bearing an Fmoc protecting group on the C6 hydroxyl known to favor the formation of the α-product was

Scheme 2. Synthesis of phosphoramidite **19** and F-Pse linked glucosyl glycerol **21**.



explored.[20-22] Coupling 6-*O*-Fmoc glucosyl imidate **12b** with glycerol **13** using Et_2O as a solvent, led to the formation of **14b** in high selectivity (α/β ~10/1). Purification by column chromatography, afforded α-glucoside **14b** in 91% yield (containing < 3% β-adduct, based on 1H-NMR analysis). Compound **14b** was then treated with DBU in DCM, and benzylation of the intermediate alcohol **15** led to tetrabenzylglucosyl derivative **16** in 87% yield. In the next step the allyl ether was removed by iridium catalyzed isomerisation, followed by oxidative cleavage of the intermediate enol ether, giving alcohol **17** in 87% yield. Installation of the DMT ether and desilylation led to building block **18**, which was transformed into the phosphoramidite synthon **19** using *N*,*N*-diisopropyl-2-cyanoethylchlorophosphoramidite and Et_3N. Alternatively, **18** was reacted with succinic anhydride and Et_3N in DCM, giving succinyl ester **20** in 96% yield (scheme 2). Coupling of **20** with perfluorooctylpropylamine, using BOP as a condensing agent, was followed by detritylation to give the crude fluorous glucosyl glycerol **21**, which was purified by F-SPE to give the pure target compound in 90%

Scheme 3. Light fluorous assembly of **2**.

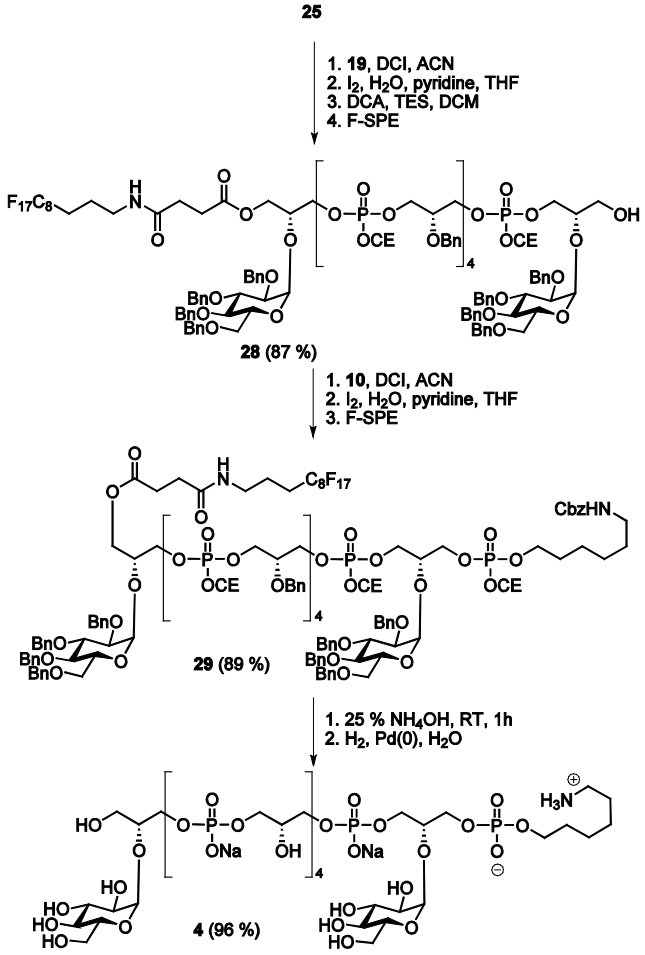


yield. This molecule was elongated in a step-wise manner with glycerol phosphoramidite **6** using a five-fold repetition of the 4-step elongation process described above leading to hexamer **26** (scheme 3). The aminohexyl-spacer was then introduced to give the fully protected hexamer **27**. Deprotection by 25% aqueous ammonia (1 hr, RT), was followed by hydrogenolysis to give 40 mg of target compound **2** (92%).

To broaden the palette of TA fragments, and further explore the effectiveness of the light fluorous chemistry, the synthesis was continued with the assembly of hexamer **4**, containing two glucosyl moieties (Scheme 4). Pentamer **25** was coupled to glucosylglycerol phosphoramidite **19**, resulting in bis glucosylated hexamer **28** in 87%. Also this compound was uneventfully purified by F-SPE. After introduction of the spacer, the resulting hexamer **29** was deprotected using the aforementioned conditions, to yield the bis-glucosyl TA fragment **4** in 96% yield.

Hexameric TAs **3** and **4** were compared with TAs **1** and **2** on their ability to bind to rabbit antibodies raised against enterococcal LTA in an OPIA, that was performed as described before.[1-4] Surprisingly, TA **3** bearing an extra phosphate moiety compared to one of the lead fragments (**1**) showed no inhibitory activity at all, even when administered at a concentration of 400 μg/ml. This indicates that the terminal phosphate moiety is at least disadvantageous to the immunogenicity of the TA. Compound **4**, which bears a second glucose moiety but lacks the terminal phosphate, showed some inhibitory potency. However, when compared to lead TA **2** the

Scheme 4. Light fluorous assembly of **29**.



antibody binding was considerably reduced, indicating that an extra glucosyl moiety has a detrimental effect on antigenicity (see figure 2).

Conclusion

In summary, this chapter describes the development of two complementary fluorous linker systems for the assembly of glucosylated TA fragments. The first linker, perfluorooctylpropyl-sulfonylethyl, is used as a phosphate protecting group and allows the assembly of TA fragments featuring a terminal phosphate monoester (hexamer **3**). The second linker, a perfluorooctyl-propyl succinyl system, is used as a hydroxyl protecting functionality and leads to the formation of TA structures terminating in an alcohol functionality (structures **2** and **4**). Acid stable tetra-*O*-benzyl glucosyl building block **19**, allowing the incorporation of a glucosyl substitution at any position of the TA chain was

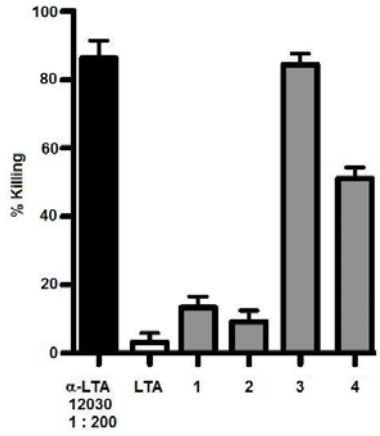


Figure 2. Hexamers **3,4** showing dimished inhibitory activity in the OPIA compared to previously made hexamers **1** and **2**. The left bar represents killing by the serum without addition of inhibitor. The second bar from the left (LTA) represents the positive control where native LTA is added as the inhibitor.

prepared and applied in the assembly of TAs **1** and **2**. The presence of two lipophilic tetrabenzylglucosyl moieties in the fully protected precursor (**29**) of **4** did not have a negative effect on the F-SPE purifications. Fluorous chemistry is an efficient means for the assembly of (glycosylated) TA fragments and allows the construction of pure TA oligomers in multi milligram quantities, sufficient for most initial biochemical studies.

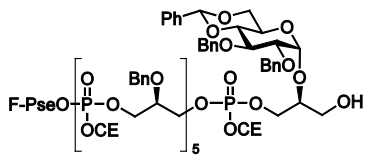
Experimental section

General Procedures and Material: All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript, Fluorous Technologies) 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 $(NH_4)_6Mo_7O_{24}$•4 H_2O 25 g/l and $(NH_4)_4Ce(SO_4)_4$•2 H_2O 10 g/l, in 10% aqueous H_2SO_4 followed by charring at +/- 140 ^oC. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. 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. ^{31}P, ^1H, and ^{13}C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 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. When D_2O was used, ^1H-NMR spectra were recorded with chemical shift relative (δ) to HDO (4.755 ppm), ^{31}P spectra were measured with chemical shift relative to 85% H_3PO_4 (external standard) and ^{13}C-NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and either 0.1% formic acid or 10mM ammonium formate for the oligomers) 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 275 ^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).

General procedure for phosphoramidite coupling, oxidation, detritylation and FSPE on a typical scale (0.1-0.25 mmol): Starting alcohol was dissolved in ACN (0.1M). DCI (0.25M solution in CH_3CN, 2 eq compared to phosphoramidite) was added, together with freshly activated MS3Å and the mixture was stirred under argon for 15 minutes. Phosphoramidite (0.175M in ACN, 1.3 - 4.0 eq) was added and the reaction was stirred until TLC analysis revealed full conversion of the starting material into a higher running spot (~1 hr). Added were, respectively, H_2O (~1 ml) and I_2 (0.2 M in THF/pyr 4/1), and the mixture was stirred for an additional 5 min. The mixture was diluted with EtOAc (~50 ml) and washed with, respectively, sat. aq. $Na_2S_2O_3$ (~20 ml), 0.5 M $KHSO_4$ (~20 ml) and a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (~20 ml). The organic layer was dried over Na_2SO_4 (s) and concentrated under reduced pressure. The residue was coevaporated once with toluene (10 ml) before it was redissolved in DCM. Triethylsilane and dichloroacetic acid were added and the mixture was stirred until the bright orange color fully dissapeared (~30 min). DCM (~40 ml) was added and the organic layer was washed with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (~20 ml, check if pH >7), before it was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was taken up in 4/1

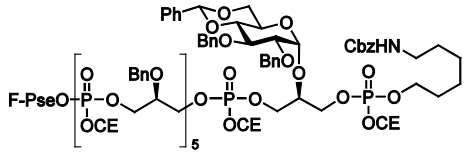
ACN/H_2O (10 ml) and washed with hexane (50 ml). The hexane layer was extracted twice with 4/1 ACN/H_2O (2 x 10 ml) and the combined ACN/H_2O layers were concentrated under reduced pressure in a 100 ml pear shaped flask. The residue was taken up in 0.5 ml ACN and applied to a small column containing fluoroflash™ fluorous silica (4g) which was preeluted with 1/1 ACN H_2O. The column was eluted with 1/1 ACN/H_2O until all the non-fluorous byproducts (DMT-H, phosphates, DCI) were removed. Subsequently the fluorous product was eluted from the column with, respectively, CH_3CN and acetone.

Global deprotection and purification of oligomers: The fully protected oligomer was treated with a 9/1 mixture of 28% NH_4OH (aq)/1,4-dioxane at a concentration of 5 mg/ml at 40 - 45 °C overnight in a sealed flask or tube in case of of **11**. In the synthesis of oligomers **2** and **4**, the corresponding protected hexamers (**27** and **29**, respectively) were treated with a 9/1 mixture of 28% NH_4OH (aq)/1,4-dioxane at a concentration of 5 mg/ml at room temperature for 1h. Next, in all cases, the mixture was washed with Et_2O (equal volume) and the ether layer was extracted twice with H_2O. The aqueous layer was concentrated under reduced pressure after which NMR and HRMS analysis confirmed full conversion to the semiprotected intermediate. The intermediate was then treated with Pd (0)/H_2 in a slightly acidic (pH ~2.7) mixture of dioxane/water (1/4, containing ~1% AcOH). After stirring for three days the mixture was filtered and concentrated *in vacuo*. The residue was purified by size exclusion chromatography (Sephadex HW40, eluent: 0.15 M NH_4OAc). After repeated lyophilisation, 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 fully deprotected oligomer of which the integrity and purity was confirmed by HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.



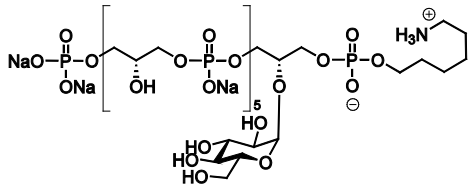
Glucosyl-2-*O*-benzylglycerol phosphate hexamer (9)
Glycerol phosphate pentamer **7** (286 mg, 139 μmol) and DCI (0.25M solution in CH_3CN, 2.22 ml, 556 μmol) were dissolved in CH_3CN (2.0 ml) together with freshly activated MS3Å and stirred for 15 min under argon. Subsequently, glucosyl-glycerol phosphoramidite **8** (0.1M in CH_3CN, 2.30 ml, 230 μmol) was added and the mixture stirred for 30 min at RT. H_2O (1.0 ml) was added after which the oxidation step was performed according to the general procedure. The crude intermediate was redissolved in a 1/1 mixture of DCM and MeOH (40 ml) and treated with PPTS (40 mg, 0.16 mmol) for 8 hrs under gentle stirring. The mixture was diluted with DCM (80 ml) and washed with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (50 ml). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*, after which the crude product was purified with FSPE, according to the general procedure. Glucosylated hexamer **9** (277 mg, 103 μmol, 74%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.3 (2P), -1.1 - -0.9 (3P); ^1H NMR (400 MHz): δ = 2.09 - 2.35 (m, 4H, $F_{17}C_8CH_2CH_2CH_2CH_2SO_2$-, $F_{17}C_8CH_2CH_2CH_2CH_2SO_2$-), 2.47 - 2.77 (m, 13H, 6 x CH_2 cyanoethyl, CH_2-O*H*), 3.05 - 3.13 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2SO_2$-), 3.24 - 3.34 (m, 2H, -$OCH_2CH_2CH_2SO_2$-), 3.51 - 3.72 (m, 5H, H-2, H-4, H-6, CH_2 glycerol), 3.77 - 3.88 (m, 6H, 6 x CH glycerol), 3.98 - 4.34 (m, 37H, H-3, H-5, H-6', 11 x CH_2 glycerol, 6 x CH_2 cyanoethyl), 4.42 - 4.50 (m, 2H, -$OCH_2CH_2SO_2$-), 4.56 - 4.65 (m, 10H, 5 x CH_2 Bn), 4.70 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.80 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 4.87 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.91 (d, 1H, *J* = 3.7 Hz, H-1), 4.95 (d, 1H, *J* = 11.3 Hz, CH*H* Bn), 5.55 (s, 1H, CH benzylidene), 7.25 - 7.40 (m, 38H, H_{arom}), 7.44 - 7.48 (m, 2H, H_{arom}); ^{13}C NMR (100 MHz): δ = 13.3 ($F_{17}C_8CH_2CH_2CH_2SO_2$-), 19.2 - 19.5 (12 x CH_2 cyanoethyl), 29.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2SO_2$-), 53.0, 53.1 ($F_{17}C_8CH_2CH_2CH_2SO_2$-, -$OCH_2CH_2SO_2$-), 60.9 ($CH_2$ glycerol), 61.3 (-$OCH_2CH_2SO_2$-), 62.0 - 62.4 (6 x CH_2 cyanoethyl), 62.8 (C-5), 65.4 - 66.0 (10 x CH_2 glycerol),

67.2 (CH_2 glycerol), 68.7 (C-6), 72.0 - 72.1 (5 x CH_2 Bn), 74.3 (CH_2 Bn), 75.0 (CH_2 Bn), 75.2 - 75.4 (5 x CH glycerol), 78.6 - 78.8 (C-2, C-3, CH glycerol), 82.0 (C-4), 98.6, 98.9 (C-1), 100.9 (CH benzylidene), 116.6 - 116.7 (6 x C_q cyanoethyl), 125.7 (CH_{arom}), 127.6 - 128.9 (CH_{arom}), 137.1 - 137.4 (7 x C_q Bn), 138.4 (C_q benzylidene); HRMS: $C_{111}H_{127}F_{17}N_6O_{38}P_6S$ + NH_4^+ requires 2710.6403, found 2710.6393.



Glucosyl-2-*O*-benzylglycerol phosphate hexamer aminohexyl spacer (11)

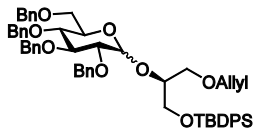
Hexamer **9** (272 mg, 101 μmol) was coupled to spacer phosphoramidite **10** (4 eq), oxidized and purified (FSPE) using the general procedure as described above. Fully protected hexamer **11** (267 mg, 87.2 μmol, 86%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.3 - -1.0 (6P); ^{1}H NMR (400 MHz): δ = 1.27 - 1.37 (m, 4H, 2 x CH_2 hexylspacer), 1.42 - 1.51 (m, 2H, CH_2 hexylspacer), 1.60 - 1.68 (m, 2H, CH_2 hexylspacer), 2.09 - 2.34 (m, 4H, $F_{17}C_8CH_2C\mathit{H}_2CH_2SO_2$-, $F_{17}C_8C\mathit{H}_2CH_2CH_2SO_2$-), 2.39 - 2.69 (m, 14H, 7 x CH_2 cyanoethyl), 3.05 - 3.17 (m, 4H, $F_{17}C_8CH_2CH_2C\mathit{H}_2SO_2$-, CH_2-N hexylspacer), 3.24 - 3.33 (m, 2H, -$OCH_2C\mathit{H}_2SO_2$-), 3.57 - 3.72 (m, 3H, H-2, H-4, H-6), 3.75 - 3.85 (m, 5H, 5 x CH glycerol), 3.94 - 4.33 (m, 44H, H-3, H-5, H-6', CH glycerol, 12 x CH_2 glycerol, 7 x CH_2 cyanoethyl, CH_2-O hexylspacer), 4.43 - 4.50 (m, 4H, -$OC\mathit{H}_2CH_2SO_2$-, CH_2 Bn), 4.53 - 4.64 (m, 10H, 5 x CH_2 Bn), 4.71 - 4.77 (m, 2H, CH_2 Bn), 4.81, (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.92, (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.99 - 5.12 (m, 4H, H-1, NH CBz, CH_2 CBz), 5.55 (s, 1H, CH benzylidene), 7.26 - 7.38 (m, 43H, H_{arom}), 7.44 - 7.48 (m, 2H, H_{arom}); ^{13}C NMR (100 MHz): δ = 13.3 ($F_{17}C_8CH_2\mathit{C}H_2CH_2SO_2$-), 19.2 - 19.5 (7 x CH_2 cyanoethyl), 24.8, 25.9 (2 x CH_2 hexylspacer), 29.3 - 29.9 (2 x CH_2 hexylspacer, $F_{17}C_8\mathit{C}H_2CH_2CH_2SO_2$-), 40.7 ($CH_2$-N hexylspacer), 53.0, 53.1 ($F_{17}C_8CH_2CH_2\mathit{C}H_2SO_2$-, -$OCH_2\mathit{C}H_2SO_2$-), 61.3 (-$O\mathit{C}H_2CH_2SO_2$-), 61.8 - 62.4 (7 x CH_2 cyanoethyl), 62.8 (C-5), 65.3 - 66.0 (11 x CH_2 glycerol), 66.3 (CH_2 CBz), 68.4 - 68.6 (C-6, CH_2 glycerol), 72.0 - 72.2 (5 x CH_2 Bn), 73.4 - 73.5 (CH_2 Bn), 75.0 (CH_2 Bn), 75.2 - 75.5 (6 x CH glycerol), 78.0 - 78.1 (C-3), 78.9 (C-2), 81.7 - 81.8 (C-4), 97.4 - 97.7 (C-1), 100.8 (CH benzylidene), 116.6 - 116.7 (7 x C_q cyanoethyl), 125.7 (CH_{arom}), 127.5 - 128.9 (CH_{arom}), 136.6 (C_q Bn), 137.1 - 137.3 (6 x C_q Bn), 137.9 (C_q Bn), 138.5 (C_q benzylidene), 156.3 (C_q CBz); HRMS: $C_{128}H_{150}F_{17}N_8O_{43}P_7S$ + NH_4^+ requires 3076.7748, found 3076.7789.



Glucosyl-glycerolphosphate hexamer (3)

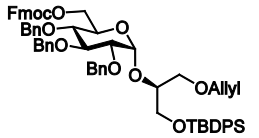
Protected hexamer **11** (99.5 mg, 32.5 μmol) was treated with aqueous ammonia as described above. Additionally, the compound was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use) and, subsequently, lyophilized, yielding the intermediate semiprotected hexamer (75.2 mg, 32.5 μmol, 100%) as an amorphous white solid. Analytical data intermediate: ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 - 1.1 (6P), 2.9 (1P, phosphomonoester); ^{1}H NMR (400 MHz, D_2O): δ = 0.95 - 1.24 (m, 6H, 3 x CH_2 hexylspacer), 1.34 - 1.44 (m, 2H, CH_2 hexylspacer), 2.85 - 2.94 (m, 2H, CH_2-N hexylspacer), 3.51 - 4.15 (m, 38H, H-2, H-3, H-4, H-5, H-6, H-6', CH_2-O hexylspacer, 6 x CH glycerol, 12 x CH_2 glycerol), 4.29 - 4.41 (m, 10H, 5 x CH_2 Bn), 4.47 - 4.58 (m, 4H, 2 x CH_2 Bn), 4.89 (s, 2H, CH_2 CBz), 5.29 (d, 1H, *J* = 3.6 Hz, H-1), 5.47 (s, 1H, CH benzylidene), 6.98 - 7.37 (m, 45H, H_{arom}); HRMS: $[C_{94}H_{120}NO_{41}P_7 + 2H]^{2+}$ requires 1068.7822, found 1068.7828. A portion of the intermediate (75.1 mg, 32.5 μmol) was deprotected with Pd (0)/H_2 using the standard

procedure. Monoglucosylated hexamer **3** (45.5 mg, 31.7 μmol, 98%) was obtained as an amorphous white solid. ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 (1P), 1.2 - 1.3 (4P), 1.4 (1P), 4.7 (1P, phosphomonoester); ^{1}H NMR (600 MHz, D_2O): δ = 1.38 - 1.43 (m, 4H, 2 x CH_2 hexylspacer), 1.60 - 1.68 (m, 4H, 2 x CH_2 hexylspacer), 2.97 (t, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.37 (at, 1H, *J* = 9.6 Hz, H-4), 3.49 (dd, 1H, *J* = 3.8 Hz, 9.9 Hz, H-2), 3.71 - 3.77 (m, 3H, H-3, H-6, CH*H* glycerol), 3.79 - 4.04 (m, 32H, H-5, H-6', 5 x CH glycerol, 11 x CH_2 glycerol, CH*H* glycerol, CH_2-O hexylspacer), 4.06 - 4.09 (m, 1H, CH glycerol), 5.14 (d, 1H, *J* = 3.7 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6 (3 x CH_2 hexylspacer), 30.4 (d, *J* = 6.8 Hz, CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.5 (C-6), 65.2 (d, *J* = 6.0 Hz, CH_2 glycerol), 65.7 (d, *J* = 4.5 Hz, CH_2 glycerol), 66.1 (d, *J* = 5.2 Hz, CH_2 glycerol), 67.1 - 67.3 (8 x CH_2 glycerol, CH_2-O hexylspacer), 67.7 (d, *J* = 5.5 Hz, CH_2 glycerol), 70.5 (t, *J* = 7.7 Hz, 4 x CH glycerol), 70.7 (C-4), 71,3 (t, *J* = 7.3 Hz, CH glycerol), 72.5 (C-2), 72.8 (C-5), 73.9 (C-3), 76.4 (t, *J* = 8.0 Hz, CH glycerol), 98.7 (C-1); HRMS: $C_{30}H_{68}NO_{39}P_7$ + H^+ requires 1284.1605, found 1284.1610.



3-*O*-Allyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (14a)

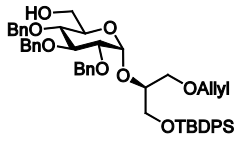
To a cooled (0 °C) solution of donor **12a** (171 mg, 0.250 mmol) and semiprotected glycerol **13** (111 mg, 0.300 mmol) in a 4/1 mixture of Et_2O/DCM (5.0 ml) was added TMSOTf (2.25 μl, 12.4 μmol). After stirring 40 min, Et_3N (3 drops) was added and the mixture diluted with DCM (10 ml). After washing once with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (10 ml), the organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (EtOAc/PE) gave pseudodisaccharide **14a** (168 mg, 0.188 mmol, 75%) as an inseparable mixture of anomers (α/β ratio of ~4/1, based on ^{1}H-NMR analysis. This was ~2/1 when the reaction was performed in pure DCM). For analytical data of the pure α-isomer see the synthesis of compound **16**.



3-*O*-Allyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethyloxycarbonyl]-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (14b)

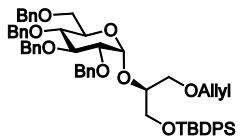
To a cooled (0 °C) solution of donor **12b** (7.51 g, 8.90 mmol) and semiprotected glycerol **13** (3.96 g, 10.7 mmol) in Et_2O (180 ml) was added TfOH (157 μl, 1.78 mmol). After stirring 25 min, sat. aq. $NaHCO_3$ (75 ml) was added and the layers separated. The ether layer was washed once with brine (50 ml) before it was dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (EtOAc/PE) gave pseudodisaccharide **14b** (8.30 g, 8.09 mmol, 91%) as a colourless oil containing a minor amount (< 3%, based on ^{1}H-NMR analysis) of the β-product. $[\alpha]_D^{20}$ ($CHCl_3$): +32.0; IR: 1007, 1072, 1254, 1450, 1748, 2859; ^{1}H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.58 - 3.63 (m, 3H, H-2, H-4, CH*H* glycerol), 3.69 - 3.80 (m, 3H, CH*H* glycerol, CH_2 glycerol), 3.94 - 4.14 (m, 6H, H-3, H-5, CH glycerol, CH*H* FMOc, CH_2 allyl), 4.20 - 4.26 (m, 2H, CH*H* FMOc, CH FMOc), 4.31 - 4.40 (m, 2H, H-6, H-6'), 4.55 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.69 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.76 - 4.79 (m, 3H, 1 x CH_2 Bn, CH*H* Bn), 4.88 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 5.00 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 5.16 (ad, 1H, *J* = 10.8 Hz, CH*H* allyl), 5.25 (dd, 1H, *J* = 1.4 Hz, 17.4 Hz, CH*H* allyl), 5.32 (d, 1H, *J* = 3.6 Hz, H-1), 5.87 (ddd, 1H, *J* = 5.5 Hz, 10.7 Hz, 22.6 Hz, CH allyl), 7.22 - 7.40 (m, 25H, H_{arom}), 7.58 (d, 1H, *J* = 7.5 Hz, H_{arom}), 7.61 (d, 1H, *J* = 7.5 Hz, H_{arom}), 7.66 (d, 4H, *J* = 7.1 Hz, H_{arom}), 7.75 (d, 2H, *J* = 7.6 Hz, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 (C_q *t*-Bu), 26.8 (3 x CH_3 TBDPS), 46.6 (CH FMOc), 63.8 (CH_2 glycerol), 66.2 (CH_2 FMOc), 68.5 (C-5), 69.9 (C-6), 70.6 (CH_2 glycerol), 72.2 (CH_2 allyl), 72.3 (CH_2 Bn), 75.0 (CH_2 Bn), 75.7 (CH_2 Bn), 75.8 (CH glycerol), 77.1 (C-4), 79.5 (C-2), 81.7 (C-3), 95.7 (C-1), 116.9 (CH_2 allyl), 125.1, 125.2 (CH_{arom}), 127.1 - 128.6 (CH_{arom}), 129.7 (CH_{arom}), 133.1, 133.2 (C_q phenyl), 134.6 (CH

allyl), 135.5 (CH_{arom}), 138.1, 138.2, 138.7, 138.8 (3 x C_q Bn), 141.2, 141.2 (2 x C_q FMOc), 143.2, 143.4 (2 x C_q FMOc), 155.0 (C=O FMOc); HRMS: $C_{64}H_{68}O_{10}Si + NH_4^+$ requires 1042.4920, found 1042.4933.



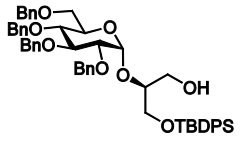
3-*O*-Allyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (15)

To a solution of compound **14b** (3.40 g, 3.32 mmol) in DCM (65 ml) was added DBU (165 µl, 1.10 mmol). After stirring for 15 min the solvent was removed under reduced pressure and the residue purified by silica gel column chromatography (EtOAc/PE) giving alcohol **15** (2.50 g, 3.11 mmol, 94%) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +33.6; IR: 737, 1026, 1072, 1454, 2928; ^{1}H NMR (400 MHz): δ = 1.03 (s, 9H, *t*-Bu TBDPS), 3.48 - 3.80 (m, 9H, H-2, H-4, H-5, H-6, H-6', 2 x CH_2 glycerol), 3.97 - 4.04 (m, 4H, H-3, CH glycerol, CH_2 allyl), 4.61 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.68 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.76 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.78 (d, 1H, *J* = 10.4 Hz, CH*H* Bn), 4.86 (d, 1H, *J* = 11.2 Hz, CH*H* Bn), 4.97 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 5.16 (dd, 1H, *J* = 1.6 Hz, 10.4 Hz, CH*H* allyl), 5,24 - 5.28 (m, 2H, H-1, CH*H* allyl), 5.88 (ddd, 1H, *J* = 5.5 Hz, 10.7 Hz, 22.6 Hz, CH allyl), 7.24 - 7.42 (m, 21H, H_{arom}), 7.64 - 7.67 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-Bu), 26.8 (3 x CH_3 TBDPS), 61.4 (C-6), 63.8 (CH_2 glycerol), 70.7 (C-5), 70.9 (CH_2 glycerol), 72.2 (CH_2 allyl), 72.3 (CH_2 Bn), 74.9 (CH_2 Bn), 75.6 (CH_2 Bn), 76.0 (CH glycerol), 77.1 (C-4), 79.6 (C-2), 81.6 (C-3), 96.0 (C-1), 116.9 (CH_2 allyl), 127.5 - 128.4 (CH_{arom}), 129.7 (CH_{arom}), 133.1, 133.2 (C_q phenyl), 134.6 (CH allyl), 135.5 (CH_{arom}), 138.2, 138.3, 138.8 (3 x C_q Bn); HRMS: $C_{49}H_{58}O_8Si + Na^+$ requires 825.3793, found 825.3784.



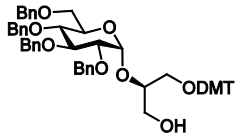
3-*O*-Allyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (16)

A solution of alcohol **15** (2.521 g, 3.14 mmol) together with BnBr (0.94 ml, 7.85 mmol) in DMF (20 ml) was stirred for 5 minutes at 0 °C, after which NaH (60% dispersion in mineral oil, 0.314 g, 7.85 mmol) was added. The resulting mixture was stirred for 75 min and allowed to slowly warm up to RT, before MeOH (5.0 ml) was added. After stirring for 15 min, H_2O (30 ml) was added and the mixture extracted with Et_2O (50 ml). The organic layer was washed twice with H_2O (20 ml) and once with brine (20 ml) before it was dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residual oil by silica gel column chromatography (EtOAc/PE) furnished perbenzylglucosyl glycerol derivative **16** (2.429 g, 2.72 mmol, 87%) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +31.8; IR: 737, 1026, 1069, 1454, 2928; ^{1}H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.36 (dd, 1H, *J* = 1.7 Hz, 10.6 Hz, H-6), 3.54 - 3.84 (m, 8H, H-2, H-4, H-5, H-6', 2 x CH_2 glyc), 3.95 - 4.00 (m, 3H, CH_2 allyl, H-3), 4.06 (m, 1H, CH glycerol), 4.36 (d, 1H, *J* = 12.4 Hz, CH*H* Bn), 4.43 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 4.55 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.69 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.74 - 4.82 (m, 3H, CH_2 Bn, CH*H* Bn), 4.97 (d, 1H, *J* = 10.8 Hz, CH*H* Bn), 5.15 (dd, 1H, *J* = 1.4 Hz, 10.6 Hz, CH*H* allyl), 5.24 - 5.29 (m, 2H, CH*H* allyl, H-1), 5.88 (ddd, 1H, *J* = 5.5 Hz, 10.7 Hz, 22.7 Hz, CH allyl), 7.08 - 7.11 (m, 2H, H_{arom}), 7.20 - 7.38 (m, 24H, H_{arom}), 7.64 - 7.67 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-Bu), 26.8 (3 x CH_3 TBDPS), 63.8 (CH_2 glycerol), 68.0 (C-6), 70.1 (C-5), 70.5 (CH_2 glycerol), 72.2 (CH_2 allyl), 72.2 (CH_2 Bn), 73.3 (CH_2 Bn), 74.8 (CH_2 Bn), 75.5 (CH_2 Bn), 76.0 (CH glycerol), 77.4 (C-4), 79.5 (C-2), 81.8 (C-3), 96.1 (C-1), 116.7 (CH_2 allyl), 127.4 - 128.2 (CH_{arom}), 129.6 (CH_{arom}), 133.1, 133.3 (C_q phenyl), 134.6 (CH allyl), 135.5 (CH_{arom}), 137.9, 138.3, 138.4, 138.8 (4 x C_q Bn); HRMS: $C_{56}H_{64}O_8Si + NH_4^+$ requires 910.4709, found 910.4718.



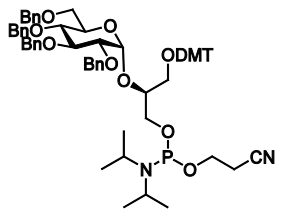
2-*O*-(2,3,4,6-tetra-*O*-Benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (17)

A solution of glycoside **16** (2.37 g, 2.65 mmol) in freshly distilled THF (18 ml) was stirred under argon for 30 min. After the addition of $Ir(COD)(Ph_2MeP)_2PF_6$ (112 mg, 0.133 mmol) the solution was purged with H_2 (g) for ~15s. After stirring under argon for 2 hrs, the mixture was diluted with THF (20 ml) and sat. aq. $NaHCO_3$ (20 ml). Upon addition of I_2 (1.01 g, 3.98 mmol), the mixture was allowed to stir for 1.5 hrs at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, sat. aq. NaS_2O_3 (30 ml) and brine (40 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (EtOAc/PE) afforded **17** (1.97 g, 2.31 mmol, 87%) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +21.4; IR: 737, 1026, 1069, 1454, 1724, 2928, 3449; ^{1}H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.11 (bs, 1H, CH_2OH), 3.32 (dd, 1H, *J* = 1.5 Hz, 10.6 Hz, H-6), 3.54 - 3.57 (m, 2H, H-2, H-6'), 3.62 - 3.70 (m, 3H, H-4, 2 x CH glycerol), 3.77 - 3.86 (m, 4H, H-5, CH glycerol, 2 x CH glycerol), 3.97 (t, 1H, *J* = 9.3 Hz, H-3), 4.33 (d, 1H, *J* = 12.2 Hz, CH Bn), 4.44 (d, 1H, *J* = 10.9 Hz, CH Bn), 4.52 (d, 1H, *J* = 12.2 Hz, CH Bn), 4.66 (d, 1H, *J* = 11.6 Hz, CH Bn), 4.77 - 4.85 (m, 3H, CH_2 Bn, CH Bn), 4.90 - 4.93 (m, 2H, H-1, CH Bn), 7.08 - 7.12 (m, 2H, H_{arom}), 7.20 - 7.39 (m, 24H, H_{arom}), 7.62 - 7.64 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.0 (C_q *t*-Bu), 26.7 (3 x CH_3 TBDPS), 62.6 (CH_2 glycerol), 63.7 (CH_2 glycerol), 67.8 (C-6), 70.4 (C-5), 73.3, 73.8, 74.7, 75.5 (4 x CH_2 Bn), 77.4 (C-4), 79.4 (C-2), 80.8 (CH glycerol), 82.0 (C-3), 98.4 (C-1), 127.4 - 128.4 (CH_{arom}), 129.6 (CH_{arom}), 132.9, 133.0 (C_q phenyl), 135.4 (CH_{arom}), 137.4, 137.6, 138.1, 138.5 (C_q Bn); HRMS: $C_{53}H_{60}O_8Si + Na^+$ requires 875.3950, found 875.3946.



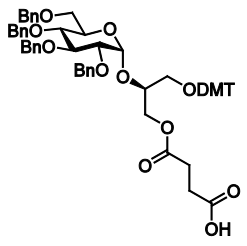
2-*O*-(2,3,4,6-tetra-*O*-Benzyl-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (18)

To a cooled (0 °C) solution of alcohol **17** (1.85 g, 2.17 mmol) and Et_3N (0.45 ml, 3.3 mmol) in DCM (11 ml) was added DMTr-Cl (881 mg, 2.60 mmol). The mixture was stirred for 2.5 hrs before MeOH (1.0 ml) was added. After stirring for an additional 15 minutes the reaction mixture was diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (30 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residual oil was redissolved in THF (15ml) and, subsequently, TBAF (1M solution in THF, 7.8 ml) was added. The mixture was stirred for 3 hrs after which the volatiles were removed *in vacuo* and the residual oil was purified by silica gel column chromatography (EtOAc/PE/Et_3N) yielding mono-alcohol **18** (1.77 g, 1.93 mmol, 89%) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +40.6; IR: 737, 1030, 1065, 1250, 1508, 1609, 2927, 3487; ^{1}H NMR (400 MHz): δ = 2.85 (at, 1H, *J* = 5.0 Hz, CH_2OH), 3.21 (dd, 1H, *J* = 6.1 Hz, 9.6 Hz, CH glycerol), 3.35 (dd, 1H, *J* = 5.5 Hz, 9.6 Hz, CH glycerol), 3.53 - 3.57 (m, 2H, H-2, H-4), 3.61 - 3.67 (m, 3H, H-6, H-6', CH glycerol), 3.71 (s, 6H, 2 x OMe), 3.76 - 3.82 (m, 1H, CH glycerol), 3.86 (m, 1H, CH glycerol), 3.96 - 4.04 (m, 2H, H-3, H-5), 4.46 (d, 1H, *J* = 10.8 Hz, CH Bn), 4.47 (d, 1H, *J* = 12.4 Hz, CH Bn), 4.58 (d, 1H, *J* = 11.6 Hz, CH Bn), 4.63 (d, 1H, *J* = 12.0 Hz, CH Bn), 4.80 (d, 1H, *J* = 10.4 Hz, CH Bn), 4.82 (d, 1H, *J* = 10.4 Hz, CH Bn), 4.96 (d, 1H, *J* = 10.8 Hz, CH Bn), 4.99 (d, 1H, *J* = 3.6 Hz, H-1), 6.78 - 6.81 (m, 4H, H_{arom}), 7.11 - 7.13 (m, 2H, H_{arom}), 7.18 - 7.36 (m, 25H, H_{arom}), 7.46 (d, 2H, *J* = 7.4 Hz, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.0 (2 x OMe), 63.4 (CH_2 glycerol), 63.9 (CH_2 glycerol), 68.5 (C-6), 70.5 (C-5), 72.6, 73.4, 75.0, 75.6 (4 x CH_2 Bn), 77.7 (C-4), 79.5 (C-2), 80.0 (CH glycerol), 81.8 (C-3), 86.3 (C_q DMTr), 96.8 (C-1), 113.0 (CH_{arom}), 126.7 - 129.0 (CH_{arom}), 130.0 (CH_{arom}), 135.8, 137.5, 137.9, 138.0, 138.6, 144.7, 158.4 (4 x C_q Bn, 5 x C_q DMTr); HRMS: $C_{58}H_{60}O_{10} + Na^+$ requires 939.4079, found 939.4090.



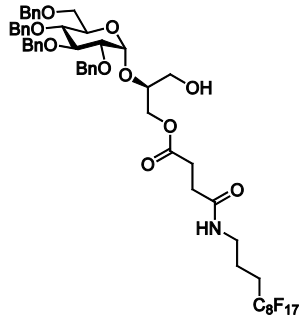
1-*O*-([*N,N*-diisopropyl]-2-cyanoethyl-phosphoramidite)-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (19)

To a cooled (0 °C) solution of alcohol **18** (801 mg, 0.873 mmol) and Et_3N (0.19 ml, 1.4 mmol) in DCM (6.0 ml) was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (258 mg, 1.09 mmol). After stirring 30 min, the reaction was quenched by the addition of H_2O (1.0 ml), diluted with DCM (20 ml) and washed with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (20 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et_3N) gave phosphoramidite **19** (722 mg, 0.646 mmol, 74%) as a colourless oil. IR: 1030, 1250, 1508, 1605, 2928; ^{31}P NMR (161.7 MHz, CD_3CN): δ = 149.0, 149.4 (diastereoisomers); ^1H NMR (400 MHz, CD_3CN, mixture of diastereoisomers): δ = 1.04 - 1.12 (m, 12H, 4 x CH_3 isopropylamino), 2.40 - 2.42 (m, 2H, CH_2 cyanoethyl), 3.16 - 3.25 (m, 2H, CH_2 glycerol), 3.44 - 3.55 (m, 4H, H-2, H-4, 2 x CH isopropylamino), 3.60 - 3.88 (m, 13H, H-3, H-6, H-6', 2 x OMe, CH_2 glycerol, CH_2 cyanoethyl), 3.91 - 4.02 (m, 2H, H-5, CH glycerol), 4.48 - 4.61 (m, 5H, CH_2 Bn), 4.72 - 4.80 (m, 2H, C_2 Bn), 4.86 - 4.90 (m, 1H, CH*H* Bn), 5.15 - 5.18 (m, 1H, H-1), 6.80 (d, 4H, *J* = 8.9 Hz, H_{arom}), 7.12 - 7.36 (m, 27H, H_{arom}), 7.44 - 7.47 (m, 2H, H_{arom}); ^{13}C NMR (100 MHz): δ = 24.9 - 25.1 (4 x CH_3 isopropylamino), 43.7 - 43.8 (2 x CH isopropylamino), 55.8 (2 x OMe), 59.3 - 59.6 (CH_2 glycerol), 64.3 - 64.4 (CH_2 glycerol, CH_2 cyanoethyl), 69.9 (C-6), 71.4 - 71.5 (C-5), 72.8 - 72.9, 73.9, 75.4 - 75.5, 76.0 (4 x CH_2 Bn), 77.5 - 77.7 (CH glycerol), 78.8 (C-4), 81.0 (C-2), 82.5 (C-3), 87.2 (C_q DMTr), 97.1 - 97.3 (C-1), 114.0 (CH_{arom}), 127.7 - 129.3 (CH_{arom}), 131.0 (CH_{arom}), 136.9, 139.5, 139.5, 139.7, 139.8, 140.1, 146.1, 159.6 (4 x C_q Bn, 5 x C_q DMTr, C_q cyanoethyl); HRMS: $C_{67}H_{77}N_2O_{11}P$ + H^+ requires 1117.5338, found 1117.5337.



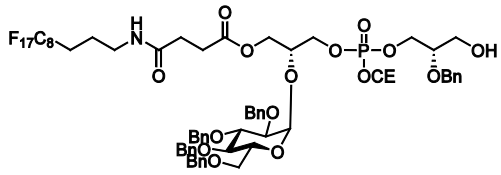
2-*O*-(2,3,4,6-tetra-*O*-Benzyl-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-1-*O*-succinyl-*sn*-glycerol (20)

To a cooled (0 °C) solution of alcohol **18** (914 mg, 0.997 mmol) and Et_3N (1.52 ml, 11.0 mmol) in DCM (10 ml) was added succinic anhydride (498 mg, 4.98 mmol). After stirring for 1 h the mixture was concentrated under reduced pressure, after which column chromatography (EtOAc/PE/Et_3N) gave succinyl ester **20** (966 mg, 0.963 mmol, 96%) as a pale yellow oil. $[\alpha]_D^{20}$ ($CHCl_3$): +36.6; IR: 1030, 1153, 1246, 1508, 1609, 1713, 1736, 2928; ^1H NMR (400 MHz, CD_3CN): δ = 2.49 (s, 4H, 2 x CH_2 succinyl), 3.22 - 3.31 (m, 2H, CH_2 glycerol), 3.50 (dd, 1H, *J* = 3.5 Hz, 9.7 Hz, H-2), 3.57 (at, 1H, *J* = 9.5 Hz, H-4), 3.68 - 3.77 (m, 8H, H-6, H-6', 2 x OMe), 3.90 (at, 1H, *J* = 9.3 Hz, H-3), 3.93 - 3.98 (m, 1H, H-5), 4.01 - 4.08 (m, 1H, CH glycerol), 4.23 - 4.32 (m, 2H, CH_2 glycerol), 4.49 - 4.61 (m, 5H, CH*H* Bn, 2 x CH_2 Bn), 4.78 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.83 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 4.91 (d, 1H, *J* = 11.1 Hz, CH*H* Bn), 5.12 (d, 1H, *J* = 3.5 Hz, H-1), 6.84 (d, 4H, *J* = 8.9 Hz, H_{arom}), 7.16 - 7.39 (m, 27H, H_{arom}), 7.48 (d, 2H, *J* = 7.4 Hz, H_{arom}); ^{13}C NMR (100 MHz, CD_3CN): δ = 29.8, 30.3 (2 x CH_2 succinyl), 56.5 (2 x OMe), 64.1 (CH_2 glycerol), 65.9 (CH_2 glycerol), 70.5 (C-6), 72.2 (C-5), 73.7, 74.5, 76.1 (3 x CH_2 Bn), 76.2 (CH glycerol), 76.6 (CH_2 Bn), 79.4 (C-4), 81.5 (C-2), 82.9 (C-3), 87.9 (C_q DMTr), 97.6 (C-1), 114.7 (CH_{arom}), 128.4 - 129.9 (CH_{arom}), 131.6 (CH_{arom}), 137.3, 137.4, 139.9, 140.0, 140.2, 140.7, 146.6, 160.2 (5 x C_q DMTr, 4 x C_q Bn), 173.5, 174.9 (2 x C=O succinyl) ; HRMS: $C_{62}H_{64}O_{13}$ + Na^+ requires 1039.4239, found 1039.4237.



2-*O*-(2,3,4,6-tetra-*O*-Benzyl-α-D-glucopyranosyl)-1-*O*-(*N*-[3-perfluorooctylpropyl]-succinamidyl-*sn*-glycerol (21)

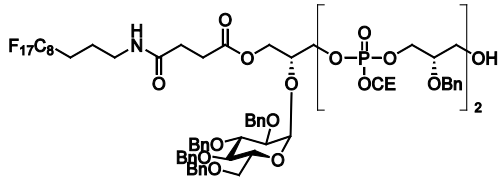
To a solution of compound **20** (351 mg, 0.350 mmol), perfluorooctylpropylamine (119 mg, 0.250 mmol) and *N,N*-diisopropylethylamine (0.366 ml, 2.10 mmol) in a 2/1 mixture of DCM/DMF (5.0 ml) was added BOP (310 mg, 0.700 mmol). The mixture was stirred for 1.5 h before it was diluted with EtOAc (100 ml) and, subsequently, washed with sat. aq. $NaHCO_3$ (2 x 50 ml), H_2O (2 x 50 ml) and brine (50 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure, after which the residue was taken up in DCM (5.0 ml). After the addition of, respectively, triethylsilane (0.605 ml, 3.75 mmol) and dichloroacetic acid (0.308 ml, 3.75 mmol) the mixture was stirred 30 min and, subsequently, diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq. $NaHCO_3$ and brine (20 ml). The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*, after which the residue was partitioned between 80/20 acetonitrile/water and hexane and purified by FSPE as described in the general procedure. Fluorous compound **21** (263 mg, 0.224 mmol, 90%) was isolated as an amorphous solid. $[\alpha]_D^{20}$ ($CHCl_3$): +23.8; IR: 1026, 1065, 1146, 1200, 1547, 1644, 1736, 2924; ^{1}H NMR (400 MHz): δ = 1.71 - 1.79 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.99 - 2.13 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.38 (t, 2H, *J* = 6.7 Hz, CH_2 succinyl), 2.63 (t, 2H, *J* = 6.7 Hz, CH_2 succinyl), 3.24 (dd, 2H, *J* = 6.8 Hz, 13.3 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.75 (m, 6H, H-2, H-4, H-6, H-6', CH_2 glycerol), 3.83 - 3.89 (m, 1H, CH glycerol), 3.91 - 3.96 (m, 1H, H-5), 4.00 (at, 1H, *J* = 9.4 Hz, H-3), 4.13 - 4.16 (m, 2H, CH_2 glycerol), 4.46 - 4.50 (m, 2H, 2 x CH*H* Bn), 4.59 (d, 1H, *J* = 12.1 Hz, CH*H* Bn), 4.67 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.80 - 4.90 (m, 4H, H-1, 3 x CH*H* Bn), 4.95 (d, 1H, *J* = 11.0 Hz, CH*H* Bn), 5.85 (t, 1H, *J* = 5.9 Hz, NH), 7.12 - 7.15 (m, 2H, H_{arom}), 7.24 - 7.37 (m, 18H, H_{arom}); ^{13}C NMR (100 MHz): δ = 20.8 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 28.3 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.7 (2 x CH_2 succinyl), 38.5 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 61.8 ($CH_2$ glycerol), 64.1 (CH_2 glycerol), 68.4 (C-6), 70.9 (C-5), 73.5, 74.2, 75.1, 75.6 (4 x CH_2 Bn), 77.7 (C-4), 78.8 (CH glycerol), 79.5 (C-2), 82.1 (C-3), 98.6 (C-1), 127.6 - 128.6 (CH_{arom}), 137.4, 137.7, 138.0, 138.5 (4 x C_q Bn), 171.5, 172.6 (2 x C=O succinyl); HRMS: $C_{52}H_{52}F_{17}NO_{10}$ + Na^+ requires 1196.3212, found 1196.3210.



Glucosyl glycerol phosphate dimer (22)

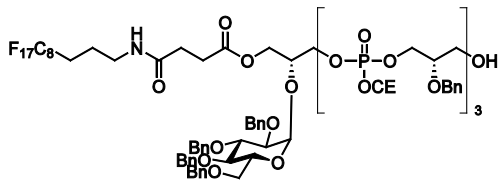
Monomer **21** (133 mg, 113 μmol) was coupled to glycerol phosphoramidite **6** (1.5 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Dimer **22** (153 mg, 104 μmol, 92%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -0.9, -0.9 (1P); ^{1}H NMR (400 MHz): δ = 1.70 - 1.78 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.99 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.26 - 2.67 (m, 7H, 2 x CH_2 succinyl, CH_2 cyanoethyl, CH_2OH), 3.22 (dd, 2H, *J* = 6.7 Hz, 13.0 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.73 (m, 6H, H-4, H-6, H-6', CH glycerol, CH_2 glycerol), 3.84 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.31 (m, 9H, CH glycerol, 3 x CH_2 glycerol, CH_2 cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CH*H* Bn), 4.56 - 4.73 (m, 5H, CH*H* Bn, 2 x CH_2 Bn), 4.78 - 4.83 (m, 2H, 2 x CH*H* Bn), 4.92 - 4.96 (m, 1H, CH*H* Bn), 5.01 - 5.03 (m, 1H, H-1), 6.01 - 6.05 (m,1H, NH), 7.12 - 7.14 (m, 2H, H_{arom}), 7.25 - 7.37 (m, 23H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.3 - 19.4 (CH_2 cyanoethyl), 20.7 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 28.3 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.4, 30.7 (2 x CH_2 succinyl), 38.5 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 60.5 (d, *J* = 5 Hz, CH_2 glycerol), 62.0 (d, *J* = 5 Hz, CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 66.1 - 66.6 (2 x CH_2 glycerol), 68.3 (C-6), 70.9 (C-5), 72.0, 72.0 (CH_2 Bn), 73.1, 73.2 (CH_2 Bn), 73.5 (CH_2 Bn), 73.9 - 74.1 (CH glycerol), 75.1, 75.5 (2 x CH_2 Bn), 77.4 - 77.6 (C-4, CH glycerol), 79.6, 79.7 (C-2),

81.5 (C-3), 96.9, 97.1 (C-1), 116.4, 116.5 (C_q cyanoethyl), 127.6 - 128.5 (CH_{arom}), 137.6 - 137.7, 137.9, 138.6 (5 x C_q Bn), 171.5, 172.3 (2 x C=O succinyl); HRMS: $C_{65}H_{68}F_{17}N_2O_{15}P$ + Na^+ requires 1493.3978, found 1493.3978.



Glucosyl glycerol phosphate trimer (23)

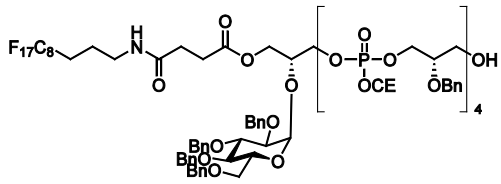
Dimer **22** (149 mg, 101 μmol) was coupled to glycerol phosphoramidite **6** (1.5 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Trimer **23** (161 mg, 91.0 μmol, 90%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4, -1.3, -1.3, -1.3 (1P), -1.0, -0.9 (1P); ^1H NMR (400 MHz): δ = 1.69 - 1.77 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.98 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.17 - 2.69 (m, 9H, 2 x CH_2 succinyl, 2 x CH_2 cyanoethyl, CH_2OH), 3.18 - 3.26 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.55 - 3.60 (m, 1H, H-2), 3.61 - 3.74 (m, 6H, H-4, H-6, H-6', CH glycerol, CH_2 glycerol), 3.76 - 3.82 (m, 1H, CH glycerol), 3.86 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.31 (m, 15H, CH glycerol, 5 x CH_2 glycerol, 2 x CH_2 cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CH*H* Bn), 4.56 - 4.65 (m, 5H, CH*H* Bn, 2 x CH_2 Bn), 4.69 - 4.72 (m, 2H, 2 x CH*H* Bn), 4.77 - 4.83 (m, 2H, 2 x CH*H* Bn), 4.91 - 4.95 (m, 1H, CH*H* Bn), 5.01 - 5.04 (m, 1H, H-1), 6.16 - 6.22 (m,1H, NH), 7.12 - 7.15 (m, 2H, H_{arom}), 7.25 - 7.37 (m, 28H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 - 19.4 (2 x CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 28.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.6 (2 x CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 60.4, 60.5 ($CH_2$ glycerol), 62.0 - 62.2 (2 x CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5 - 66.7 (4 x CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 72.0 (CH_2 Bn), 72.1, 72.2 (CH_2 Bn), 73.0, 73.1 (CH_2 Bn), 73.4 (CH_2 Bn), 73.8 - 74.1 (CH glycerol), 75.1 (CH_2 Bn), 75.2 - 75.4 (CH glycerol), 75.5 (CH_2 Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.9, 97.0 (C-1), 116.5 - 116.6 (2 x C_q cyanoethyl), 127.5 - 128.5 (CH_{arom}), 137.1, 137.7 - 138.0, 138.5 (6 x C_q Bn), 171.5, 172.3 (2 x C=O succinyl); HRMS: $C_{78}H_{84}F_{17}N_3O_{20}P_2$ + Na^+ requires 1790.4744, found 1790.4744.



Glucosyl glycerol phosphate tetramer (24)

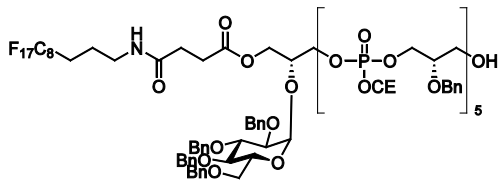
Trimer **23** (157 mg, 88.7 μmol) was coupled to glycerol phosphoramidite **6** (1.5 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Tetramer **23** (169 mg, 82.0 μmol, 92%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.2 (2P), -0.9, -0.9, -0.9 (1P); ^1H NMR (400 MHz): δ = 1.68 - 1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.98 - 2.19 (m, 3H, $F_{17}C_8CH_2CH_2CH_2N$, CH_2OH), 2.35 - 2.67 (m, 10H, 2 x CH_2 succinyl, 3 x CH_2 cyanoethyl), 3.18 - 3.25 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.75 (m, 6H, H-4, H-6, H-6', CH glycerol, CH_2 glycerol), 3.76 - 3.83 (m, 2H, 2 x CH glycerol), 3.85 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.30 (m, 21H, CH glycerol, 7 x CH_2 glycerol, 3 x CH_2 cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CH*H* Bn), 4.56 - 4.66 (m, 7H, CH*H* Bn, 3 x CH_2 Bn), 4.69 - 4.72 (m, 2H, 2 x CH*H* Bn), 4.76 - 4.83 (m, 2H, 2 x CH*H* Bn), 4.91 - 4.95 (m, 1H, CH*H* Bn), 5.01 - 5.04 (m, 1H, H-1), 6.12 - 6.19 (m,1H, NH), 7.10 - 7.15 (m, 2H, H_{arom}), 7.23 - 7.38 (m, 33H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 - 19.5 (3 x CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 28.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.6 (2 x CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 60.4, 60.5 ($CH_2$ glycerol), 62.0 - 62.2 (3 x CH_2 cyanoethyl), 63.1 (CH_2 glycerol), 65.5 - 66.6 (6 x CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 72.0 (CH_2 Bn), 72.1 - 72.2 (2 x CH_2 Bn), 73.0, 73.1 (CH_2 Bn), 73.4 (CH_2 Bn), 73.7 - 74.2 (CH glycerol), 75.1 (CH_2 Bn), 75.2 - 75.4 (2 x CH glycerol), 75.5 (CH_2 Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.6 (C-3), 96.8, 97.0 (C-1), 116.6 - 116.7 (3 x C_q cyanoethyl), 127.6 - 128.5 (CH_{arom}), 137.2, 137.7 - 138.0,

138.5 (7 x C_q Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: $[C_{91}H_{100}F_{17}N_4O_{25}P_3 + 2Na]^{2+}$ requires 1055.2701, found 1055.2705.



Glucosyl glycerol phosphate pentamer (25)

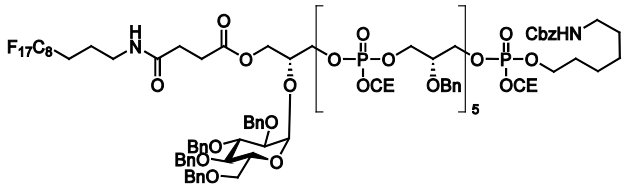
Tetramer **24** (165 mg, 79.9 μmol) was coupled to glycerol phosphoramidite **6** (1.8 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Pentamer **25** (176 mg, 74.3 μmol, 93%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.1 (3P), -0.9, -0.9, -0.9 (1P); ^{1}H NMR (400 MHz): δ = 1.68 - 1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.98 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.26 - 2.67 (m, 13H, 2 x CH_2 succinyl, 4 x CH_2 cyanoethyl, CH_2OH), 3.18 - 3.26 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.75 (m, 6H, H-4, H-6, H-6', CH glycerol, CH_2 glycerol), 3.76 - 3.83 (m, 3H, 3 x CH glycerol), 3.86 - 3.95 (m, 2H, H-3, H-5), 4.00 - 4.30 (m, 27H, CH glycerol, 9 x CH_2 glycerol, 4 x CH_2 cyanoethyl), 4.43 - 4.49 (m, 2H, 2 x CH*H* Bn), 4.56 - 4.67 (m, 9H, CH*H* Bn, 4 x CH_2 Bn), 4.69 - 4.71 (m, 2H, 2 x CH*H* Bn), 4.76 - 4.83 (m, 2H, 2 x CH*H* Bn), 4.91 - 4.95 (m, 1H, CH*H* Bn), 5.01 - 5.04 (m, 1H, H-1), 6.14 - 6.20 (m,1H, NH), 7.10 - 7.15 (m, 2H, H_{arom}), 7.23 - 7.39 (m, 38H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.3 - 19.4 (4 x CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 28.2 (t, *J* = 23 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.6 (2 x CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 60.4, 60.5 ($CH_2$ glycerol), 62.0 - 62.2 (4 x CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5 - 66.6 (8 x CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH_2 Bn), 72.1 - 72.2 (3 x CH_2 Bn), 73.0, 73.1 (CH_2 Bn), 73.4 (CH_2 Bn), 73.7 - 74.0 (CH glycerol), 75.1 (CH_2 Bn), 75.1 - 75.4 (3 x CH glycerol), 75.5 (CH_2 Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.6 - 116.7 (4 x C_q cyanoethyl), 127.5 - 128.4 (CH_{arom}), 137.2, 137.7 - 138.0, 138.5 (8 x C_q Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: $[C_{104}H_{116}F_{17}N_5O_{30}P_4 + 2Na]^{2+}$ requires 1204.3101, found 1204.3100.



Glucosyl glycerol phosphate hexamer (26)

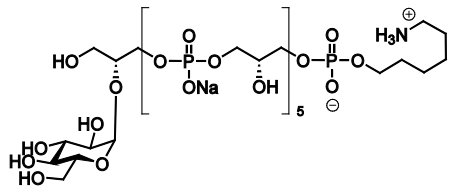
Pentamer **25** (149 mg, 63.1 μmol) was coupled to glycerol phosphoramidite **6** (2.0 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Hexamer **26** (151 mg, 56.8 μmol, 90%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.1 (4P), -0.9, -0.9, -0.9 (1P); ^{1}H NMR (400 MHz): δ = 1.68 - 1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.98 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.31 - 2.67 (m, 15H, 2 x CH_2 succinyl, 5 x CH_2 cyanoethyl, CH_2OH), 3.17 - 3.25 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.59 (m, 1H, H-2), 3.61 - 3.74 (m, 6H, H-4, H-6, H-6', CH glycerol, CH_2 glycerol), 3.76 - 3.84 (m, 4H, 4 x CH glycerol), 3.85 - 3.94 (m, 2H, H-3, H-5), 4.00 - 4.31 (m, 33H, CH glycerol, 11 x CH_2 glycerol, 5 x CH_2 cyanoethyl), 4.44 - 4.48 (m, 2H, 2 x CH*H* Bn), 4.57 - 4.66 (m, 11H, CH*H* Bn, 5 x CH_2 Bn), 4.68 - 4.71 (m, 2H, 2 x CH*H* Bn), 4.77 - 4.82 (m, 2H, 2 x CH*H* Bn), 4.90 - 4.95 (m, 1H, CH*H* Bn), 5.01 - 5.04 (m, 1H, H-1), 6.17 - 6.23 (m,1H, NH), 7.11 - 7.14 (m, 2H, H_{arom}), 7.23 - 7.38 (m, 43H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 - 19.4 (5 x CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 28.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.5 (2 x CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 60.4, 60.5 ($CH_2$ glycerol), 62.0 - 62.2 (5 x CH_2 cyanoethyl), 63.1 (CH_2 glycerol), 65.5 - 66.6 (10 x CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH_2 Bn), 72.1 - 72.2 (4 x CH_2 Bn), 73.0, 73.0 (CH_2 Bn), 73.4 (CH_2 Bn), 73.8 - 74.0 (CH glycerol), 75.1 (CH_2 Bn), 75.2 - 75.5 (4 x CH glycerol), 75.5 (CH_2 Bn), 77.4 - 77.6 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.6 - 116.8 (5 x C_q cyanoethyl), 127.5 - 128.5 (CH_{arom}), 137.2,

137.7 - 138.0, 138.5 (9 x C_q Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: $[C_{117}H_{132}F_{17}N_6O_{35}P_5 + 2Na]^{2+}$ requires 1352.8484, found 1352.8479.



Glucosyl glycerol phosphate hexamer spacer (27)

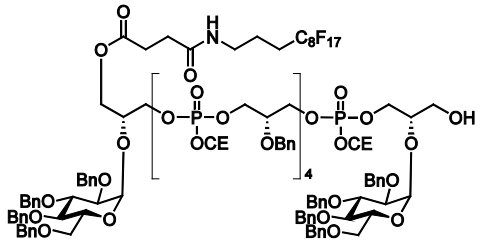
Hexamer **26** (145 mg, 54.6 μmol) was coupled to spacer phosphoramidite **10** (2.5 eq), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **27** (147 mg, 48.7 μmol, 89%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.1 (6P); ^{1}H NMR (400 MHz): δ = 1.25 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.44 - 1.52 (m, 2H, CH_2 hexylspacer), 1.60 - 1.68 (m, 2H, CH_2 hexylspacer), 1.68 - 1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.98 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.35 - 2.67 (m, 16H, 2 x CH_2 succinyl, 6 x CH_2 cyanoethyl), 3.12 - 3.25 (m, 4H, CH_2-N hexylspacer, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.59 (m, 1H, H-2), 3.61 - 3.68 (m, 2H, H-4, H-6), 3.70 - 3.75 (m, 1H, H-6'), 3.76 - 3.84 (m, 5H, 5 x CH glycerol), 3.85 - 3.94 (m, 2H, H-3, H-5), 4.00 - 4.31 (m, 39H, CH glycerol, 12 x CH_2 glycerol, 6 x CH_2 cyanoethyl, CH_2-O hexylspacer), 4.44 - 4.48 (m, 2H, 2 x CH*H* Bn), 4.57 - 4.65 (m, 11H, CH*H* Bn, 5 x CH_2 Bn), 4.68 - 4.71 (m, 2H, 2 x CH*H* Bn), 4.76 - 4.83 (m, 2H, 2 x CH*H* Bn), 4.90 - 4.95 (m, 1H, CH*H* Bn), 5.01 - 5.13 (m, 4H, H-1, CH_2 Cbz, NH Cbz), 6.11 - 6.18 (m,1H, NH), 7.11 - 7.14 (m, 2H, H_{arom}), 7.22 - 7.39 (m, 48H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.2 - 19.5 (6 x CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 24.8 ($CH_2$ hexylspacer), 25.9 (CH_2 hexylspacer), 28.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3 ($CH_2$ succinyl), 29.6 (CH_2 hexylspacer), 29.9 (d, *J* = 7 Hz, CH_2 hexylspacer), 30.5 (CH_2 succinyl), 38.3 ($F_{17}C_8CH_2CH_2CH_2CH_2N$), 40.7 ($CH_2$-N hexylspacer), 61.8 - 62.1 (6 x CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5 - 66.4 (11 x CH_2 glycerol, CH_2 Cbz), 68.3 - 68.4 (C-6, CH_2-O hexylspacer), 70.8 (C-5), 72.1 - 72.2 (6 x CH_2 Bn), 72.9, 73.0 (CH_2 Bn), 73.4 (CH_2 Bn), 73.7 - 74.0 (CH glycerol), 75.0 (CH_2 Bn), 75.3 - 75.5 (5 x CH glycerol), 77.4 (C-4), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.5 - 116.7 (6 x C_q cyanoethyl), 127.5 - 128.5 (CH_{arom}), 136.6, 137.2, 137.7 - 138.0, 138.5 (9 x C_q Bn, C_q Cbz), 156.3 (C=O Cbz), 171.3, 172.2 (2 x C=O succinyl); HRMS: $[C_{134}H_{155}F_{17}N_8O_{40}P_6 + 2Na]^{2+}$ requires 1535.9156, found 1535.9153.



Glucosyl glycerol phosphate hexamer (2)

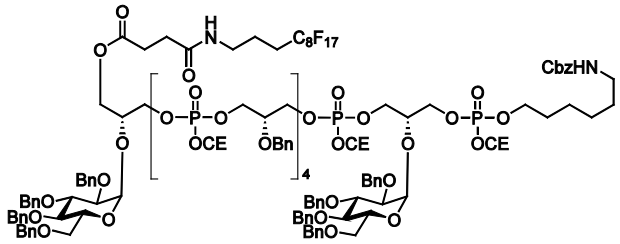
Protected hexamer **27** (139 mg, 46.3 μmol) was treated with aqueous ammonia as described above. The intermediate hexamer (98.5 mg, 44.1 μmol, 95%) was obtained as an amorphous white solid. Analytical data intermediate: ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 - 1.1 (5P), 1.2 (1P); ^{1}H NMR (400 MHz, D_2O): δ = 0.80 - 1.10 (m, 6H, 3 x CH_2 hexylspacer), 1.21 - 1.36 (m, 2H, CH_2 hexylspacer), 2.59 - 2.78 (m, 2H, CH_2-N hexylspacer), 3.24 - 4.05 (m, 38H, H-2, H-3, H-4, H-5, H-6, H-6', 12 x CH_2 glycerol, 6 x CH glycerol, CH_2-O hexylspacer), 4.16 - 5.02 (m, 22H, H-1, 9 x CH_2 Bn, CH_2 Cbz, NH Cbz), 6.66 - 7.14 (m, 50H, H_{arom}); HRMS: $[C_{101}H_{127}NO_{38}P_6 + 2NH_4]^{2+}$ requires 1092.3586, found 1092.3590. A portion of the intermediate (34.5 mg, 15.4 μmol) was deprotected with Pd (0)/H_2 using the standard procedure. Glucosylated hexamer **2** (19.7 mg, 15.0 μmol, 97%) was obtained as an amorphous white solid. ^{31}P NMR (161.7 MHz, D_2O): δ = 1.2 (1P), 1.2 - 1.3 (4P), 1.3 (1P); ^{1}H NMR (600 MHz, D_2O): δ = 1.39 - 1.44 (m, 4H, 2 x CH_2 hexylspacer), 1.61 - 1.70 (m, 4H, 2 x CH_2 hexylspacer), 2.99 (t, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.39 (at, 1H, *J* = 9.6 Hz, H-4), 3.52 (dd, 1H, *J* = 3.9 Hz, 9.9 Hz, H-2), 3.71 - 3.76 (m, 4H, H-3, H-6, CH_2 glycerol), 3.80 - 4.05 (m, 32H, H-5, H-6', 6 x CH

glycerol, 11 x CH_2 glycerol, CH_2-O hexylspacer), 4.14 - 4.17 (m, 1H, CH glycerol), 5.15 (d, 1H, *J* = 3.8 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.3, 26.0, 27.5, 30.3 (4 x CH_2 hexylspacer), 40.3 (CH_2-N hexylspacer), 61.4 (C-6), 62.2 (CH_2 glycerol), 65.2 (d, *J* = 6 Hz, CH_2 glycerol), 66.9 - 67.1 (CH_2-O hexylspacer, 11 x CH_2 glycerol), 70.3 - 70.5 (5 x CH glycerol,C-4), 72.4 (C-2), 72.9 (C-5), 73.8 (C-3), 77.7 (d, *J* = 8 Hz, CH glycerol), 98.7 (C-1); HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1948.



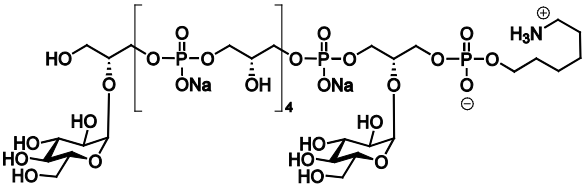
bis-Glucosyl glycerol phosphate hexamer (28)

Pentamer **25** (22.5 mg, 9.52 μmol) was coupled to glucosyl-glycerol phosphoramidite **19** (3.0 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Bis-glucosylated hexamer **28** (25.7 mg, 8.31 μmol, 87%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.3 (2P), -1.2 , -1.0 (3P); ^1H NMR (400 MHz): δ = 1.66 - 1.82 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.97 - 2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.28 - 2.68 (m, 15H, 2 x CH_2 succinyl, 5 x CH_2 cyanoethyl, CH_2OH), 3.17 - 3.28 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.51 - 3.98 (m, 19H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 5 x CH glycerol, CH_2 glycerol), 4.00 - 4.31 (m, 33H, CH glycerol, 11 x CH_2 glycerol, 5 x CH_2 cyanoethyl), 4.42 - 4.48 (m, 4H, 4 x CH*H* Bn), 4.56 - 4.64 (m, 10H, 2 x CH*H* Bn, 4 x CH_2 Bn), 4.67 - 4.71 (m, 3H, 3 x CH*H* Bn), 4.76 - 4.84 (m, 5H, 5 x CH*H* Bn), 4.90 - 4.95 (m, 3H, H-1, 2 x CH*H* Bn), 5.01 - 5.03 (m, 1H, H-1), 6.02 - 6.09 (m, 1H, NH), 7.09 - 7.16 (m, 4H, H_{arom}), 7.22 - 7.38 (m, 56H, H_{arom}); HRMS: $[C_{144}H_{160}F_{17}N_6O_{40}P_5 + 2Na]^{2+}$ requires 1568.9452, found 1568.9454.



bis-Glucosylglycerol phosphate hexamer spacer (29)

Hexamer **28** (24.5 mg, 7.92 μmol) was coupled to spacer phosphoramidite **10** (5 eq), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **29** (24.3 mg, 7.06 μmol, 89%) was obtained as a colorless oil. ^{31}P NMR (161.7 MHz): δ = -1.4 - -1.0 (6P); ^1H NMR (400 MHz): δ = 1.26 - 1.37 (m, 4H, 2 x CH_2 hexylspacer), 1.41 - 1.49 (m, 2H, CH_2 hexylspacer), 1.59 - 1.68 (m, 2H, CH_2 hexylspacer), 1.68 - 1.77 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 1.97 - 2.11 (m, 2H, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 2.30 - 2.66 (m, 16H, 2 x CH_2 succinyl, 6 x CH_2 cyanoethyl), 3.10 - 3.25 (m, 4H, CH_2-N hexylspacer, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 3.54 - 3.94 (m, 18H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 4 x CH glycerol), 4.00 - 4.30 (m, 40H, 2 x CH glycerol, 12 x CH_2 glycerol, 6 x CH_2 cyanoethyl, CH_2-O hexylspacer), 4.40 - 4.48 (m, 4H, 4 x CH*H* Bn), 4.55 - 4.64 (m, 10H, 2 x CH*H* Bn, 4 x CH_2 Bn), 4.68 - 4.71 (m, 4H, 4 x CH*H* Bn), 4.76 - 4.83 (m, 4H, 4 x CH*H* Bn), 4.90 - 4.95 (m, 2H, 2 x CH*H* Bn), 5.01 - 5.13 (m, 5H, 2 x H-1, CH_2 Cbz, NH Cbz), 6.04 - 6.11 (m,1H, NH), 7.08 - 7.15 (m, 4H, H_{arom}), 7.22 - 7.38 (m, 61H, H_{arom}); HRMS: $[C_{161}H_{183}F_{17}N_8O_{45}P_6 + 2H]^{2+}$ requires 1730.0305, found 1730.0312.



***bis*-Glucosyl glycerol phosphate hexamer (4)**

Protected hexamer **29** (22.5 mg, 6.54 μmol) was treated with aqueous ammonia as described above. The intermediate hexamer (17.1 mg, 6.42 μmol, 98%) was obtained as an amorphous white solid. Analytical data intermediate: ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 - 1.2 (6P); ^{1}H NMR (400 MHz, D_2O): δ = 0.76 - 1.10 (m, 6H, 3 x CH_2 hexylspacer), 1.21 - 1.34 (m, 2H, CH_2 hexylspacer), 2.55 - 2.76 (m, 2H, CH_2-N hexylspacer), 3.11 - 4.14 (m, 44H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 12 x CH_2 glycerol, 6 x CH glycerol, CH_2-O hexylspacer), 4.15 - 4.84 (m, 28H, 2 x H-1, 12 x CH_2 Bn, CH_2 Cbz), 4.96 - 5.04 (m, 1H, NH Cbz), 6.61 - 7.17 (m, 65H, H_{arom}); HRMS: $[C_{128}H_{155}NO_{43}P_6 + 2NH_4]^{2+}$ requires 1308.4554, found 1308.4563. A portion of the intermediate (15.6 mg, 5.85 μmol) was deprotected with Pd (0)/H_2 using the standard procedure. Bis-glucosylated hexamer **4** (8.43 mg, 5.71 μmol, 98%) was obtained as an amorphous white solid. ^{31}P NMR (161.7 MHz, D_2O): δ = 0.9 (1P), 1.2 - 1.3 (5P); ^{1}H NMR (600 MHz, D_2O): δ = 1.40 - 1.44 (m, 4H, 2 x CH_2 hexylspacer), 1.62 - 1.70 (m, 4H, 2 x CH_2 hexylspacer), 2.99 (t, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.36 - 3.41 (m, 2H, 2 x H-4), 3.48 - 3.53 (m, 2H, 2 x H-2), 3.71 - 3.77 (m, 6H, 2 x H-3, 2 x H-6, CH_2 glycerol), 3.80 - 4.05 (m, 33H, 2 x H-5, 2 x H-6', 5 x CH glycerol, 11 x CH_2 glycerol, CH_2-O hexylspacer), 4.07 - 4.10 (m, 1H, CH glycerol), 5.15 (d, 2H, *J* = 3.4 Hz, 2 x H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.5, 26.1, 27.6, 30.5 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.2 (2 x C-6), 61.7 (CH_2 glycerol), 65.3 (d, *J* = 5 Hz, CH_2 glycerol), 66.1 (d, *J* = 5 Hz, CH_2 glycerol), 67.1 - 67.3 (CH_2-O hexylspacer, 10 x CH_2 glycerol), 70.4 - 70.7 (4 x CH glycerol,2 x C-4), 72.5, 72.5 (2 x C-2), 72.9, 73.0 (2 x C-5), 73.9, 74.0 (2 x C-3), 76.4 (t, *J* = 8 Hz, CH glycerol), 77.8 (d, *J* = 8 Hz, CH glycerol), 98.7, 98.8 (2 x C-1); HRMS: $C_{36}H_{77}NO_{41}P_6$ + H^+ requires 1366.2469, found 1366.2474.

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