Carbohydrate Research 356 (2012) 142–151

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Light fluorous synthesis of glucosylated glycerol teichoic acids

W.F.J. Hogendorf [a], A. Kropec [b], D.V. Filippov [a], H.S. Overkleeft [a], J. Huebner [b], G.A. van der Marel [a,*], J.D.C. Codée [a,*]

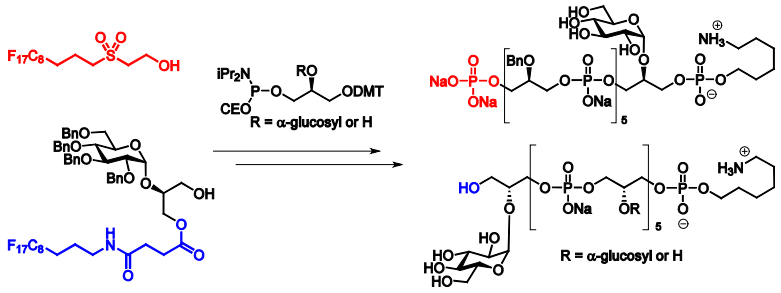
[a] Leiden Institute of Chemistry, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands
[b] Division of Infectious Diseases, Department of Medicine, University Medical Center Freiburg, Hugstetter Strasse 55, 79106 Freiburg, Germany

ARTICLE INFO

Article history:
Received 20 January 2012
Accepted 24 February 2012
Available online 6 March 2012

Keywords:
Teichoic acid
Fluorous
Glycerol
Glucose
Phosphate
Phosphoramidite

ABSTRACT



We here describe the synthesis of glucosylated teichoic acid (TA) fragments using two complementary fluorous scaffolds. The use of a perfluorooctylpropylsulfonylethyl (F-Pse) linker in combination with (glucosyl)glycerol phosphoramidite building blocks allows for the assembly of TA fragments with a terminal phosphate mono-ester, whereas the use of a perfluorooctylsuccinyl spacer delivers TA oligomers featuring a terminal alcohol functionality. These complementary linker systems have been developed because the nature of the TA chain terminus can play a role in the biological activity of the synthetic TAs. A novel α-glucosylated glycerolphosphoramidite building block is introduced to allow for a robust light fluorous synthetic protocol.

1. Introduction

The cell walls of pathogenic and non-pathogenic Gram-positive bacteria are characterized by a thick peptidoglycan layer, in which polyanionic glycopolymers are present. An important class of these cell surface polymers is comprised by the teichoic acids (TAs), which can be divided in wall teichoic acids (WTAs), covalently linked to the peptidoglycan layer and lipoteichoic acids (LTAs), which are anchored in the bacterial membrane. The repeating units of both types of TA are generally alditol (glycerol, ribitol) phosphates of which the hydroxyl functions are randomly equipped with carbohydrate or D-alanine residues. TAs perform vital functions as exemplified by their role in membrane integrity and permeability and their task as scaffold for various extracytoplasmic enzymes.[1] TAs also mediate extracellular interactions and are recognized by the mammalian adaptive and innate immune system, thus functioning as antigenic structures and immunostimulatory ligands for Toll-like receptors, respectively.[2] To establish structure–activity relationships of teichoic acids we started a programme aimed at the development of synthetic strategies to generate well-defined TA structures to help elucidate their mode of action at the molecular level.[3]

Enterococcus faecalis is a commensal, Gram-positive bacterium, responsible for many hospital related infections and represents a significant health threat especially to immunocompromised patients. The advent of strains resistant against multiple antibiotics is a strong stimulus for the development of alternative prophylactic and therapeutic strategies.[4] *E. faecalis* LTA has been shown to be a target of protective antibodies, and as such it represents a potential candidate for future vaccine development.[5] To identify potent

* Corresponding authors.
E-mail addresses: marel_g@chem.leidenuniv.nl (G.A. van der Marel), jcodee@chem.leidenuniv.nl (J.D.C. Codée).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2012.02.023

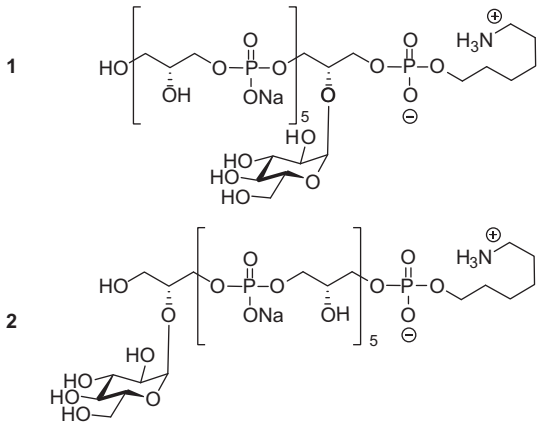


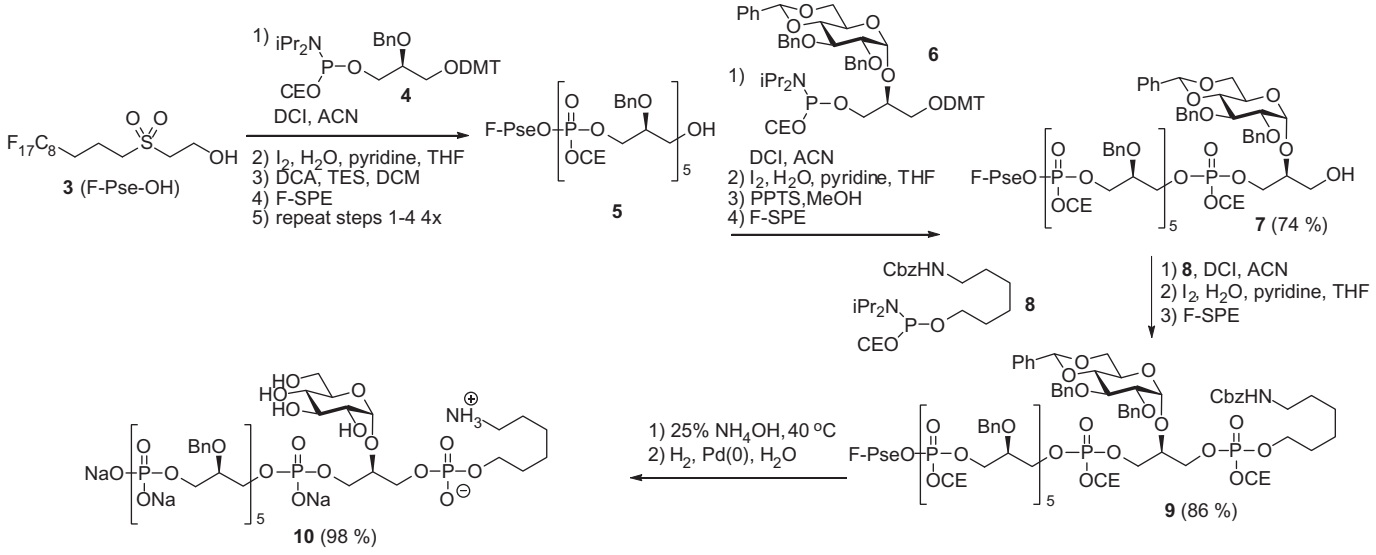
Figure 1. Lead structures **1** and **2**.

antigenic fragments of the microheterogenic *E. faecalis* TA we developed an automated solid phase synthesis approach, in which well-defined TA fragments were assembled on a commercially available DNA synthesizer using suitably protected glycerol phosphoramidite building blocks.[3b] Through this methodology we generated a small library of TA fragments, which allowed us to identify two potent antigenic TA structures in an opsonophagocytic killing inhibition assay (OPIA). In this assay compounds are screened for their ability to inhibit the complement-assisted killing of bacteria though opsonic antibodies.[5] These two compounds, **1** and **2** (Fig. 1), are characterized by the presence of an α-glucosyl substituent on either of the two terminal glycerolphosphate residues. Notably, this type of substitution is common in *Bacillus subtilis*[1c,6] and several *Staphylococcus* species[1c,7] but has not been encountered in native *E. faecalis* LTA. To further investigate these antigenic lead compounds we required an amount of these structures, larger than automated solid phase synthesis could provide us with and therefore we set out to develop a complementary synthesis strategy based on the use of 'light fluorous' chemistry.[3c,8]

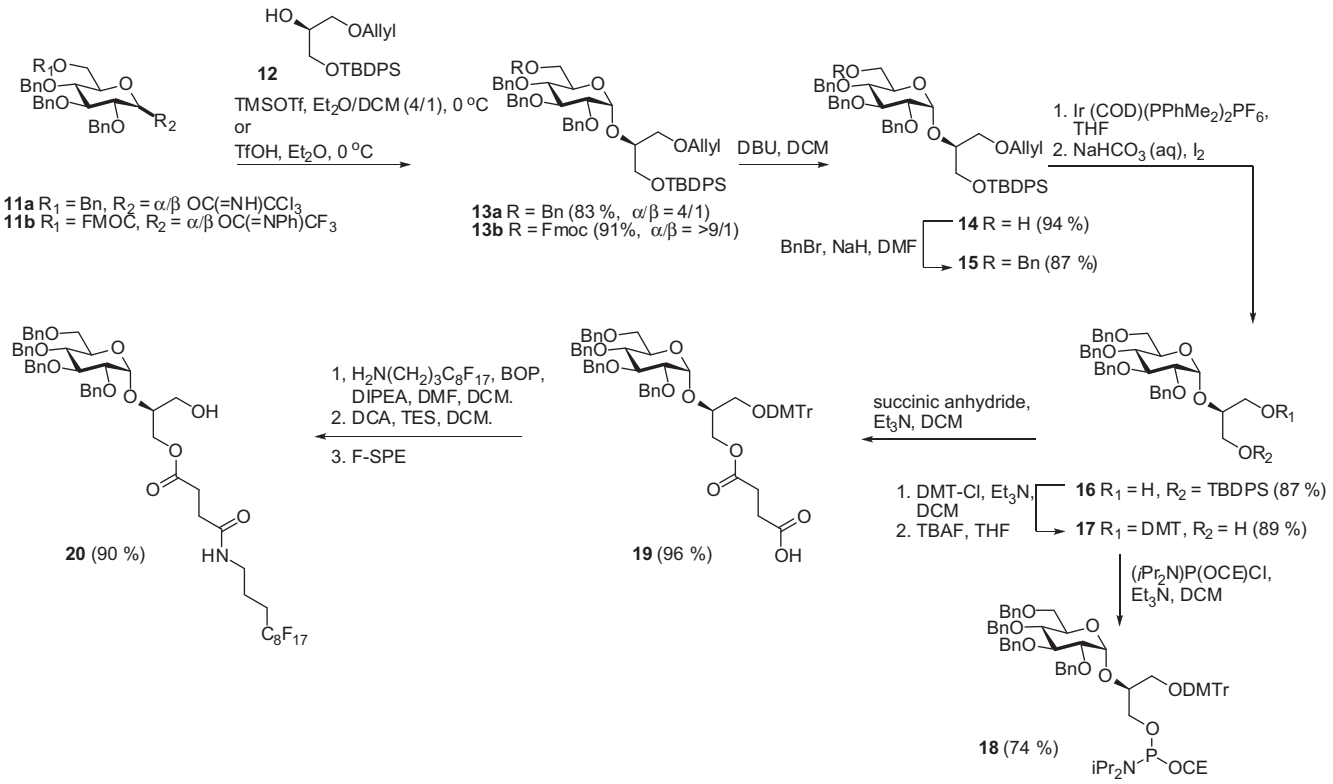
2. Results and discussion

We have recently disclosed that 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.[3c,8g] Therefore we initially explored the synthesis of hexamer **10**, a phosphorylated analogue of TA-fragment **1**, using this linker-system. Thus, F-Pse **3** was elongated in a stepwise manner (Scheme 1) with glycerol phosphoramidite **4**[3a] in a four-step elongation process, in which: (1) The phosphoramidite was coupled with the alcohol using dicyanoimidazole (DCI) as an activator in acetonitrile. (2) The resulting phosphite intermediate was oxidized using I_2 in a mixture of THF/H_2O/pyridine. (3) From the elongated fragment the 4,4′-dimethoxytrityl (DMT) ether was cleaved using dichloroacetic acid (DCA) and triethylsilane (TES) in DCM. (4) Fluorous solid phase extraction (F-SPE) was employed to separate the fluorous products from the non-fluorous side products. Before the F-SPE purification the mixture was partitioned between acetonitrile/water (80/20) and hexane to remove the bulk of TES and DMT-H to simplify the F-SPE purification, as described previously.[3c] Repeating this process four times led to pentamer **5** which was then elongated with benzylidene protected glucosylglycerol phosphoramidite **6** under the agency of DCI and oxidized. As the 4,6-*O*-benzylidene moiety is unstable towards DCA/TES, detritylation of the intermediate hexamer was effected using the milder PPTS/MeOH cocktail.[3a,9] The presence of the lipophilic 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 **7** and phosphoramidite **8** was followed by oxidation and F-SPE to give the fully protected construct **9** in 86% yield. Deprotection of hexamer **9** 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 eliminated fluorous scaffold (perfluorooctylpropylsulfonylethene) using a Et_2O/H_2O extraction. Subsequently, the benzylidene moiety, benzyl ethers and benzyl carbamate function were all removed by means of hydrogenolysis (Pd/H_2), leading to the target hexamer **10** in 98% yield. Surprisingly, in sharp contrast to its close analogue **1**, hexamer **10** proved to be inactive in the OPIA inhibition assay, indicating that the presence of the terminal phosphate is detrimental to the antigenicity of the synthetic TA fragment.[10]

To allow the light-fluorous assembly of TA fragments without a terminal phosphate moiety we set out to probe a hydroxyl



Scheme 1. Assembly of TA-fragment **10**.



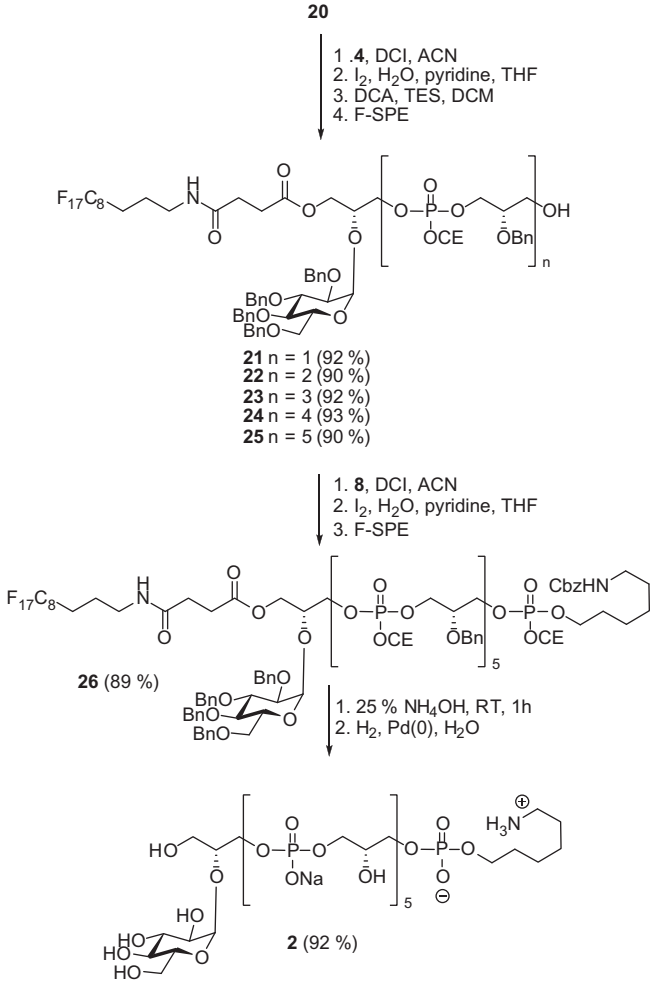
Scheme 2. Synthesis of phosphoramidite **18** and perfluorooctylpropyl succinyl linked glycosyl glycerol **20**.

protecting fluorous linker. Inspired by contemporary DNA synthesis methods, a succinyl type linker was deemed suitable because of its stability towards coupling, oxidation and detritylation conditions.[11] The base lability of a fluorous succinyl linker allows the same deprotection strategy as employed in the synthesis of hexamer **10**. At the same time we were interested in developing a more acid-stable glucosyl glycerol synthon. As described above, the benzylidene acetal is sensitive to standard detritylation conditions, necessitating the use of a carefully controlled procedure in the removal of the temporary DMT group. Introduction of the benzylidene glucosyl glycerol synthon early on in the synthesis therefore significantly affects the robustness of the assembly process. We therefore implemented tetra-*O*-benzyl glucosyl synthon **18** in our subsequent syntheses (Schemes 2 and 3). This building block was prepared as described in Scheme 2. We first explored the use of per benzylated glucosyl imidate **11a**[12] for the construction of the crucial α-glucosyl linkage. Condensation of this donor with glycerol acceptor **12** in DCM led to formation of product **13a** with poor selectivity ($\alpha/\beta = 2:1$). The use of ether as co-solvent improved the α/β-ratio, but the anomeric mixture proved to be inseparable. To circumvent this problem, we explored the use of a glucosyl donor bearing a bulky Fmoc protecting group on the C6 hydroxyl, as these are known to favour the formation of the α-product.[13] Coupling 6-*O*-Fmoc glucosyl imidate **11b** with glycerol **12** using Et_2O as a solvent, led to the formation of **13b** in high selectivity ($\alpha/\beta \sim 10/1$). The major amount of β-glucoside was removed by column chromatography, affording glucoside **13b** in 91% yield (containing <3% β-adduct, based on ^{1}H NMR analysis). Compound **13b** was then treated with DBU in DCM, and benzylation of the intermediate alcohol **14** led to tetrabenzylglucosyl derivative **15** in 87% yield. In the next step the allyl ether was removed by iridium catalysed isomerization, followed by oxidative cleavage of the intermediate enol ether, giving alcohol **16** in 87% yield. Installation of the DMT ether and desilylation led to building block **17**, which was transformed into the phosphoramidite synthon **18** using *N,N*-diisopropyl-2-cyanoethyl-chlorophosphoramidite and Et_3N. Alternatively, **17** was reacted with succinic anhydride and Et_3N in DCM, giving succinyl ester **19** in 96% yield. Subsequently, **19** was coupled to perfluorooctylpropylamine, using BOP as a coupling agent and detritylated to give the crude fluorous glucosylglycerol **20**, which was purified by F-SPE to give the pure target compound in 90% yield. This molecule was elongated in a step-wise manner with glycerol phosphoramidite **5** using the chemistry described above leading to hexamer **25**. The aminohexylspacer was then introduced to give the fully protected hexamer **26**. Deprotection by 25% aqueous ammonia (1 h, rt), was followed by hydrogenolysis to give 40 mg of target compound **2** (92%).

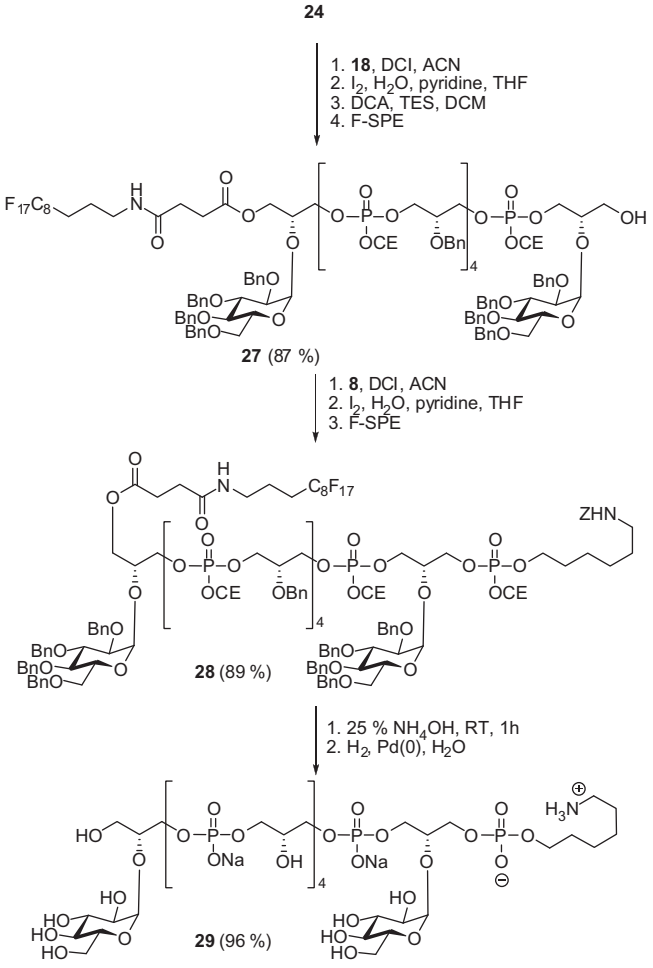
To broaden the palette of TA fragments, and further explore the effectiveness of the light fluorous chemistry, we continued with the assembly of hexamer **29**, containing two glucosyl moieties (Scheme 4). Pentamer **24** was coupled to glucosylglycerol phosphoramidite **18**, resulting in bis-glucosylated hexamer **27** in 87%. Also this compound was uneventfully purified by F-SPE. After introduction of the spacer, the resulting hexamer **28** was deprotected using the aforementioned conditions, to yield the bis-glucosyl TA fragment **29** in 96% yield.

3. Conclusion

We have described the development of two complementary fluorous linker systems for the assembly of TA fragments. The first linker, perfluorooctylpropylsulfonylethyl, is used as a phosphate protecting group and allows the assembly of TA fragments featuring a terminal phosphate monoester. The second linker, a perfluorooctylpropyl succinyl system, is used as a hydroxyl protecting functionality and leads to the formation of TA structures terminating in an alcohol functionality. Light fluorous chemistry is an efficient means for the assembly of TA fragments and allows the construction of pure TA oligomers in multi milligram quantities, sufficient for most initial biochemical studies. Full immunochemical evaluation of the compounds is ongoing and will be reported in due course.



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



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

4. Experimental section

4.1. General

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 $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ 10 g/l, in 10% aqueous H_2SO_4 followed by charring at +/− 140 °C. 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, ^{1}H 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 a chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. When D_2O was used, ^{1}H 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 10 mM 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 °C) with resolution R = 60,000 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).

4.1.1. General procedure for phosphoramidite coupling, oxidation, detritylation and F-SPE

Starting alcohol was dissolved in ACN (0.1 M). DCI (0.25 M solution in CH_3CN, 2 equiv compared to phosphoramidite) was added, together with freshly activated MS 3 Å and the mixture was stirred under argon for 15 min. Phosphoramidite (0.175 M in ACN, 1.3–4.0 equiv) was added and the reaction was stirred until TLC analysis revealed full conversion of the starting material into a higher running spot (~1 h). 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, satd aq $Na_2S_2O_3$ (~20 ml), 0.5 M $KHSO_4$ (~20 ml) and a 1/1 mixture of satd 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 colour fully disappeared (~30 min). DCM (~40 ml) was added and the organic layer was washed with a 1/1 mixture of satd 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 × 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 (4 g) 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 (DMTr, phosphates, DCI) were removed. Subsequently the fluorous product was eluted from the column with, respectively, CH_3CN and acetone.

4.1.2. 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 **10**. In the synthesis of oligomers **1** and **29**, the corresponding protected hexamers (**26** and **28**, 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 2.5 h. 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 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 fully deprotected oligomer of which the integrity and purity was confirmed by HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.

4.1.3. Glucosyl-2-*O*-benzylglycerol phosphate hexamer (7)

Glycerol phosphate pentamer **5** (286 mg, 139 μmol) and DCI (0.25 M solution in CH_3CN, 2.22 ml, 556 μmol) were dissolved in CH_3CN (2.0 ml) together with freshly activated MS 3 Å and stirred for 15 min under argon. Subsequently, glucosyl-glycerol phosphoramidite **6** (0.1 M 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 h under gentle stirring. The mixture was diluted with DCM (80 ml) and washed with a 1/1 mixture of satd 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 **7** (277 mg, 103 μmol, 74%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.9, −1.8 (1P), −1.3 (2P), −1.1 to −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 × 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, –$OCH_2CH_2SO_2$–), 3.51–3.72 (m, 5H, H-2, H-4, H-6, CH_2 glycerol), 3.77–3.88 (m, 6H, 6 × CH glycerol), 3.98–4.34 (m, 37H, H-3, H-5, H-6′, 11 × CH_2 glycerol, 6 × CH_2 cyanoethyl), 4.42–4.50 (m, 2H, –$OCH_2CH_2SO_2$–), 4.56–4.65 (m, 10H, 5 × 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 ($F_{17}C_8CH_2CH_2CH_2CH_2SO_2$–), 19.2–19.5 (12 × CH_2 cyanoethyl), 29.2 (t, *J* = 22 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2SO_2$–), 53.0, 53.1 ($F_{17}C_8CH_2CH_2CH_2CH_2SO_2$–, –$OCH_2CH_2SO_2$–), 60.9 ($CH_2$ glycerol), 61.3 (–$OCH_2CH_2SO_2$–), 62.0–62.4 (6 × CH_2 cyanoethyl), 62.8 (C-5), 65.4–66.0 (10 × CH_2 glycerol), 67.2 (CH_2 glycerol), 68.7 (C-6), 72.0–72.1 (5 × CH_2 Bn), 74.3 (CH_2 Bn), 75.0 (CH_2 Bn), 75.2–75.4 (5 × 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 × C_q cyanoethyl), 125.7 (CH_{arom}), 127.6–128.9 (CH_{arom}), 137.1–137.4 (7 × 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.

4.1.4. Glucosyl-2-*O*-benzylglycerol phosphate hexamer-amino hexyl spacer (9)

Hexamer **7** (272 mg, 101 μmol) was coupled to spacer phosphoramidite **8** (4 equiv), oxidized and purified (FSPE) using the general procedure as described above. Fully protected hexamer **9** (267 mg, 87.2 μmol, 86%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.9, −1.8 (1P), −1.3 to −1.0 (6P); ^1H NMR (400 MHz): δ = 1.27–1.37 (m, 4H, 2 × 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, $F_{17}C_8CH_2CH_2CH_2SO_2$–, $F_{17}C_8CH_2CH_2CH_2CH_2SO_2$–), 2.39–2.69 (m, 14H, 7 × CH_2 cyanoethyl), 3.05–3.17 (m, 4H, $F_{17}C_8CH_2CH_2CH_2SO_2$–, CH_2–N hexylspacer), 3.24–3.33 (m, 2H, –$OCH_2CH_2SO_2$–), 3.57–3.72 (m, 3H, H-2, H-4, H-6), 3.75–3.85 (m, 5H, 5 × CH glycerol), 3.94–4.33 (m, 44H, H-3, H-5, H-6′, CH glycerol, 12 × CH_2 glycerol, 7 × CH_2 cyanoethyl, CH_2–O hexylspacer), 4.43–4.50 (m, 4H, –$OCH_2CH_2SO_2$–, CH_2 Bn), 4.53–4.64 (m, 10H, 5 × 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 ($F_{17}C_8CH_2CH_2CH_2SO_2$–), 19.2–19.5 (7 × CH_2 cyanoethyl), 24.8, 25.9 (2 × CH_2 hexylspacer), 29.3–29.9 (2 × CH_2 hexylspacer, $F_{17}C_8CH_2CH_2CH_2SO_2$–), 40.7 ($CH_2$–N hexylspacer), 53.0, 53.1 ($F_{17}C_8CH_2CH_2CH_2SO_2$–, –$OCH_2CH_2SO_2$–), 61.3 (–$OCH_2CH_2SO_2$–), 61.8–62.4 (7 × CH_2 cyanoethyl), 62.8 (C-5), 65.3–66.0 (11 × CH_2 glycerol), 66.3 (CH_2 CBz), 68.4–68.6 (C-6, CH_2 glycerol), 72.0–72.2 (5 × CH_2 Bn), 73.4–73.5 (CH_2 Bn), 75.0 (CH_2 Bn), 75.2–75.5 (6 × 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 × C_q cyanoethyl), 125.7 (CH_{arom}), 127.5–128.9 (CH_{arom}), 136.6 (C_q Bn), 137.1–137.3 (6 × 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.

4.1.5. Glucosyl-glycerolphosphate hexamer (10)

Protected hexamer **9** (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); ^1H NMR (400 MHz, D_2O): δ = 0.95–1.24 (m, 6H, 3 × 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 × CH glycerol, 12 × CH_2 glycerol), 4.29–4.41 (m, 10H, 5 × CH_2 Bn), 4.47–4.58 (m, 4H, 2 × 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 **10** (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 × CH_2 hexylspacer), 1.60–1.68 (m, 4H, 2 × 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 × CH glycerol, 11 × 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 × 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 × 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 × 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.

4.1.6. 3-*O*-Allyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (13a)

To a cooled (0 °C) solution of donor **11a** (171 mg, 0.250 mmol) and semiprotected glycerol **12** (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 for 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 satd 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 **13a** (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 **15**.

4.1.7. 3-*O*-Allyl-2-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-[9-fluorenylmethyloxycarbonyl]-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (13b)

To a cooled (0 °C) solution of donor **11b** (7.51 g, 8.90 mmol) and semiprotected glycerol **12** (3.96 g, 10.7 mmol) in Et_2O (180 ml) was added TfOH (157 μl, 1.78 mmol). After stirring for 25 min, satd 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 **13b** (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 × 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 (d, 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, 17.1 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 × 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 × C_q Bn), 141.2, 141.2 (2 × C_q FMOc), 143.2, 143.4 (2 × C_q FMOc), 155.0 (C═O FMOc); HRMS: $C_{64}H_{68}O_{10}Si + NH_4^+$ requires 1042.4920, found 1042.4933.

4.1.8. 3-*O*-Allyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (14)

To a solution of compound **13b** (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 **14** (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 × 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, 17.1 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 × 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 × C_q Bn); HRMS: $C_{49}H_{58}O_8Si + Na^+$ requires 825.3793, found 825.3784.

4.1.9. 3-*O*-Allyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (15)

A solution of alcohol **14** (2.521 g, 3.14 mmol) together with BnBr (0.94 ml, 7.85 mmol) in DMF (20 ml) was stirred for 5 min 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 was 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 **15** (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 × 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, 17.1 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 × 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 × C_q Bn); HRMS: $C_{56}H_{64}O_8Si + NH_4^+$ requires 910.4709, found 910.4718.

4.1.10. 2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (16)

A solution of glycoside **15** (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 ~15 s. After stirring under argon for 2 h, the mixture was diluted with THF (20 ml) and satd 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 h at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, satd aq $Na_2S_2O_3$ (30 ml) and brine (40 ml). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (EtOAc/PE) afforded **16** (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 × CH*H* glycerol), 3.77–3.86 (m, 4H, H-5, CH glycerol, 2 × CH*H* glycerol), 3.97 (t, 1H, *J* = 9.3 Hz, H-3), 4.33 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.44 (d, 1H, *J* = 10.9 Hz, CH*H* Bn), 4.52 (d, 1H, *J* = 12.2 Hz, CH*H* Bn), 4.66 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.77–4.85 (m, 3H, CH_2 Bn, CH*H* Bn), 4.90–4.93 (m, 2H, H-1, CH*H* 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 × 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 × 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.

4.1.11. 2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-(4,4′-dimethoxytrityl)-*sn*-glycerol (17)

To a cooled (0 °C) solution of alcohol **16** (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 h before MeOH (1.0 ml) was added. After stirring for an additional 15 min the reaction mixture was diluted with DCM (40 ml) and washed with a 1/1 mixture of satd aq $NaHCO_3$ and brine (30 ml). The aqueous layer was extracted with DCM (2 × 10 ml) and the combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residual oil was redissolved in THF (15 ml) and, subsequently, TBAF (1 M solution in THF, 7.8 ml) was added. The mixture was stirred for 3 h 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 **17** (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*H* glycerol), 3.35 (dd, 1H, *J* = 5.5 Hz, 9.6 Hz, CH*H* glycerol), 3.53–3.57 (m, 2H, H-2, H-4), 3.61–3.67 (m, 3H, H-6, H-6′, CH*H* glycerol), 3.71 (s, 6H, 2 × OMe), 3.76–3.82 (m, 1H, CH*H* 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*H* Bn), 4.47 (d, 1H, *J* = 12.4 Hz, CH*H* Bn), 4.58 (d, 1H, *J* = 11.6 Hz, CH*H* Bn), 4.63 (d, 1H, *J* = 12.0 Hz, CH*H* Bn), 4.80 (d, 1H, *J* = 10.4 Hz, CH*H* Bn), 4.82 (d, 1H, *J* = 10.4 Hz, CH*H* Bn), 4.96 (d, 1H, *J* = 10.8 Hz, CH*H* 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 × 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 × 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 × C_q Bn, 5 × C_q DMTr); HRMS: $C_{58}H_{60}O_{10} + Na^+$ requires 939.4079, found 939.4090.

4.1.12. 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 (18)

To a cooled (0 °C) solution of alcohol 17 (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 for 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 satd aq $NaHCO_3$ and brine (20 ml). The aqueous layer was extracted with DCM (2 × 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 18 (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); ^{1}H NMR (400 MHz, CD_3CN, mixture of diastereoisomers): δ = 1.04–1.12 (m, 12H, 4 × 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 × CH isopropylamino), 3.60–3.88 (m, 13H, H-3, H-6, H-6′, 2 × 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 × CH_3 isopropylamino), 43.7–43.8 (2 × CH isopropylamino), 55.8 (2 × 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 × 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 × C_q Bn, 5 × C_q DMTr, C_q cyanoethyl); HRMS: $C_{67}H_{77}N_2O_{11}P + H^+$ requires 1117.5338, found 1117.5337.

4.1.13. 2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-(4,4′-dimethoxytrityl)-1-*O*-succinyl-*sn*-glycerol (19)

To a cooled (0 °C) solution of alcohol **17** (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 **19** (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; ^{1}H NMR (400 MHz, CD_3CN): δ = 2.49 (s, 4H, 2 × 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 × 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 × 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 × CH_2 succinyl), 56.5 (2 × 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 × 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 × C_q DMTr, 4 × C_q Bn), 173.5, 174.9 (2 × C=O succinyl); HRMS: $C_{62}H_{64}O_{13} + Na^+$ requires 1039.4239, found 1039.4237.

4.1.14. 2-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-1-*O*-(*N*-[3-perfluorooctylpropyl]-succinamidyl-*sn*-glycerol (20)

To a solution of compound **19** (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 satd aq $NaHCO_3$ (2 × 50 ml), H_2O (2 × 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 for 30 min and, subsequently, diluted with DCM (40 ml) and washed with a 1/1 mixture of satd 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 **20** (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, $F_{17}C_8CH_2CH_2CH_2N$), 1.99–2.13 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 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, $F_{17}C_8CH_2CH_2CH_2N$), 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 × 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 × 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 ($F_{17}C_8CH_2CH_2CH_2N$), 28.3 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 29.3, 30.7 (2 × CH_2 succinyl), 38.5 ($F_{17}C_8CH_2CH_2CH_2N$), 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 × 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 × C_q Bn), 171.5, 172.6 (2 × C=O succinyl); HRMS: $C_{52}H_{52}F_{17}NO_{10} + Na^+$ requires 1196.3212, found 1196.3210.

4.1.15. Glucosyl glycerol phosphate dimer (21)

Monomer **20** (133 mg, 113 μmol) was coupled to glycerol phosphoramidite **4** (1.5 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Dimer **21** (153 mg, 104 μmol, 92%) was obtained as a colourless 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, $F_{17}C_8CH_2CH_2CH_2N$), 1.99–2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.26–2.67 (m, 7H, 2 × CH_2 succinyl, CH_2 cyanoethyl, CH_2OH), 3.22 (dd, 2H, J = 6.7 Hz, 13.0 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH_2 glycerol, CH_2 cyanoethyl), 4.44–4.49 (m, 2H, 2 × CH*H* Bn), 4.56–4.73 (m, 5H, CH*H* Bn, 2 × CH_2 Bn), 4.78–4.83 (m, 2H, 2 × 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 ($F_{17}C_8CH_2CH_2CH_2N$), 28.3 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 29.4, 30.7 (2 × CH_2 succinyl), 38.5 ($F_{17}C_8CH_2CH_2CH_2N$), 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 × 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 × 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 × C_q Bn), 171.5, 172.3 (2 × C=O succinyl); HRMS: $C_{65}H_{68}F_{17}N_2O_{15}P + Na^+$ requires 1493.3978, found 1493.3978.

4.1.16. Glucosyl glycerol phosphate trimer (22)

Dimer **21** (149 mg, 101 μmol) was coupled to glycerol phosphoramidite **4** (1.5 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Trimer **22** (161 mg, 91.0 μmol, 90%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4, −1.3, −1.3, −1.3 (1P), −1.0, −0.9 (1P); ^{1}H 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 × CH_2 succinyl, 2 × CH_2 cyanoethyl, CH_2OH), 3.18–3.26 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH_2 glycerol, 2 × CH_2 cyanoethyl), 4.44–4.49 (m, 2H, 2 × CH*H* Bn), 4.56–4.65 (m, 5H, CH*H* Bn, 2 × CH_2 Bn), 4.69–4.72 (m, 2H, 2 × CH*H* Bn), 4.77–4.83 (m, 2H, 2 × 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 × CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 28.2 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 29.3, 30.6 (2 × CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2N$), 60.4, 60.5 (CH_2 glycerol), 62.0–62.2 (2 × CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5–66.7 (4 × 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 × C_q cyanoethyl), 127.5–128.5 (CH_{arom}), 137.1, 137.7–138.0, 138.5 (6 × C_q Bn), 171.5, 172.3 (2 × C=O succinyl); HRMS: $C_{78}H_{84}F_{17}N_3O_{20}P_2 + Na^+$ requires 1790.4744, found 1790.4744.

4.1.17. Glucosyl glycerol phosphate tetramer (23)

Trimer **22** (157 mg, 88.7 μmol) was coupled to glycerol phosphoramidite **4** (1.5 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Tetramer **23** (169 mg, 82.0 μmol, 92%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.2 (2P), −0.9, −0.9, −0.9 (1P); ^{1}H NMR (400 MHz): δ = 1.68–1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.98–2.19 (m, 3H, $F_{17}C_8CH_2CH_2CH_2N$, CH_2OH), 2.35–2.67 (m, 10H, 2 × CH_2 succinyl, 3 × CH_2 cyanoethyl), 3.18–3.25 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH glycerol), 3.85–3.95 (m, 2H, H-3, H-5), 4.01–4.30 (m, 21H, CH glycerol, 7 × CH_2 glycerol, 3 × CH_2 cyanoethyl), 4.44–4.49 (m, 2H, 2 × CH*H* Bn), 4.56–4.66 (m, 7H, CH*H* Bn, 3 × CH_2 Bn), 4.69–4.72 (m, 2H, 2 × CH*H* Bn), 4.76–4.83 (m, 2H, 2 × 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 × CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 28.2 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 29.3, 30.6 (2 × CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2N$), 60.4, 60.5 (CH_2 glycerol), 62.0–62.2 (3 × CH_2 cyanoethyl), 63.1 (CH_2 glycerol), 65.5–66.6 (6 × CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 72.0 (CH_2 Bn), 72.1–72.2 (2 × 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 × 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 × C_q cyanoethyl), 127.6–128.5 (CH_{arom}), 137.2, 137.7–138.0, 138.5 (7 × C_q Bn), 171.4, 172.3 (2 × C=O succinyl); HRMS: $[C_{91}H_{100}F_{17}N_4O_{25}P_3 + 2Na]^{2+}$ requires 1055.2701, found 1055.2705.

4.1.18. Glucosyl glycerol phosphate pentamer (24)

Tetramer **23** (165 mg, 79.9 μmol) was coupled to glycerol phosphoramidite **4** (1.8 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Pentamer **24** (176 mg, 74.3 μmol, 93%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.1 (3P), −0.9, −0.9, −0.9 (1P); ^{1}H NMR (400 MHz): δ = 1.68–1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.98–2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.26–2.67 (m, 13H, 2 × CH_2 succinyl, 4 × CH_2 cyanoethyl, CH_2OH), 3.18–3.26 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH glycerol), 3.86–3.95 (m, 2H, H-3, H-5), 4.00–4.30 (m, 27H, CH

glycerol, 9 × CH_2 glycerol, 4 × CH_2 cyanoethyl), 4.43–4.49 (m, 2H, 2 × CH*H* Bn), 4.56–4.67 (m, 9H, CH*H* Bn, 4 × CH_2 Bn), 4.69–4.71 (m, 2H, 2 × CH*H* Bn), 4.76–4.83 (m, 2H, 2 × 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 × CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 28.2 (t, J = 23 Hz, $F_{17}C_8CH_2CH_2CH_2CH_2N$), 29.3, 30.6 (2 × 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 × CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5–66.6 (8 × CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH_2 Bn), 72.1–72.2 (3 × 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 × 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 × C_q cyanoethyl), 127.5–128.4 (CH_{arom}), 137.2, 137.7–138.0, 138.5 (8 × C_q Bn), 171.4, 172.3 (2 × C=O succinyl); HRMS: $[C_{104}H_{116}F_{17}N_5O_{30}P_4 + 2Na]^{2+}$ requires 1204.3101, found 1204.3100.

4.1.19. Glucosyl glycerol phosphate hexamer (25)

Pentamer **24** (149 mg, 63.1 μmol) was coupled to glycerol phosphoramidite **4** (2.0 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Hexamer **25** (151 mg, 56.8 μmol, 90%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.1 (4P), −0.9, −0.9, −0.9 (1P); ^1H NMR (400 MHz): δ = 1.68–1.76 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.98–2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.31–2.67 (m, 15H, 2 × CH_2 succinyl, 5 × CH_2 cyanoethyl, CH_2OH), 3.17–3.25 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH glycerol), 3.85–3.94 (m, 2H, H-3, H-5), 4.00–4.31 (m, 33H, CH glycerol, 11 × CH_2 glycerol, 5 × CH_2 cyanoethyl), 4.44–4.48 (m, 2H, 2 × CH*H* Bn), 4.57–4.66 (m, 11H, CH*H* Bn, 5 × CH_2 Bn), 4.68–4.71 (m, 2H, 2 × CH*H* Bn), 4.77–4.82 (m, 2H, 2 × 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 × CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 28.2 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 29.3, 30.5 (2 × CH_2 succinyl), 38.4 ($F_{17}C_8CH_2CH_2CH_2N$), 60.4, 60.5 (CH_2 glycerol), 62.0–62.2 (5 × CH_2 cyanoethyl), 63.1 (CH_2 glycerol), 65.5–66.6 (10 × CH_2 glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH_2 Bn), 72.1–72.2 (4 × 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 × 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 × C_q cyanoethyl), 127.5–128.5 (CH_{arom}), 137.2, 137.7–138.0, 138.5 (9 × C_q Bn), 171.4, 172.3 (2 × C=O succinyl); HRMS: $[C_{117}H_{132}F_{17}N_6O_{35}P_5 + 2Na]^{2+}$ requires 1352.8484, found 1352.8479.

4.1.20. Glucosyl glycerol phosphate hexamer-spacer (26)

Hexamer **25** (145 mg, 54.6 μmol) was coupled to spacer phosphoramidite **8** (2.5 equiv), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **26** (147 mg, 48.7 μmol, 89%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.1 (6P); ^1H NMR (400 MHz): δ = 1.25–1.40 (m, 4H, 2 × 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, $F_{17}C_8CH_2CH_2CH_2N$), 1.98–2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.35–2.67 (m, 16H, 2 × CH_2 succinyl, 6 × CH_2 cyanoethyl), 3.12–3.25 (m, 4H, CH_2–N hexylspacer, $F_{17}C_8CH_2CH_2CH_2N$), 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 × CH glycerol), 3.85–3.94 (m, 2H, H-3, H-5), 4.00–4.31 (m, 39H, CH glycerol, 12 × CH_2 glycerol, 6 × CH_2 cyanoethyl, CH_2–O hexylspacer), 4.44–4.48 (m, 2H, 2 × CH*H* Bn), 4.57–4.65 (m, 11H, CH*H* Bn, 5 × CH_2 Bn), 4.68–4.71 (m, 2H, 2 × CH*H* Bn), 4.76–4.83 (m, 2H, 2 × 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 × CH_2 cyanoethyl), 20.6 ($F_{17}C_8CH_2CH_2CH_2N$), 24.8 (CH_2 hexylspacer), 25.9 (CH_2 hexylspacer), 28.2 (t, J = 22 Hz, $F_{17}C_8CH_2CH_2CH_2N$), 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 ($F_{17}C_8CH_2CH_2CH_2N$), 40.7 (CH_2–N hexylspacer), 61.8–62.1 (6 × CH_2 cyanoethyl), 63.0 (CH_2 glycerol), 65.5–66.4 (11 × 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 × 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 × 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 × C_q cyanoethyl), 127.5–128.5 (CH_{arom}), 136.6, 137.2, 137.7–138.0, 138.5 (9 × C_q Bn, C_q Cbz), 156.3 (C=O Cbz), 171.3, 172.2 (2 × C=O succinyl); HRMS: $[C_{134}H_{155}F_{17}N_8O_{40}P_6 + 2Na]^{2+}$ requires 1535.9156, found 1535.9153.

4.1.21. Glucosyl-glycerol phosphate hexamer (2)

Protected hexamer **26** (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); ^1H NMR (400 MHz, D_2O): δ = 0.80–1.10 (m, 6H, 3 × 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 × CH_2 glycerol, 6 × CH glycerol, CH_2–O hexylspacer), 4.16–5.02 (m, 22H, H-1, 9 × 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); ^1H NMR (600 MHz, D_2O): δ = 1.39–1.44 (m, 4H, 2 × CH_2 hexylspacer), 1.61–1.70 (m, 4H, 2 × 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 × CH glycerol, 11 × 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 × 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 × CH_2 glycerol), 70.3–70.5 (5 × 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.

4.1.22. Bis-glucosyl-glycerol phosphate hexamer (27)

Pentamer **24** (22.5 mg, 9.52 μmol) was coupled to glucosyl-glycerol phosphoramidite **18** (3.0 equiv), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Bis-glucosylated hexamer **27** (25.7 mg, 8.31 μmol, 87%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.3 (2P), −1.2, −1.0 (3P); ^1H NMR (400 MHz): δ = 1.66–1.82 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 1.97–2.12 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 2.28–2.68 (m, 15H, 2 × CH_2 succinyl, 5 × CH_2 cyanoethyl, CH_2OH), 3.17–3.28 (m, 2H, $F_{17}C_8CH_2CH_2CH_2N$), 3.51–3.98 (m, 19H, 2 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 2 × H-6, 2 × H-6′, 5 × CH glycerol, CH_2 glycerol), 4.00–4.31 (m, 33H, CH glycerol, 11 × CH_2 glycerol, 5 × CH_2 cyanoethyl), 4.42–4.48 (m, 4H, 4 × CH*H* Bn), 4.56–4.64 (m, 10H, 2 × CH*H* Bn, 4 × CH_2 Bn), 4.67–4.71 (m, 3H, 3 × CH*H* Bn), 4.76–4.84 (m, 5H, 5 × CH*H* Bn), 4.90–4.95 (m, 3H, H-1, 2 × 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.

4.1.23. Bis-glucosyl-glycerol phosphate hexamer-spacer (28)

Hexamer **27** (24.5 mg, 7.92 μmol) was coupled to spacer phosphoramidite **8** (5 equiv), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **28** (24.3 mg, 7.06 μmol, 89%) was obtained as a colourless oil. ^{31}P NMR (161.7 MHz): δ = −1.4 to −1.0 (6P); ^{1}H NMR (400 MHz): δ = 1.26–1.37 (m, 4H, 2 × 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 × CH_2 succinyl, 6 × 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 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 2 × H-6, 2 × H-6′, 4 × CH glycerol), 4.00–4.30 (m, 40H, 2 × CH glycerol, 12 × CH_2 glycerol, 6 × CH_2 cyanoethyl, CH_2–O hexylspacer), 4.40–4.48 (m, 4H, 4 × CH*H* Bn), 4.55–4.64 (m, 10H, 2 × CH*H* Bn, 4 × CH_2 Bn), 4.68–4.71 (m, 4H, 4 × CH*H* Bn), 4.76–4.83 (m, 4H, 4 × CH*H* Bn), 4.90–4.95 (m, 2H, 2 × CH*H* Bn), 5.01–5.13 (m, 5H, 2 × 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.

4.1.24. Bis-glucosyl-glycerol phosphate hexamer (29)

Protected hexamer **28** (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 × 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 × H-2, 2 × H-3, 2 × H-4, 2 × H-5, 2 × H-6, 2 × H-6′, 12 × CH_2 glycerol, 6 × CH glycerol, CH_2–O hexylspacer), 4.15–4.84 (m, 28H, 2 × H-1, 12 × 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 **29** (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 × CH_2 hexylspacer), 1.62–1.70 (m, 4H, 2 × CH_2 hexylspacer), 2.99 (t, 2H, J = 7.5 Hz, CH_2–N hexylspacer), 3.36–3.41 (m, 2H, 2 × H-4), 3.48–3.53 (m, 2H, 2 × H-2), 3.71–3.77 (m, 6H, 2 × H-3, 2 × H-6, CH_2 glycerol), 3.80–4.05 (m, 33H, 2 × H-5, 2 × H-6′, 5 × CH glycerol, 11 × CH_2 glycerol, CH_2–O hexylspacer), 4.07–4.10 (m, 1H, CH glycerol), 5.15 (d, 2H, J = 3.4 Hz, 2 × H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.5, 26.1, 27.6, 30.5 (4 × CH_2 hexylspacer), 40.4 (CH_2–N hexylspacer), 61.2 (2 × 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 × CH_2 glycerol), 70.4–70.7 (4 × CH glycerol,2 × C-4), 72.5, 72.5 (2 × C-2), 72.9, 73.0 (2 × C-5), 73.9, 74.0 (2 × C-3), 76.4 (t, J = 8 Hz, CH glycerol), 77.8 (d, J = 8 Hz, CH glycerol), 98.7, 98.8 (2 × C-1); HRMS: $C_{36}H_{77}NO_{41}P_6 + H^+$ requires 1366.2469, found 1366.2474.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2012.02.023.

References

1. (a) Fischer, W. *Adv. Microb. Physiol.* **1988**, *29*, 233–302; (b) Naumova, I. B.; Shashkov, A. S.; Tul'skaya, E. M.; Streshinskaya, G. M.; Kozlova, Y. I.; Potekhina, N. V.; Evtushenko, L. I.; Stackebrandt, E. *FEMS Microbiol. Rev.* **2001**, *25*, 269–283; (c) Neuhaus, F. C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686–723; (d) Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, *6*, 276–287.
2. (a) Morath, S.; Geyer, A.; Hartung, T. *J. Exp. Med.* **2001**, *193*, 393–397; (b) Morath, S.; Geyer, A.; Spreitzer, I.; Hermann, C.; Hartung, T. *Infect. Immun.* **2002**, *70*, 938–944; (c) Morath, S.; Von Aulock, S.; Hartung, T. *J. Endotoxin Res.* **2005**, *11*, 348–356.
3. (a) 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; (b) 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; (c) Hogendorf, W. F. J.; Lameijer, L. N.; Beenakker, T. J. M.; Overkleeft, H. S.; Filippov, D. V.; Codée, J. D. C.; Van der Marel, G. A. *Org. Lett.* **2012**, *14*, 848–851.
4. (a) Theilacker, C.; Krueger, W. A.; Kropec, A.; Huebner, J. *Vaccine* **2004**, *22*, S31–S38; (b) Koch, S.; Hufnagel, M.; Huebner, J. *Expert Opin. Biol. Ther.* **2004**, *4*, 1519–1531; (c) Sava, I. G.; Heikens, E.; Huebner, J. *Clin. Microbiol. Inf.* **2010**, *16*, 533–540.
5. (a) Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703–5712; (b) Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839; (c) Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J. D. C.; Hogendorf, W. F. J.; Van der Marel, G. A.; Huebner, J. *J. Infect. Dis.* **2012**, *205*, 1076–1085.
6. Pollack, J. H.; Neuhaus, F. C. *J. Bacteriol.* **1994**, *176*, 7252–7259.
7. Endl, J.; Seidl, H. P.; Fiedler, F.; Schleifer, K. H. *Arch. Microbiol.* **1983**, *135*, 215–223.
8. (a)*Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horváth, I. T., Eds.; Wiley-VCH: Weinheim, 2004; (b) Curran, D. P. *Aldrichim. Acta* **2006**, *39*, 3–9; (c) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837–11865; (d) Zhang, W.; Cai, C. *Chem. Commun.* **2008**, 5686–5694; (e) Zhang, W. *Chem. Rev.* **2009**, *109*, 749–795; (f) Zhang, W. *Green Chem.* **2009**, *11*, 911–920; For an example of a fluorous phosphate protecting group see: (g) Liu, L.; Pohl, N. L. B. *Org. Lett.* **2011**, *13*, 1824–1827.
9. Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946–7968.
10. Complete immunological evaluation studies, including OPA inhibition assays will be reported in due course.
11. (a) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223–2311; (b) Reese, C. B. *Org. Biomol. Chem.* **2005**, *3*, 3851–3868.
12. (a) Schmidt, R. R. *Angew. Chem.* **1986**, *98*, 213–236; *Angew. Chem., Int. Ed.* **1986**, *25*, 215–235; For a recent example see: (b) Pedersen, C. M.; Figueroa-Perez, I.; Lindner, B.; Ulmer, A. J.; Zähringer, U.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 1–7.
13. (a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Shiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573–5577; (b) Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479–6482; (c) Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; Van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990–8002.