Synthesis and applications of cell wall glycopolymer fragments from Staphilococci and Enterococci

Berni, F.

Chapter 4

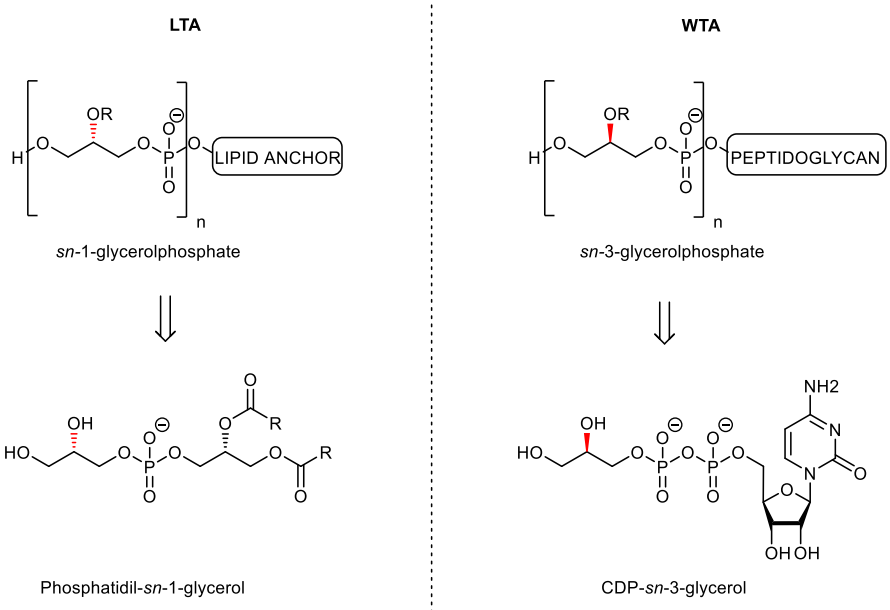
Synthesis of glucosyl sn-1-glycerolphosphate teichoic acids: glycerol stereochemistry affects synthesis and antibody interaction

F. Berni, L. Wang, E. Kalfopoulou, L. D. Nguyen, D. van der Es, J. Huebner, H. S. Overkleeft, C. H. Hokke, G. A. van der Marel, A. van Diepen, J. D. C. Codée; *RSC Chem. Biol*, **2021**, 2: 187-191.

INTRODUCTION

Teichoic acids (TA) are anionic polymeric structures, found in the cell-wall of Gram-positive bacterial species.[1] Among the several biological functions, it has been observed that TA are highly immunogenic and therefore, considered as good antigen candidate for vaccine development against different opportunistic pathogens.[2] As described in Chapter 1, they are divided in lipoteichoic acids (LTA) and wall teichoic acids (WTA) upon the way and location of interconnection with the cell wall. The chemical structure is widely diverse among the different species, but generally most Enterococci and Staphylococci bear a Type I LTA, which is composed by an *sn*-glycerol-1-phosphate (GroP) backbone with either D-Alanine or glycosylic substituents at the C-2 position (Figure 1).[3, 4] The stereospecificity of the GroP chain has been previously carried out based on the differences in the biosynthetic precursors and degradation products compared to GroP based wall teichoic acids (WTA). Indeed, Type I glycerolphosphate based LTA are not only biologically distinct from WTA, but they are structurally enantiomeric polymers composed respectively by *sn*-Gro1P or *sn*-Gro3P.[5] Biochemical evidence of the stereochemical difference between GroP based WTA and LTA has been carried out recently using a stereospecific exo acting sn-glycerol-3-phosphodiesterase (GlpQ) from B. *subtilis* strain 168.[6] It was observed that GlpQ was able to cleave off *sn*-glycerol-3-phosphate repeating unit from the exposed end of WTA but such hydrolysis activity was not occurring with LTA substrate having opposite stereochemistry.

Figure 1*: General structure of sn-Gro-1-P LTA and sn-Gro-3-P WTA and their biosynthesis precursors phosphatidyl-sn-1-glycerol and CDP-sn-3-glycerol*



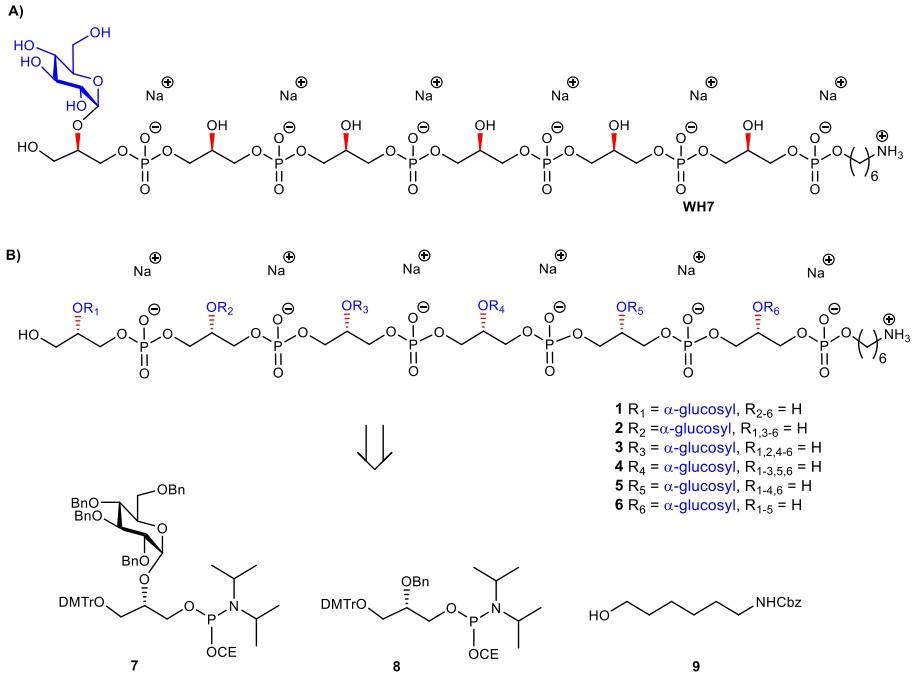
Because of the microheterogenicity of TAs, resulting from the different glycosylation and D-alanylation patterns, it has been difficult to determine the precise antigenic elements

at the molecular level using isolated TAs.[7] Synthetic chemistry can provide instead well-defined structures to establish structure-immunogenicity relationship,[8] and several groups have reported on strategies to assemble both LTA and WTA fragments (See Chapter1).[9] Here attention was focused on glucosyl substituted fragments showing how antibody-TA interaction can be influenced not only for the position of the carbohydrate appendage but also by the stereochemistry of the glycerol unit. The synthetic route towards the pivotal glucosyl-glycerol building block has been improved by employing a recent methodology developed by Wang *et al.* for the construction of 1,2 cis glycosidic linkage.[10] Interestingly the glycosylation outcome was different upon the nature of protecting groups and stereogenic center of the glycerol acceptor.

RESULTS AND DISCUSSION

Different approaches have been described to assemble LTA fragments with well-defined glycosylation patterns. These fragments were equipped with a linker to attach them to either carrier proteins, fluorescent labels or affinity tags as well as microarray surfaces (See Chapter 3).[11] The linker previously was attached to the side of the oligomers, formally generating *sn*-Gro-3-P LTAs. From the pool of synthetic LTA oligomers, a glucosylated fragment was selected as a lead antigen, and this structure, **WH7** (See Figure 2A), was attached to a carrier protein (bovine serum albumin, BSA) to provide a model TA-conjugate vaccine.[11a, 12] Realizing that the chirality of the GroP chains may play a role in the interaction with antibodies, the generation of a set of glucosylated *sn*-Gro-1-P LTA-hexamers **1**-**6** is here described, differing in the position of the α-glucose substituent (Figure 2B).

Figure 2: *A) Lead compound **WH7**; B) The new set of TA hexamers and the building blocks used for their synthesis.*

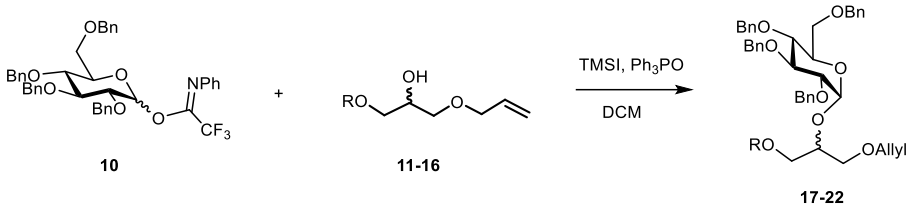


The required LTA-hexamers were assembled using phosphoramidite building blocks **7** and **8** and linker **9** (Figure 2B). The glycerol phosphoramidite building blocks **7** and **8** carry a base labile cyanoethyl protecting group (OCE) and a temporary dimethoxytrityl (DMTr) protecting group to enable the assemble of the target TA hexamers using well-established and highly efficient nucleic acid chemistry.[13-16] The remaining hydroxyl are all substituted with benzyl type protecting group to facilitate final deprotection via hydrogenolysis. While synthesis of compounds **8** and **9** were already optimized previously in our group, attention was focused on the synthesis of building block **7**. The crucial step in the synthesis is the introduction of the 1,2-cis glycosidic linkage. To deliver the desired α-glucosyl glycerol intermediate with good stereoselectivity, previously a glucosyl imidate donor building block carrying a bulky fluorenylmethoxycarbonyl protecting group at the C-6 position was employed.[12] The use of a glucosyl donor, carrying solely benzyl ether protecting groups, would reduce instead the number of required protecting group manipulations.

Among the several strategies for the formation of the 1,2-cis glycosidic linkages, the use of an additive-mediated glycosylation was explored to assemble compound **7**. Recently it has been described that a combination of trimethylsilyl iodide (TMSI) and an excess of triphenylphosphine oxide (Ph_3PO) can be used to glycosylate nucleophilic alcohols with a perbenzylated glucosyl imidate donor in a highly stereoselective manner.[10] This strategy was applied here in the coupling of donor **10** and glycerol acceptor **11**, providing compound **17** in 72% yield. Unfortunately, the stereoselectivity was relatively poor (see

Table 1, entry 1, α/β = 1.3/1). We therefore explored the use of acceptor **12** having the same protecting groups but opposite chirality. As shown in entry 2, the stereoselectivity significantly improved, indicating double stereodifferentiation[17] to play an important role in the union of donor **10** and acceptor **11/12**. This finding is quite unexpected as the acceptor used is relatively flexible and small (as compared to other carbohydrate acceptors, for which this phenomenon has been observed). Upon scale up of the reaction, the yield of the glycosylation dropped to 45%, because of loss of the silyl group, and therefore different protecting groups at this position were probed. Since the stereochemistry of the glycerol acceptor had a strong impact on the stereoselectivity of the glycosylation reactions, we examined both enantiomers of the glycerol acceptor bearing either a para-methoxybenzyl (PMB) ether or a benzoyl (Bz) ester (**13-16**). The results of the glycosylations are summarized in Table 1, showing that the stereoselectivity is actually affected by both chirality and type of substituent. In the case of PMB protecting groups (entry 3 and 4) no double differentiation was observed and the β-by product was detected by ^{1}H-NMR as minor impurity. When the protecting group was replaced with a benzoate ester (entry 5 and 6), the stereoselectivity was good with acceptor **16** (6:1), while no traces of the β anomer were detected in the case of acceptor **15**. The desired α-product (**21**) could be isolated in 68% yield and by extending the reaction time (36h) the yield was further improved to 86%, which was also reproducible on a large scale (up to 15 mmol, Table 1, entry 7).

Table 1: *Glycosylations of donor **10** and glycerol acceptors **11-16**.*[a]



Entry	R	OH	Acc.	Prod.	Yield	α : β
1	TBDPS	OH	**11**	**17**	72%	1.3:1
2	TBDPS	OH	**12**	**18**	68%	>10:1
3	PMB	OH	**13**	**19**	65%	>10:1
4	PMB	OH	**14**	**20**	66%	>10:1
5	Bz	OH	**15**	**21**	68%	>10:1
6	Bz	OH	**16**	**22**[18]	70%	6:1
7[b]	Bz	OH	**15**	**21**	86%[b]	>10:1

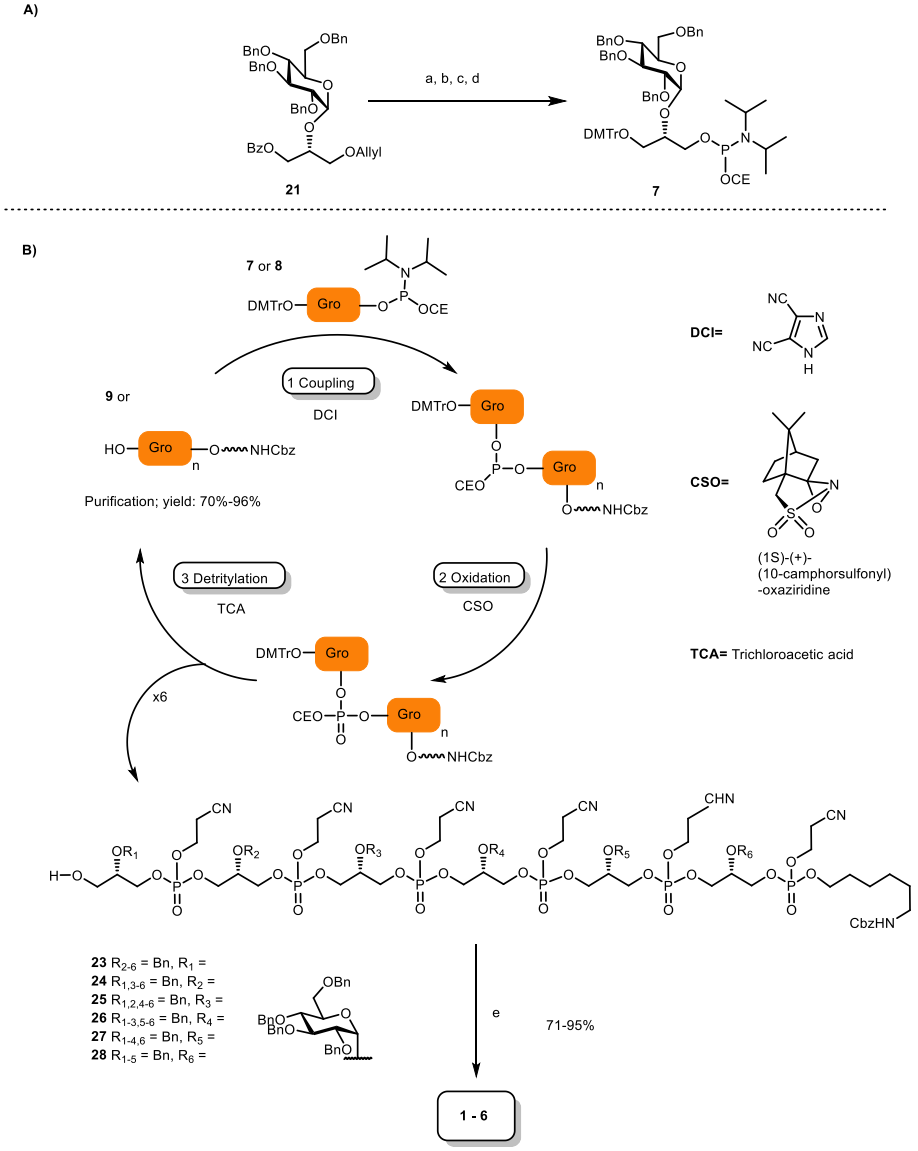
[a] Donor (1 eq), acceptor (0.7 eq), TMSI (1 eq), Ph_3PO (6 eq), DCM (0.1 M), r.t., 24 h.

[b] Reaction time 36h (15 mmol scale)

Next compound **21** was transformed into the required building block phosphoramidite **7** as shown in Scheme 1A. Briefly, the benzoate ester in **21** was exchanged for the required DMTr-ether, after which the allyl ether was removed and the cyanoethyl-protected phosphoramidite installed. With building block **7**, **8** and **9** in hand, the assembly of the

GroP hexamers was performed using repetitive coupling cycles in solution (Scheme 1B). The alcohols, *i.e.* alcohol spacer **9** or the oligomer intermediates, were coupled with phosphoramidite building block **7** or **8** using DCI (4,5-Dicyanoimidazole) as activating agent, followed by CSO [(1S)-(+)-(10-camphorsulfonyl)-oxaziridine] mediated oxidation of the so-formed phosphite triester. After aqueous work up, the DMTr was removed under mild acidic conditions (0.18 M trichloroacetic acid in DCM). The generated alcohol was then purified and used for the subsequent coupling. All coupling-deprotection cycles proceeded uneventfully delivering the elongated structures in 60-96% yield. After construction of the fully protected hexamers **23**-**28**, they were deprotected by first removing the cyanoethyl protecting groups under basic conditions, followed by Pd black catalyzed hydrogenolysis of all benzyl groups and the Cbz carbamate.

Scheme 1: *A) Synthesis of building block **7**. B) Assembly of hexamers **1**-**6***

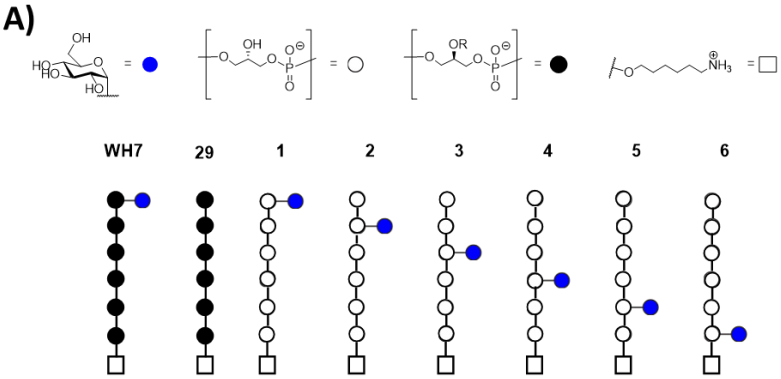


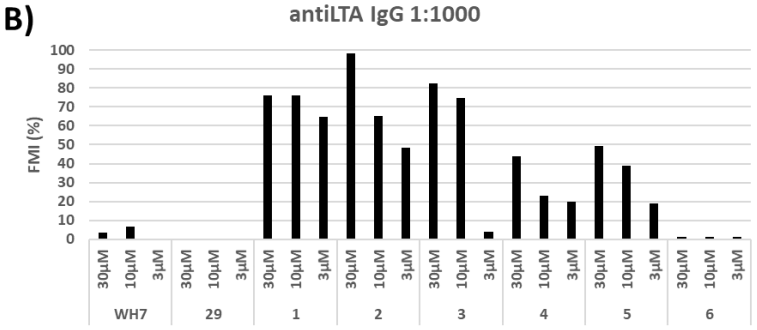
Reagents and conditions: *a) $Na_{(s)}$, MeOH, quant.; b) DMTrCl, TEA, DCM, 88%; c) (i) $Ir(COD)(PPh_2Me)_2PF_6$, H_2, THF; (ii) $NaHCO_{3(aq)}$, I_2, THF, 92%; d) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, TEA, DCM, 70%; e) (i) NH_4OH, H_2O, Dioxane; (ii) Pd black, H_2, H_2O, AcOH.*

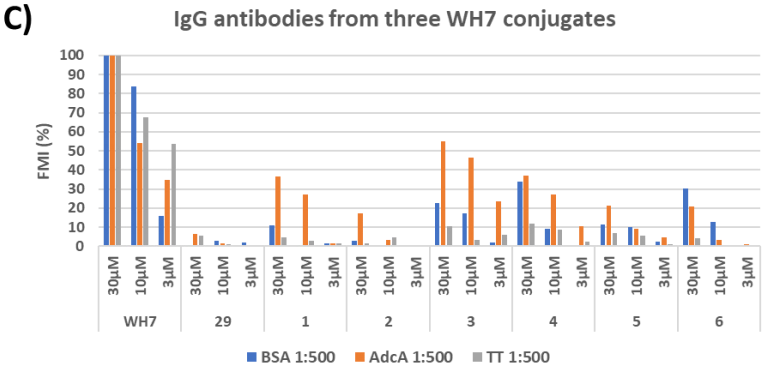
After the generation of the target hexamers, anti-LTA antibodies binding was evaluated using the newly generated library of sn-1-GroP. Chapter 3 dealt with the development of a TA-microarray, which allowed the screening of a library of synthetic TA-fragments for binding with mono- or polyclonal antibodies raised

against isolated LTA from *E. faecalis* **12030** or **WH7** based glycoconjugates vaccine.[11a, 18, 19] It was shown that sera obtained by immunization with native LTA from *E. faecalis* **12030**[20] showed preferential binding to glycosylated TA-fragments. The serum raised against the WH7-BSA glycoconjugate specifically recognized TA-fragments encompassing the **WH7** structure.[11b]
Thus, the six glucosyl hexamers **1**-**6**, the lead antigen **WH7** and an unsubstituted hexamer (**29**, Figure 3A), previously generated,[16] were immobilized on epoxy-silane functionalized glass-slides at three different concentrations (30 μM, 10 μM and 3 μM). The microarrays were then used to probe binding of the serum raised against the native LTA from *E. faecalis* **12030** (Figure 3B) and different **WH7**-conjugate forms as shown in Figure 3C: BSA (blue), AdcA (orange) and TT (grey). IgG binding was visualized using a fluorescently labelled (DyLight550) goat anti-Rabbit IgG antibody. In order to compare the relative signal towards the synthetic fragments among the polyclonal sera, the average of three datapoints from the fluorescence read-out is normalized to the highest peak value (Compound **2** at 30 μM for anti-LTA and **WH7** at 30 μM for sera against the synthetic conjugates). It becomes immediately apparent that IgG binding is influenced not only by the presence of the glucose substituent and its position, but also by the stereochemistry of the Gro-P backbone. The anti-LTA serum did not recognize the bare *sn*-Gro-3-P-backbone nor the **WH7** antigen. In contrast, it bound well to the *sn*-Gro-1-P-hexamers bearing an α-glucosyl moiety. The antibodies seem to show a slightly better binding to fragments that display the glucosyl moiety further away from the linker. Perhaps the display of the glycosylated antigen close to the microarray surface prohibits binding of the antibody. The IgG antibodies present in the sera raised against WH7-conjugates strongly recognized the sn-Gro-3-P-antigen **WH7**, while the signal is significantly attenuated for its sn-Gro-1-P-counterpart **1**, as well as for the other sn-Gro-1-P-hexamers. These results clearly reveal that the stereochemistry of the LTA GroP-backbone is a crucial determinant for antibody binding. From the array results it can be concluded that glycosylated GroP-fragments represent important natural epitopes and anti-LTA antibodies can discriminate between glycosylated *sn*-1 and *sn*-3-glycerol fragments. This exquisite recognition implies that the position of the linker in the synthetic antigens is an important element in the design and construction of synthetic LTA-conjugate vaccines. Also, the position of the glucose appendage plays a major role in recognition by the antibodies, which need sufficient space for binding. The results highlight that a very specific antibody response can be elicited using conjugate vaccines carrying single well-defined synthetic LTA-fragment epitopes.

Figure 3: *A) Overview of the TA-fragments tested; B) IgG binding in rabbit serum raised against native LTA from E. faecalis* ***12030*** *(1:1000 dilution); C) IgG binding in rabbit serum raised against WH7-BSA (blue), WH7-AdcA (orange), WH7-TT (grey). FMI (%): median fluorescent intensity normalized to the highest peak.*







CONCLUSION

In conclusion, the synthesis of a new set of glucosylated GroP-LTA-fragments is here reported, featuring a *sn*-Gro-1-P backbone with an α-glucosyl substituent at different positions along the chain. The synthesis of the pivotal building block **7** was achieved by employing an additive-mediated glycosylation strategy. The stereochemistry of the glycerol acceptor proved to be important for the stereochemistry of the glycosylation reaction linking the glucose moiety to the glycerol alcohol. Evaluation of the set of glucosylated *sn*-Gro-1-P hexamers alongside an unsubstituted *sn*-Gro-3-P LTA hexamer and a glucosylated *sn*-Gro-3-P hexamer (**WH7**) for interactions with anti-LTA antibodies showed that the stereochemistry of the Gro-P backbone plays a decisive role. The position of the α-glucosyl substituent also influenced binding of the antibodies. In the design of conjugate vaccines or diagnostic tools using synthetic TA-fragments, it is therefore important to position the linker connecting the TA fragments to its carrier at the site of the fragment that mimics the natural linkage to the bacterial cell wall.

EXPERIMENTAL SECTION

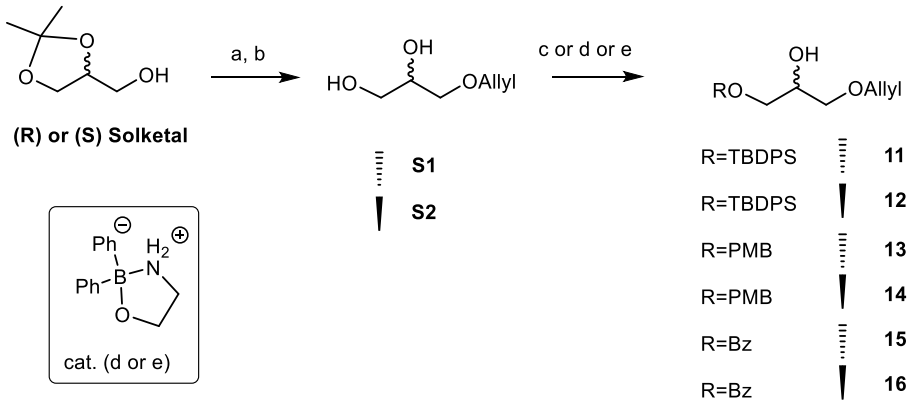
General

All chemicals (Acros, Fluka, Merck, Sigma-Aldrich, etc.) 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 or with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water followed by charring at +/- 140 °C. 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 and the reported value was calculated as the mean of 10 measurements. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker AV 400 (400, 101 and 162 MHz respectively), a Bruker AV 500 (500, 125 and 202 MHz respectively) or a Bruker DMX 850 (850, 214 and 344 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane for both ^1H and ^{13}C. When D_2O or CD_3CN were used, ^1H-NMR were recorded with chemical shift (δ) relative to the proton of residual solvent (4.75 ppm and 1.94 ppm respectively). ^{13}C-NMR spectra were recorded with chemical shift (δ) relative to TMS (external standard) in case of D_2O and 1.32 ppm as residual solvent in CD_3CN.The ^{31}P- NMR spectra were recorded with chemical shift (δ) relative to H_3PO_4. (external standard). High resolution mass

spectra were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at *m/z* 400 (mass range *m/z* = 150-2000) and dioctylphthalate (*m/z* = 391.28428) as a lock mass. High resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Synthesis of acceptors 11-16

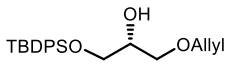
Scheme 2: *Synthetic strategy for synthesis of acceptors* ***11-16****.*



Reagents and conditions: a) AllylBr, NaH, DMF 96%; b) AcOH, H_2O, 50 °C, 300mbar, quant; c) TBDPSCl, Imidazole, DMF, 82% (**11**), 80% (**12**); d) cat., PMBCl, KI, K_2CO_3, ACN, 60 °C, quant (**13**), 96% (**14**); e) cat., BzCl, DIPEA, CAN, 98% (**15**), quant. (**16**).

Note: for experimental procedure and data analysis of steps (a) and (b) see J. Shin, D. H. Thompson, *JOC*, **2003**, 68, 17, 6760-6766

(S)-1-*O*-allyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (11)

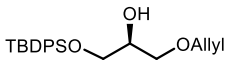


Diol **S1** (0.86 mmol) was diluted in DMF (8.6 ml, 0.1 M) and Imidazole (1 mmol, 1.15 eq) and TBDPSCl (0.86 mmol, 1 eq) were added. After two hours stirring at room temperature, TLC analysis (DCM:MeOH, 95:5) showed complete conversion of the starting material. The reaction mixture was diluted with Et_2O (10 mL) and washed with H_2O (10mL x 3). The aqueous phase was reextracted with Et_2O and the combined organic layers were washed once with brine, dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. Compound **11** was isolated by column chromatography (Pentane:EtOAc, 9:1; R_f: 0.31) as transparent oil in 82% yield (0.71 mmol).

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.70-7.62 (4H, H_{arom}, m), 7.47-7.35 (6H, H_{arom}, m), 5.95-5.82 (1H, H_{allyl}, m), 5.29-5.15 (2H, H_{2_allyl}, m), 4.03-3.97 (2H, CH_{2_allyl}, m), 3.95-3.86 (1H, $CH_{glycerol}$,

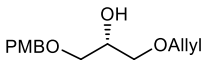
m), 3.71 (2H, $CH_{2\_glycerol}$, J_{CH2-CH}=5.4 Hz, d), 3.58-3.44 (2H, $CH_{2\_glycerol,}$ m), 2.49 (1H, OH, J_{OH-CH}=5.1Hz, d), 1.06 (9H, tBu, s).
^{13}C-NMR (101 MHz, $CDCl_3$), δ: 135.7 (CH_{arom}), 134.7 (CH_{allyl}), 133.1 (C_q), 129.9 (CH_{arom}), 127.9 (CH_{arom}), 117.3 (CH_{allyl}), 72.5 (CH_{2_allyl}), 71.0 ($CH_{2\_glycerol}$), 70.9 ($CH_{glycerol}$), 64.9 ($CH_{2\_glycerol}$), 27.0 (CH_{3_tBu}), 18.9 (C_{q_tBu}).
$([\alpha]_D^{20}$($CHCl_3$): -4.1
HRMS: $C_{22}H_{30}O_3Si$ + Na^+ required 359.1856, found 359.1901.

(R)-1-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-allyl-*sn*-glycerol (12)



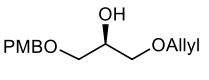
Starting with diol **S2** (1.00 mmol), compound **12** was obtained following the procedure described for **11** in 80% yield (0.80 mmol).
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.70-7.62 (4H, H_{arom}, m), 7.47-7.35 (6H, H_{arom}, m), 5.95-5.82 (1H, H_{allyl}, m), 5.29-5.15 (2H, 2 x H_{allyl}, m), 4.03-3.97 (2H, CH_{2_allyl}, m), 3.95-3.86 (1H, $CH_{glycerol}$, m), 3.71 (2H, $CH_{2\_glycerol}$, J_{CH2-CH}=5.4 Hz, d), 3.58-3.44 (2H, $CH_{2glycerol,}$ m), 2.49 (1H, OH, J_{OH-CH}=5.1Hz, d), 1.06 (9H, tBu, s).
^{13}C-NMR (101 MHz, $CDCl_3$), δ: 135.7 (CH_{arom}), 134.7 (CH_{allyl}), 133.1 (C_q), 129.9 (CH_{arom}), 127.9 (CH_{arom}), 117.3 (CH_{allyl}), 72.5 (CH_{2_allyl}), 71.0 ($CH_{2\_glycerol}$), 70.9 ($CH_{glycerol}$), 64.9 ($CH_{2\_glycerol}$), 27.0 (CH_{3_tBu}), 18.9 (C_{q_tBu})
$[\alpha]_D^{20}$($CHCl_3$): +3.5
HRMS: $C_{22}H_{30}O_3Si$ + Na^+ required 359.1856, found 359.1901.

(R)-1-*O*-allyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (13)



Diol **S1** (1.00 mmol) was coevaporated three times with toluene and dissolved under inert atmosphere in dry ACN (2.5 mL, 0.4 M) and the flask was wrapped in aluminium foil. After 10 minutes stirring, PMBCl (1.10 mmol, 1.1 eq) was added followed by K_2CO_3 (1.10 mmol, 1.1 eq) and KI (1 mmol, 1 eq). The reaction was heated to 60 °C and after stirring overnight TLC analysis (DCM:MeOH; 95:5) showed complete consumption of starting material. The reaction mixture was cooled to r.t., diluted with EtOAc and washed with H_2O. The water layer was extract with EtOAc and the combined organic layers were washed with Brine, dried over MgSO4 and concentrated *in vacuo*. The resulting crude was purified by column chromatography (8:2→7:3 Pentane:EtOAc) yielding **13** as a colorless oil in quantitative yield (1.00 mmol).
TLC analysis: R_f= 0.35 (Pentane:EtOAc; 7:3)
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.28-7.23 (2H, H_{arom}, m), 6.91-6.86 (2H, H_{arom}, m), 5.96-5.84 (1H, H_{allyl}, m), 5.31-5.15 (2H, H_{2_allyl}, m), 4.49 (2H, CH_{2_PMB}, s), 4.04-3.95 (3H, CH_{2_allyl}, $CH_{glycerol}$, m), 3.80 (2H, CH_{3_OMe}, s), 3.57-3.43 (4H, $CH_{2\_glycerol}$, m), 2.46 (1H, OH, J_{OH-CH} =4.2 Hz, d).
^{13}C-NMR(101 MHz, $CDCl_3$), δ: 134.5 (CH_{allyl}), 130.1 (C_q), 129.4 (CH_{arom}), 117.3 (CH_{allyl}), 113.9 (CH_{arom}), 73.1 (CH_{2_PMB}), 72.3 (CH_{2_allyl}), 71.3 ($CH_{2\_glycerol}$), 71.04 ($CH_{2\_glycerol}$), 69.6 ($CH_{2\_glycerol}$), 55.3 (CH_{3_OMe}).
$[\alpha]_D^{20}$($CHCl_3$): -7.1
HRMS: $C_{14}H_{20}O_4$ + Na^+ required 275.1254, found 275.1259

(S)-1-*O*-(4-methoxybenzyl)-3-*O*-allyl-*sn*-glycerol (14)



Starting from diol **S2** (1.00), compound **14** was obtained as colorless oil in 96% yield (0.96 mmol) following the procedure described for compound **13**.

TLC analysis: R_f= 0.35 (Pentane:EtOAc; 7:3)

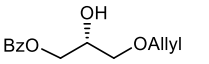
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.28-7.23 (2H, H_{arom}, m), 6.91-6.86 (2H, H_{arom}, m), 5.96-5.84 (1H, H_{allyl}, m), 5.31-5.15 (2H, 2 x H_{allyl}, m), 4.49 (2H, CH_{2_PMB}, s), 4.04-3.95 (3H, CH_{2_allyl}, $CH_{glycerol}$, m), 3.80 (2H, CH_{3_OMe}, s), 3.57-3.43 (4H, $CH_{2\_glycerol}$, m), 2.46 (1H, OH, J=4.2 Hz, d).

^{13}C-NMR(101 MHz, $CDCl_3$), δ: 134.5 (CH_{allyl}), 130.1 (C_q), 129.4 (CH_{arom}), 117.3 (CH_{allyl}), 113.9 (CH_{arom}), 73.1 (CH_{2_PMB}), 72.3 (CH_{2_allyl}), 71.3 ($CH_{2\_glycerol}$), 71.04 ($CH_{2\_glycerol}$), 69.6 ($CH_{2\_glycerol}$), 55.3 (CH_{3_OMe}).

$[\alpha]_D^{20}$($CHCl_3$): +7.5

HRMS: $C_{14}H_{20}O_4$ + Na^+ required 275.1254, found 275.1259

(R)-1-*O*-allyl-3-*O*-benzoyl-*sn*-glycerol (15)



Diol **S1** (10 mmol) was coevaporated with toluene three times and dissolved under inert atmosphere in dry ACN (25 mL, 0.4M). The flask was wrapped in aluminium foil and after ten minutes stirring, BzCl (11 mmol, 1.1 eq), DiPEA (12 mmol, 1.2 eq) and 2-Aminoethyl diphenylborinate (0.1 mmol, 0.01 eq) were subsequently added. The reaction was left to stir at room temperature and after 2h TLC analysis (DCM:MeOH; 95:5) showed complete consumption of starting material. The reaction mixture was diluted with EtOAc and washed with H_2O. The water layer was reextracted with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude was purified by column chromatography (85:15→7:3, pentane:EtOAc) yielding **15** in 98% yield (9.8 mmol).

TLC analysis: R_f= 0.35 (Pentane:EtOAc; 75:25)

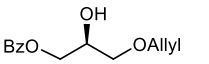
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 8.09-8.03 (2H, H_{arom}, m), 7.61-7.55 (1H, H_{arom}, m), 7.49-7.41 (2H, H_{arom}, m), 5.97-5.85 (1H, H_{allyl}, m), 5.33-5.18 (2H, H_{2_allyl}, m), 4.48-4.36 (2H, $CH_{2\_glycerol}$, m), 4.22-4.11 (1H, $CH_{glycerol}$, m), 4.08-4.03 (2H, CH_{2_allyl}, m), 3.65-3.53 (2H, $CH_{2\_glycerol}$, m), 2.64-2.56 (1H, OH, bs).

^{13}C-NMR(101 MHz, $CDCl_3$), δ: 166.7 (C_q), 134.2 (CH_{allyl}), 133.2 (CH_{arom}), 129.9 (C_q), 129.7 (CH_{arom}), 128.4 (CH_{arom}), 117.6 (CH_{allyl}), 72.5 (CH_{2_allyl}), 710.9 ($CH_{2\_glycerol}$), 69.0 ($CH_{glycerol}$), 66.0 ($CH_{2\_glycerol}$).

$[\alpha]_D^{20}$ ($CHCl_3$) : -5.6

HRMS:$C_{13}H_{16}O_4$ + Na^+ required 259.0941, found 259.1002

(S)-1-*O*-benzoyl-3-*O*-allyl-*sn*-glycerol (16)



Starting from diol **S2** (1 mmol), compound **16** was obtained as colourless oil in quantitative yield (1.00 mmol), following the procedure described for compound **15**.

TLC analysis: R_f= 0.35 (Pentane:EtOAc; 75:25)

^{1}H-NMR (400 MHz, $CDCl_3$), δ: 8.09-8.03 (2H, H_{arom}, m), 7.61-7.55 (1H, H_{arom}, m), 7.49-7.41 (2H, H_{arom}, m), 5.97-5.85 (1H, H_{allyl}, m), 5.33-5.18 (2H, 2 x H_{allyl}, m), 4.48-4.36 (2H,

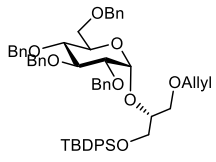
$CH_{2glycerol,}$ m), 4.22-4.11 (1H, $CH_{glycerol}$, m), 4.08-4.03 (2H, CH_{2_allyl}, m), 3.65-3.53 (2H, $CH_{2\_glycerol}$, m), 2.64-2.56 (1H, OH, bs).
^{13}C-NMR(101 MHz, $CDCl_3$), δ: 166.7 (C_q), 134.2 (CH_{allyl}), 133.2 (CH_{arom}), 129.9 (C_{q_arom}), 129.7 (CH_{arom}), 128.4 (CH_{arom}), 117.6 (CH_{allyl}), 72.5 (CH_{2_allyl}), 710.9 ($CH_{2\_glycerol}$), 69.0 ($CH_{glycerol}$), 66.0 ($CH_{2\_glycerol}$).
$[\alpha]_D^{20}$($CHCl_3$): +4.7
HRMS:$C_{13}H_{16}O_4$ + Na^+ required 259.0941, found 259.0993

Glycosylation using TMSI/Ph_3PO.

General procedure

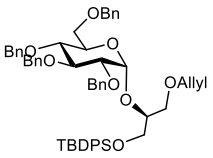
Donor (1 eq) and acceptor (0.75 eq) were co-evaporated three times with toluene. Under argon atmosphere, they were dissolved in dry DCM (0.1M) and after 10 minutes stirring Ph_3PO (6 eq) was added, followed by slow addition of TMSI (1 eq). The reaction mixture was allowed to stir at r.t. overnight. The reaction mixture was diluted with DCM, washed with $Na_2S_2O_3$, H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was subjected to size exclusion gel chromatography (DCM:MeOH=1:1, for purification of the final product obtained as mixture of anomers (unless otherwise stated). The ratio α/β was calculated by ^{1}H-NMR.

(S)-1-*O*-allyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-(*ter*t-butyldiphenylsilyl-*sn*-glycerol (17)



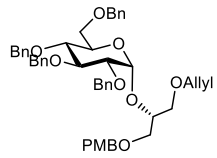
On a scale of 0.10 mmol of donor **10**, following the general procedure, compound **17** was obtained in 72% yield (0.072 mmol) as colourless syrup in a α/β mixture (1.5:1).
TLC analysis: R_f= 0.48 (Pentane:EtOAc; 9:1)
^{1}H-NMR (400 MHz, $CDCl_3$), δ(α): 7.68-7.64 (4H, H_{arom}, m), 7.47-7.07 (26H, H_{arom}, m), 5.94-5.81 (1H, H_{allyl}, m), 5.33-5.07 (3H, 2 x H_{allyl}, H_1 m), 5.02-4.90 (1H, CHH_{Bn}, m), 4.84-4.62 (2H, 3 x CHH_{Bn}, m), 4.68-4.43 (4H, 4 x CHH_{Bn}, m), 4.12-4.05 (1H, H_5, m), 4.01-3.86 (4H, H_3, $CH_{glycerol}$, CH_{2_allyl}, m), 3.82-3.50 (8H, 2 x $CH_{2\_glycerol}$, 2 x H_6, H_4, H_2, m), 1.08 (9H, tBu, s).
^{13}C-NMR (101 MHz, $CDCl_3$), δ(α): 139.1, 138.6, 138.2 (C_q), 135.7, 135.7 (CH_{arom}), 134.9 (CH_{allyl}), 133.1 (C_q), 129.9, 128.5, 128.1, 127.9, 127.8, 127.7 (CH_{arom}), 116.7 (CH_{2_allyl}), 96.2 (C_1), 82.1 (C_3), 79.9 (C_2), 77.8 (C_4), 77.3 ($CH_{glycerol}$), 75.8, 75.1, 73.6, 73.0 (CH_{2_Bn}), 72.2 (CH_{2_Allyl}), 70.4 (C_6), 70.2 (C_5), 68.8, 63.2 ($CH_{2\_glycerol}$), 27.0 (CH_{3_tBu}), 19.4 (C_q).
$[\alpha]_D^{20}$($CHCl_3$): +26.3
HRMS: $C_{56}H_{64}O_8Si$ + Na^+ required 915.4263, found 915.4265

(R)-1-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (18)



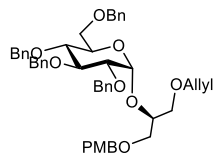
On a scale of 0.1 mmol of donor **10**, following the general procedure described above, compound **18** was isolated as colourless oil in a mixture of α/β anomers (>10:1) in 68% yield (0.068 mmol).
Analytical data in accordance with the one reported in: W. F. J. Hogendorf, L. J. van den Bos, H. S. Overkleeft, J. D. C. Codee, G. A. van der Marel, *Bioorg, Med. Chem.,* **2010**, 18, 3668-3678.

(R)-1-*O*-allyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (19)



On a scale of 0.1 mmol of donor **10**, following the general procedure, compound **19** was obtained in 65% yield (0.065 mmol) as colourless syrup in a α/β mixture (>10:1).
TLC analysis: R_f= 0.34 (Pentane:EtOAc; 8:2)
^{1}H-NMR (101 MHz, $CDCl_3$), δ(α): 7.42-7.22 (20H, H_{arom}, m), 7.203-7.12 (2H, H_{arom}, m), 6.90-6.84 (2H, H_{arom}, m), 5.94-5.81 (1H, H_{allyl}, m), 5.30-5.12 (3H, 2 x H_{allyl}, H_1, m), 5.01 (1H, CH*H*$_{Bn}$, J=10.9 Hz, d), 4.88-4.78 (2H, 2 x CH*H*$_{Bn}$, m), 4.71-4.61 (3H, 2 x CH*H*$_{Bn}$, m) 4.54-4.45 (4H, 4 x CH*H*$_{Bn}$, m), 4.17-4.08 (1H, $CH_{glycerol}$, m), 4.07-3.93 (4H, H_5, H_3, CH_{2_allyl}, m), 3.81 (3H, CH_{3_OMe}, s), 3.76 (1H, CH*H*$_{glycerol}$, $J_{CHH\text{-}CHH}$=10.7 Hz, $J_{CHH\text{-}CH}$=3.4 Hz, dd), 3.72-3.51 (7H, CH*H*$_{glycerol}$, $CH_{2\_glycerol}$, 2 x H_6, H_4, H_2, m).
^{13}C-NMR(400 MHz, $CDCl_3$), δ(α): 139.0, 138.5, 138.2, 138.0 (C_q), 134.7 (CH_{allyl}), 130.3 (C_q), 129.2, 128.3, 128.0 x 2, 127.9 x 2, 127.7, 127.6 x 2, 127.5 (CH_{arom}), 117.0 (CH_{2_allyl}), 96.2 (C_1), 81.9 (C_3), 79.5 (C_2), 77.7 (C_4), 75.7, 75.0 (CH_{2_Bn}), 74.7 ($CH_{glycerol}$), 73.5, 73.0, 72.3 (CH_{2_Bn}), 72.2 x 2 (CH_{2_Allyl}, CH_{2_Bn}), 70.4 ($CH_{2\_glycerol}$), 70.2 (C_5), 69.7 (C_6), 55.3 (CH_{3_OMe})
$[\alpha]_D^{20}$($CHCl_3$): +31.2
HRMS: $C_{48}H_{54}O_9$ + Na^+ required 9797.3660, found 797.3667

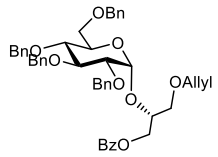
(S)-1-*O*-(4-methoxybenzyl)-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (20)



On a scale of 0.1 mmol of donor **10**, following the general procedure, compound **20** was obtained in 66% (0.066 mmol) yield as colourless syrup in a α/β mixture (9:1).
TLC analysis: R_f= 0.34 (Pentane:EtOAc; 8:2)
^{1}H-NMR (400 MHz, $CDCl_3$), δ(α): 7.39-7.16 (20H, H_{arom}, m), 7.15-7.08 (2H, H_{arom}, m), 6.80-6.73 (2H, H_{arom}, m), 5.93-5.81 (1H, H_{allyl}, m), 5.29-5.11 (3H, 2 x H_{allyl}, H_1, m), 4.98 (1H, CH*H*$_{Bn}$, J=10.8 Hz, d), 4.84-4.77 (2H, 2 x CH*H*$_{Bn}$, m), 4.74 (1H, CH*H*$_{Bn}$, J=12.0 Hz, d), 4.69 (1H, CH*H*$_{Bn}$, J=12.0 Hz, d), 4.57 (1H, CH*H*$_{Bn}$, J=12.1 Hz, d), 4.48-4.34 (4H, 4 x CH*H*$_{Bn}$, m), 4.12-3.94 (5H, $CH_{glycerol}$, H_5, H_3, CH_{2_allyl}, m), 3.79-3.71 (4H, CH*H*$_{glycerol}$, CH_{3_OMe}, s), 3.67-3.51 (6H, 2 x CH*H*$_{glycerol}$, 2 x H_6, H_4, H_2, m), 3,45 (1H, CH*H*$_{glycerol}$, $J_{CHH\text{-}CHH}$=10.6 Hz, $J_{CHH\text{-}CH}$=2,1 Hz, dd),
^{13}C-NMR(101 MHz, $CDCl_3$), δ(α): 139.1, 138.7, 138.5, 138.2 (C_q), 134.8 (CH_{allyl}), 130.4 (C_q), 129.6, 128.5 x 3, 128.2, 128.1, 128.0, 127.8 x 2, 127.7, 127.6 (CH_{arom}), 117.1 (CH_{2_allyl}), 113.8 (CH_{arom}), 96.3 (C_1), 82.1 (C_3), 79.8 (C_2), 77.8 (C_4), 75.8, 75.1 (CH_{2_Bn}), 74.8

($CH_{glycerol}$), 73.6, 73.1, 72.6 (CH_{2_Bn}), 72.5 (CH_{2_Allyl}), 70.8, 70.3 ($CH_{2\_glycerol}$), 70.2 (C_5), 69.6 (C_6), 55.4 (CH_{3_OMe})
$[\alpha]_D^{20}$($CHCl_3$): +19.4
HRMS: $C_{48}H_{54}O_9$ + Na^+ required 797.3660, found 797.3664

(R)-1-*O*-allyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-benzoyl-*sn*-glycerol (21)

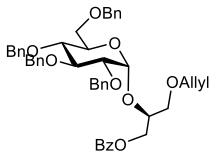


On a scale of 15 mmol of donor **10**, following the general procedure and leaving the reaction stirring for 3 days, compound **21** was obtained in 86% yield (12.9 mmol) as colourless syrup (no presence of β anomer was detected).

TLC analysis: R_f= 0.31 (Pentane:EtOAc; 8:2)

^{1}H-NMR (400 MHz, $CDCl_3$), δ(α): 8.05-8.00 (2H, H_{arom}, m), 7.57-7.52 (1H, H_{arom}, m), 7.43-7.23 (15H, H_{arom}, m), 7.18 (5H, H_{arom}, s), 7.15-7.10 (2H, H_{arom}, m), 5.90-5.79 (1H, H_{allyl}, m), 5.28-5.12 (3H, 2 x H_{allyl}, H_1, m), 4.95 (1H, CH*H*$_{Bn}$, J=10.8 Hz, d), 4.86-4.76 (2H, 2 x CH*H*$_{Bn}$, m), 4.65-4.58 (3H, 3 x CH*H*$_{Bn}$, m), 4.54 (1H, CH*H*$_{glycerol}$, $J_{CH\textit{H}\text{-}\textit{CH}H}$=10.8 Hz, $J_{CH\textit{H}\text{-}CH}$=4.03 Hz, dd), 4.50-4.40 (3H, 2 x CH*H*$_{Bn}$, CH*H*$_{glycerol}$, m*),* 4.28-4.19 (1H, $CH_{glycerol}$, m), 4.04-3.94 (4H, H_5, H_3, CH_{2_allyl}, m), 3.78-3.55 (6H, $CH_{2\_glycerol}$, 2 x H_6, H_4, H_2, m).
^{13}C-NMR(101 MHz, $CDCl_3$), δ(α): 166.5 (C_q), 139.0, 138.5, 138.1, 138.0 (C_q), 134.5 (CH_{allyl}), 133.2 (CH_{arom}), 130.0 (C_q), 129.8, 128.7, 128.6, 128.5, 128.4, 128.0 x 2, 127.9, 127.8 x 2, 127.7, 127.1 (CH_{arom}, 117.4 (CH_{2_allyl}), 96.4 (C_1), 82.0 (C_3), 79.8 (C_2), 77.7 (C_4), 75.7, 75.2 (CH_{2_Bn}), 73.9 ($CH_{glycerol}$), 73.7, 72.9 (CH_{2_Bn}), 72.4 (CH_{2_Allyl}), 70.6 (C_5), 70.0 (C_6), 68.6, 64.7 ($CH_{2\_glycerol}$).
$[\alpha]_D^{20}$($CHCl_3$): +22.4
HRMS: $C_{47}H_{50}O_9$ + Na^+ required 781.3347, found 781.3354

(S)-1-*O*-benzoyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (22)



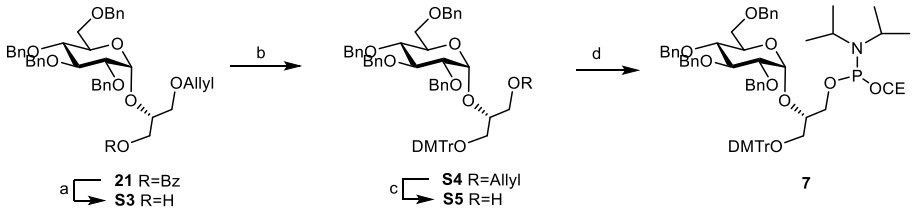
On a scale of 0.1 mmol of donor **10**, following the general procedure, compound **22** was obtained in 70% yield (0.070 mmol) as colourless syrup in a α/β mixture (6:1).

TLC analysis: R_f= 0.31 (Pentane:EtOAc; 8:2)

^{1}H-NMR (400 MHz, $CDCl_3$), δ(α): 8.02-7.97 (2H, H_{arom}, m), 7.53-7.47 (1H, H_{arom}, m), 7.39-7.17 (20H, H_{arom}, m), 7.11-7.03 (2H, H_{arom}, m), 5.94-5.81 (1H, H_{allyl}, m), 5.31-5.15 (3H, 2 x H_{allyl}, H_1, m), 4.97 (1H, CH*H*$_{Bn}$, J=10.8 Hz, d), 4.84-4.76 (2H, 2 x CH*H*$_{Bn}$, m), 4.73 (2H, CH_{2_Bn}, J=2.9 Hz, d) 4.58-4.49 (2H, CH*H*$_{Bn}$, CH*H*$_{glycerol}$, m), 4.44-4.34 (2H, CH*H*$_{Bn}$, CH*H*$_{glycerol}$, m), 4.28 (1H, CH*H*$_{Bn}$, J=12.1 Hz, d), 4.26-4.20 (1H, $CH_{glycerol}$, m), 4.05-3.94 (4H, H_5, H_3, CH_{2_allyl}, m), 3.71-3.53 (4H, 2 x H_6, H_4, H_2, m). 3.49 (1H, *CH*H$_{glycerol}$, $J_{CH\textit{H}\text{-}\textit{CH}H}$ =10.6 Hz, $J_{CH\textit{H}\text{-}CH}$=3.1 Hz, dd), 3.34 (1H, *CH*H$_{glycerol}$, $J_{CH\textit{H}\text{-}CH}$=2.1 Hz, dd).
^{13}C-NMR(101 MHz, $CDCl_3$), δ(α): 166.4 (C_q), 139.0, 138.5, 138.4, 137.9 (C_q), 134.5 (CH_{allyl}), 133.1 ()CH_{arom}), 130.0 (C_q), 129.8, 128.6 x 2, 128.5 x 2, 128.4, 128.2, 128.1, 128.0 x 2, 127.8, 127.7 x 2, (CH_{arom}), 117.4 (CH_{2_allyl}), 96.3 (C_1), 82.0 (C_3), 79.7 (C_2), 77.6 (C_4), 75.8, 75.1 (CH_{2_Bn}), 73.8 ($CH_{glycerol}$), 73.6, 72.8 (CH_{2_Bn}), 72.5 (CH_{2_Allyl}), 70.5 (C_5), 69.8 (C_6), 68.2, 65.2 ($CH_{2\_glycerol}$).
$[\alpha]_D^{20}$($CHCl_3$): +35.6

HRMS: $C_{47}H_{50}O_9$ + Na^+ requires 781.3347, found 781.3357

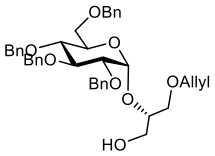
Synthesis of building block 7 from 21



*Scheme S1: Synthetic strategy towards compound **7**.* a) $Na_{(s)}$, MeOH, quant.; b) DMTrCl, TEA, DCM, 88%; c) (i) $Ir(COD)(PPh_2Me)_2PF_6$, H_2, THF; (ii) $NaHCO_3$(aq), I_2, THF, 92%; d) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, TEA, DCM, 70%.

(R)-1-*O*-allyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-*sn*-glycerol (S3)

Compound **21** (12 mmol) was dissolved in dry MeOH (60 mL, 0,2 M) and a piece of $Na_{(s)}$ was added. The reaction was stirred for 1 hour, until TLC analysis (Pentane:EtOAc, 8:2) showed complete consumption of the starting material. The reaction mixture was neutralized by addition of Amberlite IR-120 (H^+ form), filtered and concentrated *in vacuo*. The product was obtained quantitatively (12 mmol) and used directly in the subsequent step without further purification.



TLC analysis: R_f= 0.32 (Pentane:EtOAc; 7:3)

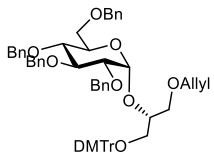
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.41-7.20 (18H, H_{arom}, m), 7.19-7.07 (2H, H_{arom}, m), 5.90-5.75 (1H, H_{allyl}, m), 5.22 (1H, CHH_{allyl}, J = 17.3, 1.7 Hz, dd), 5.14 (1H, CHH_{allyl}, J = 10.4, 1.8 Hz, dd), 4.98-4.89 (2H, H_1, CHH_{Bn}, m), 4.89-4.75 (3H, 3 x CHH_{Bn}, m), 4.67 (1H, CHH_{Bn}, J=11.6 Hz, d), 4.60 (1H, CHH_{Bn}, J=12.1 Hz, d), 4.52-4.41 (2H,2 x CHH_{Bn}, m), 4.05-3.87 (4H, H_3, H_5, $CH_{2\text{-Allyl}}$, m), 3.87-3.78 (1H, $CH_{glycerol}$, m), 3.77-3.38 (8H, 2 x H_6, H_4, H_2, 2 x $CH_{2\_glycerol}$ m), 3.17-3.04 (1H, OH, bs).

^{13}C-NMR(101 MHz, $CDCl_3$), δ: 138.8, 138.3, 138.0, 137.7 (C_q), 134.6 (CH_{allyl}), 128.7, 128.5 x 3, 128.4 x 2, 128.3, 128.2, 128.1 x 2, 128.0 x 2, 127.9, 127.8 x 2, 127.7 (CH_{arom}), 117.3 (CH_{2_allyl}), 98.8 (C_1), 82.4 (C_3), 79.9 (C_2, $CH_{glycerol}$), 77.9 (C_4), 75.7, 75.2, 74.2, 73.6 (CH_{2_Bn}), 72.4 (CH_{2_Allyl}), 70.8 (C_5), 70.3 (C_6), 68.5, 63.0 ($CH_{2\_glycerol}$).

$[\alpha]_D^{20}$($CHCl_3$): +26.7

HRMS: $C_{40}H_{46}O_8$ + H^+ required 655.3265, found 655.3271

(R)-1-*O*-allyl-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*(4,4'-dimethoxytrityl)-*sn*-glycerol (S4)



Compound **S3** (12 mmol) was dissolved in dry DCM (60mL, 0.2M) and under inert atmosphere Et_3N (18 mmol, 1.5eq) and DMTrCl (13.8 mmol, 1.15 eq) were added. The reaction mixture stirred for 3 hours until TLC analysis (Pentane:EtOAc:Et_3N, 7:3:0.1) showed complete consumption of starting material. The reaction was quenched by addition of MeOH (1 mL), diluted with DCM and washed with a 1:1 mixture of $NaHCO_3$ and brine. The aqueous layer was extracted with DCM twice and the combined organic layer were dried with $Na_2S_2O_4$, filtered and concentrated *in vacuo*. Compound **S4** was isolated in 88% yield (10.6 mmol) after column chromatography (Pentane:EtOAc:Et_3N, 97:2:1→80:19:1).

TLC analysis: R_f= 0.31 (Pentane:EtOAc; 8:2)

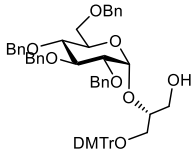
^{1}H-NMR (400 MHz, $CDCl_3$), δ: 7.47-7.38 (2H, H_{arom}, m), 7.37-7.04 (27H, H_{arom}, m), 6.84-6.73 (4H, H_{arom}, m), 5.90-5.73 (1H, H_{allyl}, m), 5.36-5.06 (3H, 2 x H_{allyl}, H_1, m), 4.95 (1H, CH*H*$_{Bn}$, J=10.7 Hz, d), 4.86-4.73 (2H, 2 x CH*H*$_{Bn}$, m), 4.64 (1H, CH*H*, J=12.0 Hz, d) 4.58-4.53 (2H, CH*H*$_{Bn}$, m), 4.51-4.41 (2H, 2 x CH*H*$_{Bn}$, m), 4.19-4.01 (2H, $CH_{glycerol}$, H_5, m), 4.01-3.86 (3H, H_3, CH_{2_allyl}, m), 3.81-3.43 (12H, 2 x CH_{3_OMe}, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$, m). 3.25 (2H, $CH_{2\_glycerol}$, $J_{CH\textit{H}-CH}$=5.7 Hz, dd).

^{13}C-NMR(101 MHz, CD_3CN), δ: 159.6, 146.2, 140.1, 139.8, 139.6, 139.5, 137.0 x 2 (C_q), 136.1 (CH_{allyl}), 131.0, 130.0, 129.3 x 2, 129.2 x 3, 129.0, 128.9 x 2, 128.8 x 4, 128.4 x 2, 127.8 (CH_{arom}), 118.3 (CH_{2_allyl}), 114.0 (CH_{arom}), 97.0 (C_1), 82.5 (C_3), 81.0 (C_2), 78.9 (C_4), 76.8 ($CH_{glycerol}$), 76.09, 75.5, 73.9, 72.9 (CH_{2_Bn}), 72.6 (CH_{2_Allyl}), 71.4 (C_5), 71.2 (C_6), 70.1, 64.5 ($CH_{2\_glycerol}$), 55.9 (2 x CH_{3_OMe}).

$[\alpha]_D^{20}$($CHCl_3$): +21.8

HRMS: $C_{61}H_{60}O_{10}$ + Na^+ required 979.4392, found 979.4401

(R)-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*(4,4'-dimethoxytrityl)-*sn*-glycerol (S5)



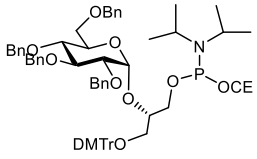
Compound **S4** (10.2 mmol) was dissolved in freshly distilled dry THF (68 mL, 0.15 M). After bubbling $Ar_{(g)}$ for 20 minutes, $Ir(COD)(PPh_2Me)PF_6$ (0,1 mmol, 0.01 eq) was added to the reaction mixture. $Ar_{(g)}$was bubbled for 10 minutes, followed by $H_{2(g)}$ purge for not more than 10 seconds, after which a change in the catalyst colour was observed from red to yellow. After 1 hour TLC analysis (Pentane:Toluene:EtOAc, 85:5:10) showed complete conversion of the starting material to the isomerized intermediate. The reaction mixture was diluted with THF (20 mL) and a sat. aq. solution of $NaHCO_3$ (20 mL) was added together with I_2 (15.9 mmol, 1.6 eq). TLC analysis showed complete consumption of the isomerized intermediate after 18 hours of stirring and the reaction mixture was diluted with EtOAc and washed with $NaS_2O_{3(sat)(aq)}$, $NaHCO_{3(sat.)(aq)}$, H_2O and brine. The organic layer was dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. The desired product **S5** was isolated after purification with column chromatography (Pentane:EtOAc:Et_3N, 70:25:5) in 92% yield (9.4 mmol) as colourless syrup.

TLC analysis: R_f= 0.31 (Pentane:EtOAc; 8:2)

^{1}H-NMR (400 MHz, CD_3CN), δ: 7.50-7.44 (2H, H_{arom}, m), 7.38-7.13 (27H, H_{arom}, m), 6.84-6.74 (4H, H_{arom}, m), 5.17 (1H, H_1, J=3.6 Hz, d), 4.92 (1H, CH*H*$_{Bn}$, J=11.0 Hz, d), 4.83-4.74

(2H, 2 x CHH_{Bn}, m), 4.64-4.47 (5H, CHH_{Bn}, m), 4.05-3.98 (1H, H_5, m), 3.94-3.82 (2H, H_3, $CH_{glycerol}$, m), 3.75-3.63 (9H, 2 x CH_{3_OMe}, 2 x H_6, CH$H_{glycerol}$, m). 3.62-3.53 (1H, CH$H_{glycerol}$, m), 3.53-3.44 (2H, H_4, H_2, m), 3.25-3.13 (2H, $CH_{2\_glycerol}$, m), 3.04-2.97 (1H, OH, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 158.6, 145.3, 139.2, 138.7, 138.5 x 2, 136.1, 136.0 (C_q), 130.1 x 2, 128.4, 128.3 x 2, 128.1, 128.0, 127.9 x 2, 127.8, 127.7, 127.6 x 2, 127.5, 126.8 (CH_{arom}), 96.3 (C_1), 81.6 (C_3), 80.1 (C_2), 78.9 ($CH_{glycerol}$), 78.1 (C_4), 75.1, 74.7, 72.9, 72.0 (CH_{2_Bn}), 70.6 (C_5), 69.1 (C_6), 63.6, 62.5 ($CH_{2\_glycerol}$), 54.9 (2 x CH_3).
$[\alpha]_D^{20}$($CHCl_3$): +27.3
HRMS: $C_{58}H_{60}O_{10}$ + Na^+ required 939.4079, found 939.4090

(S)-1-*O*-([*N,N*-diisopropyl]-2-cyanoethyl-phosphoramidite)-2-*O*-(2,3,4,6-*O*-benzyl-α-D-glucopyranosyl)-3-*O*(4,4'-dimethoxytrityl)-*sn*-glycerol (7)



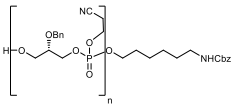
Compound **S5** (8.5 mmol) was dissolved in dry DCM (85 mL, 0.1 M) and Et_3N (12.75 mmol, 1.5 eq) was added. At 0 °C 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (10.2 mmol, 1.2 eq) was added and the reaction was left for 2 hours after which TLC analysis (Pentane:EtOAc:Et_3N, 7:3:0,1) showed complete consumption of the starting material. After diluting the reaction mixture with DCM, a wash with a mixture of $NaHCO_3$ and brine (1:1) was performed and the organic layer was dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. The desired product was purified by column chromatography (Pentane:EtOAc:Et_3N, 90:19:1 → 75:25:0), affording compound **7** in 70% (5.95 mmol) as a colourless oil.
Analytical data in accordance with the one reported in W. F. J. Hogendorf, L. J. van den Bos, H. S. Overkleeft, J. D. C. Codee, G. A. van der Marel, *Bioorg, Med. Chem.,* **2010**, 18, 3668-3678.

Phosphoramidite couplings

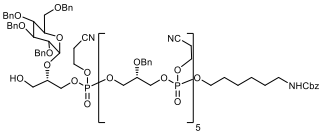
General procedure

The starting material alcohol is co-evaporated three times with dry ACN. Once dissolved in dry ACN (0.1M), a solution of DCI in ACN (0.25 M, 1.5-2.5 eq) is added together with 3Å MS and the reaction mixture is stirred for 15 min at room temperature. A solution of phosphoramidite **7** or **8** (0.176 M in ACN) is added (1.2-2.0 eq) under inert atmosphere. After TLC analysis shows complete consumption of starting material, a solution of CSO (0.5 M in ACN) is added (2.0-3.0 eq) and the reaction is allowed to stir at r.t. for 15 min, after which the reaction is diluted with EtOAc and washed once with a mixture of $NaHCO_3$ and brine (1:1). The organic layer is dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. The crude is then dissolved in DCM (0.1 M) and a solution of TCA (0.18 M in DCM) is added (5 eq). Once TLC analysis show complete conversion to a lower running spot, the reaction mixture is diluted in DCM and washed with a solution of $NaHCO_3$ and brine (1:1), dried over $Na_2S_2O_4$, filtered and concentrated *in vacuo*. The desired product is isolated by column chromatography.

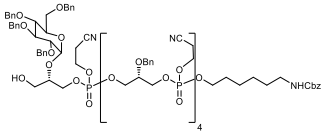
List of intermediates from phoshoramidite couling



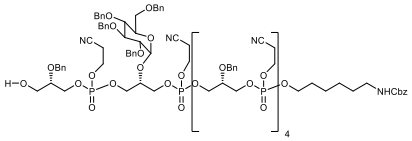
n=1, **S6**
n=2, **S7**
n=3, **S8**
n=4, **S9**
n=5, **S10**



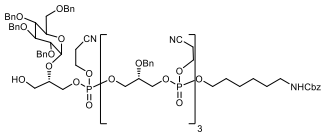
23



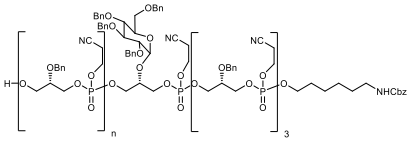
S11



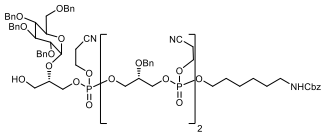
24



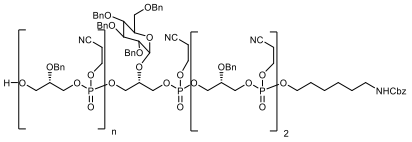
S12



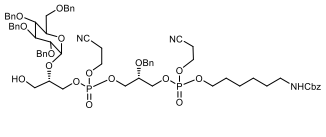
n=1, **S13**
n=2, **25**



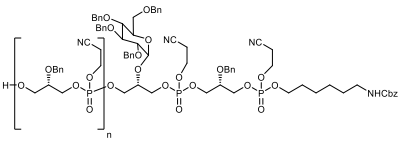
S14



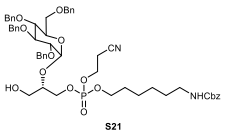
n=1, **S15**
n=2, **S16**
n=3, **26**



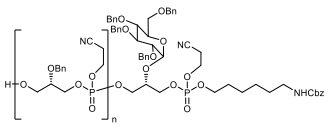
S17



n=1, **S18**
n=2, **S19**
n=3, **S20**
n=4, **27**

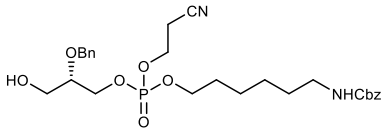


S21



n=1, **S22**
n=2, **S23**
n=3, **S24**
n=4, **S25**
n=5, **28**

(Protected) (GroP)-Spacer or Monomer S6



Alcohol spacer **9** (1.1 mmol) was coupled with phosphoramidite **8** (1.67 mmol, 1.5 eq) following the general procedure. Compound **S6** was obtained after column chromatography (DCM:Acetone, 7.5:2.5) in 90% yield (0.99 mmol).

TLC analysis, R_f:0.48 (DCM:Acetone, 7:3)

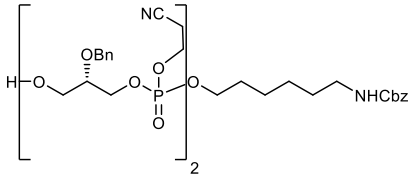
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.42-7.23 (10H, H_{arom}, m), 5.68-5.54 (1H, NH, b), 5.03 (2H, CH_{2_Cbz}, s), 4.63 (2H, CH_{2_Bn}, s), 4.25-3.97 (6H, CH_{2_OCE}, $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.71-3.54 (3H, $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.07 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 3.02-2.92 (1H, OH, b), 2.78-2.68 (2H, CH_{2_OCE}, m), 1.68-1.57 (2H, CH_{2_spacer}, m), 1.51-1.23 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.4, 139.6, 138.4 (C_q), 129.4, 129.2, 128.8, 128.7, 128.6, 128.5 (CH_{arom}), 118.6 (C_q), 79.2-79.1 ($CH_{glycerol}$), 72.4 (CH_{2_Bn}), 69.0 ($CH_{2\_Ospacer}$), 67.6-67.5 ($CH_{2\_glycerol}$), 66.7 (CH_{2_Cbz}), 63.2-63.1 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.32, -1.29

HRMS: $C_{27}H_{37}N_2O_8P$ + H^+ required 549.2360, found 549.2361

(Protected) (GroP)$_2$-Spacer or Dimer S7



Alcohol **S6** (0.75 mmol) was coupled with phosphoramidite **8** (1.1 mmol, 1.5 eq) following the general procedure. Compound **S7** was obtained after column chromatography (DCM:Acetone, 6.5:3.5) in quantitative yield (0.75 mmol).

TLC analysis, R_f: 0.43 (DCM:Acetone, 6:4)

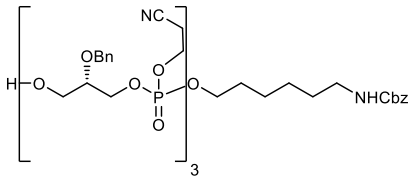
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.23 (15H, H_{arom}, m), 5.95-5.86 (1H, NH, b), 5.06 (2H, CH_{2_Cbz}, s), 4.71-4.58 (4H, CH_{2_Bn}, m), 4.34-3.98 (12H, 2 x CH_{2_OCE}, 3 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.93-3.81 (1H, $CH_{glycerol}$, m), 3.72-3.56 (3H, $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.50-3.35 (1H, OH, b), 3.09 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 2.77-2.67 (2H, 2 x CH_{2_OCE}, m), 1.71-1.55 (2H, CH_{2_spacer}, m), 1.52-1.22 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.6, 139.1 (C_q), 129.4, 129.3, 128.9, 128.8, 128.7, 128.5 (CH_{arom}), 118.6 (C_q), 79.1-79.0 ($CH_{glycerol}$), 76.8-76.7 ($CH_{glycerol}$), 72.7, 72.4 (CH_{2_Bn}), 69.1-69.0 ($CH_{2\_Ospacer}$), 67.0-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.34, -0.33, -0.31, -0.13, -0.10.

HRMS $C_{40}H_{53}N_3O_{13}P_2$ + H^+ required 846.3126, found 846.3119

(Protected) (GroP)$_3$-Spacer or Trimer S8



Alcohol **S7** (0.83 mmol) was coupled with phosphoramidite **8** (1.4 mmol, 1.7 eq) following the general procedure. Compound **S8** was obtained after column chromatography (DCM:Acetone, 6:4) in 97% yield (0.80 mmol).

TLC analysis, R_f: 0.39 (DCM:Acetone, 6.5:3.5)

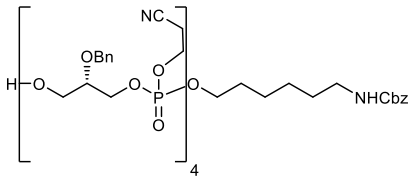
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.23 (20H, H_{arom}, m), 5.95-5.86 (1H, NH, b), 5.06 (2H, CH_{2_Cbz}, s), 4.71-4.58 (6H, CH_{2_Bn}, m), 4.34-3.98 (18H, 3 x CH_{2_OCE}, 5 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.93-3.81 (2H, 2 x $CH_{glycerol}$, m), 3.72-3.56 (3H, $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.09 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 3.10-2.94 (1H, OH, b), 2.77-2.67 (6H, 3 x CH_{2_OCE}, m), 1.71-1.55 (2H, CH_{2_spacer}, m), 1.52-1.22 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.6, 139.1 (C_q), 129.4, 129.3, 128.9, 128.8, 128.7, 128.5 (CH_{arom}), 118.6 (C_q), 79.1-79.0 ($CH_{glycerol}$), 76.8-76.7 ($CH_{glycerol}$), 72.7, 72.4 (CH_{2_Bn}), 69.1-69.0 ($CH_{2\_Ospacer}$), 67.0-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.48, -1.47, -1.43, -1.42, -1.41, -1.23, -1.21, -1.19.

HRMS: $C_{53}H_{69}N_4O_{18}P_3$ + H^+ required 1143.3893, found 1143.3900

(Protected) (GroP)$_4$-Spacer or Tetramer S9



Alcohol **S8** (0.12 mmol) was coupled with phosphoramidite **8** (0.24 mmol, 2 eq) following the general procedure. Compound **S9** was obtained after column chromatography (DCM:Acetone, 1:1) in 83% yield (0.1 mmol).

TLC analysis, R_f:0.32 (DCM:Acetone, 1:1)

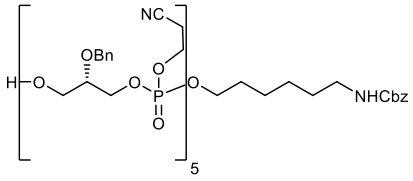
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.23 (25H, H_{arom}, m), 5.82-5.69 (1H, NH, b), 5.03 (2H, CH_{2_Cbz}, s), 4.67-4.54 (8H, CH_{2_Bn}, m), 4.34-3.98 (24H, 4 x CH_{2_OCE}, 7 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.93-3.81 (3H, 3 x $CH_{glycerol}$, m), 3.72-3.56 (3H, $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.23-3.13 (1H, OH, b), 3.07 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 2.77-2.67 (8H, 4 x CH_{2_OCE}, m), 1.71-1.55 (2H, CH_{2_spacer}, m), 1.52-1.22 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.6, 139.1 (C_q), 129.4 x 2, 129.3, 128.9 x 2, 128.8 x 2, 128.7, 128.5 (CH_{arom}), 118.6 (C_q), 79.1-79.0 ($CH_{glycerol}$), 76.8-76.7 ($CH_{glycerol}$), 72.7, 72.4 (CH_{2_Bn}), 69.1-69.0 ($CH_{2\_Ospacer}$), 67.0-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.66, -1.64, -1.62, -1.60, -1.58, -1.40, -1.37.

HRMS: $C_{66}H_{85}N_5O_{23}P_4$ + H^+ required 1440.4659, found 1440.4656

(Protected) (GroP)$_5$-Spacer or Pentamer S10



Alcohol **S9** (35 µmol) was coupled with phosphoramidite **8** (86 µmol, 2.5 eq) following the general procedure. Compound **S10** was obtained after column chromatography (DCM:Acetone, 1:1) in 65% yield (23 µmol).

TLC analysis, R_f: 0.27 (DCM:Acetone, 6:4)

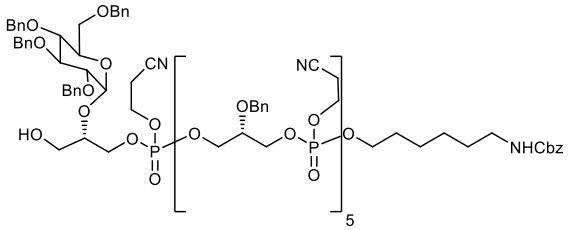
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.23 (30H, H_{arom}, m), 5.82-5.69 (1H, NH, b), 5.03 (2H, CH_{2_Cbz}, s), 4.67-4.54 (10H, CH_{2_Bn}, m), 4.34-3.98 (30H, 5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.93-3.81 (4H, 4 x $CH_{glycerol}$, m), 3.72-3.56 (3H, $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.20-3.02 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.67 (10H, 5 x CH_{2_OCE}, m), 1.71-1.55 (2H, CH_{2_spacer}, m), 1.52-1.22 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.6, 139.1 (C_q), 129.4 x 2, 129.3, 128.9 x 2, 128.8 x 2, 128.7, 128.5 (CH_{arom}), 118.6 (C_q), 79.1-79.0 ($CH_{glycerol}$), 76.8-76.7 ($CH_{glycerol}$), 72.7, 72.4 (CH_{2_Bn}), 69.1-69.0 ($CH_{2\_Ospacer}$), 67.0-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.67, -1.64, -1.63, -1.61, -1.58, -1.44, -1.40, -1.37.

HRMS: $C_{79}H_{101}N_6O_{28}P_5$ + H^+ required 1737.5425, found 1737.5428

(Protected) (GlcGroP)(GroP)$_5$-Spacer or Hexamer 23



Alcohol **S10** (22 µmol) was coupled with phosphoramidite **7** (32 µmol, 1.5 eq) following the general procedure. Compound **23** was obtained after column chromatography (DCM:Acetone, 1:1) in 65% yield (14 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

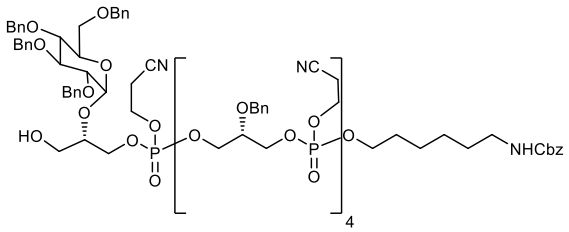
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.45-7.16 (50H, H_{arom}, m), 5.79-5.69 (1H, NH, b), 5.21-5.14 (1H, H_1, m), 5.06 (2H, CH_{2_Cbz}), 4.91-4.45 (18H, CH_{2_Bn}, m), 4.31-3.99 (36H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.99-3.78 (8H, 6 x $CH_{\_glycerol}$, H_5, H_3, m), 3.78-3.48 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.28-3.27 (1H, OH, b), 3.09 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 2.78-2.58 (12H, 6 x CH_{2_OCE}, m), 1.71-1.55 (2H, CH_{2_spacer}, m), 1.52-1.22 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.8, 139.6, 139.1 (C_q), 129.4 x 2, 129.3 X 2, 129.1 x 2, 129.0, 128.9, 128.8 x 2, 128.7, 128.6, 128.4 (CH_{arom}), 118.6 (C_q), 98.4 (C_1), 82.5 (C_3), 81.0 (C_2), 78.7 (C_4), 77.9 ($CH_{glycerol}$), 77.9 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 72.7 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 66.8-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.5 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.9-30.8, 30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.66, -1.63, -1.62, -1.60, -1.58, -1.44, -1.38.

HRMS: $C_{119}H_{145}N_7O_{38}P_6$ + H^+ required 2466.8128, found 2466.8129

(Protected) (GlcGroP)(GroP)$_4$-Spacer or Pentamer S11



Alcohol **S9** (35 µmol) was coupled with phosphoramidite **7** (53 µmol, 1.5 eq) following the general procedure. Compound **S11** was obtained after column chromatography (DCM:Acetone, 1:1) in 67% yield (24 µmol).

TLC analysis, R_f: 0.33 (DCM:Acetone, 6:4)

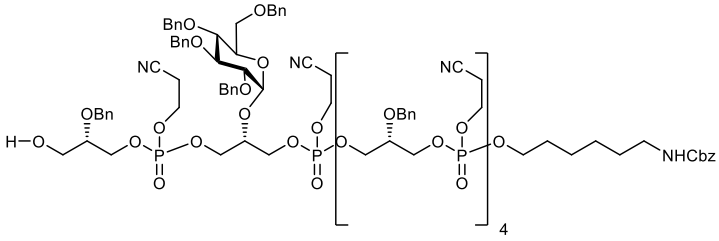
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.45-7.11 (45H, H_{arom}, m), 5.71-5.58 (1H, NH, b), 5.16 (1H, H_1, J=3.6 Hz, d), 5.01 (2H, CH_{2_Cbz}, s), 4.89-4.81 (1H, CH_{2_Bn}, m), 4.80-4.66 (3H, CH_{2_Bn}, m), 4.66-4.43 (12H, CH_{2_Bn}, m), 4.31-3.94 (30H, 5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (6H, 4 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.13-2.94 (3H, OH, $CH_{2\_Nspacer}$, m), 2.74-2.54 (10H, 5 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.2, 140.0, 139.6, 139.4, 139.0 (C_q), 129.4 x 2, 129.3 X 2, 129.2 x 2, 129.1, 129.0 x 2, 128.9 x 2, 128.8 x 3, 128.7 x 2, 128.6, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.0 (C_1), 82.3 (C_3), 80.7 (C_2), 79.0 (C_4), 78.6 ($CH_{glycerol}$), 76.7 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 73.0, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.0 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.41, -0.36, -0.34, -0.32, -0.30, -0.29, -0.14, -0.11

HRMS: $C_{106}H_{129}N_6O_{33}P_5$ + H^+ required 2169.7361, found 2169.7368

(Protected) (GroP)(GlcGroP)(GroP)$_4$-Spacer or Hexamer 24



Alcohol **S11** (20 µmol) was coupled with phosphoramidite **8** (50 µmol, 2.5 eq) following the general procedure. Compound **24** was obtained after column chromatography (DCM:Acetone, 1:1) in 77% yield (15 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

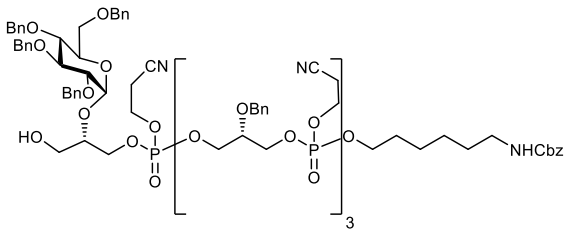
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.16 (50H, H_{arom}, m), 5.71-5.58 (1H, NH, b), 5.16 (1H, H_1, J=3.6 Hz, d), 5.01 (2H, CH_{2_Cbz}, s), 4.89-4.66 (4H, CH_{2_Bn}, m), 4.66-4.43 (14H, CH_{2_Bn}, m), 4.30-3.94 (36H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (7H, 5 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.13-2.94 (3H, OH, $CH_{2\_Nspacer}$, m), 2.74-2.54 (12H, 6 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.2, 140.1, 139.6 x 2, 139.5, 139.0 (C_q), 129.4 x 2, 129.3 X 2, 129.2 x 2, 129.1, 129.0 x 4, 128.9 x 2, 128.8 x 3, 128.7 x 2, 128.6, 128.5 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.0 (C_1), 82.3 (C_3), 80.7 (C_2), 79.1-79.0 ($CH_{glycerol}$), 78.6 (C_4), 76.7 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.0, 72.7, 72.4 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.44, -0.43, -0.40, -0.38, -0.34, -0.32, -0.18, -0.15.

HRMS: $C_{119}H_{145}N_7O_{38}P_6$ + H^+ required 2466.8128, found 2466.8133

(Protected) (GlcGroP)(GroP)$_3$-Spacer or Tetramer S12



Alcohol **S8** (59 µmol) was coupled with phosphoramidite **7** (88 µmol, 1.5 eq) following the general procedure. Compound **S12** was obtained after column chromatography (DCM:Acetone, 5.5:4.5) in 86% yield (51 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

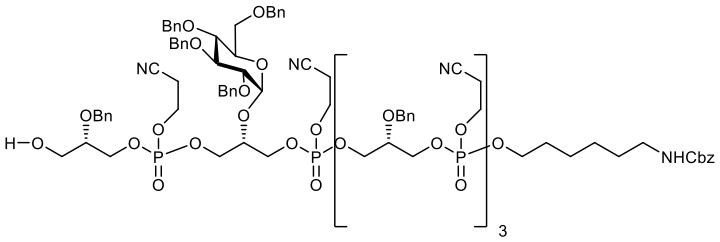
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.50-7.08 (40H, H_{arom}, m), 5.77-5.64 (1H, NH, b), 5.18-5.12 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.92-4.39 (14H, CH_{2_Bn}, m), 4.31-3.97 (34H, 4 x CH_{2_OCE}, 7 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.75 (6H, 4 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.45 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.26-3.16 (1H, OH, b), 3.06 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 2.78-2.50 (8H, 4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.0, 139.6, 139.5, 139.4, 139.1 (C_q), 129.4 x 2, 129.3 X 2, 129.2, 129.1 x 2, 129.0, 128.9 x 3, 128.8 x 3, 128.7 x 2, 128.6, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 80.9 (C_2), 78.9 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.8-76.7 ($CH_{glycerol}$), 75.9, 75.6, 73.8, 73.5, 72.7 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.1 ($CH_{2\_Ospacer}$), 68.8-66.06($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.8-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.3-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.39, -0.37, -0.35, -0.33, -0.31, -0.17, -0.10.

HRMS: $C_{93}H_{113}N_5O_{28}P_4$ + H^+ required 1872.6595, found 1872.6603

(Protected) (GroP)(GlcGroP)(GroP)$_3$-Spacer or Pentamer S13



Alcohol **S12** (40 µmol) was coupled with phosphoramidite **8** (100 µmol, 2.5 eq) following the general procedure. Compound **S13** was obtained after column chromatography (DCM:Acetone, 1:1) in 76% yield (30 µmol).

TLC analysis, R_f: 0.38 (DCM:Acetone, 4:6)

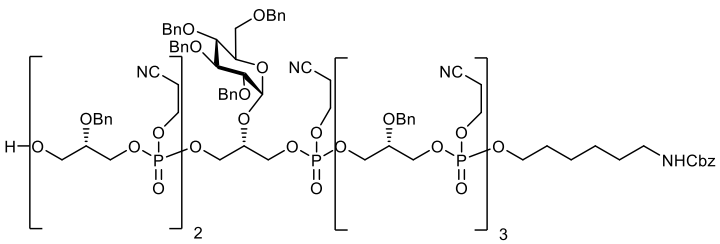
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.16 (45H, H_{arom}, m), 5.74-5.61 (1H, NH, b), 5.16 (1H, H_1, J=3.6 Hz, d), 5.01 (2H, CH_{2_Cbz}, s), 4.89-4.41 (18H, CH_{2_Bn}, m), 4.30-3.93 (30H, 5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (5H, 3 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.22-3.10 (1H, OH, m), 3.08-2.94 (2H, $CH_{2\_Nspacer}$, m), 2.74-2.53 (10H, 5 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.2, 140.0, 139.6, 139.4, 139.0 (C_q), 129.4 x 2, 129.3 X 2, 129.2 x 2, 129.1, 129.0 x 2, 128.9 x 2, 128.8 x 3, 128.7 x 2, 128.6, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.0 (C_1), 82.3 (C_3), 80.7 (C_2), 79.0 (C_4), 78.6 ($CH_{glycerol}$), 76.7 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 73.0, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.0 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.41, -0.36, -0.34, -0.32, -0.30, -0.29, -0.14, -0.11

HRMS: $C_{106}H_{129}N_6O_{33}P_5 + H^+$ required 2169.7361, found 2169.7355

(Protected) $(GroP)_2(GlcGroP)(GroP)_3$-Spacer or Hexamer 25



Alcohol **S13** (10 µmol) was coupled with phosphoramidite **8** (25 µmol, 2.5 eq) following the general procedure. Compound **25** was obtained after column chromatography (DCM:Acetone, 1:1) in 72% yield (7.2 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

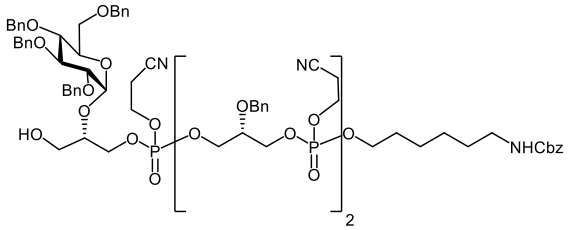
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.50-7.16 (50H, H_{arom}, m), 5.76-5.65 (1H, NH, b), 5.20-5.12 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.89-4.39 (18H, CH_{2_Bn}, m), 4.30-3.94 (36H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (7H, 5 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.17-2.98 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.48 (12H, 6 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.2, 140.0, 139.6, 139.5, 139.1 (C_q), 129.4 x 3, 129.3, 129.2 x 2, 129.1, 129.0, 128.9, 128.8 x 2,128.7, 128.6, 128.4 (CH_{arom}), 118.6 (C_q), 98.1 (C_1), 82.3 (C_3), 80.7 (C_2), 79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.8 ($CH_{glycerol}$), 76.0, 75.7, 73.8, 73.1, 72.7, 72.4 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.1 ($CH_{2\_Ospacer}$), 67.8-66.9 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.77, -1.71, -1.68, -1.63, -1.59, -1.56, -1.43, -1.13, -1.11, -1.03, -1.01.

HRMS: $C_{119}H_{145}N_7O_{38}P_6$ + H^+ required 2466.8128, found 2466.8137

(Protected) (GlcGroP)(GroP)$_2$-Spacer or Trimer S14



Alcohol **S7** (98 µmol) was coupled with phosphoramidite **7** (147 µmol, 1.5 eq) following the general procedure. Compound **S14** was obtained after column chromatography (DCM:Acetone, 5.5:4.5) in 86% yield (84 µmol).

TLC analysis, R_f: 0.35 (DCM:Acetone, 6:4)

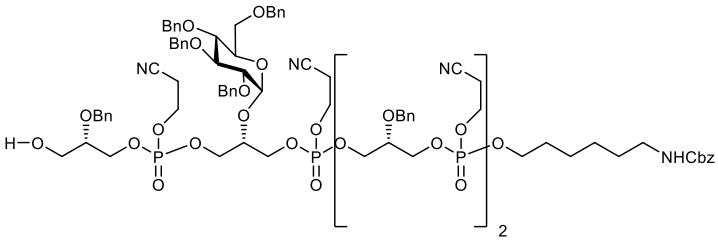
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.44-7.11 (35H, H_{arom}, m), 5.77-5.62 (1H, NH, b), 5.17-5.11 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.90-4.41 (12H, 6 x CH_{2_Bn}, m), 4.29-3.96 (18H, 3 x CH_{2_OCE}, 5 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (5H, 3 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.26-3.14 (1H, OH, b), 3.05 (2H, $CH_{2\_Nspacer}$, J=6.6 Hz, q), 2.77-2.53 (6H, 3 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.4, 140.1, 139.6, 139.5, 139.4, 139.1, 139.0, 138.6 (C_q), 129.4 x 3, 129.3 x 2, 129.2, 129.1, 129.0, 128.9, 128.8 x 2,128.7, 128.6, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 81.0 (C_2), 78.7 (C_4), 78.0, 76.0 ($CH_{glycerol}$), 76.0, 75.6 x 2, 73.8, 73.6, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.1 ($CH_{2\_Ospacer}$), 68.3, 67.8-66.9 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.67, -1.65, -1.64, -1.62, -1.61, -1.58, -1.45, -1.42, -1.39, -1.38

HRMS: $C_{80}H_{97}N_4O_{23}P_3$ + H^+ required 1575.5829, found 1575.5833

(Protected) (GroP)(GlcGroP)(GroP)$_2$-Spacer or Tetramer S15



Alcohol **S14** (11 µmol) was coupled with phosphoramidite **8** (28 µmol, 2.5 eq) following the general procedure. Compound **S15** was obtained after column chromatography (DCM:Acetone, 1:1) in 83% yield (9.1 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

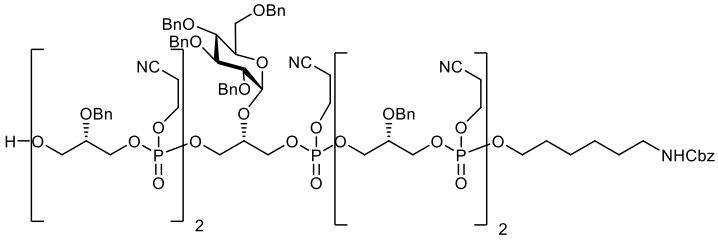
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.11 (40H, H_{arom}, m), 5.73-5.62 (1H, NH, b), 5.19-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.89-4.42 (14H, 7 x CH_{2_Bn}, m), 4.30-3.96 (24H, 4 x CH_{2_OCE}, 7 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (5H, 3 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (8H, 4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.7, 139.6, 139.5, 139.1 (C_q), 129.4 x 3, 129.3 x 2, 129.2, 129.1 x 2, 129.0 x 4, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.1 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{gltcerol}$), 78.6 (C_4), 76.9-76.8 ($CH_{glycerol}$), 76.0, 75.6 x 2, 73.9, 73.1, 72.4 (CH_{2_Bn}), 71.8 (C_5), 69.7 (C_6), 69.1 ($CH_{2\_Ospacer}$), 67.9, 67.8, 67.0, 66.7, 66.6, 66.5 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.3 (CH_{2_OCE}), 61.2 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.71, -1.69, -1.65, -1.63, -1.61, -1.59, -1.55, -1.53, -1.43, -1.42, -1.39, -1.36

HRMS: $C_{93}H_{113}N_5O_{28}P_4$ + H^+ required 1872.6595, found 1872.6594

(Protected) (GroP)$_2$(GlcGroP)(GroP)$_2$-Spacer or Pentamer S16

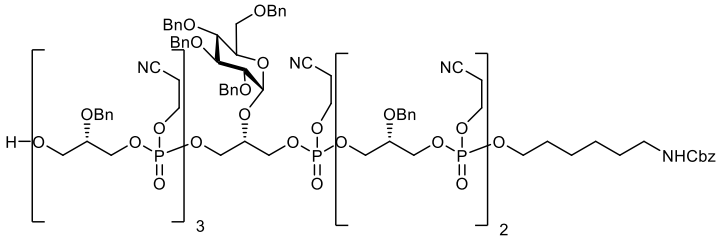


Alcohol **S15** (24 µmol) was coupled with phosphoramidite **8** (60 µmol, 2.5 eq) following the general procedure. Compound **S16** was obtained after column chromatography (DCM:Acetone, 1:1) in 82% yield (19 µmol).

TLC analysis, R_f: 0.38 (DCM:Acetone, 4:6)

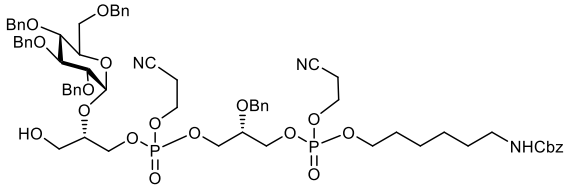
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.12 (45H, H_{arom}, m), 5.77-5.64 (1H, NH, b), 5.20-5.13 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.87-4.40 (16H, 7 x CH_{2_Bn}, m), 4.30-3.94 (30H, 5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (5H, 3 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.19-2.99 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (10H, 5 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6 x 2, 139.5, 139.0 (C_q), 129.4 x 2, 129.3 x 2, 129.2 x 2, 129.1, 129.0 x 4, 128.9 x 2, 128.8 x 3,128.7 x 3, 128.6, 128.5 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.0 (C_1), 82.3 (C_3), 80.7 (C_2), 79.1-79.0 ($CH_{gltcerol}$), 78.6 (C_4), 76.7 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.1, 72.7, 72.4 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.68, -1.65, -1.62, -1.60, -1.42, -1.39.
HRMS: $C_{106}H_{129}N_6O_{33}P_5$ + H^+ required 2169.7361, found 2169.7365

(Protected) $(GroP)_3(GlcGroP)(GroP)_2$-Spacer or Hexamer 26



Alcohol **S16** (35 µmol) was coupled with phosphoramidite **8** (86 µmol, 2.5 eq) following the general procedure. Compound **26** was obtained after column chromatography (DCM:Acetone, 1:1) in 65% yield (23 µmol).
TLC analysis, R_f: 0.41 (DCM:Acetone, 7:3)
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.43-7.12 (50H, H_{arom}, m), 5.75-5.64 (1H, NH, b), 5.20-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.88-4.41 (18H, 7 x CH_{2_Bn}, m), 4.30-3.94 (36H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.94-3.74 (6H, 4 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (8H, 2 x $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.17-2.99 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (12H, 6 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6 x 2, 139.5, 139.0 (C_q), 129.4 x 2, 129.3 x 3, 129.2, 129.1, 129.0 x 2, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.0 (C_1), 82.3 (C_3), 80.7 (C_2), 79.1-79.0 ($CH_{gltcerol}$), 78.6 (C_4), 76.7 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.1, 72.7, 72.4 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.69, -1.67, -1.64, -1.63, -1.61, -1.58, -1.44, -1.40, 1.37.
HRMS: $C_{119}H_{145}N_7O_{38}P_6$ + H^+ required 2466.8128, found 2466.8125

(Protected) (GlcGroP)(GroP)-Spacer or Dimer S17



Alcohol **S6** (160 µmol) was coupled with phospharamidite **7** (200 µmol, 1.3 eq) following the general procedure. Compound **S17** was obtained after column chromatography (DCM:Acetone, 7:3) in 64% yield (102 µmol).

TLC analysis, R_f: 0.38 (DCM:Acetone, 7:3)

^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.11 (30H, H_{arom}, m), 5.73-5.62 (1H, NH, b), 5.19-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.89-4.42 (10H, 5 x CH_{2_Bn}, m), 4.30-3.96 (12H, 2 x CH_{2_OCE}, 3 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (4H, 2 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (4H, 2 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.7, 139.6, 139.5, 139.4 (C_q), 129.4 x 2, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 2,128.7, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 81.0 (C_2), 79.2 ($CH_{gltcerol}$), 78.7 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.0 ($CH_{2\_Ospacer}$), 68.3-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.4-63.1 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

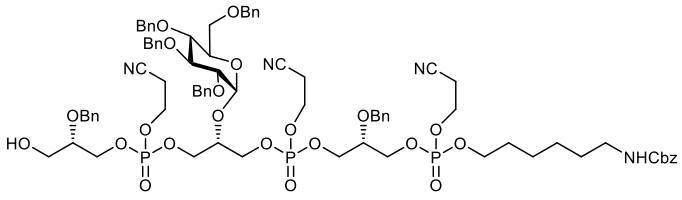
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.64, -1.61, -1.60, -1.48, -1.46, -1.44, -1.43, -1.39, -1.32, -1.28.

HRMS: $C_{67}H_{81}N_3O_{18}P_2 + H^+$ required 1278.5063, found 1278.5064

(Protected) (GroP)(GlcGroP)(GroP) -Spacer or Trimer S18

Alcohol **S17** (86 µmol) was coupled with phosphoramidite **8** (215 µmol, 2.5 eq) following the general procedure. Compound **S18** was obtained after column chromatography (DCM:Acetone, 6:4) in 68% yield (58 µmol).

TLC analysis, R_f: 0.35 (DCM:Acetone, 6:4)



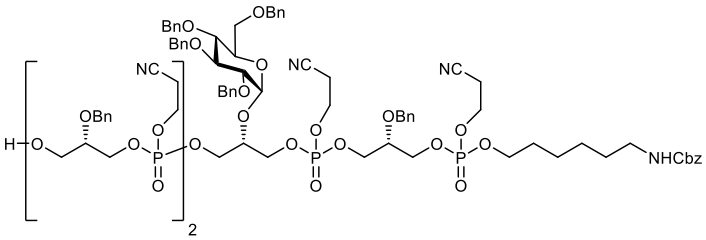
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.11 (35H, H_{arom}, m), 5.73-5.62 (1H, NH, b), 5.19-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.89-4.42 (12H, 6 x CH_{2_Bn}, m), 4.30-3.96 (18H, 3 x CH_{2_OCE}, 5 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (4H, 2 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (6H, 3 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.0, 139.6 x 2, 139.5, 139.1 (C_q), 129.4 x 2, 129.3 x 3, 129.2, 129.1 x 2, 129.0 x 3, 128.9 x 3, 128.8 x 3,128.7 x 3, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.1 (C_1), 82.3 (C_3), 80.7 (C_2), 79.1-79.0 ($CH_{gltcerol}$), 78.6 (C_4), 76.8 ($CH_{glycerol}$), 76.0, 75.6 x 2, 73.9, 73.1, 72.4 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.1 ($CH_{2\_Ospacer}$), 67.9-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.6-63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.43, -0.42, -0.40, -0.39, -0.37, -0.36, -0.35, -0.12, -0.09, -0.07

HRMS: $C_{80}H_{97}N_4O_{23}P_3$ + H^+ required 1575.5829, found 1575.5832

(Protected) $(GroP)_2(GlcGroP)(GroP)$ -Spacer or Tetramer S19



Alcohol **S18** (41 µmol) was coupled with phosphoramidite **8** (102 µmol, 2.5 eq) following the general procedure. Compound **S19** was obtained after column chromatography (DCM:Acetone, 1:1) in 65% yield (27 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 1:1)

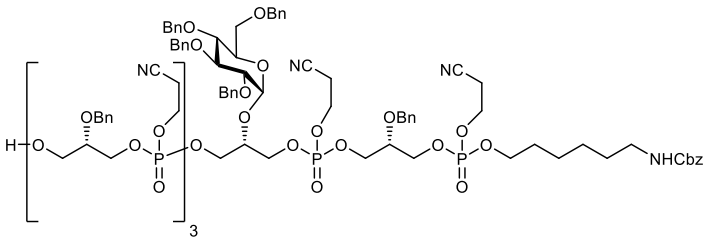
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.42-7.11 (40H, H_{arom}, m), 5.72-5.61 (1H, NH, b), 5.20-5.13 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.89-4.42 (14H, 7 x CH_{2_Bn}, m), 4.30-3.96 (24H, 4 x CH_{2_OCE}, 7 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (4H, 2 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (8H, 4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.7, 139.6, 139.5, 139.4 (C_q), 129.4 x 2, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 2,128.7, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 81.0 (C_2), 79.2 ($CH_{gltcerol}$), 78.7 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.0 ($CH_{2\_Ospacer}$), 68.3-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.4-63.1 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.42, -0.40, -0.36, -0.34, -0.32, -0.14, -0.12.

HRMS: $C_{93}H_{113}N_5O_{28}P_4$ + H^+ required 1872.6595, found 1872.6598

(Protected) $(GroP)_3(GlcGroP)(GroP)$ -Spacer or Pentamer S20



Alcohol **S19** (20 µmol) was coupled with phosphoramidite **8** (50 µmol, 2.5 eq) following the general procedure. Compound **S20** was obtained after column chromatography (DCM:Acetone, 1:1) in 77% yield (15 µmol).

TLC analysis, R_f: 0.38 (DCM:Acetone, 4:6)

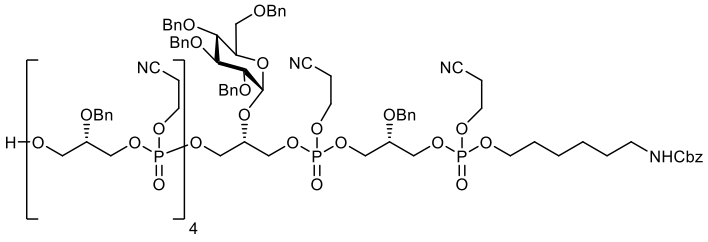
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.11 (45H, H_{arom}, m), 5.73-5.62 (1H, NH, b), 5.19-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.89-4.42 (16H, 8 x CH_{2_Bn}, m), 4.30-3.96 (30H, 5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (4H, 3 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (4H, 2 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.7, 139.6, 139.5, 139.4 (C_q), 129.4 x 2, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 2,128.7, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 81.0 (C_2), 79.2 ($CH_{gltcerol}$), 78.7 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.0 ($CH_{2\_Ospacer}$), 68.3-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.4-63.1 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.41, -0.36, -0.34, -0.32, -0.30, -0.29, -0.14, -0.11.

HRMS: $C_{106}H_{129}N_6O_{33}P_5 + H^+$ required 2169.7361, found 2169.7363

(Protected) (GroP)$_4$(GlcGroP)(GroP) -Spacer or Hexamer 27

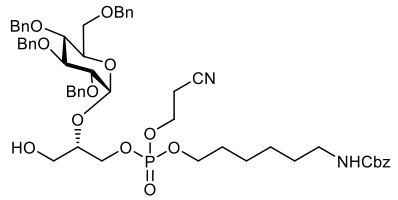


Alcohol **S20** (13 µmol) was coupled with phosphoramidite **8** (40 µmol, 2.5 eq) following the general procedure. Compound **27** was obtained after column chromatography (DCM:Acetone, 1:1) in 72% yield (9.4 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 4:6)

^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.11 (50H, H_{arom}, m), 5.73-5.62 (1H, NH, b), 5.19-5.13 (1H, H_1, m), 5.03 (2H, CH_{2_Cbz}, s), 4.89-4.42 (18H, 9 x CH_{2_Bn}, m), 4.30-3.96 (36H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{2\_Ospacer}$, m), 3.96-3.75 (6H, 4 x $CH_{\_glycerol}$, H_5, H_3, m), 3.74-3.42 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-3.00 (3H, OH, $CH_{2\_Nspacer}$, m), 2.77-2.53 (4H, 2 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 139.7, 139.6, 139.5, 139.4 (C_q), 129.4 x 2, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 2,128.7, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.4 (C_1), 82.5 (C_3), 81.0 (C_2), 79.2 ($CH_{gltcerol}$), 78.7 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5, 72.7, 72.4 (CH_{2_Bn}), 71.6 (C_5), 69.7 (C_6), 69.0 ($CH_{2\_Ospacer}$), 68.3-66.6 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.4-63.1 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25,7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -0.41, -0.36, -0.34, -0.32, -0.30, -0.29, -0.14, -0.11.
HRMS: $C_{119}H_{145}N_7O_{38}P_6$ + H^+ required 2466.8128, found 2466.8130

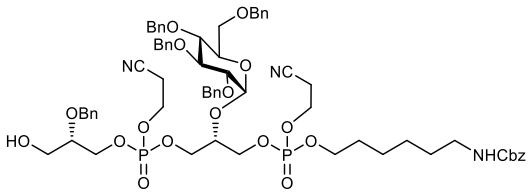
(Protected) GlcGroP-Spacer or Monomer S21



Alcohol spacer **9** (0.26 mmol) was coupled with phosphoramidite **7** (0.35 mmol, 1.3 eq) following the general procedure. Compound **S21** was obtained after column chromatography (DCM:Acetone, 7.5:2.5) in 81% yield (0.21 mmol). TLC analysis, R_f: 0.45 (DCM:Acetone, 7:3

^{1}H-NMR (400 MHz, CD_3CN), δ: 7.47-7.12 (25H, H_{arom}, m), 5.68-5.54 (1H, NH, b), 5.16 (1H, H_1, J=3.6 Hz, d), 5.03 (2H, CH_{2_Cbz}, s), 4.88 (1H, CH*H*$_{\_Bn}$, J=10.6 Hz, d), 4.82-4.61 (4H, 2 x CH_{2_Bn}, m), 4.58-4.43 (3H, CH*H*$_{\_Bn}$), 4.22-3.06 (4H, CH_{2_OCE}, $CH_{2\_glycerol}$), 4.05-3.80 (5H, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, H_3, m), 3.75-3.46 (6H, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.15-2.98 (3H, OH, $CH_{2\_Nspacer}$, m), 2.68-2.57 (2H,CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6, 139.5, 139.4 (C_q), 129.4, 129.3 x 2, 129.2 x 2, 129.1, 128.9 x 2, 128.8 x 2,128.7 x 2, 128.5, 128.4 (CH_{arom}), 118.6 (C_q), 97.3 (C_1), 82.5 (C_3), 81.0 (C_2), 78.7 (C_4), 78.0-77.8 ($CH_{glycerol}$), 76.0, 75.6, 73.8, 73.5 (CH_{2_Bn}), 71.5 (C_5), 69.7 (C_6), 69.0 ($CH_{2\_Ospacer}$), 68.1-68.0 ($CH_{2\_glycerol}$), 66.6 CH_{2_Cbz}), 63.2 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.44, -1.37
HRMS: $C_{54}H_{65}N_2O_{13}P$ + H^+ required 981.4297, found 981.4296

(Protected) (GroP)(GlcGroP)-Spacer or Dimer S22



Alcohol **S21** (0.17 mmol) was coupled with phosphoramidite **8** (0.26 mmol, 1.5 eq) following the general procedure. Compound **S22** was obtained after column chromatography (DCM:Acetone, 6:4) in 82% yield (0.14 mmol).

TLC analysis, R_f: 0.32 (DCM:Acetone, 1:1).

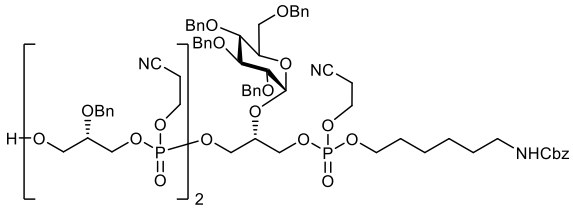
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.10 (30H, H_{arom}, m), 5.71-5.55 (1H, NH, b), 5.16 (1H, H_1, J=3.6 Hz, d), 5.02 (2H, CH_{2_Cbz}, s), 4.90-4.83 (1H, $CH\textit{H}_{\_Bn}$, m), 4.80-4.69 (3H, 3 x $CH\textit{H}_{\_Bn}$, m), 4.66-4.56 (3H, 3 x $CH\textit{H}_{\_Bn}$, m), 4.58-4.43 (3H, $CH\textit{H}_{\_Bn}$), 4.30-4.04 (11H,2 x CH_{2_OCE}, 3 x $CH_{2\_glycerol}$, $CH_{glycerol}$), 4.05-3.96 (2H, $CH_{2\_Ospacer}$, m), 3.95-3.86 (H_5), 3.86-3.76 (1H, H_3, m), 3.75-3.46 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.09-2.97 (3H, OH, $CH_{2\_Nspacer}$, m), 2.78-2.52 (4H,2 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.7, 139.6, 139.5 (C_q), 129.4 x3, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.1-97.0 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.0, 75.6 (CH_{2_Bn}), 75.1-74.2 ($CH_{glycerol}$), 73.9, 73.1, 72.5 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -1.72, -1.70, -1.67, -1.66 -1.47, -1.41

HRMS: $C_{67}H_{81}N_3O_{18}P_2$ + H^+ required 1278.5063, found 1278.5067

(Protected) $(GroP)_2$(GlcGroP)-Spacer or Trimer S23



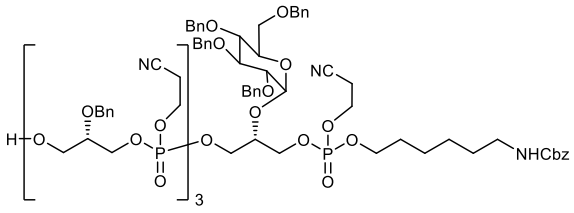
Alcohol **S22** (0.12 mmol) was coupled with phosphoramidite **8** (0.24 mmol, 2.0 eq) following the general procedure. Compound **S23** was obtained after column chromatography (DCM:Acetone, 1:1) in 77% yield (0.92 mmol).

TLC analysis, R_f: 0.38 (DCM:Acetone, 4:6)

^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.10 (35H, H_{arom}, m), 5.71-5.56 (1H, NH, b), 5.18-5.15 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.90-4.81 (1H, $CH\textit{H}_{\_Bn}$, m), 4.80-4.69 (3H, 3 x $CH\textit{H}_{\_Bn}$, m), 4.65-4.45 (8H, 8 x $CH\textit{H}_{\_Bn}$, m), 4.28-3.94 (17H,3 x CH_{2_OCE}, 5 x $CH_{2\_glycerol}$, $CH_{glycerol}$), 3.94-3.75 (5H, $CH_{2\_Ospacer}$, H_5, H_3, $CH_{glycerol}$, m), 3.75-3.46 (7H, $CH_{glycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.13-2.97 (3H, OH, $CH_{2\_Nspacer}$, m), 2.73-2.58 (6H,3 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

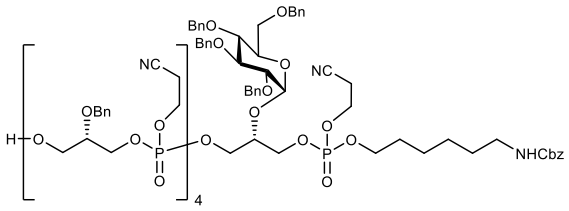
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.7, 139.6, 139.5 (C_q), 129.4 x3, 129.3 x 2, 129.2, 129.1 x 2, 129.0, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.1-97.0 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.0, 75.6 (CH_{2_Bn}), 75.1-74.2 ($CH_{glycerol}$), 73.9, 73.1, 72.5 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.72, -1.71, -1.69, -1.68, -1.66, -1.65, -1.64, -1.61, -1.40, -1.38, -1.35
HRMS: $C_{80}H_{97}N_4O_{23}P_3$ + H^+ required 1575.5829, found 1575.5827

(Protected) $(GroP)_3$(GlcGroP)-Spacer or Tetramer S24



Alcohol **S23** (80 µmol) was coupled with phosphoramidite **8** (160 µmol, 2.0 eq) following the general procedure. Compound **S24** was obtained after column chromatography (DCM:Acetone, 1:1) in 81% yield (65 µmol).
TLC analysis, R_f: 0.33 (DCM:Acetone, 4:6)
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.10 (35H, H_{arom}, m), 5.74-5.56 (1H, NH, b), 5.18-5.15 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.90-4.81 (1H, $CH\mathit{H}_{\_Bn}$, m), 4.80-4.69 (3H, 3 x $CH\mathit{H}_{\_Bn}$, m), 4.65-4.45 (10H, 10 x $CH\mathit{H}_{\_Bn}$, m), 4.28-3.94 (23H,4 x CH_{2_OCE}, 7 x $CH_{2\_glycerol}$, $CH_{glycerol}$), 3.94-3.75 (6H, $CH_{2\_Ospacer}$, H_5, H_3,2 x $CH_{glycerol}$, m), 3.75-3.46 (7H, $CH_{gòycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.16-2.97 (3H, OH, $CH_{2\_Nspacer}$, m), 2.73-2.58 (8H,4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).
^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6 x 2, 139.5. 139.1 (C_q), 129.4 x3, 129.3 x 2, 129.2 x 2, 129.1 x 2, 129.0 x 3, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.0-96.9 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.0, 75.6 (CH_{2_Bn}), 75.1-74.2 ($CH_{glycerol}$), 73.9, 73.1, 72.5 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR(162 MHz, CD_3CN), δ: -1.67, -1.66, -1.64, -1.63, -1.61, -1.59, -1.57, -1.39, -1.37.
HRMS: $C_{93}H_{113}N_5O_{28}P_4$ + H^+ required 1872.6595, found 1872.6601

(Protected) $(GroP)_4$(GlcGroP)-Spacer or Pentamer S25



Alcohol **S24** (48 µmol) was coupled with phosphoramidite **8** (120 µmol, 2.5 eq) following the general procedure. Compound **S25** was obtained after column chromatography (DCM:Acetone, 1:1) in 76% yield (36 µmol).

TLC analysis, R_f: 0.31 (DCM:Acetone, 4:6)

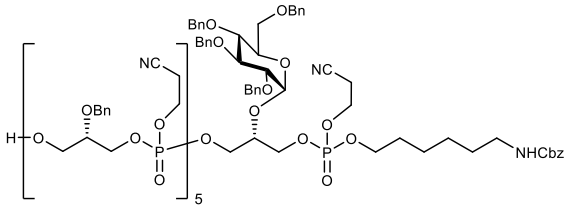
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.10 (35H, H_{arom}, m), 5.74-5.56 (1H, NH, b), 5.18-5.15 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.90-4.81 (1H, CH*H*$_{\_Bn}$, m), 4.80-4.69 (3H, 3 x CH*H*$_{\_Bn}$, m), 4.65-4.45 (12H, 12 x CH*H*$_{\_Bn}$, m), 4.28-3.94 (29H,5 x CH_{2_OCE}, 9 x $CH_{2\_glycerol}$, $CH_{glycerol}$), 3.94-3.75 (7H, $CH_{2\_Ospacer}$, H_5, H_3,3 x $CH_{glycerol}$, m), 3.75-3.46 (7H, $CH_{gòycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.16-2.97 (3H, OH, $CH_{2\_Nspacer}$, m), 2.73-2.58 (8H,4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6 x 2, 139.5. 139.1 (C_q), 129.4 x3, 129.3 x 2, 129.2 x 2, 129.1 x 2, 129.0 x 3, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.0-96.9 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.0, 75.6 (CH_{2_Bn}), 75.1-74.2 ($CH_{glycerol}$), 73.9, 73.1, 72.5 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).

^{31}P-NMR(162 MHz, CD_3CN), δ: -0.44, -0.43, -0.40, -0.38, -0.34, -0.32, -0.18, -0.15.

HRMS: $C_{106}H_{129}N_6O_{33}P_5$ + H^+ required 2169.7361, found 2169.7358

(Protected) $(GroP)_5$(GlcGroP)-Spacer or Hexamer 28



Alcohol **S25** (23 µmol) was coupled with phosphoramidite **8** (58 µmol, 2.5 eq) following the general procedure. Compound **28** was obtained after column chromatography (DCM:Acetone, 1:1) in 65% yield (15 µmol).

TLC analysis, R_f: 0.28 (DCM:Acetone, 4:6)

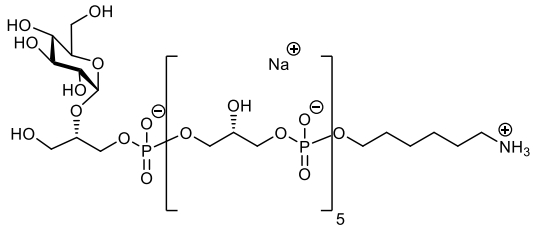
^{1}H-NMR (400 MHz, CD_3CN), δ: 7.48-7.10 (35H, H_{arom}, m), 5.74-5.56 (1H, NH, b), 5.18-5.15 (1H, H_1, m), 5.02 (2H, CH_{2_Cbz}, s), 4.90-4.81 (1H, CH*H*$_{\_Bn}$, m), 4.80-4.69 (3H, 3 x CH*H*$_{\_Bn}$, m), 4.65-4.45 (14H, 14 x CH*H*$_{\_Bn}$, m), 4.28-3.94 (35H, 6 x CH_{2_OCE}, 11 x $CH_{2\_glycerol}$, $CH_{glycerol}$), 3.94-3.75 (8H, $CH_{2\_Ospacer}$, H_5, H_3,4 x $CH_{glycerol}$, m), 3.75-3.46 (7H, $CH_{gòycerol}$, 2 x H_6, H_4, H_2, $CH_{2\_glycerol}$), 3.16-2.97 (3H, OH, $CH_{2\_Nspacer}$, m), 2.73-2.58 (8H,4 x CH_{2_OCE}, m), 1.65-1.49 (2H, CH_{2_spacer}, m), 1.45-1.14 (6H, 3 x CH_{2_spacer}, m).

^{13}C-NMR (101 MHz, CD_3CN), δ: 157.3, 140.1, 139.6 x 2, 139.5. 139.1 (C_q), 129.4 x3, 129.3 x 2, 129.2 x 2, 129.1 x 2, 129.0 x 3, 128.9 x 2, 128.8 x 3,128.7 x 2, 128.6 x 2, 128.4 (CH_{arom}), 118.6 (C_q), 97.0-96.9 (C_1), 82.3 (C_3), 80.8 (C_2), 79.2-79.1 ($CH_{glycerol}$), 78.6 (C_4), 76.0, 75.6 (CH_{2_Bn}), 75.1-74.2 ($CH_{glycerol}$), 73.9, 73.1, 72.5 (CH_{2_Bn}), 71.7 (C_5), 69.7 (C_6), 69.2 ($CH_{2\_Ospacer}$), 67.8-66.4 ($CH_{2\_glycerol}$, CH_{2_Cbz}), 63.5-63.3 (CH_{2_OCE}), 61.1 ($CH_{2\_glycerol}$), 41.4 ($CH_{2\_Nspacer}$), 30.7-30.4, 26.8, 25.7 (CH_{2_spacer}), 20.2-20.1 (CH_{2_OCE}).
^{31}P-NMR (162 MHz, CD_3CN), δ: -0.41, -0.36, -0.34, -0.32, -0.30, -0.29, -0.14, -0.11.
HRMS: $C_{119}H_{145}N_7O_{38}P_6 + H^+$ required 2466.8128, found 2466.8133

Final deprotections

The oligomer is dissolved in dioxane (2mM) and upon the addition of ammonia solution in H_2O (33%) the reaction mixture turns turbid. Once the solution becomes transparent (1-3 hours) the reaction mixture is concentrated *in vacuo*. After checking by ^{1}H-NMR the disappearing of the cyanoethyl group, the residue is flushed over Dowex Na^+ cation-exchange resin (type 50WX4-200, stored in 0.5M NaOH in MilliQ, flushed with MeOH and MilliQ before use) column. After evaporation, the residue is dissolved in MilliQ (2mM) and 2 drops of AcOH are added. $Ar_{(g)}$ is bubbled in the reaction mixture for 20 minutes while sonicating, Pd-black (≈10 mg) is added and after an additional 10 minutes of $Ar_{(g)}$ bubbling, the solution is left stirring under $H_{2(g)}$ atmosphere for 1 week. After filtration over Celite®, the reaction mixture is concentrated *in vacuo*. The final compound is purified by sixe-exclusion chromatography (HW40, dimensions: 16/60 mm, eluent: 0.15M NH_4OAc). After several co-evaporation with MilliQ, the product is eluted through a small column containing Dowex Na^+ cation-exchange resin (type 50WX4-200, stored in 0.5M NaOH in MilliQ, flushed with MeOH and MilliQ before use).

(GlcGroP)(GroP)$_5$-Spacer or Hexamer (1)



Compound **23** (6 µmol) was deprotected following the general procedure. The final product **1** was obtained in 78% yield (4.7 µmol).

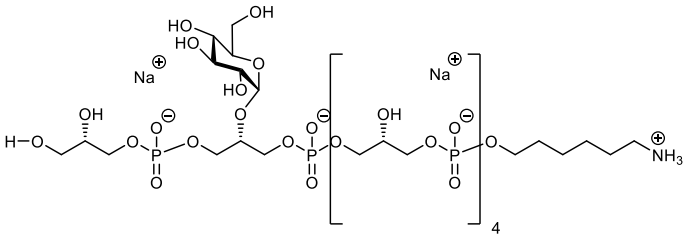
^{1}H-NMR (850 MHz, CD_3CN), δ: 5.07 (1H, H_1, J=3.8 Hz, d), 4.05-3.95 (7H, 5 x $CH_{glycerol}$, $CH_{2\_glycerol}$, m), 3.95-3.78 (24H, 10 $CH_{2\_glycererol,}$ $CH\mathit{H}_{glycerol}$, $CH_{2\_Ospacer}$, H_5, m), 3.77-3.64 (4H, 2 x H_6, H_3, $CH\mathit{H}_{glycerol}$, m), 3.49 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.38-3.30 (1H, H_4, m), 3.00-2.91 (2H, $CH_{2\_Nspacer}$, m), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 100.4 (C_1), 79.7 ($CH_{glycerol}$), 75.6 (C_3), 74.5 (C_5), 74.2 (C_2), 72.3 (C_4), 72.2-72.1 ($CH_{glycerol}$), 68.9-68.6 ($CH_{2\_glycerol}$), 67.8-67.7 ($CH_{2\_glycerol}$), 63.1 (C_6), 62.8 ($CH_{2\_glycerol}$), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.78, 1.89, 1.93, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ required 1204.1941, found 1204.1951

(GroP)(GlcGroP)(GroP)$_4$-Spacer or Hexamer (2)



Compound **24** (11 µmol) was deprotected following the general procedure. The final product **2** was obtained in 73% yield (8 µmol).

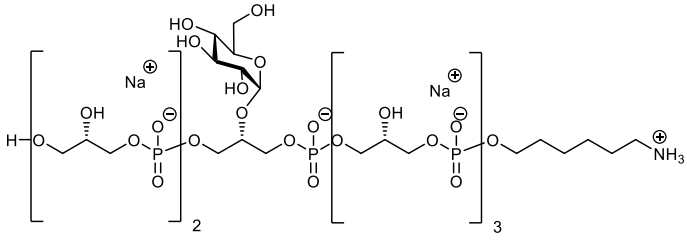
^{1}H-NMR (850 MHz, CD_3CN), δ: 5.14 (1H, H_1, J=3.8 Hz, d), 4.11-4.04 (1H, $CH_{glycerol}$, m), 4.05-3.95 (8H, 4 x $CH_{glycerol}$, 2 x $CH_{2\_glycerol}$, m), 3.95-3.78 (23H, 9 x $CH_{2\_glycererol,}$ H_6, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, m), 3.76-3.68 (2H, H_6, H_3, m), 3.64 (1H, $CH\mathit{H}_{glycerol}$, J=4.3 Hz, J=11.8 Hz, dd), 3.56 (1H, $CH\mathit{H}_{glycerol}$, J=6.1 Hz, J=11.8 Hz, dd) 3.50 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.35 (1H, H_4, J=9.6 Hz, t), 2.96 (2H, $CH_{2\_Nspacer}$, J=7.5 Hz, t), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 98.6 (C_1), 76.2-76.1 ($CH_{glycerol}$), 73.8 (C_3), 72.7 (C_5), 72.4 (C_2), 71.7-71.6 ($CH_{glycerol}$), 70.6 (C_4), 70.5-70.3 ($CH_{glycerol}$), 67.3-66.9 ($CH_{2\_glycerol}$), 66.1 ($CH_{2\_glycerol}$), 65.3 ($CH_{2glycerol}$), 62.8 ($CH_{2\_glycerol}$), 61.4 (C_6), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.62, 1.84, 1.94, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ required 1204.1941, found 1204.1956

$(GroP)_2(GlcGroP)(GroP)_3$-Spacer or Hexamer (3)



Compound **25** (6 µmol) was deprotected following the general procedure. The final product **3** was obtained in 62% yield (3.7 µmol).

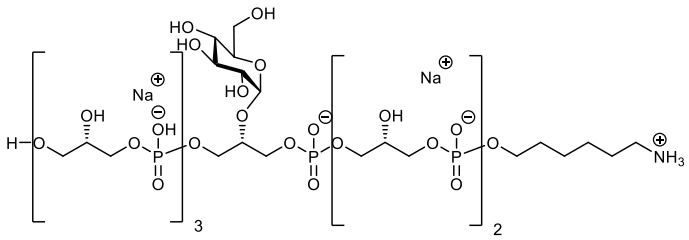
^{1}H-NMR (850 MHz, CD_3CN), δ: 5.14 (1H, H_1, J=3.8 Hz, d), 4.11-4.04 (1H, $CH_{glycerol}$, m), 4.05-3.95 (8H, 4 x $CH_{glycerol}$, 2 x $CH_{2\_glycerol}$, m), 3.95-3.78 (23H, 9 x $CH_{2\_glycererol,}$ H_6, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, m), 3.76-3.68 (2H, H_6, H_3, m), 3.64 (1H, $CH\mathit{H}_{glycerol}$, J=4.3 Hz, J=11.8 Hz, dd), 3.56 (1H, $CH\mathit{H}_{glycerol}$, J=6.1 Hz, J=11.8 Hz, dd) 3.50 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.35 (1H, H_4, J=9.6 Hz, t), 2.96 (2H, $CH_{2\_Nspacer}$, J=7.5 Hz, t), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 98.6 (C_1), 76.2-76.1 ($CH_{glycerol}$), 73.8 (C_3), 72.7 (C_5), 72.4 (C_2), 71.7-71.6 ($CH_{glycerol}$), 70.6 (C_4), 70.5-70.3 ($CH_{glycerol}$), 67.3-66.9 ($CH_{2\_glycerol}$), 66.1 ($CH_{2\_glycerol}$), 65.3 ($CH_{2glycerol}$), 62.8 ($CH_{2\_glycerol}$), 61.4 (C_6), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.62, 1.84, 1.94, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1951

$(GroP)_3(GlcGroP)(GroP)_2$-Spacer or Hexamer (4)



Compound **26** (9 µmol) was deprotected following the general procedure. The final product **4** was obtained in 81% yield (7.3 µmol).

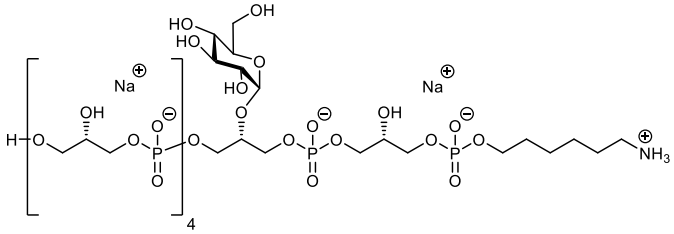
^{1}H-NMR (850 MHz, CD_3CN), δ: 5.14 (1H, H_1, J=3.8 Hz, d), 4.11-4.04 (1H, $CH_{glycerol}$, m), 4.05-3.95 (8H, 4 x $CH_{glycerol}$, 2 x $CH_{2\_glycerol}$, m), 3.95-3.78 (23H, 9 x $CH_{2\_glyceregerol,}$ H_6, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, m), 3.76-3.68 (2H, H_6, H_3, m), 3.64 (1H, $CHH_{glycerol}$, J=4.3 Hz, J=11.8 Hz, dd), 3.56 (1H, $CHH_{glycerol}$, J=6.1 Hz, J=11.8 Hz, dd) 3.50 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.35 (1H, H_4, J=9.6 Hz, t), 2.96 (2H, $CH_{2\_Nspacer}$, J=7.5 Hz, t), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 98.6 (C_1), 76.2-76.1 ($CH_{glycerol}$), 73.8 (C_3), 72.7 (C_5), 72.4 (C_2), 71.7-71.6 ($CH_{glycerol}$), 70.6 (C_4), 70.5-70.3 ($CH_{glycerol}$), 67.3-66.9 ($CH_{2\_glycerol}$), 66.1 ($CH_{2\_glycerol}$), 65.3 ($CH_{2glycerol}$), 62.8 ($CH_{2\_glycerol}$), 61.4 (C_6), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.62, 1.84, 1.94, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ required 1204.1941, found 1204.1949

$(GroP)_4(GlcGroP)(GroP)$ -Spacer or Hexamer (5)



Compound **27** (16 µmol) was deprotected following the general procedure. The final product **5** was obtained in 78% yield (12 µmol).

^{1}H-NMR (850 MHz, CD_3CN), δ: 5.14 (1H, H_1, J=3.8 Hz, d), 4.11-4.04 (1H, $CH_{glycerol}$, m), 4.05-3.95 (8H, 4 x $CH_{glycerol}$, 2 x $CH_{2\_glycerol}$, m), 3.95-3.78 (23H, 9 x $CH_{2\_glyceregerol,}$ H_6, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, m),

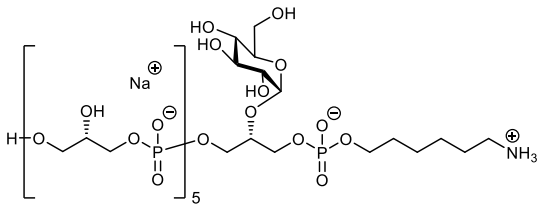
3.76-3.68 (2H, H_6, H_3, m), 3.64 (1H, CH$H_{glycerol}$, J=4.3 Hz, J=11.8 Hz, dd), 3.56 (1H, CH$H_{glycerol}$, J=6.1 Hz, J=11.8 Hz, dd) 3.50 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.35 (1H, H_4, J=9.6 Hz, t), 2.96 (2H, $CH_{2\_Nspacer}$, J=7.5 Hz, t), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 98.6 (C_1), 76.2-76.1 ($CH_{glycerol}$), 73.8 (C_3), 72.7 (C_5), 72.4 (C_2), 71.7-71.6 ($CH_{glycerol}$), 70.6 (C_4), 70.5-70.3 ($CH_{glycerol}$), 67.3-66.9 ($CH_{2\_glycerol}$), 66.1 ($CH_{2\_glycerol}$), 65.3 ($CH_{2glycerol}$), 62.8 ($CH_{2\_glycerol}$), 61.4 (C_6), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.62, 1.84, 1.94, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ required 1204.1941, found 1204.1957

(GroP)$_5$(GlcGroP)-Spacer or Hexamer (6)



Compound **28** (21 µmol) was deprotected following the general procedure. The final product **6** was obtained in 68% yield (14 µmol).

^{1}H-NMR (850 MHz, CD_3CN), δ: 5.14 (1H, H_1, J=3.8 Hz, d), 4.11-4.04 (1H, $CH_{glycerol}$, m), 4.05-3.95 (8H, 4 x $CH_{glycerol}$, 2 x $CH_{2\_glycerol}$, m), 3.95-3.78 (23H, 9 x $CH_{2\_glycererol}$, H_6, $CH_{2\_Ospacer}$, H_5, $CH_{glycerol}$, m), 3.76-3.68 (2H, H_6, H_3, m), 3.64 (1H, CH$H_{glycerol}$, J=4.3 Hz, J=11.8 Hz, dd), 3.56 (1H, CH$H_{glycerol}$, J=6.1 Hz, J=11.8 Hz, dd) 3.50 (1H, H_2, J=3.8 Hz, J=9.9 Hz, dd), 3.35 (1H, H_4, J=9.6 Hz, t), 2.96 (2H, $CH_{2\_Nspacer}$, J=7.5 Hz, t), 1.69-1.56 (4H, CH_{2_spacer}, m), 1.45-1.34 (4H, CH_{2_spacer}, m).

^{13}C-NMR(101 MHz, CD_3CN), δ: 98.6 (C_1), 76.2-76.1 ($CH_{glycerol}$), 73.8 (C_3), 72.7 (C_5), 72.4 (C_2), 71.7-71.6 ($CH_{glycerol}$), 70.6 (C_4), 70.5-70.3 ($CH_{glycerol}$), 67.3-66.9 ($CH_{2\_glycerol}$), 66.1 ($CH_{2\_glycerol}$), 65.3 ($CH_{2glycerol}$), 62.8 ($CH_{2\_glycerol}$), 61.4 (C_6), 42.1 ($CH_{2\_Nspacer}$), 32.0, 29.2, 27.7, 27.1 ($CH_{2spacer}$).

^{31}P-NMR(162 MHz, CD_3CN), δ: 1.62, 1.84, 1.94, 2.04.

HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1948

Generation and Serum analysis of microarrays

The amino-spacer equipped GTA-fragments were dissolved in spotting buffer (Nexterion Spot, Schott Nexterion) with 10% DMSO in 384-wells V-bottom plates (Genetix, New Milton, UK). The GTA-fragments were printed in three final concentrations (30µM, 10µM and 3µM) in triplicate on epoxysilane-coated glass slides (Slide E, Schott, Nexterion) by contact printing using the Omnigrid 100 microarrayer (Genomic Solutions, Ann Arbor, MI) equipped with SMP3 pins with uptake channels that deposit 0.7 nl at each contact. The slides were rested in a high humidity chamber for 18 hours and were stored in the dark until used. The slides were washed with PBS (3x) and subsequently all unreacted sites on the arrays were blocked by shaking the slides for 1 hour with ethanolamine (0.25 ml, 0.05M in PBS containing 20 mg/ml of BSA). The slides were flushed with PBS containing 5% of Tween® 20 and PBS containing 1% of Tween® 20 subsequently. After removal of the PBS containing 1% of Tween® 20, the arrays were shaken with the primary antibody dilutions (0.25 ml, diluted with PBS containing 1% of Tween® 20 and 10 mg/ml of BSA) for 60 minutes. Serum obtained from rabbits immunized with native LTA isolated from *E. faecalis* strain **12030** was used at a 1:1000 dilution, while rabbit serum raised against the previously reported BSA-WH7 at a 1:500 dilution. The slides were flushed with PBS containing 5% of Tween® 20 and PBS containing 1% of Tween® 20 subsequently. After removal of the PBS containing 1% of Tween® 20, slides were shaken with anti-rabbit-IgG secondary antibodies, labeled with DyLight 550 reporter groups (0.25 ml, 0.5 µg/ml final dilution in PBS containing 1% of Tween® 20 and 10 mg/ml of BSA) for 30 minutes in the dark. The slides were flushed with PBS containing 5% of Tween® 20, PBS and MilliQ subsequently. The slides were dried by centrifugation and were analyzed on fluorescence on 532 nm and 635 nm using a G2565BA scanner. Data and image analyses were performed with GenePix Pro 7.0 software (Molecular Devices, Sunnyvale, CA, USA) as described previously (J. Proteome Res., 8 (2009), pp. 4301–4310). Fluorescence intensities were quantified and corrected for background/non-specific antibody adhesion by subtracting the fluorescence at blank spots, where only spotting buffer was printed without GTA fragment. The average of the triplicate spots was normalized to the highest intensity on the array and visualized in bar graphs using Microsoft Excel.

References

1 a) W. Fischer; *Adv. Micro. Phys.,* **1988**, 29: 233-302. b) W. Fischer, *Glycolipids, Phosphoglycolipids, and Sulfoglycolipids,* Springer US: Boston MA, 1990, pp. 123-234. c) W. Fischer, *New Comp. Biochem.,* **1994,** 27: 199-215

2 a) C. Theilacker, Z. Kaczynski, A. Kropec, F. Fabretti, T. Sange, O. Holst, J. Huebner, *Infect. Immun.,* **2006**, 70: 5703-5712. b) D.-J Jung, J.-H. An, K. Kurokawa, Y.-C. Jung, M. J. Kim, Y. Aoyagi, M. Matsushita, S Takahashi, H.-S. Lee, K. Takahashi, B. L. Lee; *J. Immunol.* **2012**, 189: 4951. c) Q. Chen, J. Dintaman, A. Lees, G. Sen, D. Schwartz, M. E. Shirtliff, S. Park, J. C. Lee, J. J. Mond, C. M. Snapper, *Infect. Immun.,* **2013**, 81: 2554–2561. d) F. Micoli, F. Berti, R. Adamo; *FEMS Microb. Reviews,* **2018**, 42: 388-423.

3 S. Kodali, E. Vinogradov, F. Lin, N. Khoury, L. Hao, V. Pavliak, C H. Jones, D. Laverde, J. Huebner, K. U. Jansen, A. S. Anderson, R. G. K. Donald; *J. Biol. Chem*. **2015**, 290**:** 19512. b) A. R. Sanderson, J. L. Strominger, S. G. Nathenson, R. J. Sanderson, *Biol. Chem*. **1962**, 237: 3603. c) M. Cot, A. Ray, M. Gilleron, A. Vercellone, G. Larrouy-Maumus, E. Armau, S. Gauthier, G. Tiraby, G. Puzo, J. Nigou, *PLoS One,* **2011**, 6: e26316.

4 a) K. Kurokawa, D. J. Jung, J. H. An, K. Fuchs, Y. J. Jeon, N. H. Ki, X. Li, K. Tateishi, J. A. Park, G. Xia, M. Matsushita K. Takahashi, H. J. Park, A. Peschel, B. L. Lee; *J. Biol. Chem.* **2013**, 288**:** 30956-68. b) G. Xia, C. Wolz; *Infect. Gen. Evol.* **2014**, 21: 593-601. c) M. P. Chapot-Chartier; *Front. Microbiol.*, **2014**, 5: 236.

5 S. Brown, J. P. Santa Maria, S. Walker; *Annu. Rev. Microbiol.* 2013, **67**, 313–336.

6 A. Walter, S. Unsleber, J. Rismondo, A. M. Jorge, A. Peschel, A. Gründling, C Mayer; *J. Biol. Chem.*, **2020**, 295: 4024-4034.

7 S. Morath, A. Geyer, I Spreitzer, C. Hermann, T. Hartung; *Infect. Immun.*, **2002**, 70: 938-944.

8 M. A. Oberli, M. Tamburrini, Y. H. Tsai, D. B. Werz, T. Holacher, A. Adibekian, D. Gauss, H. M. Moller, G. Pluschke, P. H. Seeberger; *J. Am. Chem. Soc.*, **2010**, 132: 10239-10241.

9 D. van der Es, W. F. J. Hogendorf, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée; *Chem. Soc. Rev.*, **2017**, 46: 1464-1482.

10 a) L. Wang, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée; *J. Am. Chem. Soc.*, **2018**, 140: 4632-4638. b) L. Yang, Q. Qin, X.-S. Ye; *Asian J. Org. Chem*., **2013**, 2, 30−49. c) S. K. Mulani, W.-C. Hung, A. B. Ingle, K.-S. Shiau, K.-K. T. Mong; *Org. Biomol. Chem,* **2014**, 12: 1184−119.

11 a) D. Laverde, D. Wobser, F. Romero-Saavedra, W. Hogendorf, G. van der Marel, M. Berthold, A. Kropec, J. Codée and J. Huebner; *PLoS One*, **2014**, 9: e110953. b) D. van der Es, F. Berni, W. F. J. Hogendorf, N. Meeuwenoord, D. Laverde, A. van Diepen, H. S. Overkleeft, D. V. Filippov, C. H. Hokke, J. Huebner, G. A. van der Marel, J. D. C. Codée; *Chem. Eur. J.*, **2018**, **24**: 4014–4018.

12 W. F. J. Hogendorf, A. Kropec, H. S. Overkleeft, D. V Filippov, J. Huebner, G. A. Van der Marel and J. D. C. Codée; *Carbohydr. Res*., **2012**, 356: 142-151.

13 a) C. B. Reese; *Org. Biomol. Chem,* **2005**, 3: 3851-3868. b) S. L. Beaucage, M. H. Caruthers; *Tetrahedr. Lett.*, **1981**, 22: 1859-1862.

14 W. F. J. Hogendorf, L. J. van den Bos, H. S. Overkleeft, J. D. C. Codée, G. A. van der Marel; *Bioorg, Med. Chem.,* **2010**, 18, 3668-3678.

15 W. F. J. Hogendorf, L. N. Lameijer, T. J. M. Beenakker, H. S. Overkleeft, D. V. Filippov, J. D. C. Codee, G. A. van der Marel; *Org. Lett.,* **2012**, 14: 848-851.

16 W. F. J. Hogendorf, N. Meeuwenoord, H. S. Overkleeft, D. V Filippov, D. Laverde, A. Kropec, J. Huebner, G. A. Van der Marel and J. D. C. Codée; *Chem. Commun*., **2011**, 47: 8961–8963.

17 a) N. M. Spijker, C. A. A. van Boeckel; *Angew. Chemie Int Ed.*, **1991**, 30: 180-183. b) L. Bohé, D. Crich; *Trends Glycosci. Glycotech.*, **2010**, 22: 115 c) M. Mickeleit, T. Wieder, M. Arnold, C. C. Geilen, J. Mulzer, W Reutter; *Angew Chemie Int. Ed*, **1998**, 37: 351–353.

18 W. F. J. Hogendorf (2012), Synthetic Methods to Glycerol Teichoic Acids (PhD thesis. Leiden institute of Chemistry (LIC), Science, Leiden University).

19 D. van der Es (2016), Synthesis of phosphodiester-containing bacterial cell wall components: teichoic acids, capsular polysaccharides and phosphatidyl glycerol analogues (PhD thesis. Leiden institute of Chemistry (LIC), Science, Leiden University).

20 J. Huebner, A. Quaas, W. A. Krueger, D. A. Goldmann and G. B. Pier; *Infect. Immun.,* **2000**, 68: 4631–4636.