Bacterial glycomimetics: synthesis and applications

Enotarpi, J.

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The synthesis of well-defined alanylated LTA fragments

Introduction

Peptidoglycan and anionic glycopolymers are the main cell-wall components found on Gram-positive bacteria. The anionic glycopolymers can be divided in three major classes: teichoic acids (TAs), teichurionic acids and sugar-phosphate polymers and these can typically make up 30-70% of the dry weight of the bacterial cell-wall (Figure 1).[1, 2 3] TAs are poly-alditol phosphates that can be either covalently attached to the peptidoglycan, as is the case for wall teichoic acids (WTA), or functionalized with a glycolipid anchor that

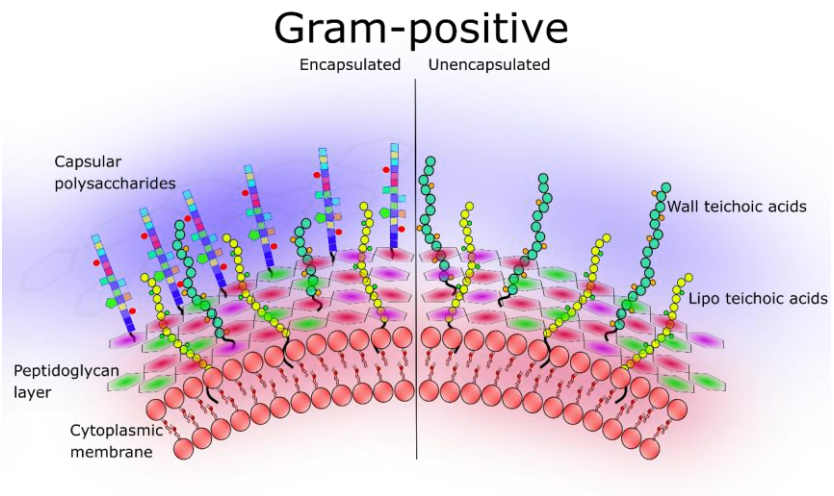


Figure 1. Gram-positive bacteria Cell-wall composition.

The work described in this chapter was supported by: C. Jimidar

connects through noncovalent bonds in the lipid bilayer, as in lipoteichoic acids (LTAs).[4] TAs are responsible for various tasks such as nutrient uptake, cation homeostasis, protection of the bacterium from external threats and binding to receptors and surfaces.[5,6] Binding of cations like Ca^{2+} and Mg^{2+} changes the conformation of the TAs, thus changing the rigidity or porosity of the cell wall. Gram-positive mutants lacking LTA synthase (LtaS), the enzyme responsible for the synthesis of the LTA polymer backbone, show susceptibility towards osmotic lysis and can only grow in osmotically stabilizing conditions, demonstrating the crucial role of LTAs for cell viability. [7, 8]

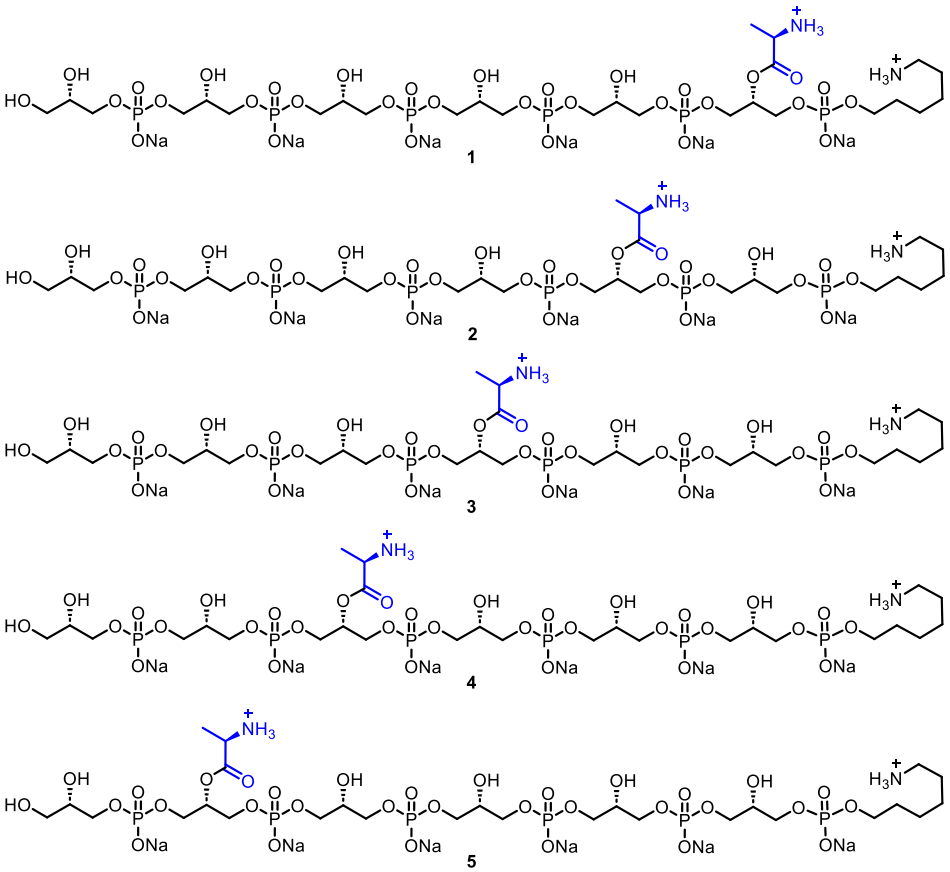


Figure 2. Target 1,3-sn-glycerolphosphate hexamers.

TAs are generally decorated with different carbohydrates and/or with D-alanine (D-Ala) appendages, and both are important for interactions with the outer environment. For example, D-Ala residues play a central role in resistance to cationic antimicrobial peptides and other microorganisms.[9, 10] Insight into the importance of the D-alanine residues at the molecular level has been gained using synthetic TA fragments in which the D-alanine

residues were replaced with L-alanine residues. This led to a significant decrease in the response of human blood leukocytes as measured by the release of pro-inflammatory cytokines.[11,12] It has been shown that TAs can be good candidates to develop glycoconjugate vaccines against Gram-positive bacteria. Both isolated TA polymers and synthetic fragments have been used to generate conjugate vaccines.[13] Synthetic TA fragments have been used to analyze the binding of antibodies in human sera and measure the immune response against invading *Staphylococcus aureus.*[14] They have also been used to probe the interaction with monoclonal antibodies at the atomic level, explaining the cross-reactivity of antibodies that recognize both β-(1,4)-*N*-acetyl glucosamine ribitol phosphate WTA and β-(1,3)-*N*-acetyl glucosamine ribitol phosphate WTA.[15] To better understand the role of the D-Ala appendages in triggering an immune response, it would be central to evaluate well-defined structures. Despite being abundant on the cell-wall of Gram-positive bacteria it is extremely difficult to isolate pure and well-defined TAs from natural sources as this results in heterogeneous mixtures. Because of the sensitivity toward basic and acidic conditions, the D-alanyl residues make it even more difficult to isolate these fragments. Therefore, organic synthesis is very attractive to procure these materials.

This chapter is focused on the synthesis of well-defined 1,3-*sn*-glycerolphosphate hexamers decorated with a single D-Ala moiety, positioned on different positions along the LTA chain as depicted in Figure 2. The aim of this work is to establish chemistry to reliably generate these fragments and study the stability of the D-alanine esters. With chemistry in place to generate these fragments, various substitution patterns can then be assembled to allow for detailed structure-activity relationship studies in the binding with different players of the immune system and explore the compounds in semi-synthetic vaccine modalities.

Results and discussion

The target library of TAs hexamers **1-5** and the correlated retrosynthesis are depicted in Figure 3. The final structures, containing one D-Ala substituent, can be obtained from their fully protected counterparts **7-11**. Benzyl ethers (Bn) have been chosen to mask glycerols and phosphate hydroxyls while *para*-methoxy benzyl ethers (PMB) temporarily block the glycerol C-2-alcohols where the *N*-Cbz-D-Ala **20** is to be installed.

The fully protected backbones can be broken down into key building blocks **12-15**, which can be used in iterative phosphoramidite coupling cycles during the elongation process. Linker building block **15** is derived from 6-amino-1-hexanol and all other glycerol building blocks **12-14** can be obtained starting from commercially available solketal **19**.

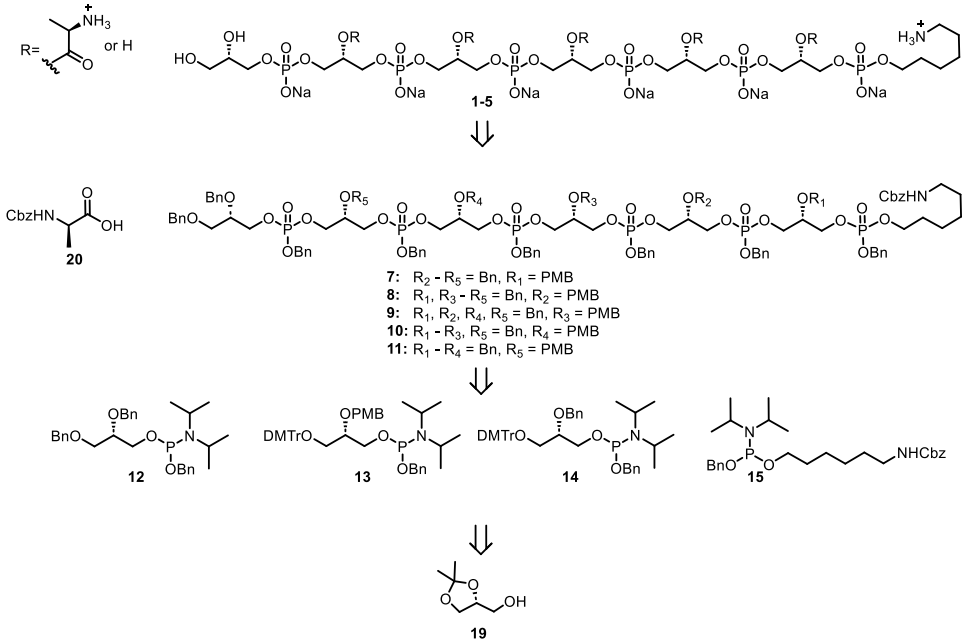
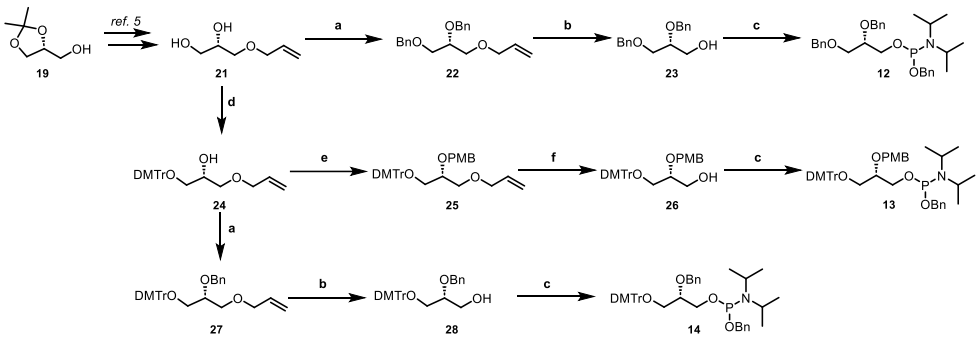


Figure 3. Retrosynthetic approach.

All glycerol phosphate building blocks were generated from solketal **19** using known procedures, comprising the initial allylation of the free primary alcohol, followed by removing the isopropylidene ketal under acidic conditions, yielding compound **21** in 90% (Scheme 1).[5] Terminal building block **12** was reached by protecting both hydroxyls on compound **21** using benzyl bromide (BnBr) and sodium hydride in a mixture of DMF/THF and subsequently removing the allyl moiety in a two-step procedure: first the allyl is isomerized to the corresponding enol ether using an iridium catalyst, which subsequently is cleaved using an aqueous solution of $NaHCO_3$ and I_2 yielding alcohol **23** in 60% over three steps. In the final step, **23** was transformed into the benzyl phosphoramidite **12**

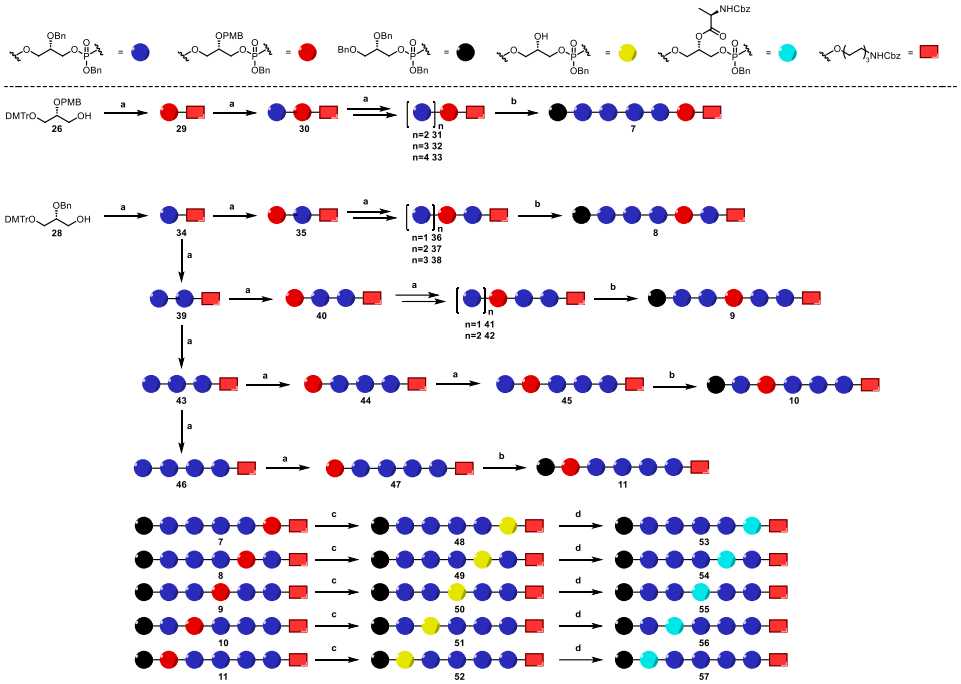
using benzyloxy bis(diisopropylamino)phosphine and diisopropylammonium tetrazol-2-ide in 63%. The second building block **13** was synthesized starting from compound **21** in four steps: first the primary alcohol was selectively protected with a dimethoxytrityl (DMTr) moiety using DMTrCl, and triethylamine (TEA) in dichloromethane (DCM) in 88% yield. At this point **24** was treated with *para*-methoxybenzyl chloride (PMBCl) and NaH in THF resulting in fully protected glycerol **25** in 88% yield. Curiously, in the following step, the isomerization of the allyl could not be achieved in satisfactory yield using the iridium catalyst as previously described, perhaps because of a competition of the electron rich PMB group with the allyl for coordination with the iridium catalyst. As an alternative the isomerization of the double bond was achieved using potassium *tert*-butoxide (*t*-BuOK) at high temperature in dimethyl sulfoxide (DMSO), followed by hydrolysis of the thus formed enol ether, giving alcohol **26** in 83% yield, which was transformed into key phosphoramidite building block **13** as described above. Lastly, **14** was synthesized from intermediate **24** by protecting the secondary alcohol as a benzyl ether, removing the allyl moiety using the iridium catalyst and by transforming the liberated alcohol into the corresponding phosphoramidite **14**.



Scheme 1. Building blocks synthesis: a) BnBr, NaH THF/DMF 9:1 ratio, 0°C to rt, ***22:*** *68%,* ***27****:87% b) i. Ir(COD)(Ph₂MeP)₂PF₆, THF, r.t., ii. I₂, NaHCO₃ THF, rt* ***23*** *88% over 2 steps,* ***28*** *90% over 2 steps c) diisopropylammonium tetrazol-2-ide, P(N(i-Pr)₂)₂OBn, DCM, rt,* ***12*** *63%,* ***13*** *82%,* ***14*** *83% d) DMTrCl, TEA, DCM, rt, 88% e) PMBCl, NaH, THF, 88% f) i. tBuOK, DMSO, 100°C, ii. I₂, NaHCO₃, THF, 83% over 2 steps.*

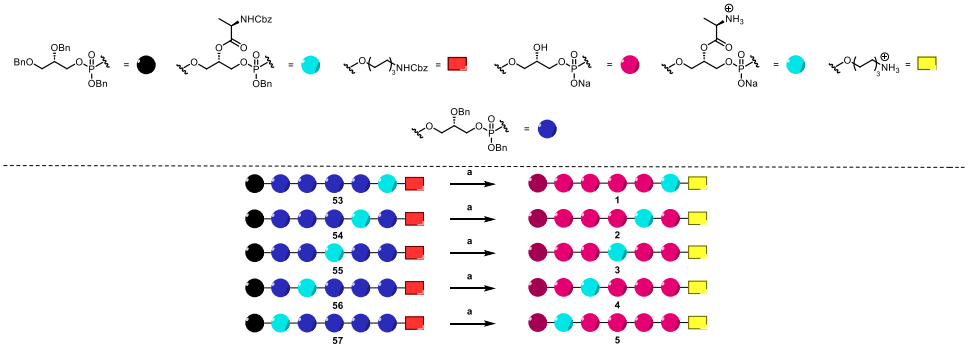
With all building blocks in hand, **7**-**11** have been prepared using the chemistry described in Chapter 2. As shown in Scheme 2, initially the linker was introduced via a phosphoramidite coupling using dicyanoimidazole (DCI) to activate the phosphoramidite, (1*S*)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) to oxidize the generated phosphite and treatment with dichloroacetic acid (DCA) to remove the DMTr moiety and liberating the hydroxyl for the subsequent coupling cycle. The PMB substituted glycerol building blocks were used for the positions where the D-Ala appendages are required and the double benzylated glycerol building block was used in the last coupling for each of the five generated fragments. The elongation proceeded smoothly for all hexamers **7**-**11** with

yields for the three-step eleongation cycles ranging from 50% to 93%. The synthesis proceeded with the selective removal of the PMB-moiety from each fragment using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a mixture of DCM/MeOH delivering compounds **48**-**52**. Subsequently D-Ala couplings were performed on the liberated hydroxy groups using derivative **20**, aided by benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-methylimidazole in acetonitrile (ACN) to obtain fully protected fragments **53**-**57**. For the final deprotection step it was decided to perform the hydrogenolysis reaction with palladium black in a mixture of 0.01% AcOH solution in MilliQ water/1,4-dioxane. The slightly acidic conditions were used to prevent deactivation of the catalyst and migration of the D-Ala moiety and dioxane was used to aid in the solubility of the starting material.



*Scheme 2. Synthesis of hexamer fragments **53**-**57**: a) 3Å ms, DCI, **13** or **14** or **15**, ACN, ii. CSO, DCM, iii. DCA, DCM **29** 71%, **30** 69%, **31** 80%, **32** 76%, **33** 62%, **34** 75%, **35** 76%, **36** 78%, **37** 56%, **38** 66%, **39** 69%, **40** 82%, **41** 74%, **42** 75%, **43** 51%, **44** 86%, **45** 86%, **46** 56%, **47** 86% b) 3Å ms, DCI, **12**, ACN, ii. CSO, DCM **7** 88%, **8** 89%, **9** 93%, **10** 80%, **11** 77% c) DDQ, DCM/MeOH **48** 41%, **49** 56%, **50** 64%, **51** 64%, **52** 58% d) Z-D-Ala, PyBOP, Me-Im, ACN **53** 70%, **54** 49%, **55** 37%, **56** 80%, **57** 78%.*

Purification of the final fragments was achieved under mild conditions using size exclusion chromatography with a neutral eluent (NaCl solution in MilliQ water), to avoid the possible migration and cleavage of the D-Ala groups. This was followed by a desalting step using a size exclusion column on MilliQ water. After final lyophilization fragments **1-5** were all successfully obtained in excellent yields ranging from 72 to 95%.



*Scheme 3. Synthesis of hexamer fragments **1-5**: a) Pd black, H_2, Dioxane/0.01% (v/v) AcOH solution in MilliQ water, **1** 88%, **2** 73%, **3** 73%, **4** 82%, **5** 95%.*

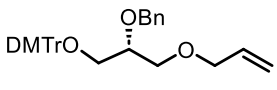
Conclusion

This Chapter has dealt with the synthesis of well-defined polyglycerol phosphates teichoic acid fragments, decorated with a D-alanine residue on pre-determined positions along the chain. Special attention has been paid to maintain the labile D-Ala moieties during the final deprotection and purification steps. The generated fragments can be tested for their immunogenicity. In the conjugation of the fragments, for example to carrier proteins, care has to be taken to use conditions that do not jeopardize the labile esters. The final Chapter of this thesis will describe possible approaches towards this end.

Experimental part

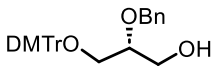
General procedures and materials: All chemicals (Acros, Biosolve, Sigma-Aldrich, TCI, etc) were used as received and all reactions were effectuated under an argon atmosphere, at ambient temperature (22°C), unless stated otherwise. For the TLC analysis were used aluminium sheets (Merck , TLC silica gel 60 F254), sprayed with a solution of H2SO4 (20%) in EtOH or with a solution of (NH4)6Mo7O24•4H2O (25 g/L) and (NH4)4Ce(SO4)4•2H2O (10g/L) in 10% aqueous H2SO4 or with a solution of KMnO4 (2%) and K2CO3 (1%) in H2O and then heated at ≈ 140°C. For the column chromatography was used 40-63 µm 60Å silica gel (SD Screening Devices). NMR spectra (1H, 13C and 31P) were recorded with a Bruker AV-400liq or a Bruker DMX-400solid or a Bruker AV-500 or a Bruker AV-600. High resolution mass spectra were recorded by direct injection on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (soure 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.

1-*O*-allyl-2-*O*-benzyl-3-*O*-(bis(4-methoxyphenyl)(phenyl))-sn-glycerol 27



Alcohol **24** (3.8 mmol, 1.65 g) was co-evaporated with toluene (3x) and dissolved in a mixture of THF/DMF (38 mL, 9:1 ratio). BnBr (4.56 mmol, 0.5 mL) was added and the mixture was cooled down to 0°C. NaH (60% dispersion in mineral oil, 9.5 mmol, 0.23 g) was slowly added and the reaction was stirred overnight at rt. H2O was added to the reaction mixture and it was diluted with Et2O (35 mL). The solution was washed with H_2O (5x) and brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **27** (1.69 g, 3.22 mmol) in 85% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.74 – 6.92 (m, 18H, aromatics), 6.10 – 5.91 (m, 1H, CH allyl), 5.39 – 5.28 (m, 2H, CH_2 allyl), 4.80 (s, 2H, CH_2 benzyl), 4.13 – 4.02 (m, 2H, CH_2 allyl), 3.96 – 3.88 (m, 1H, CH glycerol), 3.84 (s, 6H, OCH_3 DMTr), 3.75 (dd, J = 5.1, 1.9 Hz, 2H, CH_2 glycerol), 3.42 (dt, J = 5.6, 3.2 Hz, 2H, CH2 glycerol). **^{13}C NMR** (101 MHz, CD_3CN) δ = 159.6, 146.4, 140.1, 138.8, 137.2, 137.2, 136.2, 131.1, 130.0, 129.3, 129.1, 128.8, 128.7, 128.4, 127.7, 126.3, 118.1, 116.9, 114.1, 86.9, 78.7, 72.8, 72.7, 71.2, 64.6, 55.9. **HRMS** *m/z*: $[M+Na]^+$ Calcd 547.24549, founded 547.24550.

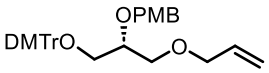
2-*O*-benzyl-3-*O*-(bis(4-methoxyphenyl)(phenyl))-sn-glycerol 28



Compound **27** (0.5 mmol, 0.26 g) was co-evaporated with toluene (3x), dissolved in freshly distilled dry THF (5.0 mL), and purged with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (0.004 mmol, 0.004 g) was added. The reaction mixture was purged with N_2, and then with H_2 for 4-5 sec, turning color from red

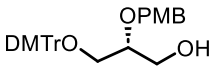
to yellow. The reaction was stirred for 70 min and diluted with a mixture of THF/$NaHCO_3$ (10.0 mL, 1:1 ratio). I_2 (0.75 mmol, 0.19 g) was added and the reaction was stirred for 1h. The solution was washed with $Na_2S_2O_3$ (1x), $NaHCO_3$ (5x), and brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **28** (0.37 mmol, 0.2 g) 74% as an orange oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.49 – 7.11 (m, 14H, aromatics), 6.89 – 6.80 (m, 4H, aromatics), 4.62 (s, 2H, CH_2Bn), 3.76 (s, 6H, 2x OMe), 3.58 (m, 3H, CH glycerol, CH_2 glycerol), 3.14 (d, J = 4.0 Hz, 2H, CH_2 glycerol), 2.69 – 2.60 (m, 1H, OH). **^{13}C NMR** (101 MHz, CD_3CN) δ = 159.5, 130.9, 129.2, 129.0, 128.7, 128.7, 128.3, 127.7, 113.9, 80.3, 72.6, 64.5, 62.7, 55.8. **HRMS** *m/z*: $[M+Na]^+$ Calcd 507.21419, founded 507.21430.

1-*O*-allyl-3-*O*-(bis(4-methoxyphenyl)(phenyl))-2-*O*-4-methoxybenzyl-sn-glycerol 25



Alcohol **24** (4.4 mmol, 1.74 g) was co-evaporated with toluene (3x) and dissolved in a mixture of THF/DMF (40.0 mL, 9:1 ratio). The mixture was cooled down to 0°C and PMBCl (6.6 mmol, 0.9 mL) was added. NaH (60% dispersion in mineral oil, 9.5 mmol, 0.23 g) was slowly added to the mixture and the reaction was stirred overnight at rt. H_2O was added to the reaction mixture and it was diluted with Et_2O (200.0 mL). The solution was washed with H_2O (5x) and brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **25** (4.14 mmol, 2.30 g) in 94% yield as a colourless oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.47 – 7.17 (m, 12H, aromatics), 6.93 – 6.79 (m, 6H, aromatics), 5.85 (m, 1H, CH allyl), 5.25 – 5.08 (m, 2H, CH_2 allyl), 4.52 (s, 2H, CH_2PMB), 3.92 (dt, J = 5.4, 1.5 Hz, 2H, OCH_2 allyl), 3.77 (d, J = 6.6 Hz, 9H, 3x OMe), 3.67 (m, 1H, CH glycerol), 3.56 – 3.48 (m, 2H, CH2 glycerol), 3.15 – 3.08 (m, 2H, CH_2 glycerol). **^{13}C NMR** (101 MHz, CD_3CN) δ = 159.5, 146.3, 137.2, 136.1, 130.9, 130.4, 129.0, 128.7, 127.7, 116.7, 114.5, 113.9, 78.1, 72.6, 72.3, 70.9, 64.4, 55.8. **HRMS** *m/z*: $[M+Na]^+$ Calcd 577.25606, founded 577.25598.

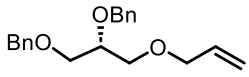
3-*O*-(bis(4-methoxyphenyl)(phenyl))-2-*O*-4-methoxybenzyl-sn-glycerol 26



Compound **25** (0.5 mmol, 0.28 g) was co-evaporated with toluene (3x) and it was dissolved in DMSO (1.0 mL). KOtBu (0.25 mmol, 0.028 g) was added to the solution and it was refluxed at 100°C for 30 min. H_2O was added to the reaction mixture and the product was extracted with Et_2O (5x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting crude was dissolved in a mixture of THF/$NaHCO_3$ (15.0 mL, 2:1 ratio). I_2 (0.75 mmol, 0.19 g) was added and the reaction was stirred overnight. The solution was washed with $Na_2S_2O_3$ (1x), $NaHCO_3$ (5x), and brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **26** (0.41 mmol, 0.21 g) in 83% yield as a yellow oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.47 – 7.18 (m, 12H, aromatics), 6.94 – 6.79 (m,

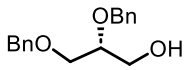
6H, aromatics), 4.53 (s, 2H, CH_2PMB), 3.77 (m, 9H, 3x OMe), 3.61 – 3.51 (m, 3H, CH glycerol, CH_2 glycerol), 3.15 – 3.06 (m, 2H, CH2 glycerol), 2.63 (t, J = 5.7 Hz, 1H, OH). **^{13}C NMR** (101 MHz, CD_3CN) δ =(101 MHz, CD_3CN) δ 130.9, 130.4, 129.0, 128.7, 127.7, 114.5, 113.9, 79.9, 72.3, 64.5, 62.7, 55.8. **HRMS** *m/z*: $[M+Na]^+$ Calcd 537.22476, founded 537.22477.

1-*O*-allyl-2,3-di-*O*-benzyl-sn-glycerol 22



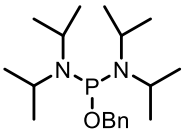
Diol **21** (23.0 mmol, 3.04 g) was co-evaporated with toluene (3x) and dissolved in a mixture of THF/DMF (230.0 mL, 9:1 ratio). The mixture was cooled down to 0°C and BnBr (55.2 mmol, 6.6 mL) was added. NaH (60% dispersion in mineral oil, 69.0 mmol, 2.76 g) was slowly added to the mixture and the reaction was stirred overnight at rt. H_2O was added to the reaction mixture and it was diluted with Et_2O (200.0 mL). The solution was washed with $Na_2S_2O_3$ (2x), $NaHCO_3$ (1x), and brine (1x). The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **22** (15.6 mmol, 4.88 g) in 68% yield as a colourless oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.43 – 7.24 (m, 10H, aromatics), 5.90 (m, 1H, CH allyl), 5.31 – 5.09 (m, 2H, CH_2 allyl), 4.64 (s, 2H, CH_2Bn), 4.51 (s, 2H, CH_2Bn), 3.97 (m, 2H, OCH_2 allyl), 3.74 (m, 1H, CH glycerol), 3.67 – 3.48 (m, 4H, 2x CH_2 glycerol). **^{13}C NMR** (101 MHz, CD_3CN) δ = 140.1, 139.7, 136.2, 129.3, 129.2, 128.6, 128.6, 128.4, 128.3, 116.7, 78.4, 73.7, 72.6, 72.5, 71.1, 71.0. **HRMS** *m/z*: $[M+Na]^+$ Calcd 335.16176, founded 335.16126.

2,3-di-*O*-benzyl-sn-glycerol 23



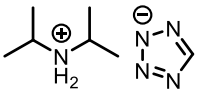
Compound **22** (0.46 mmol, 0.15 g) was co-evaporated with toluene (3x), dissolved in freshly distilled dry THF (5.0 mL), and purged with N_2. $Ir(COD)(Ph_2MeP)_2PF_6$ (0.004 mmol, 0.004 g) was added. The reaction mixture was purged with N_2, and then with H_2 for 4-5 sec, turning color from red to yellow. The reaction was stirred for 90 min and diluted with a mixture of THF/$NaHCO_3$ (10.0 mL, 1:1 ratio). I_2 (0.75 mmol, 0.19 g) was added and the reaction was stirred for 1h. The solution was washed with $Na_2S_2O_3$ (1x), $NaHCO_3$ (5x), and brine (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/Pentane) providing **23** (0.4 mmol, 0.11 g) in 88% yield as a colourless oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.22 (m, 9H, aromatics), 4.63 (s, 2H, CH_2Bn), 4.51 (s, 2H, CH_2Bn), 3.68 – 3.48 (m, 5H, 2x CH_2 glycerol, CH glycerol), 2.73 (t, J = 5.8 Hz, 1H, OH). **^{13}C NMR** (101 MHz, CD_3CN) δ = 129.2, 129.2, 128.6, 128.5, 128.4, 128.3, 79.9, 73.7, 72.4, 71.0, 62.5. **HRMS** *m/z*: $[M+Na]^+$ Calcd 295.13046, founded 295.13048.

1-(benzyloxy)-*N*,*N*,*N*',*N*'-bis(diisopropylphosphorodiamidite) 58



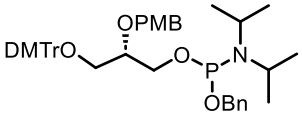
Bis(diisopropylaminochlorophosphine) (9.9 mmol, 2.63 g) was suspended in dry Et_2O (55.0 mL). Freshly activated m.s. 3Å were added to the suspension and it was cooled to 0°C. BnOH (9.9 mmol, 1.0 mL) was co-evaporated with toluene (3x) and dissolved in dry Et_2O (45.0 mL). Dry Et_3N (11.5 mmol, 1.6 mL) was added to this solution. The solution of BnOH and Et_3N was added dropwise to the suspension. The reaction mixture was stirred for 3 hours at 0°C. The mixture was filtrated and concentrated in vacuo. The crude was purified by chromatography (Et_3N/hexane 4:96) to yield a colorless oil in 79% (2.65 g, 7.8 mmol) as a mixture of bisbenzyloxy N,N-diisopropylphosphoramidite and **58** (0.13 : 1 ratio). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.42 – 7.29 (m, 5H, aromatics), 4.63 (d, J = 7.7 Hz, 2H, CH_2Bn), 3.65 – 3.53 (m, 4H, 4x CH isopropyl), 1.21 – 1.15 (m, 24H, 8x CH_3). **^{31}P NMR** (162 MHz, CD_3CN) δ = 124.5.

Diisopropylammonium tetrazol-2-ide 59



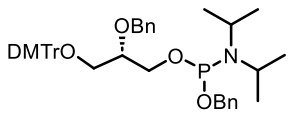
1-H-tetrazole (81 mmol, 5.6 g) was dissolved in dry ACN (145.0 mL) and DiPEA (132.03 mmol, 13.36 g) was added. The suspension was stirred for 30 min and concentrated in vacuo providing **59** (81 mmol, 13.87 g) in 95% yield. **^{1}H NMR** (400 MHz, D_2O) δ = 8.55 (s, 1H, H tetrazole), 3.44 (dt, J = 12.8, 6.3 Hz, 2H, 2x CH isopropyl), 1.24 (d, J = 6.3 Hz, 12H, 4X CH3). **^{13}C NMR** (101 MHz, D_2O) δ = 47.2, 18.2.

3-*O*-(bis(4-methoxyphenyl)(phenyl))-2-*O*-4-methoxyphenyl-1-*O*-([*N*,*N*-diisoprpylamino]-2-benzyloxy-phosphoramidite)-sn-glycerol 13



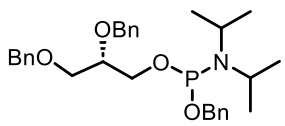
Alcohol **26** (4.1 mmol, 2.1 g) was co-evaporated with toluene (3x) and dissolved in DCM (20.5 mL). Phosphorodiamidite **58** (4.1 mmol, 20.5 mL, 0.2M solution in DCM) was added to the solution. Tetrazolide **59** (2.1 mmol, 0.36 g) was added and the suspension was stirred until the total conversion of the starting material. $NaHCO_3$ was added to the suspension and it was washed with a solution of $NaHCO_3$/brine (1:1 ratio). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/pentane) with 3% Et_3N (v/v) eluent providing **13** (3.38 mmol, 2.54 g) in 82% yield as a pale-yellow oil (mixture of diastereoisomers). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.52 – 7.14 (m, 16H, aromatics), 6.93 – 6.75 (m, 6H, aromatics), 4.65 – 4.48 (m, 4H, 2x CH_2Bn), 3.80 – 3.63 (m, 12H, CH_2 glycerol, CH glycerol, 3x OMe), 3.61 – 3.41 (m, 2H, 2x CH isopropyl), 3.23 – 3.11 (m, 2H, CH_2 glycerol), 1.16 – 1.03 (m, 12H, 4x CH_3). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.1, 159.5, 146.4, 140.7, 137.2, 137.1, 132.0, 131.0, 130.4, 129.5, 129.2, 129.0, 128.7, 128.2, 128.0, 127.7, 114.5, 113.9, 86.7, 78.9, 78.8, 72.4, 66.0, 65.8, 64.7, 64.6, 63.8, 63.7, 55.8, 43.8, 43.7, 25.1, 25.0, 24.9. **^{31}P NMR** (162 MHz, CD_3CN) δ = 148.8, 148.7.

2-*O*-benzyl-3-*O*-(bis(4-methoxyphenyl)(phenyl))-1-*O*-([*N*,*N*-diisoprpylamino]-2-benzyloxy-phosphoramidite)-sn-glycerol 14



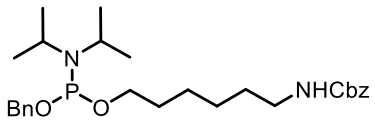
Alcohol **28** (6.0 mmol, 2.91 g) was co-evaporated with toluene (3x) and dissolved in DCM (30 mL). Phosphoramidite **58** (6 mmol, 30.0 mL, 0.2M solution in DCM) was added to the solution. Tetrazolide **59** (3.0 mmol, 0.51 g) was added and the suspension was stirred until total conversion of the starting material. $NaHCO_3$ was added to the suspension and washed with a solution of $NaHCO_3$/brine (1:1 ratio). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/pentane) with 3% Et_3N (v/v) eluent, followed by a second column of (EtOAc/pentane) with 3% Et_3N (v/v) eluent providing **14** (5 mmol, 3.6 g) in 83% yield as a colourless oil (mixture of diastereoisomers). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.51 – 6.75 (m, 23H, aromatics), 4.70 – 4.51 (m, 4H, 2x CH_2Bn), 3.83 – 3.64 (m, 9H, 2x OMe, CH glycerol, CH_2 glycerol), 3.56 (m, 2H, 2x CH isopropyl), 3.25 – 3.10 (m, 2H, CH_2 glycerol), 1.22 – 0.98 (m, 12H, 4x CH_3). **^{13}C NMR** (101 MHz, CD_3CN) δ = 159.5, 146.3, 140.0, 137.1, 137.1, 137.1, 137.0, 130.9, 130.9, 129.2, 129.2, 128.9, 128.9, 128.7, 128.7, 128.4, 128.2, 128.2, 128.0, 127.9, 127.6, 113.9, 86.7, 86.7, 79.2, 79.1, 72.6, 66.0, 65.9, 65.8, 65.8, 64.7, 64.5, 63.7, 63.6, 55.8, 43.8, 43.7, 43.6, 25.0, 24.8. **^{31}P NMR** (162 MHz, CD_3CN) δ = 148.8, 148.7.

2,3-di-*O*-benzyl-1-*O*-([*N*,*N*-diisoprpylamino]-2-benzyloxy-phosphoramidite)-sn-glycerol 12



Alcohol **23** (3.5 mmol, 0.95 g) was co-evaporated with toluene (3x) and dissolved in DCM (17.5 mL). Phosphoramidite **58** (3.5 mmol, 17.5 mL, 0.2M solution in DCM) was added to the solution. Tetrazolide **59** (1.75 mmol, 0.3 g) was added and the suspension was stirred until total conversion of the starting material. $NaHCO_3$ was added to the suspension and washed with a solution of $NaHCO_3$/brine (1:1 ratio). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/pentane) with 3% Et_3N (v/v) eluent providing **12** (2.2 mmol, 1.12 g) in 63% yield as a colourless oil (mixture of diastereoisomers). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.39 – 7.23 (m, 15H, aromatics), 4.73 – 4.58 (m, 4H, 2x CH_2Bn), 4.50 (s, 2H, CH_2Bn), 3.83 – 3.53 (m, 7H, 2x CH_2 glycerol, CH glycerol, 2x CH isopropyl), 1.16 (m, 12H, 4x CH_3). **^{13}C NMR** (101 MHz, CD_3CN) δ = 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 79.0, 73.7, 72.5, 70.8, 65.7, 63.7, 43.8, 25.0, 24.9. **^{31}P NMR** (162 MHz, CD_3CN) δ = 149.0, 148.9.

Benzyl 6-([*N*,*N*-diisopropylamino]-2-benzyloxy-phosphoramidite)-hexyl-1-carbamate (**15**)

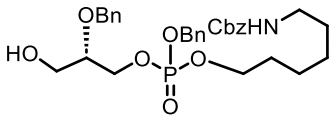


Benzyl (6-hydroxyhexyl) carbamate (4.0 mmol, 1.01 g) was co-evaporated with toluene (3x) and dissolved in DCM (20.0 mL). Phosphorodiamidite **58** (1.2 mmol, 20.0 mL, 0.2M solution in DCM) was added to the solution. Tetrazolide **59** (2.0 mmol, 0.34 g) was added and the suspension was stirred until the total conversion of the starting material. $NaHCO_3$ was added to the suspension and it was washed with a solution of $NaHCO_3$/brine (1:1 ratio). The organic phase was dried over Na_2SO_4 and concentrated in vacuo. The crude was purified by flash chromatography (EtOAc/pentane) with 3% Et_3N (v/v) eluent providing **15** (3.4 mmol, 1.67 g) in 85% yield as a colourless oil (mixture of diastereoisomers). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.43 – 7.22 (m, 10H, aromatics), 5.60 (s, 1H, NH), 5.03 (s, 2H, CH_2Cbz), 4.67 (qd, J = 12.7, 8.3 Hz, 2H, CH_2Bn), 3.71 – 3.52 (m, 4H, 2x CH isopropyl, OCH_2), 3.07 (dd, J = 13.2, 6.7 Hz, 2H, $NHCH_2$), 1.62 – 1.50 (m, 2H, CH_2 Linker), 1.50 – 1.41 (m, 2H, CH_2 Linker), 1.41 – 1.23 (m, 4H, 2x CH_2 Linker), 1.18 (m, 12H, 4x CH_3). **^{13}C NMR** (101 MHz, CD_3CN) δ = 169.3, 169.1, 157.3, 140.9, 140.8, 129.4, 129.2, 128.7, 128.6, 128.2, 128.0, 66.6, 65.9, 65.9, 64.2, 64.0, 43.7, 43.6, 41.5, 32.0, 31.9, 30.6, 27.1, 26.4, 25.0, 24.9, 1.9, 1.7, 1.5, 1.3, 1.1, 0.9, 0.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = 146.7, 146.6.

General Procedure A: phosphoramidite coupling, oxidation, and detritylation on a typical scale of 0.05 – 0.5 mmol

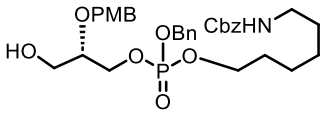
The starting material was co-evaporated with ACN (3x) and was dissolved in dry ACN (0.1-0.05 M). DCI (0.25M solution in ACN, 1.5 eq) was added to the reaction mixture and the solution was stirred for 20 min. Phosphoramidite reagent (0.2 – 0.56 M solution in ACN, 1.3 - 2 eq) was added to the reaction mixture and the solution was stirred until total conversion of the starting material (≃ 1.5h). CSO (0.5M solution in ACN, 2 eq) was added to the reaction mixture and stirred for 15 min. EtOAc was added to the reaction mixture and washed with a solution of $NaHCO_3$/brine (1:1 ratio). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was co-evaporated with ACN (3x) and dissolved in DCM (1 – 3 mL). DCA (3% DCM solution, 5eq) was added to the solution and stirred for 2h. A solution of MeOH/H_2O (1:1 ratio) was added to the reaction mixture and it was washed with a solution of $NaHCO_3$/brine (1:1 ratio). The water layer was extracted with DCM (3x). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) or by size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1).

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 34



Alcohol **28** (4.33 mmol, 2.1 g) was coupled to phosphoramidite **15** (5.63 mmol, 10.0 mL 0.56 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **34** (3.24 mmol, 1.90 g) in 75% yield. **^{1}H NMR** (400 MHz, CD3CN) δ = 7.44 – 7.22 (m, 15H, aromatics), 5.62 (s, 1H, NH), 5.06 – 4.99 (m, 4H, CH_2Bn, CH_2Cbz), 4.61 (s, 2H, CH_2Bn), 4.19 – 4.00 (m, 2H, CH_2 glycerol), 4.00 – 3.92 (m, 2H, OCH_2), 3.64 – 3.54 (m, 3H, CH2 glycerol, CH glycerol), 3.06 (m, 2H, $NHCH_2$), 3.01 – 2.94 (m, 1H, OH), 1.64 – 1.51 (m, 2H, CH_2Linker), 1.48 – 1.37 (m, 2H, CH_2Linker), 1.29 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 169.0, 157.3, 139.6, 137.7, 129.5, 129.4, 129.2, 128.8, 128.7, 128.7, 128.6, 128.4, 79.2, 79.2, 72.4, 69.8, 69.8, 68.7, 68.6, 67.2, 67.1, 66.6, 61.1, 41.3, 30.7, 30.7, 30.4, 26.7, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = 0.75, 0.73. **HRMS** *m/z*: $[M+H]^+$ Calcd 586.25641, founded 586.25643.

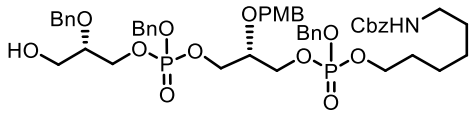
1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 29



Alcohol **26** (1.02 mmol, 0.51 g) was coupled to phosphoramidite **15** (1.33 mmol, 4.4 mL 0.29 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **29** (0.71 mmol, 0.44 g) in 71% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.43 – 6.88 (m, 14H, aromatics), 5.78 (s, 1H, NH), 5.08 – 5.00 (m, 4H, 2x CH_2Bn), 4.54 (s, 2H, CH_2PMB), 4.21 – 4.01 (m, 2H, CH_2 glycerol), 4.01 – 3.93 (m, 2H, OCH_2), 3.76 (s, 3H,

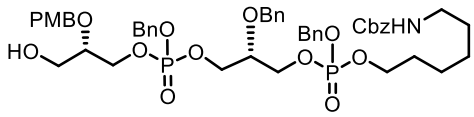
OMe), 3.65 – 3.55 (m, 3H, CH_2 glycerol, CH glycerol), 3.07 (dd, J = 13.0, 6.5 Hz, 2H, $NHCH_2$), 1.62 – 1.53 (m, 2H, CH_2Linker), 1.47 – 1.39 (m, 2H, CH_2Linker), 1.28 (s, 4H, 2x CH_2Linker). **^{13}C NMR** (126 MHz, CD_3CN) δ = 160.2, 131.7, 130.4, 129.5, 129.4, 128.9, 128.8, 128.6, 114.5, 78.9, 72.1, 69.8, 68.6, 67.2, 66.6, 61.2, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (202 MHz, CD_3CN) δ = 0.73, 0.72. **HRMS** *m/z*: $[M+H]^+$ Calcd 616.26699, founded 616.26699.

1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 30



Alcohol **29** (0.7 mmol, 0.44 g) was coupled to phosphoramidite **14** (0.91 mmol, 3.5 mL 0.26 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **30** (0.49 mmol, 0.46 g) in 69% yield. **^{1}H NMR** (400 MHz, CD3CN) δ = 7.47 – 7.22 (m, 22H, aromatics), 6.92 – 6.80 (m, 2H, aromatics), 5.90 (s, 1H, NH), 5.13 – 5.00 (m, 6H, 3x CH_2Bn), 4.65 – 4.57 (m, 2H, CH_2Bn), 4.57 – 4.48 (m, 2H, CH_2PMB), 4.27 – 4.00 (m, 6H, 3x CH_2 glycerol), 4.00 – 3.90 (m, 2H, OCH_2), 3.83 – 3.70 (m, 4H, CH glycerol, OMe), 3.68 – 3.53 (m, 3H, CH_2 glycerol, CH glycerol), 3.07 (m, 2H, $NHCH_2$), 1.55 (d, J = 5.4 Hz, 2H, CH_2Linker), 1.41 (dd, J = 15.3, 8.1 Hz, 2H, CH_2Linker), 1.36 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 138.5, 137.2, 137.2, 137.1, 137.1, 131.0, 130.5, 129.5, 129.4, 129.3, 129.2, 128.8, 128.7, 128.6, 128.6, 128.4, 118.3, 114.5, 79.2, 79.1, 76.5, 76.4, 76.4, 72.4, 72.3, 72.2, 70.0, 69.9, 69.9, 69.9, 68.7, 68.7, 67.5, 67.4, 66.5, 66.5, 66.4, 66.3, 61.1, 55.8, 41.3, 30.8, 30.7, 30.7, 30.3, 26.8, 26.7, 25.7, 25.7, 1.9, 1.9, 1.7, 1.7, 1.5, 1.5, 1.3, 1.3, 1.1, 1.0, 0.9, 0.6. **^{31}P NMR** (162 MHz, CD_3CN) δ = 0.62, 0.60, 0.41. **HRMS** *m/z*: $[M+H]^+$ Calcd 950.36400, founded 950.36400.

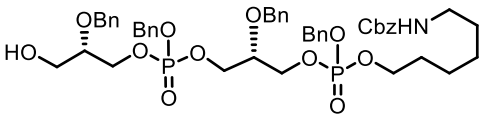
1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 35



Alcohol **34** (0.75 mmol, 0.44 g) was coupled to phosphoramidite **13** (0.98 mmol, 3.3 mL 0.3 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **35** (0.57 mmol, 0.54 g) in 76% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.45 – 7.19 (m, 22H, aromatics), 6.90 – 6.79 (m, 2H, aromatics), 5.73 (s, 1H, NH), 5.10 – 4.98 (m, 6H, 3x CH_2Bn), 4.60 – 4.56 (m, 2H, CH_2Bn), 4.49 (m, 2H, CH_2PMB), 4.21 – 3.98 (m, 6H, 3x CH_2 glycerol), 3.98 – 3.88 (m, 2H, OCH_2), 3.81 – 3.75 (m, 1H, CH glycerol), 3.73 (m, 3H, OMe), 3.62 – 3.50 (m, 3H, CH_2 glycerol, CH glycerol), 3.04 (m, 2H, $NHCH_2$), 1.61 – 1.48 (m, 2H, CH_2Linker), 1.46 – 1.34 (m, 2H, CH_2Linker), 1.26 (d, J = 3.0 Hz, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.1, 139.1, 131.5, 130.4, 129.5, 129.4, 129.3, 129.2, 128.9, 128.8, 128.7,

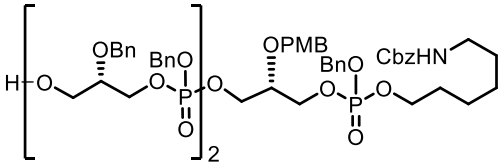
128.6, 128.6, 114.5, 78.8, 76.8, 72.6, 72.0, 70.0, 68.7, 67.5, 66.5, 61.1, 55.8, 41.3, 30.7, 30.3, 26.7, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = 0.62, 0.60, 0.40. **HRMS** *m/z*: $[M+H]^+$ Calcd 950.36400, founded 950.36400.

1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 39



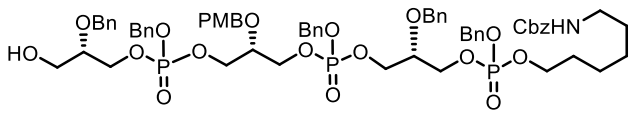
Alcohol **34** (2.4 mmol, 1.4 g) was coupled to phosphoramidite **14** (3.84 mmol, 9.6 mL 0.4 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **39** (1.6 mmol, 1.51 g) in 69% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.42 – 7.22 (m, 25H, aromatics), 5.67 (s, 1H, NH), 5.06 – 4.97 (m, 6H, 3x CH_2Bn), 4.61 – 4.55 (m, 4H, 2x CH_2Bn), 4.20 – 3.98 (m, 6H, 3x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.81 – 3.74 (m, 1H, CH glycerol), 3.63 – 3.49 (m, 3H, CH_2 glycerol, CH glycerol), 3.04 (m, 3H, $NHCH_2$, OH), 1.60 – 1.51 (m, 2H, CH_2Linker), 1.46 – 1.35 (m, 2H, CH_2Linker), 1.34 – 1.20 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 129.5, 129.4, 129.2, 129.2, 128.8, 128.8, 128.7, 128.6, 128.4, 79.1, 76.8, 72.6, 72.3, 70.0, 68.7, 67.4, 66.4, 61.1, 30.7, 30.3, 26.7, 25.6. **^{31}P NMR** (122 MHz, CD_3CN) δ = -0.86, -0.92, -0.99, -1.05, -1.11. **HRMS** *m/z*: $[M+H]^+$ Calcd 920.35344, founded 920.35344.

1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 31



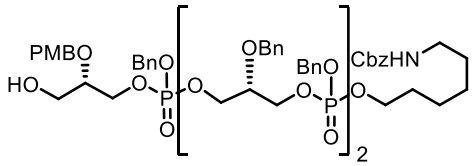
Alcohol **30** (0.48 mmol, 0.45 g) was coupled to phosphoramidite **14** (0.72 mmol, 3.6 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **31** (0.385 mmol, 0.49 g) in 80% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.40 – 7.19 (m, 32H, aromatics), 6.84 (d, J = 8.3 Hz, 2H, aromatics), 5.83 (s, 1H, NH), 5.08 – 4.97 (m, 8H, 4x CH_2Bn), 4.61 – 4.53 (m, 4H, 2x CH_2Bn), 4.52 – 4.46 (m, 2H, CH_2PMB), 4.24 – 3.98 (m, 10H, 5x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.80 – 3.73 (m, 2H, 2x CH glycerol), 3.72 (s, 3H, OMe), 3.64 – 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.05 (m, 2H, $NHCH_2$), 1.54 (m, 2H, CH_2Linker), 1.40 (m, 2H, CH_2Linker), 1.33 – 1.21 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 131.0, 130.5, 129.5, 129.4, 129.3, 129.2, 129.2, 128.8, 128.8, 128.7, 128.6, 128.6, 128.6, 128.4, 114.5, 79.1, 76.8, 76.5, 72.6, 72.3, 72.3, 70.0, 68.7, 67.5, 66.5, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.74, -0.75, -0.77, -0.97, -0.99, -1.05, -1.06. **HRMS** *m/z*: $[M+H]^+$ Calcd 1284.46101, founded 1284.46101.

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)- 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 36



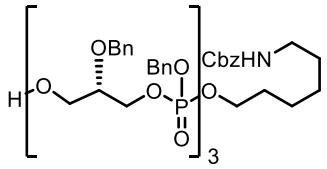
Alcohol **35** (0.55 mmol, 0.52 g) was coupled to phosphoramidite **14** (0.72 mmol ,3.6 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **36** (0.43 mmol, 0.55 g) in 78% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.48 – 7.17 (m, 32H, aromatics), 6.84 (d, J = 8.3 Hz, 2H, aromatics), 5.90 (s, 1H, NH), 5.11 – 4.98 (m, 8H, 4x CH_2Bn), 4.59 (m, 4H, 2x CH_2Bn), 4.49 (s, 2H, CH_2PMB), 4.26 – 4.00 (m, 10H, 5x CH_2 glycerol), 3.95 (m, 2H, OCH_2), 3.81 – 3.73 (m, 2H, 2x CH glycerol), 3.72 (s, 3H, OMe), 3.66 – 3.57 (m, 3H, CH glycerol, CH_2 glycerol), 3.23 (s, 1H, OH), 3.07 (m, 2H, $NHCH_2$), 1.61 – 1.49 (m, 2H, CH_2Linker), 1.46 – 1.36 (m, 2H, CH_2Linker), 1.32 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 138.4, 137.1, 137.0, 130.9, 130.5, 129.5, 129.4, 129.2, 129.2, 128.8, 128.7, 128.6, 128.4, 114.5, 79.2, 79.1, 76.8, 76.4, 72.6, 72.3, 72.2, 70.0, 69.9, 68.7, 67.5, 66.5, 66.4, 66.3, 61.1, 55.8, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (126 MHz, CD_3CN) δ = 0.82, 0.79, -1.02, -1.13. **HRMS** *m/z*: $[M+H]^+$ Calcd 1284.46101, founded 1284.46101.

1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 40



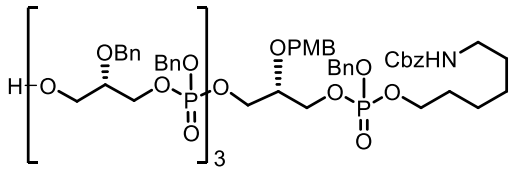
Alcohol **39** (0.59 mmol, 0.55 g) was coupled to phosphoramidite **13** (0.89 mmol ,3.0 mL 0.3 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **40** (0.48 mmol, 0.62 g) in 82% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.40 – 7.20 (m, 32H, aromatics), 6.84 (m, 2H, aromatics), 5.75 (s, 1H, NH), 5.06 – 4.97 (m, 8H, 4x CH_2Bn), 4.59 – 4.52 (m, 4H, 2x CH_2Bn), 4.52 – 4.46 (m, 2H, CH_2PMB), 4.20 – 3.96 (m, 10H, 5x CH_2 glycerol), 3.93 (m, 2H, OCH_2), 3.79 – 3.73 (m, 2H, 2x CH glycerol), 3.72 (s, 3H, OMe), 3.60 – 3.50 (m, 3H, CH glycerol, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.62 – 1.48 (m, 2H, CH_2Linker), 1.45 – 1.34 (m, 2H, CH_2Linker), 1.34 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.1, 157.2, 139.1, 136.7, 131.5, 130.4, 129.5, 129.4, 129.3, 129.2, 128.9, 128.8, 128.7, 128.6, 78.8, 78.7, 76.8, 72.6, 72.0, 70.1, 70.0, 70.0, 69.9, 69.9, 68.7, 68.7, 67.5, 66.6, 66.5, 66.4, 66.4, 61.1, 55.8, 41.3, 30.7, 30.7, 30.3, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.80, -0.82, -1.02, -1.11. **HRMS** *m/z*: $[M+H]^+$ Calcd 1284.46101, founded 1284.46101.

1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 43



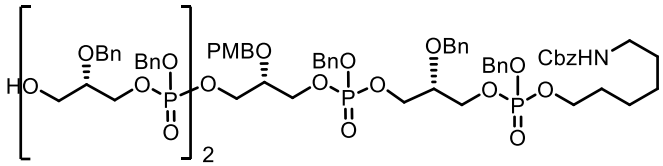
Alcohol **39** (1.04 mmol, 0.96 g) was coupled to phosphoramidite **14** (1.87 mmol, 4.7 mL 0.4 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **43** (0.53 mmol, 0.65 g) in 51% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.40 – 7.21 (m, 35H, aromatics), 5.68 (s, 1H, NH), 5.07 – 4.95 (m, 8H, 4x CH_2Bn), 4.60 – 4.50 (m, 6H, 3x CH_2Bn), 4.21 – 3.97 (m, 10H, 5x CH_2 glycerol), 3.93 (m, 2H, OCH_2), 3.75 (s, 2H, 2x CH glycerol), 3.62 – 3.50 (m, 3H, CH glycerol, CH_2 glycerol), 3.12 – 2.98 (m, 3H, $NHCH_2$, OH), 1.54 (s, 2H, CH_2Linker), 1.39 (s, 2H, CH_2Linker), 1.25 (s, 4H, 2x CH_2Linker). **^{13}C NMR** (126 MHz, CD_3CN) δ = 139.7, 139.1, 139.1, 137.3, 137.2, 129.6, 129.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.3, 81.3, 79.2, 79.2, 76.9, 76.9, 72.7, 72.4, 70.2, 70.1, 70.0, 69.9, 69.9, 68.8, 68.7, 68.7, 67.5, 67.4, 66.7, 66.6, 66.6, 66.5, 66.4, 66.3, 62.4, 61.2, 61.2, 46.7, 41.4, 30.8, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = 0.64, 0.62, 0.42, 0.33. **HRMS** *m/z*: $[M+H]^+$ Calcd 1254.40045, founded 1254.40045.

1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 32



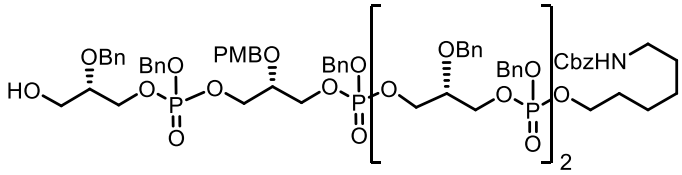
Alcohol **31** (0.39 mmol, 0.49 g) was coupled to phosphoramidite **14** (0.77 mmol, 3.9 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **32** (0.29 mmol, 0.47 g) in 76% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.21 (m, 42H, aromatics), 6.83 (d, J = 8.3 Hz, 2H, aromatics), 5.85 (s, 1H, NH), 5.01 (m, 10H, 5x CH_2Bn), 4.56 (m, 6H, 3x CH_2Bn), 4.49 (m, 2H, CH_2PMB), 4.23 – 3.98 (m, 14H, 7x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.75 (s, 3H, 3x CH glycerol), 3.71 (s, 3H, OMe), 3.64 – 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.05 (m, 2H, $NHCH_2$), 1.54 (s, 2H, CH_2Linker), 1.46 – 1.35 (m, 2H, CH_2Linker), 1.33 – 1.16 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 131.0, 130.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 128.8, 128.8, 128.7, 128.6, 128.6, 128.4, 114.5, 113.9, 79.2, 79.1, 76.9, 76.4, 72.6, 72.3, 72.3, 70.1, 69.8, 68.7, 68.7, 68.6, 67.5, 67.4, 66.6, 66.3, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.73, -0.74, -0.76, -0.96, -1.01, -1.05. **HRMS** *m/z*: $[M+H]^{2+}$ Calcd 809.78265, founded 809.78265.

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)- 3-*O*-di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 37



Alcohol **36** (0.41 mmol, 0.53 g) was coupled to phosphoramidite **14** (0.62 mmol, 3.1 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **37** (0.23 mmol, 0.41 g) in 56% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.42 – 7.19 (m, 42H, aromatics), 6.82 (d, J = 8.4 Hz, 2H, aromatics), 5.84 (s, 1H, NH), 5.06 – 4.97 (m, 10H, 5x CH_2Bn), 4.60 – 4.52 (m, 6H, 3x CH_2Bn), 4.47 (m, 2H, CH_2PMB), 4.23 – 3.97 (m, 14H, 7x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.76 (m, 3H, 3x CH glycerol), 3.71 (s, 3H, OMe), 3.64 – 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.05 (m, 2H, $NHCH_2$), 1.58 – 1.48 (m, 2H, CH_2Linker), 1.44 – 1.35 (m, 2H, CH_2Linker), 1.31 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 139.0, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 130.9, 130.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.8, 128.7, 128.7, 128.6, 128.6, 128.4, 114.5, 79.2, 79.1, 76.9, 76.4, 72.6, 72.3, 72.3, 70.1, 69.8, 68.7, 68.6, 67.5, 67.4, 66.6, 66.3, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.72, -0.74, -0.76, -0.96, -1.02. **HRMS** *m/z*: $[M+H]^+$ Calcd 1618.55801, founded 1618.55803.

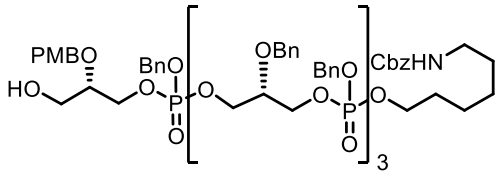
1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 41



Alcohol **40** (0.46 mmol, 0.59 g) was coupled to phosphoramidite **14** (0.69 mmol ,3.5 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **41** (0.34 mmol, 0.55 g) in 74% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.43 – 7.21 (m, 42H, aromatics), 6.84 (dd, J = 8.5, 1.2 Hz, 2H, aromatics), 5.92 (s, 1H, NH), 5.10 – 4.99 (m, 10H, 5x CH_2Bn), 4.63 – 4.54 (m, 6H, 3x CH_2Bn), 4.51 – 4.47 (m, 2H, CH_2PMB), 4.25 – 4.00 (m, 14H, 7x CH_2 glycerol), 3.95 (m, 2H, OCH_2), 3.77 (m, 3H, 3x CH glycerol), 3.74 – 3.70 (m, 3H, OMe), 3.66 – 3.57 (m, 3H, CH glycerol, CH_2 glycerol), 3.07 (m, 2H, $NHCH_2$), 1.59 – 1.48 (m, 2H, CH_2Linker), 1.47 – 1.36 (m, 2H, CH_2Linker), 1.32 – 1.16 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 139.0, 138.5, 137.2, 137.2, 137.1, 137.0, 137.0, 130.9, 130.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.8, 128.7, 128.7, 128.4, 114.5, 79.2, 79.1,

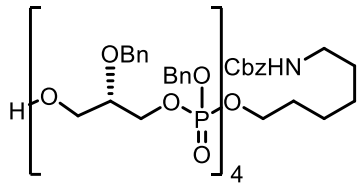
76.8, 76.3, 72.6, 72.3, 70.1, 69.8, 68.7, 67.5, 67.4, 66.6, 66.2, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7, 1.9, 1.7, 1.7, 1.5, 1.5, 1.3, 1.3, 1.1, 1.1, 0.9, 0.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.70, -0.72, -0.92, -0.97, -1.01, -1.02. **HRMS** *m/z*: $[M+H]^+$ Calcd 1618.55801, founded 1618.55803.

1-*O*-(tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 44



Alcohol **43** (0.29 mmol, 0.37 g) was coupled to phosphoramidite **13** (0.38 mmol ,1.7 mL 0.26 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **44** (0.25 mmol, 0.40 g) in 86% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.21 (m, 42H, aromatics), 6.88 – 6.81 (m, 2H, aromatics), 5.86 (s, 1H, NH), 5.07 – 4.98 (m, 10H, 5x CH_2Bn), 4.59 – 4.53 (m, 6H, 3x CH_2Bn), 4.53 – 4.47 (m, 2H, CH_2PMB), 4.22 – 3.98 (m, 14H, 7x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.80 – 3.74 (m, 3H, 3x CH glycerol), 3.74 – 3.70 (m, 3H, OMe), 3.63 – 3.53 (m, 3H, CH glycerol, CH_2 glycerol), 3.06 (m, 2H, $NHCH_2$), 1.59 – 1.48 (m, 2H, CH_2Linker), 1.45 – 1.34 (m, 2H, CH_2Linker), 1.31 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (100 MHz, CD_3CN) δ = 159.2, 156.4, 138.2, 138.2, 138.2, 137.6, 136.4, 136.3, 136.3, 136.2, 136.2, 136.1, 130.7, 129.5, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 113.7, 113.6, 78.0, 77.9, 76.0, 71.7, 71.2, 69.3, 69.3, 69.2, 69.2, 69.1, 69.1, 69.0, 67.9, 67.9, 67.8, 67.8, 66.7, 66.7, 66.6, 65.8, 65.8, 65.8, 65.7, 65.7, 65.6, 65.5, 60.3, 60.3, 54.9, 40.5, 29.9, 29.8, 29.5, 25.9, 24.8. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.75, -0.77, -0.81, -0.97, -1.02. **HRMS** *m/z*: $[M+H]^{2+}$ Calcd 809.78265, founded 809.78265.

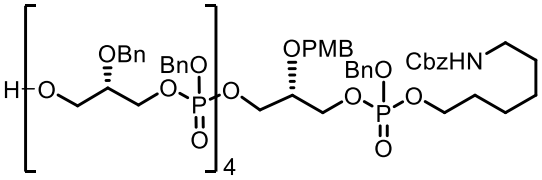
1-*O*-(tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 46



Alcohol **43** (0.3 mmol, 0.37 g) was coupled to phosphoramidite **14** (0.51 mmol, 1.7 mL 0.3 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **46** (0.17 mmol, 0.27 g) in 56% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.44 – 7.31 (m, 45H, aromatics), 5.72 (s, 1H, NH), 5.05 – 4.93 (m, 10H, 5x CH_2Bn), 4.63 – 4.47 (m, 8H, 4x CH_2Bn), 4.21 – 3.97 (m, 14H, 7x CH_2 glycerol), 3.97 – 3.84 (m, 2H, OCH_2), 3.79 – 3.66 (m, 3H, 3x CH glycerol), 3.63 – 3.47 (m, 3H, CH glycerol, CH_2 glycerol), 3.20 – 3.10 (m, 1H, OH), 3.03 (m, 2H, $NHCH_2$), 1.53 (s, 2H, CH_2Linker), 1.44 – 1.32 (m, 2H, CH_2Linker), 1.32 – 1.14 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 139.6, 139.0, 137.1, 137.0, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.8,

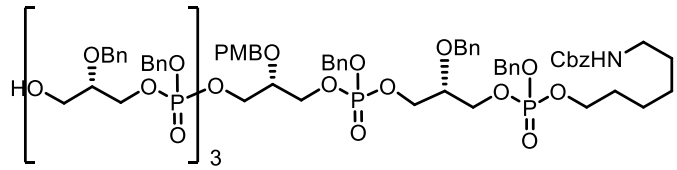
128.7, 128.7, 128.6, 128.6, 128.4, 79.2, 79.1, 76.8, 72.6, 72.4, 70.1, 70.1, 70.0, 70.0, 69.9, 69.8, 68.7, 68.7, 67.5, 67.4, 66.6, 66.5, 66.4, 66.4, 66.3, 61.1, 61.1, 41.3, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.80, -0.82, -1.02, -1.08, -1.11. **HRMS** *m/z*: $[M+H]^+$ Calcd 1588.54741, founded 1588.54746.

1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 33



Alcohol **32** (0.28 mmol, 0.45 g) was coupled to phosphoramidite **14** (0.56 mmol, 2.8 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **33** (0.17 mmol, 0.34 g) in 62% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ 7.61 – 6.94 (m, 52H, aromatics), 6.89 – 6.78 (m, 2H, aromatics), 5.82 (s, 1H, NH), 5.09 – 4.93 (m, 12H, 6x CH_2Bn), 4.63 – 4.39 (m, 10H, 4x CH_2Bn, CH_2PMB), 4.25 – 3.85 (m, 20H, 9x CH_2 glycerol, OCH_2), 3.79 – 3.65 (m, 7H, 4x CH glycerol, OMe), 3.65 – 3.51 (m, 3H, CH glycerol, CH_2 glycerol), 3.05 (m, 2H, $NHCH_2$), 1.53 (m, 2H, CH_2Linker), 1.39 (m, 2H, CH_2Linker), 1.31 – 1.21 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 131.1, 131.0, 130.8, 130.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 114.5, 113.9, 79.2, 79.1, 76.9, 76.8, 76.7, 76.5, 76.5, 76.4, 72.6, 72.3, 72.3, 70.1, 70.1, 70.0, 70.0, 69.9, 69.8, 68.7, 68.7, 67.5, 67.4, 66.6, 66.5, 66.4, 66.3, 61.1, 55.8, 55.2, 41.4, 31.5, 30.7, 30.7, 30.4, 29.7, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.74, -0.76, -0.78, -0.80, -0.97, -1.00, -1.03, -1.07. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1975.64034, founded 1975.64036.

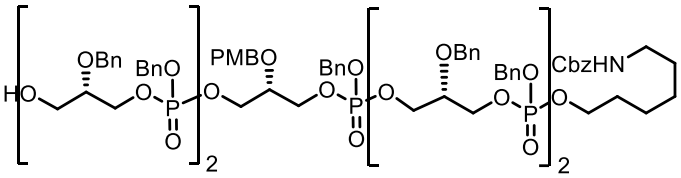
1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)- 3-*O*-tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 38



Alcohol **37** (0.23 mmol, 0.38 g) was coupled to phosphoramidite **14** (0.345 mmol, 1.7 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **38** (0.15 mmol, 0.295 g) in 66% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.55 – 7.06 (m, 52H, aromatics), 6.80 (dd, J = 8.6, 2.8 Hz, 2H, aromatics), 5.75 (s, 1H, NH), 5.10 – 4.89 (m, 12H, 6x CH_2Bn), 4.54 (m, 8H, 4x CH_2Bn), 4.45 (m, 2H, CH_2PMB), 4.23 – 3.83 (m, 20H, 9x CH_2 glycerol, OCH_2), 3.80 – 3.64 (m, 7H, 4x CH glycerol, OMe),

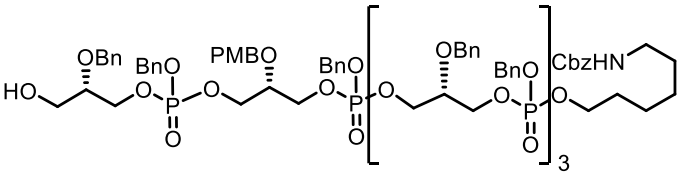
3.56 (m, 3H, CH glycerol, CH_2 glycerol), 3.29 – 3.15 (m, 1H, OH), 3.03 (m, 2H, $NHCH_2$), 1.59 – 1.46 (m, 2H, CH_2Linker), 1.46 – 1.33 (m, 2H, CH_2Linker), 1.33 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 139.0, 138.5, 137.2, 137.2, 137.1, 137.0, 137.0, 130.9, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.8, 128.7, 128.7, 128.6, 128.4, 114.5, 79.2, 76.8, 76.4, 72.6, 72.3, 70.1, 69.8, 68.7, 67.5, 66.6, 66.3, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.76, -0.79, -0.99, -1.02, -1.05. **HRMS** *m/z*: $[M+H]^+$ Calcd 1953.64034, founded 1953.64033.

1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 42



Alcohol **41** (0.33 mmol, 0.52 g) was coupled to phosphoramidite **14** (0.5 mmol, 2.5 mL 0.2 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **42** (0.247 mmol, 0.48 g) in 75% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.45 – 7.16 (m, 52H, aromatics), 6.80 (d, J = 8.4 Hz, 2H, aromatics), 5.80 (s, 1H, NH), 5.07 – 4.94 (m, 12H, 6x CH_2Bn), 4.61 – 4.51 (m, 8H, 4x CH_2Bn), 4.51 – 4.42 (m, 2H, CH_2PMB), 4.20 – 3.96 (m, 18H, 9x CH_2 glycerol), 3.93 (m, 2H, OCH_2), 3.79 – 3.71 (m, 4H, 4x CH glycerol), 3.71 – 3.66 (m, 3H, OMe), 3.62 – 3.52 (m, 3H, CH glycerol, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.59 – 1.47 (m, 2H, CH_2Linker), 1.47 – 1.33 (m, 2H, CH_2Linker), 1.33 – 1.14 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.1, 139.0, 138.9, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 137.0, 130.9, 130.9, 130.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 114.5, 79.2, 76.8, 76.4, 72.6, 72.3, 70.1, 69.8, 68.7, 67.5, 66.6, 66.3, 61.1, 55.8, 41.3, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.74, -0.75, -0.77, -0.97, -1.00, -1.03. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1975.64034, founded 1975.64036.

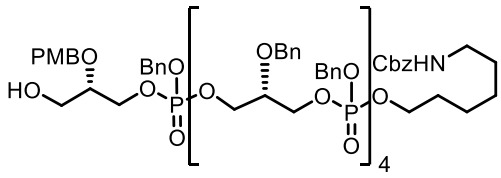
1-*O*-(tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6- hexylcarbamate 45



Alcohol **44** (0.24 mmol, 0.38 g) was coupled to phosphoramidite **14** (0.36 mmol, 1.6 mL

0.23 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **45** (0.207 mmol, 0.40 g) in 86% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.43 – 7.18 (m, 52H, aromatics), 6.84 (dd, J = 9.5, 8.0 Hz, 2H), 5.87 (s, 1H, NH), 5.11 – 4.97 (m, 12H, 6x CH_2Bn), 4.61 – 4.51 (m, 8H, 4x CH_2Bn), 4.51 – 4.44 (m, 2H, CH_2PMB), 4.24 – 3.98 (m, 18H, 9x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.81 – 3.72 (m, 4H, 4x CH glycerol), 3.72 – 3.67 (m, 3H, OMe), 3.65 – 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.17 (s, 1H, OH), 3.06 (m, 2H, $NHCH_2$), 1.60 – 1.47 (m, 2H, CH_2Linker), 1.46 – 1.35 (m, 2H, CH_2Linker), 1.32 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.6, 139.0, 139.0, 138.5, 137.2, 137.2, 137.1, 137.0, 137.0, 130.9, 130.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.4, 114.5, 79.2, 76.8, 76.3, 72.6, 72.2, 70.1, 69.8, 68.7, 67.5, 66.6, 66.2, 61.1, 55.8, 41.4, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.73, -0.75, -0.95, -0.98, -1.01. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1975.64034, founded 1975.64036.

1-*O*-(tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 47

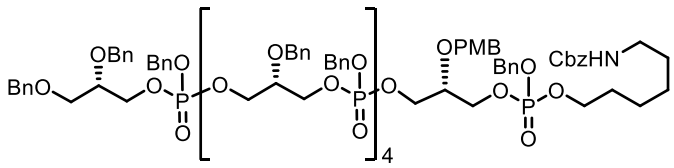


Alcohol **46** (0.148 mmol, 0.24 g) was coupled to phosphoramidite **13** (0.22 mmol, 0.85 mL 0.26 M in ACN), oxidized, and detritylated using general procedure A. The crude was purified by flash chromatography (DCM/Acetone) providing **47** (0.128 mmol, 0.25 g) in 86% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.40 – 7.20 (m, 52H, aromatics), 6.83 (m, 2H, aromatics), 5.78 (s, 1H, NH), 5.07 – 4.96 (m, 12H, 6x CH_2Bn), 4.58 – 4.51 (m, 8H, 4x CH_2Bn), 4.51 – 4.46 (m, 2H, CH_2PMB), 4.20 – 3.96 (m, 18H, 9x CH_2 glycerol), 3.92 (m, 2H, OCH_2), 3.78 – 3.72 (m, 3H, OMe), 3.72 – 3.69 (m, 4H, 4x CH glycerol), 3.60 – 3.50 (m, 3H, CH glycerol, CH_2 glycerol), 3.23 (s, 1H, OH), 3.04 (m, 2H, $NHCH_2$), 1.57 – 1.47 (m, 2H, CH_2Linker), 1.44 – 1.34 (m, 2H, CH_2Linker), 1.29 – 1.15 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = δ 160.1, 139.0, 137.1, 137.0, 131.5, 130.3, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.8, 128.7, 128.7, 128.7, 128.6, 114.5, 78.7, 76.8, 72.6, 72.0, 70.1, 69.8, 68.7, 67.5, 66.6, 66.4, 61.1, 55.8, 41.3, 30.7, 30.3, 26.7, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.77, -0.79, -0.99, -1.02, -1.05. **HRMS** *m/z*: $[M+Na]^+$ Calcd 1975.64034, founded 1975.64036.

General Procedure B: phosphoramidite coupling and oxidation on a typical scale of 0.13 – 0.23 mmol

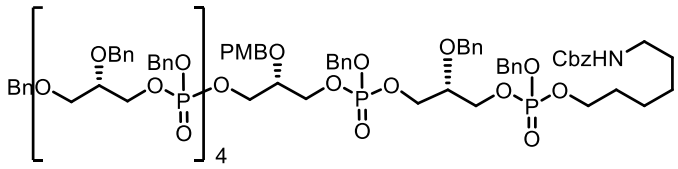
The starting material was co-evaporated with ACN (3x) and was dissolved in dry ACN (0.1-0.05 M). DCI (0.25M solution in ACN, 1.5 eq) was added to the reaction mixture and the solution was stirred for 20 min. Phosphoramidite reagent (0.2 – 0.56 M solution in ACN, 1.3 - 2 eq) was added to the reaction mixture and the solution was stirred until total conversion of the starting material (≃ 1.5h). CSO (0.5M solution in ACN, 2 eq) was added to the reaction mixture and stirred for 15 min. EtOAc was added to the reaction mixture and washed with a solution of NaHCO3/brine (1:1 ratio). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) or by size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1).

1-*O*-((2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 7



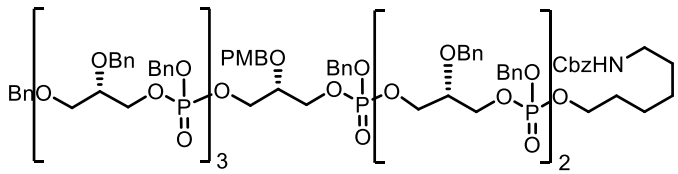
Alcohol **33** (0.17 mmol, 0.33 g) was coupled to phosphoramidite **12** (0.255 mmol, 1.3 mL 0.2 M in ACN) and oxidized using general procedure B. The crude was purified by flash chromatography (DCM/Acetone) providing **7** (0.15 mmol, 0.35 g) in 88% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.16 (m, 67H, aromatics), 6.82 (d, J = 8.4 Hz, 2H, aromatics), 5.79 (s, 1H, NH), 5.05 – 4.94 (m, 14H, 7x CH_2Bn), 4.58 – 4.49 (m, 10H, 5x CH_2Bn), 4.49 – 4.43 (m, 4H, CH_2PMB, CH_2Bn), 4.20 – 3.97 (m, 22H, 11x CH_2 glycerol), 3.93 (m, 2H, OCH_2), 3.72 (s, 6H, 6x CH glycerol), 3.70 (s, 3H, OMe), 3.56 – 3.49 (m, 2H, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.58 – 1.46 (m, 2H, CH_2Linker), 1.46 – 1.32 (m, 2H, CH_2Linker), 1.32 – 1.13 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 142.6, 139.5, 139.4, 139.0, 138.5, 137.2, 137.2, 137.1, 137.0, 137.0, 131.0, 130.5, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 117.0, 114.5, 112.3, 77.5, 77.5, 76.9, 76.8, 76.7, 76.5, 76.5, 76.4, 73.8, 72.6, 72.5, 72.3, 70.2, 70.1, 70.1, 70.0, 69.9, 69.9, 69.7, 69.7, 68.8, 68.8, 68.7, 68.7, 67.9, 67.8, 66.6, 66.6, 66.6, 66.5, 66.3, 55.8, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -1.04, -1.06, -1.09. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1189.40482, founded 1189.40483.

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)- 3-*O*-tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 8



Alcohol **38** (0.15 mmol, 0.29 g) was coupled to phosphoramidite **12** (0.3 mmol, 1.5 mL 0.2 M in ACN) and oxidized using general procedure B. The crude was purified by flash chromatography (DCM/Acetone) providing **8** (0.13 mmol, 0.32 g) in 89% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.45 – 7.18 (m, 67H, aromatics), 6.81 (d, J = 8.4 Hz, 2H, aromatics), 5.85 (s, 1H, NH), 5.08 – 4.96 (m, 14H, 7x CH_2Bn), 4.60 – 4.50 (m, 10H, 5x CH_2Bn), 4.50 – 4.43 (m, 4H, CH_2PMB, CH_2Bn), 4.23 – 3.98 (m, 22H, 11x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.74 (d, 6H, 6x CH glycerol), 3.69 (s, 3H, OMe), 3.57 – 3.49 (m, 2H, CH_2 glycerol), 3.05 (m, 2H, $NHCH_2$), 1.58 – 1.48 (m, 2H, CH_2Linker), 1.44 – 1.35 (m, 2H, CH_2Linker), 1.32 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 160.2, 157.3, 139.5, 139.3, 139.0, 139.0, 138.4, 137.2, 137.1, 137.1, 137.0, 136.9, 130.9, 130.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 129.0, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 117.2, 114.5, 113.0, 77.5, 77.4, 77.4, 76.8, 76.5, 76.4, 76.3, 73.7, 72.6, 72.5, 72.3, 70.2, 70.2, 70.1, 70.1, 70.1, 70.0, 69.9, 69.9, 69.7, 69.6, 68.8, 68.7, 67.9, 67.9, 67.8, 66.6, 66.6, 66.6, 66.5, 66.5, 66.3, 66.3, 55.8, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7, 19.3. **^{31}P NMR** (162 MHz, CD_3CN) δ = -1.05, -1.07, -1.10. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1189.40482, founded 1189.40483.

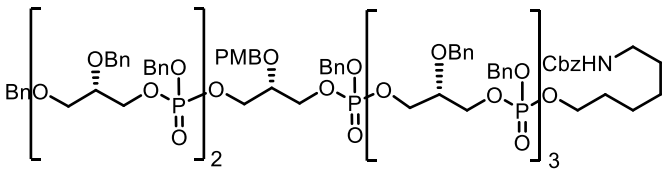
1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 9



Alcohol **42** (0.23 mmol, 0.45 g) was coupled to phosphoramidite **12** (0.345 mmol, 1.7 mL 0.2 M in ACN) and oxidized using general procedure B. The crude was purified by flash chromatography (DCM/Acetone) providing **9** 93% (0.21 mmol, 0.51 g). **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.18 (m, 67H, aromatics), 6.81 (d, J = 8.5 Hz, 2H, aromatics), 5.87 (s, 1H, NH), 5.07 – 4.97 (m, 14H, 7x CH_2Bn), 4.60 – 4.51 (m, 10H, 5x CH_2Bn), 4.50 – 4.44 (m, 4H, CH_2PMB, CH_2Bn), 4.23 – 3.99 (m, 22H, 11x CH_2 glycerol), 3.94 (m, 2H, OCH_2), 3.75 (s, 6H, 6x CH glycerol), 3.69 (s, 3H, OMe), 3.57 – 3.50 (m, 2H, CH2 glycerol), 3.06 (m, 2H, $NHCH_2$), 1.60 – 1.49 (m, 2H, CH_2Linker), 1.46 –

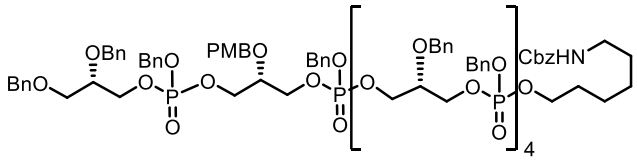
1.36 (m, 2H, CH_2Linker), 1.32 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (100 MHz, CD_3CN) δ = 160.2, 157.3, 139.5, 139.4, 139.0, 139.0, 138.5, 137.2, 137.2, 137.1, 137.0, 137.0, 130.9, 130.5, 129.5, 129.4, 129.3, 129.3, 129.2, 129.2, 129.0, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 114.5, 113.1, 77.5, 77.5, 76.9, 76.9, 76.8, 76.8, 76.7, 76.7, 76.5, 76.4, 76.4, 73.7, 72.6, 72.5, 72.3, 70.1, 70.1, 70.0, 70.0, 70.0, 69.9, 69.8, 69.7, 68.7, 68.7, 68.7, 68.6, 67.9, 67.8, 67.8, 66.6, 66.6, 66.6, 66.5, 66.5, 66.3, 55.8, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.95, -0.97, -0.98, -1.01. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1189.40482, founded 1189.40481.

1-*O*-(tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di- *O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 10



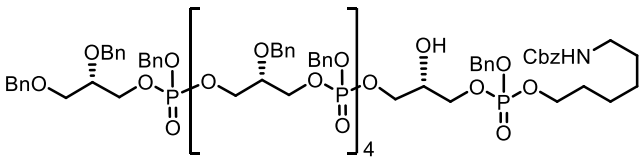
Alcohol **45** (0.128 mmol, 0.25 g) was coupled to phosphoramidite **12** (0.19 mmol, 1.0 mL 0.2 M in ACN) and oxidized using general procedure B. The crude was purified by flash chromatography (DCM/Acetone) providing **10** (0.102 mmol, 0.24 g) in 80% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.39 – 7.16 (m, 67H, aromatics), 6.79 (dd, J = 6.9, 1.6 Hz, 2H, aromatics), 5.80 (s, 1H, NH), 5.05 – 4.95 (m, 14H, 7x CH_2Bn), 4.58 – 4.49 (m, 10H, 5x CH_2Bn), 4.47 – 4.43 (m, 4H, CH_2PMB, CH_2Bn), 4.22 – 3.97 (m, 22H, 11x CH_2 glycerol), 3.93 (m, 2H, OCH_2), 3.73 (s, 6H, 6x CH glycerol), 3.68 (s, 3H, OMe), 3.55 – 3.49 (m, 2H, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.59 – 1.48 (m, 2H, CH_2Linker), 1.44 – 1.34 (m, 2H, CH_2Linker), 1.31 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (100 MHz, CD_3CN) δ = 160.2, 157.2, 139.5, 139.4, 139.0, 139.0, 137.2, 137.2, 137.1, 137.0, 130.9, 130.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.3, 129.2, 129.2, 128.8, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 114.5, 77.6, 77.5, 76.9, 76.8, 76.8, 76.5, 76.5, 76.4, 73.7, 72.6, 72.5, 72.3, 70.1, 70.1, 70.1, 70.0, 69.9, 69.9, 69.9, 69.8, 69.7, 68.7, 68.7, 68.6, 68.6, 67.8, 67.8, 67.7, 66.6, 66.6, 66.5, 66.5, 66.4, 66.3, 55.8, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.94, -0.96, -0.98, -1.01. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1189.40482, founded 1189.40481.

1-*O*-(tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-4-methoxybenzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 11



Alcohol **47** (0.128 mmol, 0.25 g) was coupled to phosphoramidite **12** (0.254 mmol, 1.3 mL 0.2 M in ACN) and oxidized using general procedure B. The crude was purified by flash chromatography (DCM/Acetone) providing **11** (0.1 mmol, 0.23 g) in 77% yield. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.38 – 7.16 (m, 67H, aromatics), 6.79 (d, J = 8.5 Hz, 2H, aromatics), 5.78 (s, 1H, NH), 5.06 – 4.94 (m, 14H, 7x CH_2Bn), 4.59 – 4.49 (m, 10H, 5x CH_2Bn), 4.48 – 4.42 (m, 4H, CH_2PMB, CH_2Bn), 4.19 – 3.96 (m, 22H, 11x CH_2 glycerol), 3.92 (m, 2H, OCH_2), 3.73 (s, 6H, 6x CH glycerol), 3.68 (s, 3H, OMe), 3.55 – 3.48 (m, 2H, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.57 – 1.46 (m, 2H, CH_2Linker), 1.44 – 1.34 (m, 2H, CH_2Linker), 1.32 – 1.17 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (100 MHz, CD_3CN) δ = 160.2, 157.3, 139.5, 139.4, 139.1, 139.0, 138.5, 137.3, 137.2, 137.1, 137.1, 137.0, 131.0, 130.5, 130.1, 129.9, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.0, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 114.5, 77.6, 77.5, 76.8, 76.5, 76.5, 76.4, 73.8, 72.6, 72.5, 72.5, 72.3, 70.1, 70.1, 70.1, 70.1, 70.0, 70.0, 70.0, 69.9, 69.9, 69.9, 69.8, 69.7, 69.7, 68.7, 68.7, 68.7, 68.6, 67.8, 67.8, 67.7, 66.6, 66.6, 66.5, 66.3, 55.8, 41.3, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.99, -1.01, -1.03, -1.06. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1189.40482, founded 1189.40483.

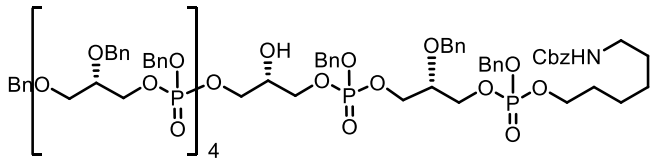
1-*O*-((1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 48



Compound **7** (67.3 µmol, 0.157 g) was dissolved in a mixture of DCM/MeOH (0.7 mL, 9:1 ratio) and cooled to 0°C. DDQ (198.0 µmol, 0.045 g) was added and the reaction mixture was stirred at r.t. until total conversion of the starting material. EtOAc was added to the reaction mixture and it was washed with Na2S2O3 (3x), NaHCO3 (3x), brine (1x). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone), followed by size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1) providing **48** (27.8 µmol, 0.063 g) in 41% yield as a colourless oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.19 (m, 65H, aromatics), 5.76 (s, 1H, NH), 5.05 – 4.95 (m, 14H, 7x CH_2Bn), 4.58 – 4.49 (m, 10H, 5x CH_2Bn), 4.45 (s, 2H, CH_2Bn), 4.20 – 3.88 (m, 24H, 11x CH_2 glycerol, OCH_2), 3.77 – 3.69 (m, 6H, 6x CH glycerol), 3.54 – 3.49 (m, 2H, CH_2 glycerol), 3.04 (m, 2H, $NHCH_2$), 1.59 – 1.50 (m, 2H, CH_2Linker), 1.44 – 1.36 (m, 2H,

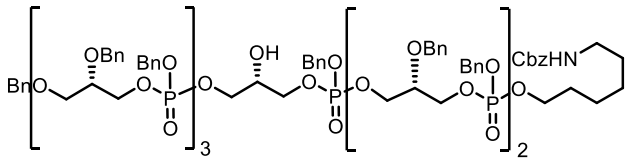
CH_2Linker), 1.33 – 1.20 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 139.5, 139.4, 139.0, 137.1, 137.0, 129.5, 129.4, 129.4, 129.4, 129.4, 129.2, 129.2, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 77.6, 77.5, 76.8, 73.8, 72.6, 72.5, 70.1, 70.1, 70.0, 70.0, 69.9, 69.9, 69.7, 69.4, 68.8, 68.7, 68.7, 68.4, 67.8, 67.8, 66.6, 41.3, 30.7, 30.7, 30.4, 26.7, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.69, -0.80, -0.82, -0.85, -0.98, -1.02, -1.05, -1.06. **HRMS** *m/z*: $[M+H]^+$ Calcd 2257.74484, founded 2257.74486.

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3- *O*-tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 49



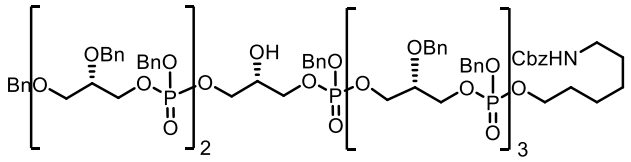
Compound **8** (50.5 µmol, 0.120 g) was dissolved in a mixture of DCM/MeOH (0.5 mL, 9:1 ratio) and cooled to 0°C. DDQ (151.5 µmol, 0.034 g) was added and the reaction mixture was stirred at r.t. until total conversion of the starting material. EtOAc was added to the reaction mixture and it was washed with Na2S2O3 (3x), NaHCO3 (3x), brine (1x). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) providing **49** (28.0 µmol, 0.063 g) in 56% yield as a colourless oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.43 – 7.16 (m, 65H, aromatics), 5.76 (s, 1H, NH), 5.07 – 4.94 (m, 14H, 7x CH_2Bn), 4.58 – 4.49 (m, 10H, 5x CH_2Bn), 4.47 – 4.42 (m, 2H, CH_2Bn), 4.20 – 3.86 (m, 24H, 11x CH_2 glycerol, OCH_2), 3.79 – 3.68 (m, 6H, 6x CH glycerol), 3.55 – 3.46 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.59 – 1.47 (m, 2H, CH_2Linker), 1.44 – 1.34 (m, 2H, CH_2Linker), 1.31 – 1.20 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 139.5, 139.4, 139.1, 139.0, 137.1, 137.0, 137.0, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 77.6, 77.5, 76.8, 73.8, 72.6, 72.5, 70.2, 70.1, 70.1, 70.1, 70.1, 70.0, 69.9, 69.7, 69.4, 68.8, 68.7, 67.8, 67.8, 66.5, 66.3, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.78, -0.82, -0.84, -0.87, -0.99, -1.02, -1.05. **HRMS** *m/z*: $[M+H]^+$ Calcd 2257.74484, founded 2257.74486.

1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 50



Compound **9** (83.3 µmol, 0.198 g) was dissolved in a mixture of DCM/MeOH (0.8 mL, 9:1 ratio) and cooled to 0°C. DDQ (250 µmol, 0.057 g) was added and the reaction mixture was stirred at r.t. until total conversion of the starting material. EtOAc was added to the reaction mixture and it was washed with Na2S2O3 (3x), NaHCO3 (3x), brine (1x). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) providing **50** (53.5 µmol, 0.121 g) in 64% yield as a pale-yellow oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.18 (m, 65H, aromatics), 5.72 (s, 1H, NH), 4.99 (m, 14H, 7x CH_2Bn), 4.59 – 4.48 (m, 10H, 5x CH_2Bn), 4.44 (m, 2H, CH_2Bn), 4.19 – 3.84 (m, 24H, 11x CH_2 glycerol, OCH_2), 3.73 (m, 6H, 6x CH glycerol), 3.54 – 3.48 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.60 – 1.47 (m, 2H, CH_2Linker), 1.44 – 1.33 (m, 2H, CH_2Linker), 1.33 – 1.18 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 139.5, 139.4, 139.0, 137.1, 137.1, 137.0, 137.0, 129.5, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 77.6, 77.5, 76.8, 76.8, 73.8, 72.6, 72.5, 70.2, 70.1, 70.0, 70.0, 69.9, 69.9, 69.7, 69.4, 68.7, 67.8, 67.8, 66.6, 66.5, 66.3, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = -0.77, -0.84, -0.99, -1.01, -1.06, -1.09. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1129.37606, founded 1129.37607.

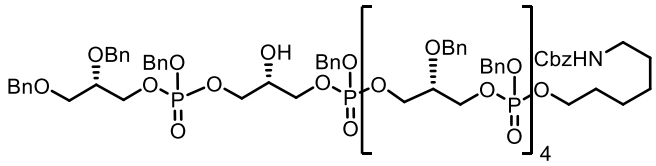
1-*O*-(tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 51



Compound **10** (49.0 µmol, 0.117 g) was dissolved in a mixture of DCM/MeOH (0.5 mL, 9:1 ratio) and cooled to 0°C. DDQ (147 µmol, 0.033 g) was added and the reaction mixture was stirred at r.t. until total conversion of the starting material. EtOAc was added to the reaction mixture and it was washed with Na2S2O3 (3x), NaHCO3 (3x), brine (1x). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) providing **51** 64% (27.8 µmol, 0.063 g) as a pale-yellow oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.40 – 7.17 (m, 65H, aromatics), 5.75 (s, 1H, NH), 5.04 – 4.96 (m, 14H,

7x CH2Bn), 4.59 – 4.50 (m, 10H, 5x CH_2Bn), 4.47 – 4.43 (m, 2H, CH_2Bn), 4.20 – 3.85 (m, 24H, 11x CH_2 glycerol, OCH2), 3.74 (s, 6H, 6x CH glycerol), 3.56 – 3.49 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.58 – 1.47 (m, 2H, CH_2Linker), 1.43 – 1.33 (m, 2H, CH_2Linker), 1.31 – 1.15 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 157.3, 139.5, 139.4, 139.0, 137.2, 137.1, 137.0, 129.8, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 77.6, 77.5, 76.8, 73.8, 72.6, 72.6, 72.5, 70.2, 70.1, 70.1, 70.1, 70.0, 69.9, 69.8, 69.7, 69.4, 68.7, 67.8, 67.8, 66.5, 66.5, 66.3, 41.3, 30.7, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (162 MHz, CD_3CN) δ = 0.71, 0.69, 0.68, 0.67, 0.64, 0.62, 0.59, 0.48, 0.46, 0.45, 0.42, 0.40, 0.37. **HRMS** *m/z*: $[M+H]^+$ Calcd 2257.74484, founded 2257.74486.

1-*O*-(tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 52

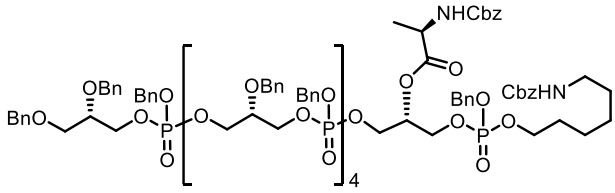


Compound **11** (49.0 µmol, 0.117 g) was dissolved in a mixture of DCM/MeOH (0.5 mL, 9:1 ratio) and cooled to 0°C. DDQ (147 µmol, 0.033 g) was added and the reaction mixture was stirred at r.t. until total conversion of the starting material. EtOAc was added to the reaction mixture and it was washed with Na2S2O3 (3x), NaHCO3 (3x), brine (1x). The organic phase was dried over Na2SO4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) providing **52** (28.2 µmol, 0.064 g) in 58% yield as a pale-yellow oil. **^{1}H NMR** (400 MHz, CD_3CN) δ = 7.41 – 7.18 (m, 65H, aromatics), 5.74 (s, 1H, NH), 5.07 – 4.93 (m, 14H, 7x CH_2Bn), 4.59 – 4.49 (m, 10H, 5x CH_2Bn), 4.46 (s, 2H, CH_2Bn), 4.21 – 3.85 (m, 24H, 11x CH_2 glycerol, OCH_2), 3.78 – 3.67 (m, 6H, 6x CH glycerol), 3.57 – 3.50 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.59 – 1.45 (m, 2H, CH_2Linker), 1.36 (m, 2H, CH_2Linker), 1.31 – 1.16 (m, 4H, 2x CH_2Linker). **^{13}C NMR** (101 MHz, CD_3CN) δ = 139.5, 139.4, 139.1, 139.0, 137.0, 129.8, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.1, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 126.2, 118.3, 77.5, 77.5, 76.8, 73.8, 72.6, 72.5, 70.1, 70.1, 70.1, 70.1, 70.0, 69.9, 69.8, 69.7, 69.4, 68.7, 68.6, 67.8, 67.8, 66.6, 66.5, 66.3, 41.3, 30.7, 30.7, 30.4, 26.7, 25.7. **^{31}P NMR** (122 MHz, CD_3CN) δ = 0.72, 0.70, 0.66, 0.64, 0.60, 0.43, 0.39, 0.38, 0.36. **HRMS** *m/z*: $[M+H]^+$ Calcd 2257.74484, founded 2257.74486.

General Procedure C: Alanine Cbz protected coupling on a typical scale of 10 – 25 µmol

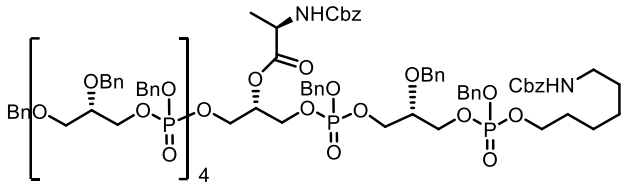
Starting alcohol was co-evaporated with ACN (3x) and dissolved in ACN (0.02 – 0.03 M). PyBOP (5 eq) and Z-D-Alanine (5 eq) were added and the reaction was stirred 15 min. NMI (5 eq) was added dropwise and the reaction mixture was stirred at r.t. overnight. The reaction mixture was diluted with DCM washed with NH_4Cl (1x). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by flash chromatography (DCM/Acetone) followed by size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing the desired product.

1-*O*-(3-*O*-(2-*O*-(*N*-Cbz-D-alanine)-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 53



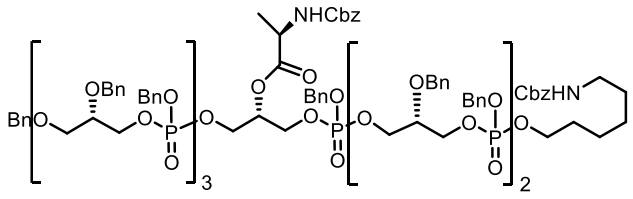
Alcohol **48** (19 µmol, 0.043 g) was coupled to Z-D-Alanine using general procedure C. The crude was purified by flash chromatography (DCM/Acetone) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing **53** (13.4 µmol, 0.033 g) in 70% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.55 – 6.99 (m, 70H, aromatics), 6.35 (s, 1H, NHAla), 5.73 (s, 1H, NHLinker), 5.17 (s, 1H, CH glycerol), 5.09 – 4.88 (m, 16H, 8x CH_2Bn), 4.62 – 4.38 (m, 12H, 6x CH_2Bn), 4.30 – 3.86 (m, 25H, 11x CH_2 glycerol, OCH_2, CHAla), 3.72 (m, 5H, 5x glycerol), 3.51 (d, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.54 (m, 2H, CH_2Linker), 1.39 (m, 2H, CH_2Linker), 1.29 (m, 7H, 2x CH_2Linker, CH_3Ala). **^{13}C NMR** (126 MHz, CD_3CN) δ = 173.1, 156.9, 139.5, 139.4, 139.1, 137.2, 137.1, 137.0, 129.6, 129.5, 129.4, 129.4, 129.4, 129.2, 129.2, 128.9, 128.9, 128.8, 128.7, 128.7, 128.6, 128.5, 128.5, 128.5, 77.6, 77.5, 77.5, 76.8, 73.8, 72.6, 72.5, 71.9, 70.3, 70.3, 70.2, 70.1, 70.1, 70.0, 70.0, 69.7, 69.7, 68.9, 68.9, 68.8, 67.9, 67.8, 67.8, 67.0, 66.8, 66.6, 66.5, 65.8, 65.8, 65.5, 50.7, 41.4, 30.7, 30.7, 30.4, 26.8, 25.7, 18.0. **^{31}P NMR** (202 MHz, CD_3CN) δ = -1.00, -1.03, -1.05, -1.12, -1.15, -1.21, -1.24. **HRMS** *m/z*: $[M+Na]^+$ Calcd 2484.80068, founded 2484.80068.

1-*O*-((2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(*N*-Cbz-D-alanine)-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 54



Alcohol **49** (10.0 µmol, 0.022 g) was coupled to Z-D-Alanine using general procedure C. The crude was purified by flash chromatography (DCM/Acetone) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing **54** (4.9 µmol, 0.012 g) in 49% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.59 – 6.99 (m, 70H, aromatics), 6.34 (s, 1H, NHAla), 5.70 (s, 1H, NHLinker), 5.16 (s, 1H, CH glycerol), 5.10 – 4.91 (m, 16H, 8x CH_2Bn), 4.62 – 4.40 (m, 12H, 6x CH_2Bn), 4.30 – 3.83 (m, 25H, 11x CH_2 glycerol, OCH_2, CHAla), 3.80 – 3.63 (m, 5H, 5x glycerol), 3.52 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.52 (m, 2H, CH_2Linker), 1.45 – 1.33 (m, 2H, CH_2Linker), 1.33 – 1.19 (m, 7H, 2x CH_2Linker, CH_3Ala). **^{13}C NMR** (126 MHz, CD_3CN) δ = 173.1, 157.2, 156.8, 139.5, 139.4, 139.1, 139.0, 138.5, 137.1, 137.0, 136.9, 136.9, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.3, 129.2, 129.2, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 77.5, 77.5, 77.5, 76.8, 76.8, 76.8, 73.7, 72.6, 72.5, 71.8, 70.3, 70.2, 70.2, 70.1, 70.0, 70.0, 69.8, 69.8, 69.7, 68.7, 68.6, 67.8, 67.8, 67.0, 66.7, 66.6, 66.6, 66.5, 66.3, 65.8, 50.7, 41.3, 30.7, 30.6, 30.3, 26.7, 25.6, 17.9. **^{31}P NMR** (202 MHz, CD_3CN) δ = -1.01, -1.04, -1.07, -1.19, -1.21, -1.24. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1231.91301, founded 1231.91301.

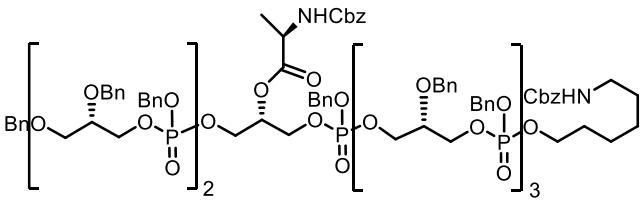
1-*O*-(di-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(*N*-Cbz-D-alanine)-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-di -(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 55



Alcohol **50** (12.0 µmol, 0.026 g) was coupled to Z-D-Alanine using general procedure C. The crude was purified by flash chromatography (DCM/Acetone) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing **55** (4.4 µmol, 0.011 g) in 37% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.52 – 7.04 (m, 70H, aromatics), 6.35 (s, 1H, NHAla), 5.67 (s, 1H, NHLinker), 5.16 (s, 1H, CH glycerol), 5.06 – 4.88 (m, 16H, 8x CH_2Bn), 4.71 – 4.41 (m, 12H, 6x CH_2Bn), 4.28 – 3.86 (m, 25H, 11x CH_2 glycerol, OCH_2, CHAla), 3.72 (s, 5H, 5x glycerol), 3.51 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, NHCH2), 1.53 (s, 2H, CH_2Linker), 1.45 – 1.33 (m, 2H, CH_2Linker), 1.27 (m, 7H, 2x CH_2Linker,

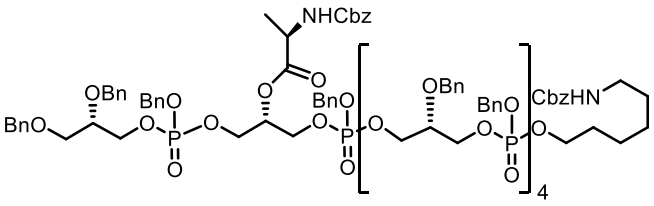
CH_3Ala). **^{13}C NMR** (126 MHz, CD_3CN) δ = 173.1, 157.2, 156.8, 139.1, 129.6, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 77.6, 77.6, 76.9, 73.8, 72.7, 72.6, 70.3, 70.1, 70.0, 69.9, 69.8, 68.7, 67.9, 67.1, 66.8, 66.7, 66.6, 66.3, 65.9, 50.8, 41.4, 30.8, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (202 MHz, CD_3CN) δ = -1.02, -1.08, -1.20, -1.23. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1231.91301, founded 1231.91301.

1-*O*-(tri-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(*N*-Cbz-D-alanine)-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2,3- di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 56



Alcohol **51** (15.0 µmol, 0.033 g) was coupled to Z-D-Alanine using general procedure C. The crude was purified by flash chromatography (DCM/Acetone) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing **56** (12.0 µmol, 0.030 g) in 80% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.56 – 6.96 (m, 70H, aromatics), 6.34 (s, 1H, NHAla), 5.70 (s, 1H, NHLinker), 5.16 (s, 1H, CH glycerol), 5.10 – 4.90 (m, 16H, 8x CH_2Bn), 4.61 – 4.39 (m, 12H, 6x CH_2Bn), 4.28 – 3.85 (m, 25H, 11x CH_2 glycerol, OCH_2, CHAla), 3.79 – 3.66 (s, 5H, 5x glycerol), 3.52 (m, 2H, CH_2 glycerol), 3.03 (m, 2H, $NHCH_2$), 1.52 (s, 2H, CH_2Linker), 1.45 – 1.34 (m, 2H, CH_2Linker), 1.28 (m, 7H, 2x CH_2Linker, CH_3Ala). **^{13}C NMR** (126 MHz, CD_3CN) δ = 173.1, 157.3, 156.9, 139.6, 139.4, 139.1, 139.1, 138.5, 137.1, 137.1, 137.0, 137.0, 129.6, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.3, 129.2, 129.0, 128.9, 128.9, 128.8, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 77.6, 77.6, 77.5, 76.9, 76.8, 73.8, 72.6, 72.5, 71.9, 70.3, 70.3, 70.3, 70.1, 70.1, 70.0, 69.9, 69.9, 69.8, 68.7, 68.7, 67.9, 67.9, 67.0, 66.8, 66.7, 66.6, 66.6, 66.3, 65.8, 50.8, 41.4, 30.8, 30.7, 30.4, 26.8, 25.7. **^{31}P NMR** (202 MHz, CD_3CN) δ = -1.01, -1.04, -1.07, -1.19, -1.21, -1.24. **HRMS** *m/z*: $[M+2H]^{2+}$ Calcd 1231.91301, founded 1231.91297.

1-*O*-(tetra-(2-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(*N*-Cbz-D-alanine)-1-*O*-benzyloxyphosphoryl-sn-glyceryl)3-*O*-(2,3-di-*O*-benzyl-1-*O*-benzyloxyphosphoryl-sn-glyceryl)) benzyl 6-hexylcarbamate 57

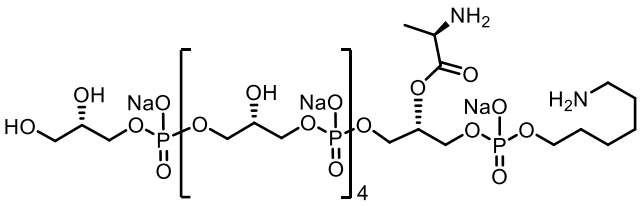


Alcohol **52** (13.0 µmol, 0.029 g) was coupled to Z-D-Alanine using general procedure C. The crude was purified by flash chromatography (DCM/Acetone) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) providing **57** (10.0 µmol, 0.025 g) in 78% yield. **^{1}H NMR** (500 MHz, CD_3CN) δ = 7.49 – 7.10 (m, 70H, aromatics), 6.25 (s, 1H, NHAla), 5.72 (s, 1H, NHLinker), 5.16 (s, 1H, CH glycerol), 5.06 – 4.92 (m, 16H, 8x CH_2Bn), 4.64 – 4.39 (m, 12H, 6x CH_2Bn), 4.26 – 3.86 (m, 25H, 11x CH_2 glycerol, OCH_2, CHAla), 3.73 (s, 5H, 5x glycerol), 3.03 (m, 2H, $NHCH_2$), 1.51 (s, 2H, CH_2Linker), 1.43 – 1.34 (m, 2H, CH_2Linker), 1.34 – 1.19 (m, 7H, 2x CH_2Linker, CH_3Ala). **^{13}C NMR** (126 MHz, CD_3CN) δ = 173.1, 156.8, 139.5, 139.4, 139.1, 139.0, 137.3, 137.1, 137.0, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.4, 129.2, 129.2, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 126.6, 77.5, 77.5, 76.8, 73.8, 72.6, 72.5, 71.9, 70.3, 70.2, 70.1, 70.1, 70.1, 70.1, 69.9, 69.8, 69.7, 68.7, 68.7, 68.7, 68.6, 68.0, 68.0, 67.9, 67.0, 66.8, 66.6, 66.6, 66.5, 66.3, 65.8, 65.7, 50.8, 49.8, 41.3, 34.4, 30.7, 30.7, 30.4, 26.8, 26.4, 26.2, 25.7, 17.9. **^{31}P NMR** (202 MHz, CD_3CN) δ = -1.00, -1.03, -1.06, -1.15, -1.19, -1.21. **HRMS** *m/z*: $[M+Na]^+$ Calcd 2484.80068, founded 2484.80068.

General Procedure D: hydrogenolysis on a typical scale of 10 – 25 µmol

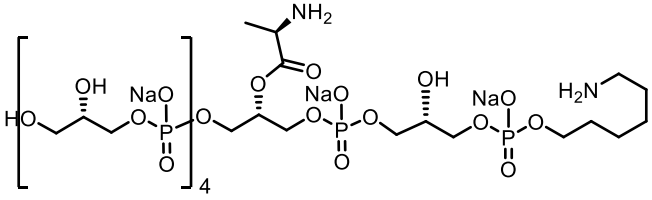
Starting compound was dissolved in a 2:1 mixture of Dioxane/0.01% (v/v) AcOH solution in MilliQ water (2 mL per 10 µmol). The reaction mixture was purged with Argon, followed by the catalytic addition of Pd black. The mixture was purged with Argon and subsequently purged with H_2 and left stirring in a H_2 atmosphere for 3 days. The reaction mixture was filtrated over a Whatman® filter. The crude was lyophilized and purified by size exclusion chromatography (Toypearl HW-40 in NaCl), lyophilized, desalted by size exclusion chromatography (Sephadex G-10 in MilliQ water) and lyophilized.

1-*O*-(3-*O*-(2-*O*-D-alanine-1-*O*-phosphoryl-sn-glyceryl) 3-*O*-penta-(1-*O*-phoryl-sn-glyceryl)) 6-hexylamine 1



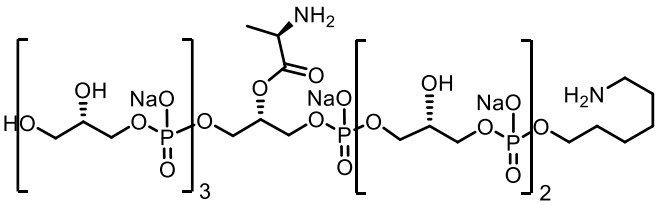
Compound **53** (4.4 µmol, 0.011 g) was deprotected using general procedure D providing **1** (3.9 µmol, 0.005 g) in 88% yield. **^{1}H NMR** (500 MHz, D_2O) δ 5.37 (dt, J = 6.2, 2.8 Hz, 1H), 4.28 (dd, J = 7.4, 3.9 Hz, 1H), 4.16 – 4.00 (m, 6H), 4.00 – 3.78 (m, 20H), 3.68 (dd, J = 11.8, 4.2 Hz, 1H), 3.60 (dd, J = 11.8, 6.1 Hz, 1H), 3.05 – 2.93 (m, 2H), 1.74 – 1.57 (m, 6H), 1.43 (d, J = 4.1 Hz, 4H). **^{13}C NMR** (214 MHz, D_2O) δ 170.9, 75.0, 73.2, 72.5, 71.6, 70.3, 70.2, 70.2, 68.4, 67.4, 67.3, 67.2, 67.1, 67.0, 66.9, 64.6, 63.4, 63.0, 61.3, 61.2, 49.8, 40.3, 30.3, 30.2, 27.5, 26.0, 25.3, 16.2. **^{31}P NMR** (202 MHz, D_2O) δ 0.81, 0.77, 0.70, 0.66, 0.62, 0.40, 0.38, 0.31. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 1135.16036, founded 1135.16034.

1-*O*-((1-*O*-phosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(D-alanine)-1-*O*-phosphoryl-sn-glyceryl) 3-*O*-tetra-(1-*O*-phosphoryl-sn-glyceryl)) 6-hexylamine 2



Compound **54** (4.5 µmol, 0.012 g) was deprotected using general procedure D providing **2** (3.3 µmol, 0.004 g) in 73% yield. **^{1}H NMR** (500 MHz, D_2O) δ 5.47 – 5.35 (m, 1H), 4.38 – 4.24 (m, 1H), 4.20 – 3.74 (m, 28H), 3.70 (dd, J = 11.8, 4.2 Hz, 1H), 3.63 (dd, J = 11.8, 6.0 Hz, 1H), 3.06 – 3.00 (m, 2H), 1.77 – 1.50 (m, 7H), 1.50 – 1.38 (m, 4H). **^{13}C NMR** (214 MHz, D_2O) δ 171.0, 75.0, 73.3, 72.5, 71.6, 70.3, 70.2, 70.2, 68.4, 67.4, 67.3, 67.2, 67.1, 67.0, 66.9, 64.5, 63.4, 62.9, 61.3, 61.2, 49.8, 40.3, 30.2, 30.2, 27.4, 25.9, 25.3, 16.2. **^{31}P NMR** (202 MHz, D_2O) δ 0.81, 0.77, 0.70, 0.66, 0.40, 0.31. **MALDI-MS** *m/z*: $[M+H]^+$ Calcd 1113.17841, founded 1113.17835.

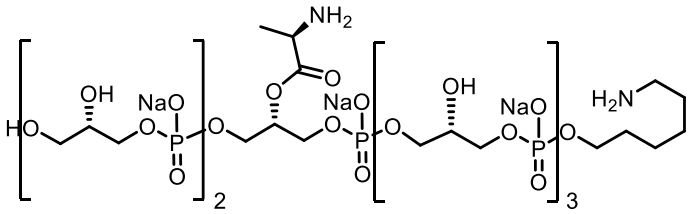
1-*O*-(3-*O*-di- (1-*O*-phosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(D-alanine)-1-*O*-phosphoryl-sn-glyceryl) 3-*O*-tri-(1-*O*-phosphoryl-sn-glyceryl)) 6-hexylamine 3



Compound **55** (4.4 µmol, 0.011 g) was deprotected using general procedure D providing **3** (3.2 µmol, 0.004 g) in 72% yield. **^{1}H NMR** (500 MHz, D_2O) δ

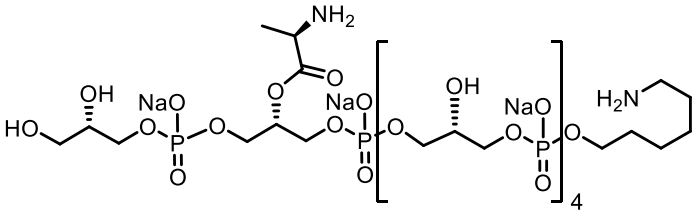
5.41 (tt, J = 6.6, 3.4 Hz, 1H), 4.30 (q, J = 7.3 Hz, 1H), 4.21 – 4.02 (m, 7H), 4.02 – 3.75 (m, 21H), 3.70 (dd, J = 11.8, 4.2 Hz, 1H), 3.63 (dd, J = 11.8, 6.1 Hz, 1H), 3.07 – 2.98 (m, 2H), 1.78 – 1.56 (m, 7H), 1.45 (t, J = 3.7 Hz, 4H). ^{13}C NMR (214 MHz, D_2O) δ 171.0, 75.0, 73.3, 72.5, 71.6, 70.3, 70.2, 70.2, 68.4, 67.4, 67.3, 67.2, 67.1, 67.0, 66.9, 64.5, 63.4, 62.9, 61.3, 61.2, 49.8, 40.3, 30.2, 30.2, 27.4, 25.9, 25.3, 16.2. ^{31}P NMR (202 MHz, D_2O) δ 0.81, 0.77, 0.70, 0.66, 0.40, 0.31. **MALDI-MS** *m/z*: $[M+H]^+$ Calcd 1113.17841, founded 1113.17856.

1-*O*-(3-*O*-tri- (1-*O*-phosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(D-alanine)-1-*O*-phosphoryl-sn-glyceryl) 3-*O*-di-(1-*O*-phosphoryl-sn-glyceryl)) 6-hexylamine 4



Compound **56** (2.8 µmol, 0.007 g) was deprotected using general procedure D providing **4** (2.3 µmol, 0.003 g) in 82% yield. **^{1}H NMR** (500 MHz, D_2O) δ 5.44 – 5.35 (m, 1H), 4.29 (dt, J = 8.1, 6.7 Hz, 1H), 4.15 – 4.00 (m, 7H), 4.00 – 3.81 (m, 22H), 3.68 (dd, J = 11.9, 4.0 Hz, 1H), 3.60 (ddd, J = 11.8, 6.1, 0.9 Hz, 1H), 3.00 (t, J = 7.5 Hz, 2H), 1.77 – 1.54 (m, 7H), 1.47 – 1.38 (m, 5H). **^{13}C NMR** (214 MHz, D_2O) δ 171.0, 75.0, 73.3, 72.5, 71.6, 70.3, 70.2, 70.2, 68.4, 67.4, 67.3, 67.2, 67.1, 67.0, 66.9, 64.5, 63.4, 62.9, 61.3, 61.2, 49.8, 40.3, 30.2, 30.2, 27.4, 25.9, 25.3, 16.2. **^{31}P NMR** (202 MHz, D_2O) δ 0.81, 0.78, 0.71, 0.66, 0.62, 0.40, 0.38, 0.31. **MALDI-MS** *m/z*: $[M+H]^+$ Calcd 1113.17841, founded 1113.17844.

1-*O*-(tetra-(1-*O*-phosphoryl-sn-glyceryl) 3-*O*-(2-*O*-(D-alanine)-1-*O*-phosphoryl-sn-glyceryl)3-*O*-(1-*O*-phosphoryl-sn-glyceryl)) 6-hexylamine 5



Compound **57** (4.0 µmol, 0.010 g) was deprotected using general procedure D providing **5** (3.8 µmol, 0.005 g) in 95% yield. **^{1}H NMR** (500 MHz, D_2O) δ 5.38 (s, 1H), 4.26 (d, J = 7.8 Hz, 1H), 4.15 – 3.99 (m, 8H), 3.99 – 3.76 (m, 27H), 3.70 – 3.63 (m, 1H), 3.59 (dd, J = 11.7, 5.8 Hz, 1H), 3.00 (t, J = 7.5 Hz, 3H), 1.79 – 1.53 (m, 8H), 1.43 (s, 5H). **^{13}C NMR** (214 MHz, D_2O) δ 171.0, 75.0, 73.3, 72.5, 71.6, 70.3, 70.2, 70.2, 68.4, 67.4, 67.3, 67.2, 67.1, 67.0, 66.9, 64.5, 63.4, 62.9, 61.3, 61.2, 49.8, 40.3, 30.2, 30.2, 27.4, 25.9, 25.3, 16.2. **^{31}P NMR** (202 MHz, D_2O) δ 0.81, 0.78, 0.71, 0.66, 0.62, 0.40, 0.38, 0.31. **MALDI-MS** *m/z*: $[M+Na]^+$ Calcd 1135.16036, founded 1135.16029.

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