Synthetic carbohydrate ligands for immune receptors

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Chapter 2

*Conjugation ready monophosphoryl lipid A-analogues for self-adjuvanting cancer peptide vaccines**

Introduction

Immunotherapy is a powerful, emerging strategy that makes use of the body's own immune system to combat malignant cancer cells. One approach is to train the immune system to recognize and destroy cancer cells through vaccination, by targeting cancer specific epitopes, such as neoantigens[1] or tumor-associated carbohydrate antigens (TACAs)[2]. To optimally direct an immune reaction against these antigens, adjuvants can be used to activate antigen presenting cells, such as dendritic cells (DCs) and macrophages, to enhance and polarize the T cell response. DCs express pattern recognition receptors (PRRs)[3], through which they recognize invading pathogens and initiate an immune response.[4] Ligands for these PRRs can be used as adjuvants and many well-defined molecular PRR-ligands have been explored for their application in cancer vaccine modalities and various classes of PRR-receptors have been targeted, including the Toll-like receptors (TLRs)[5], C-type lectins[6], and Nucleotide binding Oligomerization Domain-like receptors[7,8]. To further improve vaccine activity, the antigen and adjuvants have been combined in covalent constructs, delivering "self-

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adjuvanting" vaccines.[9,10] Several TLR agonists[9,11,12] have been conjugated to antigenic peptides, including ligands for TLR2[13–15], TLR7[16,17] and TLR9[18,19], yielding vaccine modalities with improved activity with respect to their non-conjugated counterparts. TLR4-ligands have so far not been explored in peptide-conjugate vaccine modalities. TLR4 can recognize lipopolysaccharides (LPS) and in particular lipid A (Figure 1), which can be found on the cell surface of Gram-negative bacteria.[20] Lipid A can form a complex with MD-2[21], which then binds to TLR4, resulting in the activation of the TRIF and the MyD88 signaling pathways, which induces the release of cytokines and chemokines.[22] Due to its high toxicity, lipid A cannot be used *in vivo*, but removal of the anomeric phosphate provides monophosphoryl lipid A (MPLA, Figure 1), which is significantly less toxic while maintaining the immunostimulatory activity.[23,24] MPLA has therefore been used as an adjuvant in various vaccines, and its use has been approved for human use.[25,26] It is part of the AS04 adjuvant mixture (in which it is combined with aluminum hydroxide or phosphate) in commercially available Human Papillomavirus and Hepatitis B vaccines.[27] The group of Guo, has introduced MPLA for use in several covalent glycoconjugate vaccines, in which MPLA was conjugated to a TACA or a synthetic bacterial glycan.[28–32] These latter conjugates were able to elicit a robust IgG antibody response, critical for effective anti-bacterial vaccination.[32]

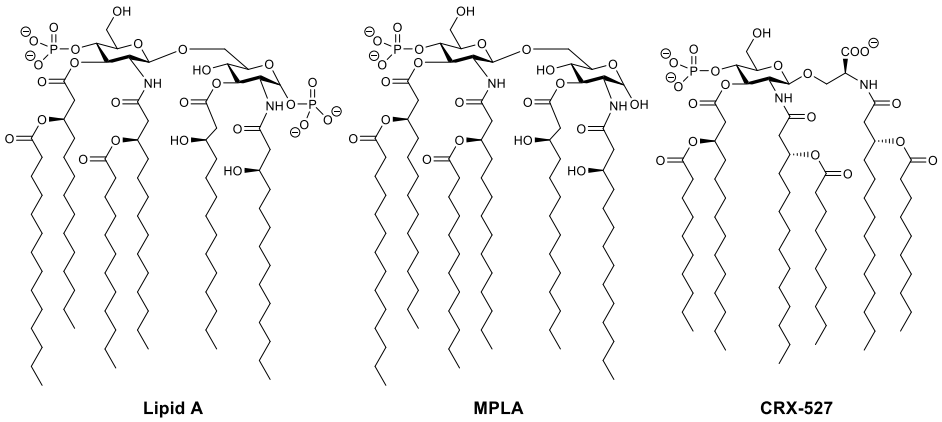


Figure 1. Structures of Lipid A and MPLA of *Salmonella enterica* serotype minnesota Re 595, and CRX-527.

One of the challenges in the generation of MPLA conjugates is to obtain sufficient quantities of the vaccine due to their complex structure and physical properties.[33–35] Based on the structure of lipid A, a new class of potent monosaccharide adjuvants has been developed, the aminoalkyl glucosamine 4-phosphates (AGPs), in which the reducing end glucosamine of lipid A is replaced for an acylated serine residue.[36] These AGPs are easier to synthesize in comparison to other MPLA derivatives, while

maintaining their well-defined and powerful immunostimulatory properties.[37–40] AGPs have been shown to be efficacious adjuvants and to be clinically safe, resulting in its use in a Hepatitis B vaccine.[41] CRX-527 (Figure 1) has been established as one of the most potent AGPs.[37]

This chapter describes the design, synthesis and immunological evaluation of TLR4-ligands **1**-**4** and the novel TLR4-ligand peptide conjugates **5**-**8** (Figure 2). A spacer equipped CRX-527 was used as the TLR4-ligand, which was conjugated with an ovalbumin derived peptide, $DEVA_5K$, comprising the MHC-I epitope SIINFEKL embedded in a longer peptide motif, serving as a model antigen in the peptide conjugates **5**-**8**. The $DEVA_5K$-peptide was equiped with a thiol functionality either at the *N*- or the *C*-terminal end and the TLR4-ligand was provided with a maleimide functionality to allow their union through thiol-maleimide chemistry. The required maleimide was installed via a linker at the *C*-6 position of the glucosamine residue in CRX-527, as this is the same position to which bacterial *O*-antigens are attached to LPS in the bacterial cell wall.[36] Previous work on anti-bacterial MPLA conjugate vaccines has shown that the adjuvant can be modified at this position without compromising adjuvant activity.[32] Two type of linkers at the *C*-6 position of CRX-527 were evaluated: an hydrophobic alkyl linker (A) and a hydrophilic triethylene glycol (TEG) linker (B). These linkers were connected to the 6-OH of CRX-527 through an ester bond,[32] or *via* a more stable amide bond to an hereto installed 6-NH_2 functionality.

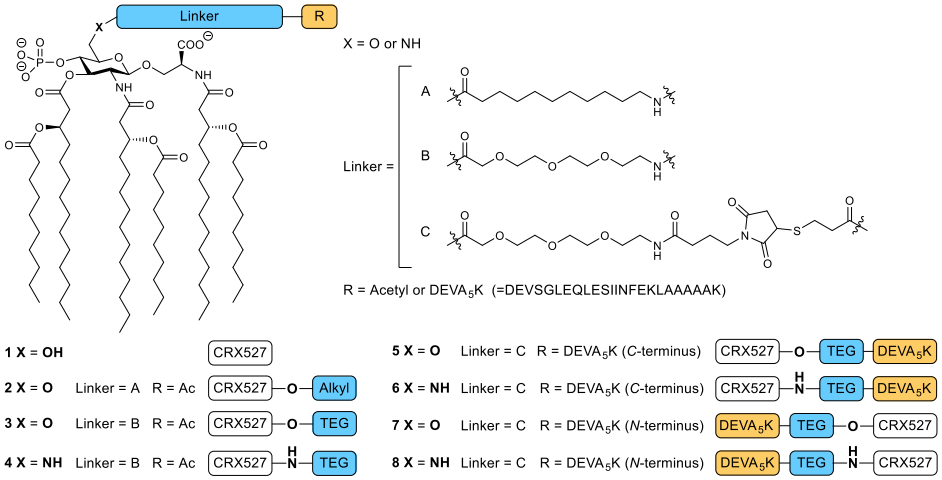
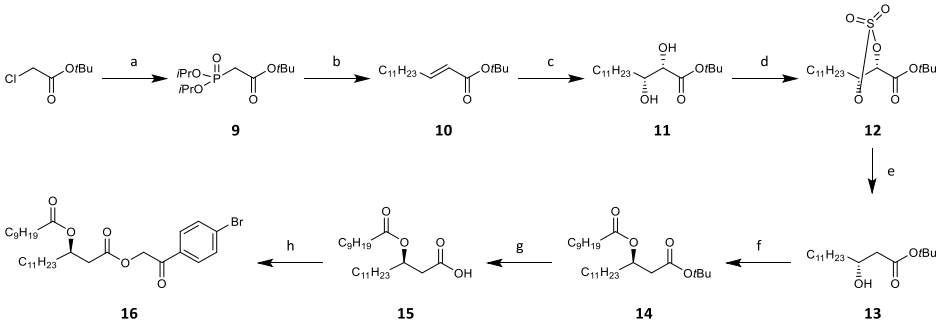


Figure 2. Structures of TLR4-ligands **1**-**4** and TLR4-ligand conjugates **5**-**8**.

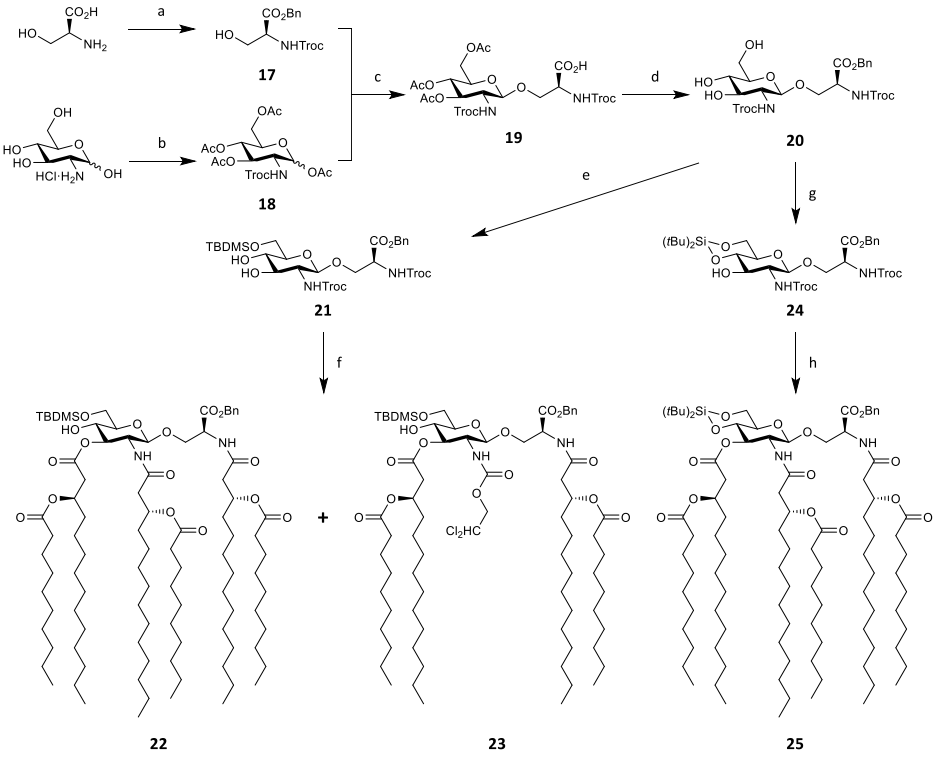
Results and Discussion

For the synthesis of ligands **1**-**4**, first the route towards (R)-3-alkyloxytetradecanoic acid **15** was optimized, to allow for a large scale synthesis (Scheme 1). Previous routes turned out to give lower yields, partly due to the formation of side products and the associated difficult separations.[42,43] The synthesis starts with the conversion of *tert*-butyl 2-chloroacetate into Horner-Wadsworth-Emmons (HWE) reagent **9**, which was obtained by vacuum distillation. Next, the HWE reaction of **9** with dodecanal led to the predominant formation of E-alkene **10** in 96% (64 mmol scale).[44] Further scaling-up of this reaction (560 mmol) led to a drop in yield (78%) due to the difficult separation of the two isomeric alkenes. Sharpless asymmetric dihydroxylation of ester **10** with OsO_4 in the presence of $(DHQD)_2PHAL$ gave diol **11** in 98% (the ee was determined at a later stage of the synthesis). Diol **11** was treated with thionyl chloride and pyridine, followed by oxidation of the intermediate cyclic sulfite with $NaIO_4$ and ruthenium trichloride to give cyclic sulfate **12**.[45–47] Regioselective nucleophilic opening of the cyclic sulfate with sodium borohydride and acidic hydrolysis of the obtained sulfate ester afforded alcohol **13**.[45] Crucial to the removal of the sulfate ester is the use of exactly two equivalents of H_2SO_4, since the use of a larger excess leads to hydrolysis of the *tert*-butyl ester. Acetylation of the hydroxyl group in **13** with decanoyl chloride, pyridine and a catalytic amount of DMAP gave **14** and subsequent TFA mediated removal of the *tert*-butyl group gave fatty acid **15** in 64% yield over 9 steps. Conversion of acid **15** into *p*-bromophenacyl ester **16** was performed to determine the *ee*, which turned out to be 98.6%.



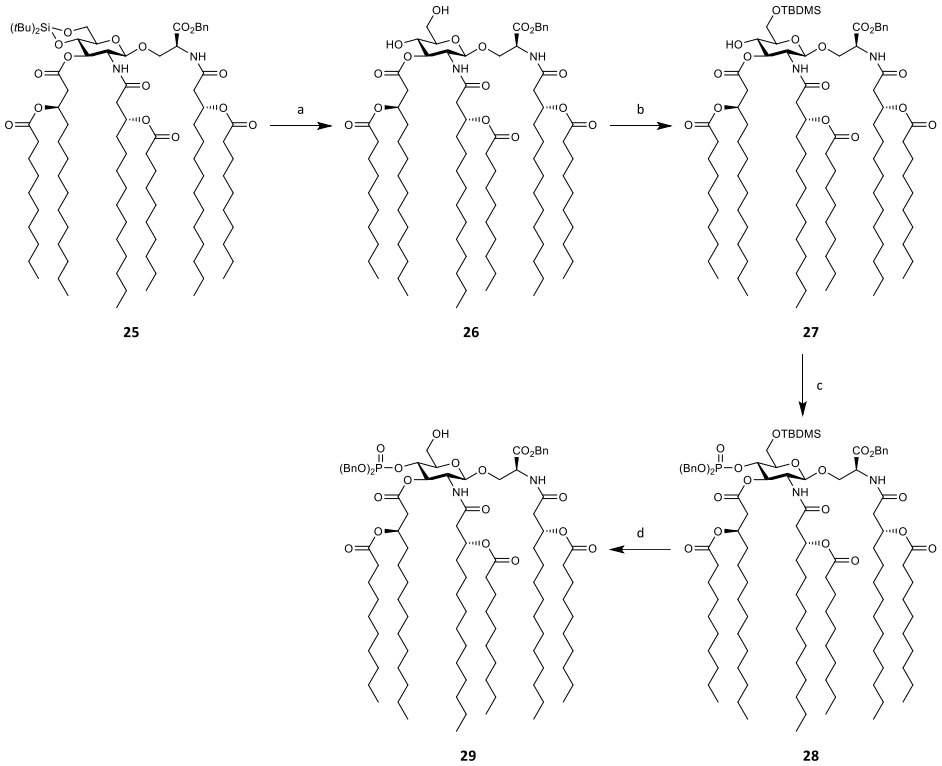
Scheme 1. Synthesis of chiral fatty acid **15**. *Reagents and conditions*: a) $P(OiPr)_3$, 130°C, quant.; b) dodecanal, *n*-BuLi, THF, 96%; c) $K_3Fe(CN)_6$, K_2CO_3, $[(DHQD)_2PHAL]$, OsO_4, methanesulfonamide, H_2O/*t*-BuOH, 98%; d) *i*. $SOCl_2$, pyridine, EtOAc, 0°C; *ii*. $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O, 95% over two steps; e) *i*. $NaBH_4$, DMF, 0°C; *ii*. H_2SO_4 (2 eq.), H_2O (2 eq.), THF, 0°C, quant. over two steps; f) decanoyl chloride, DMAP, pyridine, 0°C, 89%; g) TFA, DCM, 92%; h) 2,4'-dibromoacetophenone, Et_3N, EtOAc, 48%.

The synthesis of the required glucosaminyl serine synthon is depicted in Scheme 2.[40] First, Troc-protected serine **17** was generated by masking the amino group of benzyl L-serine with a 2,2,2-trichloroethoxycarbonyl group.[48] Next, glucosamine **18** was assembled by treatment of glucosamine with Troc-chloride and subsequent acetylation of the hydroxyl groups. Coupling of **17** and **18** under the influence of boron trifluoride etherate proceeded in a completely β-selective manner to give a mixture of the desired product and unreacted donor **18**. The mixture could be separated after hydrogenolysis of the benzyl ester giving acid **19** in 63%, on 170 mmol scale. Next all acetyls were removed with ammonia in methanol, before the benzyl ester was re-installed using phase transfer conditions to yield triol **20**. Regioselective introduction of the *tert*-butyl dimethyl silyl (TBDMS) group at the 6-OH gave compound **21**[39,40] in 81%. In previous AGP syntheses TBDMS-protected glucosamine **21** has been successfully used for the regioselective introduction of the fatty acid at the 3-OH and after Troc removal on both liberated amines.[39,40] In line with these studies, the 3-OH in **21** was acetylated and the Troc protecting groups were removed using Zn/AcOH. However, the last step was accompanied by partial reduction of the Troc groups to give 1,2-dichloroethoxycarbonyl groups. This led to the formation of several side products, such as **23**, during the acylation step and it proved to be impossible to remove these side products at any stage of the synthesis. The possibility that these side products can function as TLR4 antagonists, because of the lower number of fatty acid chains, necessitated the exploration of a different route. Therefore, building block **24**, bearing a 4,6-*O*-silylidene group, was synthesized from **20**. The removal of the Troc protecting groups of **24**, was followed by *N*,*N*,*O*-triacetylation with **15** in the presence of EDC·MeI and catalytic DMAP (0.03 eq.). Using column chromatography it was possible to separate the desired compound from the side products, derived from partial reduction of the Troc-groups, and **25** was obtained in 62% over two steps (3.0 mmol scale). Scale-up of the procedure (9.5 mmol) led to a slightly diminished yield of 57%. Of note, it was observed that increasing of the amount of DMAP (>0.1 eq.) in the acylation step, led to partial β-elimination of the fatty acids.



Scheme 2. Synthesis of building block **25**. *Reagents and conditions*: a) *i*. *p*-toluenesulfonic acid, CCl_4/benzyl alcohol, 100°C; *ii*. succinimidyl-2,2,2-trichloroethyl carbonate, Et_3N, DCM, 40% over two steps; b) *i*. 2,2,2-trichloroethoxycarbonyl chloride, $NaHCO_3$, H_2O; *ii*. Ac_2O, pyridine, 66% over two steps; c) *i*. $BF_3{\cdot}OEt_2$, DCM, 0°C to rt; *ii*. H_2, Pd/C, THF, 63% over two steps; d) *i*. NH_4OH, MeOH; *ii*. BnBr, TBAB, DCM/$NaHCO_3$ (aq. sat.), 79% over two steps; e) TBDMSCl, pyridine, 81%; f) *i*. **15**, EDC·MeI, DMAP, DCM, 84%; *ii*. Zn dust, AcOH; *iii*.) **15**, EDC·MeI, DCM; g) $(t\text{Bu})_2Si(OTf)_2$, DMF, -40°C, 94%; h) *i*. Zn dust, AcOH; *ii*.) **15**, EDC·MeI, DMAP, DCM, 62% over two steps.

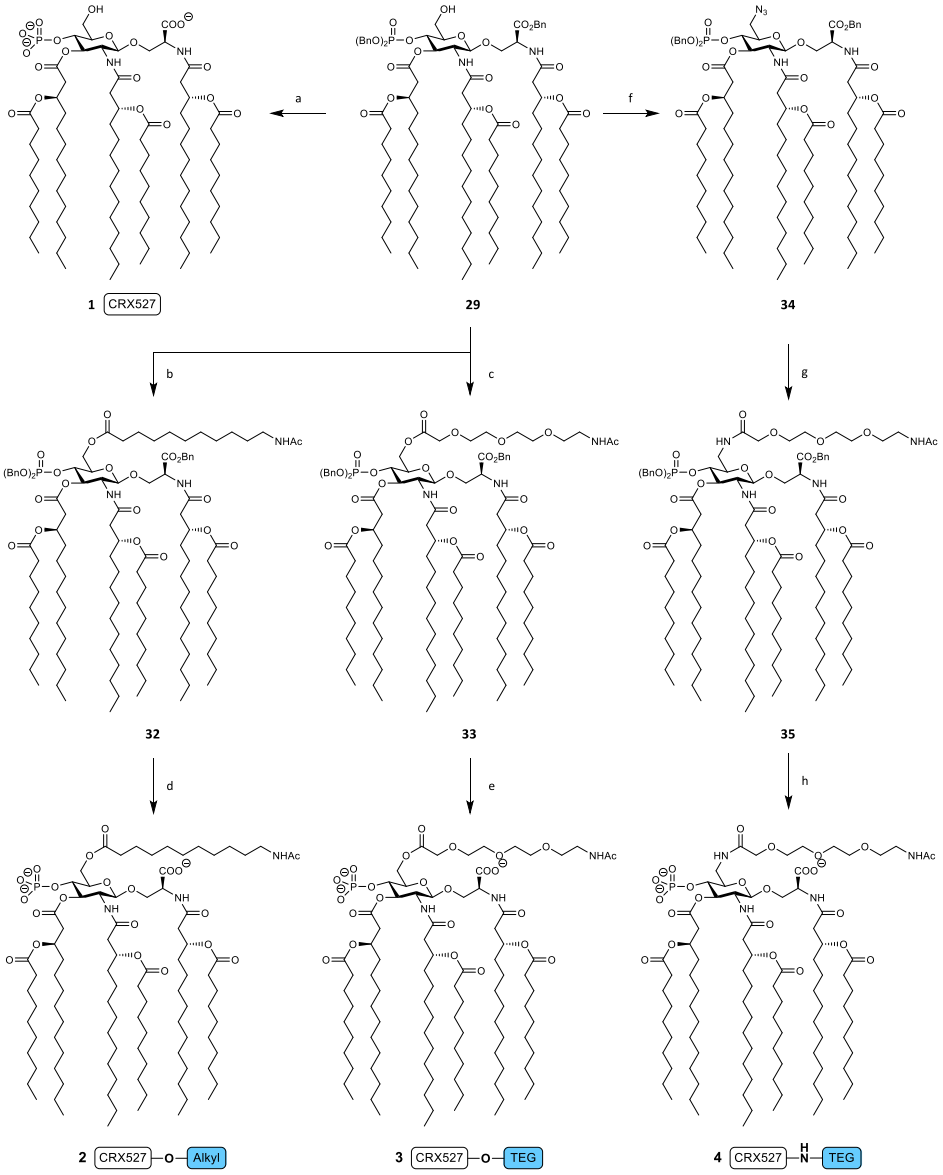
In the final stage of the synthesis of key building block **29** (Scheme 3), the silylidene was removed using $HF{\cdot}Et_3N$ to give diol **26**, of which the primary alcohol was protected with a TBDMS group. The dibenzyl phosphotriester at the *C*-4 of **27** was installed using dibenzyl di-*iso*-propylphosphoramidite and tetrazole, followed by oxidation of the intermediate phosphite triester with 3-chloroperbenzoic acid yielding **28**. Final desilylation with TFA gave key building block **29** in 13% yield over 14 steps from commercially available D-glucosamine.



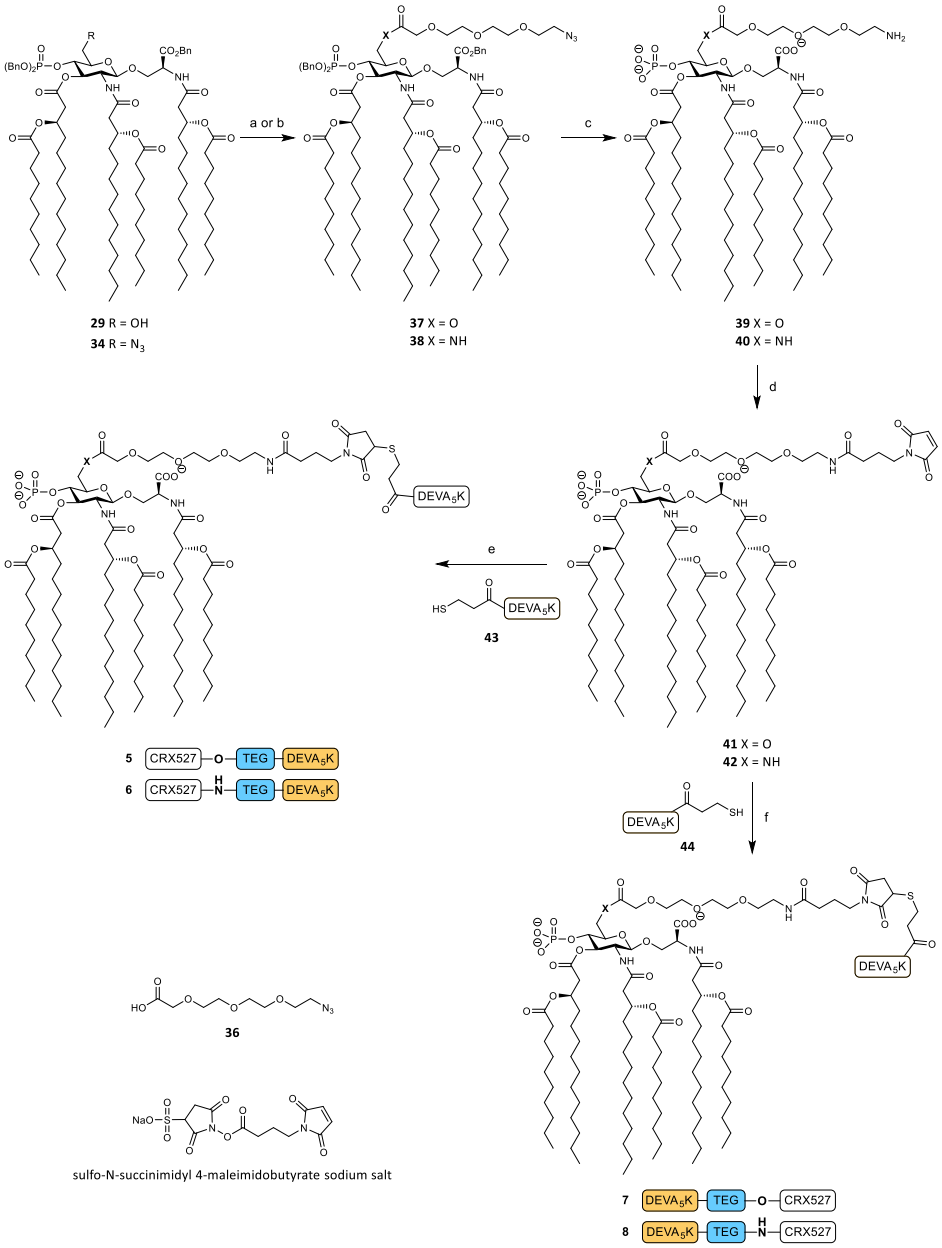
Scheme 3. Synthesis of key building block **29**. *Reagents and conditions*: a) HF·Et_3N. THF, 0°C, 92%; b) TBDMSCl, pyridine, 88%; c) *i*. dibenzyl *N*,*N*-diisopropylphosphoramidite, tetrazole, DCM, 0°, 1h; *ii*. 3-chloroperbenzoic acid, quant. over two steps; d) TFA, DCM, 84%.

With the common starting compound **29** available in sufficient amounts, attention was directed to the assembly of ligands **1**-**4,** having either an alkyl linker or a triethylene glycol (TEG) linker (Scheme 4). These ligands are needed as reference compounds in the immunological evaluation to determine the potential influence of the linker at the *C*-6 position of CRX-527. Debenzylation of **29** using Pd/C gave the original CRX-527 (**1**). Elongation of **29** with the *N*-acetylated linkers **30** and **31**, under influence of EDC·MeI and DMAP furnished **32** and **33**, in 88% and 74% yield, respectively. After hydrogenation of **32** and **33** ligands **2** and **3** were obtained. Contrary to Guo and coworkers, who noticed the 6-*O* ester bond is unstable, no hydrolysis of esters **2** and **3** is observed.[32] Ligand **4** was obtained by conversion of the primary alcohol of **29** into azide **34** using Mitsunobu conditions, reduction of the azide with Zn/NH_4Cl and condensation of the amine with linker **31** to give **35** in 40% yield over two steps. A final hydrogenation step yielded ligand **4** in 61%.

At this stage, the synthesis of the TLR4-ligand peptide conjugates **5**-**8** was undertaken (Scheme 5). Based on preliminary immunological evaluation of the ligands **1**-**4** (*vide infra*) the TEG linker was used for the assembly of the peptide antigen conjugates. The ester linker in **37** was introduced by EDC mediated condensation of **29** and linker **36** to deliver **37** in 80% yield. The amide analogue was synthesized by reduction of the azide in CRX-527 derivative **34** and condensation with linker **36** to give **38** in 56% over two steps. The maleimide functionalized TLR4-ligands **41** and **42** were acquired by hydrogenation of compounds **37** and **38** and treatment of the thus obtained **39** and **40** with sulfo-*N*-succinimidyl 4-maleimidobutyrate sodium salt. The $DEVA_5K$, antigenic peptides having a thiol function at the *N*-terminus (**43**) and at the *C*-terminus (**44**) were synthesized to allow the conjugation of a TLR4-ligand at either side of the peptide. Using a semi-automated solid phase peptide chemistry protocol, the peptides were assembled on a Tentagel S Ram resin. The thiol function in peptide **43** was introduced by a HCTU mediated condensation of the immobilized oligopeptide with 3-(tritylthio)propionic acid. This procedure could also be applied to peptide **44** by the incorporation of MMT protected lysine at the start of the sequence and bocylation of the *N*-terminal amine at the end of the solid phase peptide synthesis. Thiol functionalized peptides **43** or **44** were purified to homogeneity by RP-HPLC. The thiol-maleimide coupling was performed by dissolving **43** or **44** in DMF/H_2O followed by the addition of a solution of maleimide **41** or **42** in $CHCl_3$. After shaking for two days, LC-MS analysis confirmed full conversion of the maleimide. Initially RP-HPLC purification of the crude conjugates was performed, but ester conjugates **5** and **7** turned out to be unstable and hydrolysis of the *C*-6-ester took place during lyophilization of the purified conjugates, likely as the result of the presence of minimal amounts of acid or base. Therefore, a new purification method without any base or acid was used. Herein, the crude mixture was added to a prepacked C18 column and eluted with several solvent systems (see experimental section for details). This resulted in pure *N*-terminus conjugates **5** and **6**, and *C*-terminus conjugates **7** and **8**, of which integrity and purity were shown by analysis with LC-MS and MALD-TOF MS (Figure 3).



Scheme 4. Synthesis of TLR4-ligands **1**-**4**. *Reagents and conditions*: a) H_2, Pd/C, THF, 89%; b) **30**, EDC·MeI, DMAP, DCE, 88%; c) **31**, EDC·MeI, DMAP, DCE, 74%; d) H_2, Pd/C, THF, 56%; e) H_2, Pd/C, THF, 66%; f) PPh_3, DEAD, DPPA, THF, 67%; g) *i*. Zn, NH_4Cl, DCM/MeOH/H_2O; *ii*. **31**, EDC·MeI, DMAP, DCE, 40% over two steps; h) H_2, Pd/C, THF, 61%.



Scheme 5. Synthesis of TLR4-ligand peptide conjugates **5**-**8**. *Reagents and conditions*: a) **36**, EDC·MeI, DMAP, DCE, **37**: 80%; b) *i*. Zn, NH_4Cl, DCM/MeOH/H_2O; *ii*. **36**, EDC·MeI, DMAP, DCE, **38**: 56% over two steps; c) H_2, Pd/C, THF (**39**: 77%, **40**: 83%); d) sulfo-*N*-succinimidyl 4-maleimidobutyrate sodium salt, Et_3N, DCM or DCE (**41**: 84%, **42**: 81%); e) **43**, DMF/$CHCl_3$/H_2O, 48h (**5**: 52%, **6**: 54%); f) **44**, DMF/$CHCl_3$/H_2O, 48h (**7**: 57%, **8**: 42%).

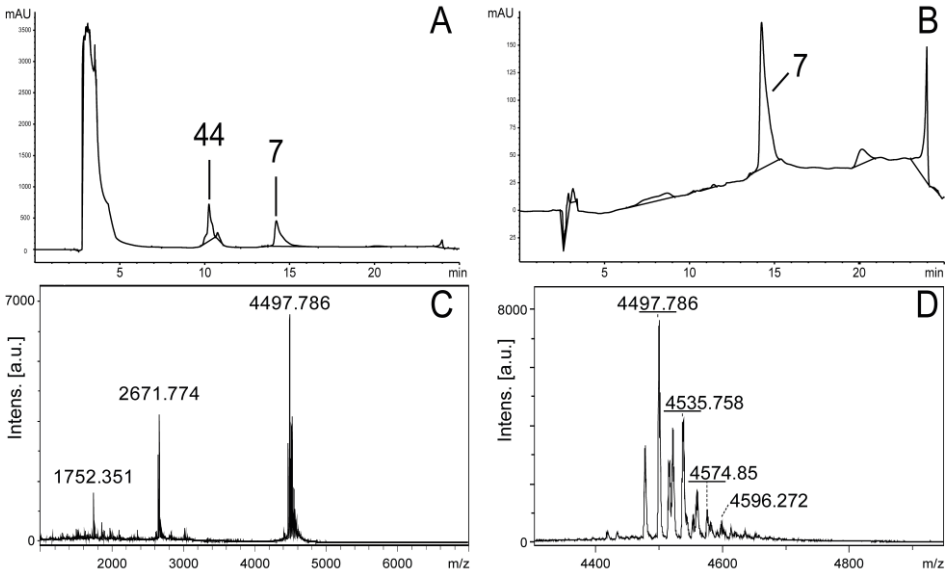


Figure 3. LC-MS traces of crude *C*-terminus conjugate **7** (A) and after purification (B) and the MALDI analysis of **7** (C and D).

Biological evaluation

Immunological evaluation of TLR4-ligands **1**-**4** and conjugates **5**-**8** was performed by assessing their ability to induce maturation of dendritic cells and antigen presentation by DCs *in vitro*.[49] For this purpose, peptide $DEVA_5K$, **45**, TLR2-ligand Pam_3CSK_4, **46**,[50] and TLR2-ligand conjugate **47** were used as relevant reference compounds (Figure 4B).[51] The outline of the experiments is depicted in Figure 4A. Binding of the conjugate to the TLR4/MD-2 complex triggers the production of inflammatory cytokines, such as interleukin-12 (IL-12) and maturation of the DCs. After uptake, the conjugates have to be processed to enable the presentation of the peptide antigen by MHC-I molecules. Binding to the T cell receptor and simultaneous activation by the excreted proinflammatory cytokines then results in the maturation $CD8^+$ T cells.[52] To probe activation of the DCs by the TLR4-ligands and conjugates, DCs were stimulated for 24h with the compounds and the amount of IL-12p40 was measured. First, the activating capacity of the CRX-527 ligand (**1**) and its close analogues **2**-**4**, was tested. As can be seen in Figure 4C the negative controls, DMSO and peptide **45**, show no activity. Stimulation of the DCs with ligands **1**, **3** and **4** gave higher IL-12 secretion than commercially available MPLA or the TLR2-ligand **46**, while ligand **2** proved incapable of DC-activation. Possibly, the hydrophobic linker leads to different binding to the MD-2/TLR4 pocket, preventing activation of the required signaling cascades. The DC-activating capacity of compounds **1**, **3** and **4** shows that functionalization at the *C*-6

position with a hydrophilic linker does not inhibit binding of the ligand to the receptor. Next, the conjugates **5**-**8** were evaluated for DC activation as shown in Figure 4D. The activity of the ester-linked conjugates, **5** and **7** is similar to the activity of the ligands **1** and **3**, while for the amide linked conjugates a slight decrease in activity was observed upon conjugation to the peptide. No difference in activity was observed between the *N*- or the *C*-terminal $DEVA_5K$ conjugates. Finally, the conjugates **5**-**8** and a mixture of peptide **45** and CRX-527 ligand **1** were evaluated for antigen presentation using a SIINFEKL-specific T cell hybridoma assay. Figure 4E shows that exposure of DCs to the ester conjugates **5** and **7**, leads to a relatively low level of antigen presentation, similar to the level induced by the stand-alone antigen and the mixture of the antigen and CRX-527 **1**. Notably, incubation with the amide conjugates **6** and **8** resulted in higher antigen presentation. It is possible that hydrolysis takes place for the ester conjugates **5** and **7** prior to uptake in the DCs, leading to diminished uptake of the peptide part of the conjugate, leading to a lower antigen presentation. The *in vitro* experiments show that the amide conjugates, **6** and **8**, are processed more efficiently than the ester conjugates, **5** and **7**, and a mix of **1** and **45**. The conjugates are not as potent however, as the TLR2-ligand conjugate **47** in terms of antigen presentation *in vitro*.

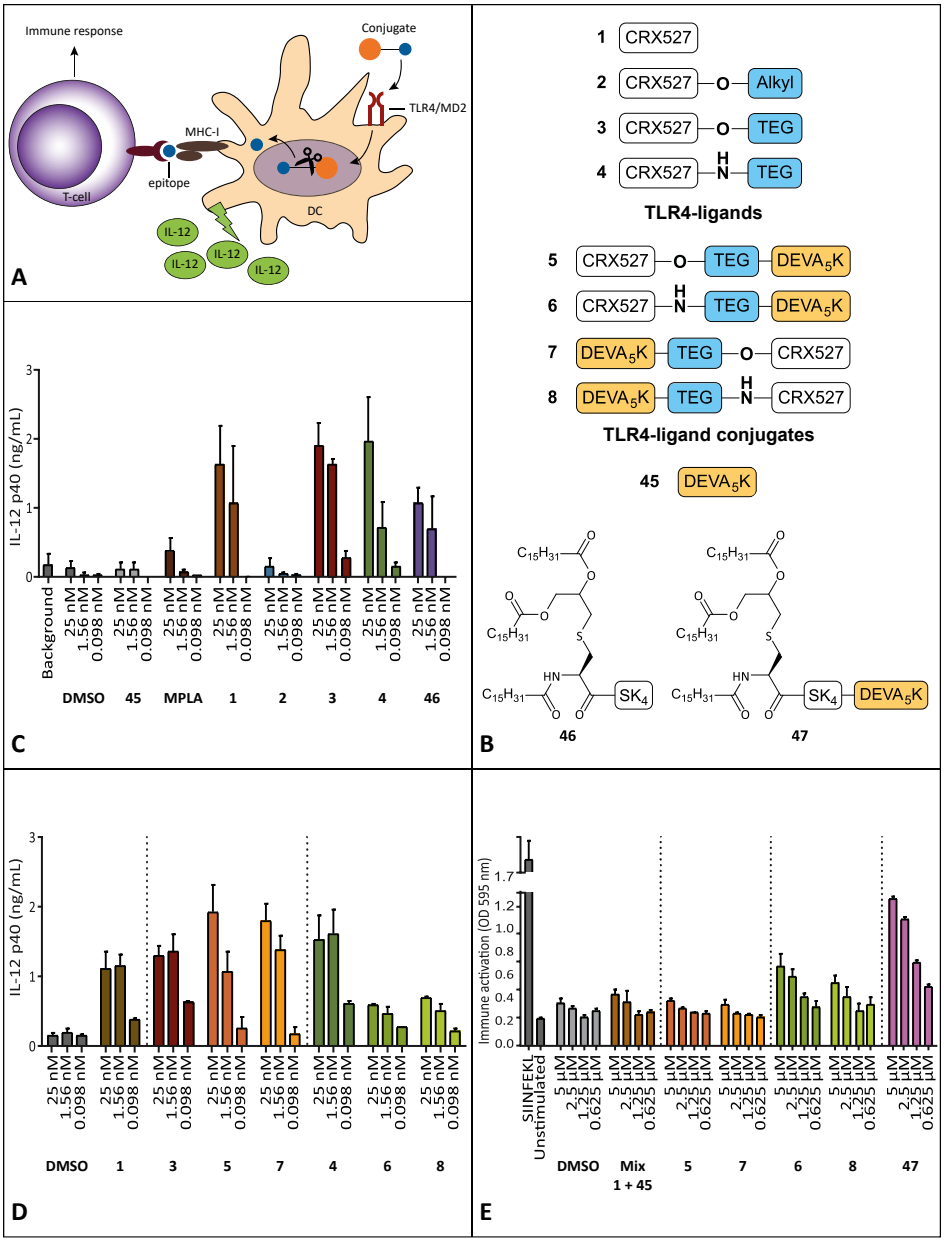


Figure 4. A) Schematic overview of stimulation of a DC with a conjugate leading to an immune response; B) Overview of the TLR4-ligands, TLR4-ligand peptide conjugates and reference compounds used in the *in vitro* experiments; C) DC activation of ligands **1**-**4** and **46**; D) DC activation of conjugates **5**-**8** and ligands **1**, **3** and **4**; E) Antigen presentation of conjugates **5**-**8**, mix of ligand **1** and peptide **45**.[49]

Conclusion

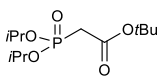
This chapter describes the first synthesis of four TLR4-ligand peptide-conjugates. In these model vaccine constructs, CRX-527, a potent MPLA analogue, was covalently linked to the *N*- or the *C*-terminal end of the $DEVA_5K$ peptide, harboring the MHC-I epitope SIINFEKL, through two different linking moieties to provide the "self adjuvanting" conjugates **5**-**8**. In order to acquire these conjugates, an efficient synthetic route was developed to generate multi-gram amounts of (R)-3-alkyloxytetradecanoic acid **15**. These chiral lipids were used in combination with a silylidene protected glucosaminyl serine building block to provide *N*,*N*,*O*-triacetylated CRX-527 derivative **29**. Different linker systems and connection modes were probed to conjugate the peptide antigen and TLR4-ligands. The conjugates with an ester bond at the *C*-6 position of CRX-527 (**5** and **7**) turned out to be relatively labile, prohibiting HPLC purification. A manual reversed phase chromatography purification protocol allowed for the purification of the conjugates delivering the pure conjugates. Biological evaluation of the ligands showed that the use of a hydrophobic linker led to an inactive ligand, while the presence of a hydrophilic linker at the *C*-6 position did not adversely affect the activity and led to the induction of IL-12 production. Stimulation of DCs with ester conjugates, **5** and **7**, resulted in higher IL-12 production than activation of the cells by the amide conjugates **6** and **8**. In contrast, conjugates **6** and **8** showed to give better antigen presentation *in vitro*. No significant difference was found between the *N*-terminus and *C*-terminus conjugates. The results presented in this Chapter show that TLR4-ligand-antigen conjugates are promising self-adjuvanting vaccine modalities and warrant the evaluation of their activity in *in vivo* experiments.

Experimental

All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3Å molecular sieves. All moisture and oxygen sensitive reactions were performed under an argon atmosphere. Column chromatography was performed on silica gel (Screening Devices BV, 40-63 µm, 60 Å). For TLC analysis, pre-coated silica gel aluminum sheets (Merck, silica gel 60, F254) were used with detection by UV-absorption (254/366 nm) where applicable. Compounds were visualized on TLC by UV absorption (245 nm), or by staining with one of the following TLC stain solutions: $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) and 10% H_2SO_4 in H_2O; bromocresol (0.4 g/L) in EtOH; $KMnO_4$ (7.5 g/L), K_2CO_3 (50 g/L) in H_2O. Staining was followed by charring at ~150°C. ^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV-300 (300/75 MHz), AV-400 (400/100/162 MHz) spectrometer, a Bruker AV-500 Ultrashield (500/126/202 MHz) spectrometer, a Bruker AV-600 (600/151 MHz) or a Bruker AV-850 (850/214 MHz) and all individual signals were assigned using 2D-NMR

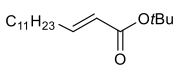
spectroscopy. Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) in $CDCl_3$ or via the solvent residual peak. Coupling constants (*J*) are given in Hz. LC-MS analysis were done on an Agilent Technologies 1260 Infinity system with a C18 Gemini 3 µm, C18, 110 Å, 50 x 4.6 mm column or a Vydac 219TP 5 µm Diphenyl, 150 x 4.6 mm column with a flow of 1, 0.8 or 0.7 ml/min. Absorbance was measured at 214 nm and 256 nm and an Agilent Technologies 6120 Quadrupole mass spectrometer was used as detector. Peptides, TLR2-ligand and conjugate were purified with a Gilson GX-281 preparative HPLC with a Gemini-NX 5u, C18, 110 Å, 250 x 10.0 mm column or a Vydac 219TP 5 µm Diphenyl, 250 x 10 mm column. Peptide fragments were synthesized with automated solid phase peptide synthesis on an Applied Biosystems 433A Peptide Synthesizer. Optical rotations were measured on an Anton Paar Modular Circular Polarimeter MCP 100/150. High resolution mass spectra were recorded on a Synapt G2-Si or a Q Exactive HF Orbitrap equipped with an electron spray ion source positive mode. Mass analysis of the TLR4-ligands and TLR4-ligand conjugates was performed on an Ultraflextreme MALDI-TOF or a 15T MALDI-FT-ICR MS system. Infrared spectra were recorded on a Perkin Elmer Spectrum 2 FT-IR. Unprotected lipid A derivatives were dissolved in a mixture of $CDCl_3$/MeOD 5/1 v/v for NMR analysis. DC activation and B3Z assay results were analysed with GraphPad Prism version 7.00 for Windows, GraphPad Software. FA = fatty acid.

tert-Butyl 2-(diisopropoxyphosphoryl)acetate (9)



A mixture of *tert*-butyl chloroacetate (0.12 L, 0.81 mol, 1.0 eq.) and triisopropyl phosphite (0.22 L, 0.90 mol, 1.1 eq.) was heated to 150°C for 3 hours, after which it was cooled down to room temperature. After purification by vacuum distillation (14 mbar, 95 °C) compound **9** was obtained in quantitative yield (245 g) as a transparent oil, which was used without further purification. $[\alpha]_D^{20}$ -1.0° (*c* = 1.3, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.57 – 4.43 (m, 2H, 2x CH *i*Pr), 2.60 (d, 2H, *J* = 21.5 Hz, CH_2), 1.23 (s, 9H, 3x CH_3 *t*Bu), 1.11 (dd, 12H, *J* = 6.3, 2.8 Hz, 4x CH_3 *i*Pr); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 164.6, 164.6 (C=O), 81.3 (C_q *t*Bu), 70.8, 70.7 (CH *i*Pr), 37.0, 35.7 (CH_2), 27.6 (CH_3 *t*Bu), 23.7, 23.7, 23.5, 23.5 (CH_3 *i*Pr); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ 18.85; FT-IR (neat, cm^{-1}): 2980, 2935, 1728, 1457, 1387, 1369, 1287, 1258, 1173, 1142, 1104, 985, 904, 889, 823, 755, 701, 617, 507; HRMS: $[M+Na]^+$ calcd. for $C_{12}H_{25}O_5PNa$: 303.1332, found 303.1337.

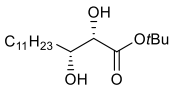
tert-Butyl (_E_)-2-tetradecanoate (10)



Compound **9** (18.1 g, 64.4 mmol, 1.3 eq.) was co-evaporated with toluene (2x), dissolved in THF (0.16 L) under an argon atmosphere and *n*-BuLi (1.6 M in hexane, 40.0 mL, 64.0 mmol 1.3 eq.) was added under an argon flow. After 1 hour, a solution of dodecanal (12.0 mL, 49.8 mmol, 1 eq.) in THF (40 mL) was added and the mixture was stirred at room temperature overnight. The obtained yellow reaction mixture was quenched by addition of H_2O and extracted with Et_2O (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0→3% Et_2O in pentane) yielded the title compound (13.5 g, 47.8 mmol, 96%). R_f: 0.71 (19.5/0.5

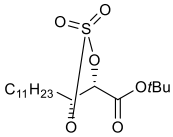
pentane/Et_2O); $[\alpha]_D^{25}$ -0.96° (*c* = 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.84 (dt, 1H, *J* = 15.6, 6.9 Hz, *H*C=CH), 5.71 (dt, 1H, *J* = 15.6, 1.6 Hz, HC=C*H*), 2.18 – 2.09 (m, 2H, CH_2), 1.46 (s, 9H, *t*Bu) , 1.44 – 1.37 (m, 2H, CH_2), 1.29 – 1.20 (m, 16H, 8x CH_2), 0.86 (d, 3H, *J* = 8.0 Hz, CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 166.3 (C=O), 148.3, 123.0 (C=C), 80.0 (C_q *t*Bu), 32.2, 32.0, 30.4, 29.8, 29.7, 29.7, 29.5, 29.5, 29.3 (CH_2), 28.3 (CH_3 *t*Bu), 28.2, 22.8 (CH_2), 14.2 (CH_3); FT-IR (neat, cm^{-1}): 2926, 2855, 2361, 1717, 1654, 1458, 1392, 1367, 1289, 1256, 1154, 1127, 979, 854; HRMS: $[M+H]^+$ calcd. for $C_{18}H_{36}O_2$: 283.26316, found 283.26289.

tert-Butyl (2*S*, 3*R*)-2,3-dihydroxytetradecanoate (11)



To a mixture of *t*BuOH/H_2O (1/1 v/v, 0.18 L) were the following chemicals subsequently added: $K_3[Fe(CN)_6]$ (35.5 g, 106 mmol, 3.0 eq.), K_2CO_3 (14.6 g, 106 mmol, 3.0 eq.), $[(DHQD)_2PHAL]$ (0.29 g, 0.35 mmol, 0.01 eq.), aq. OsO_4 (0.14 M, 1.6 mL, 0.22 mmol, 0.006 eq.), and methanesulfonamide (3.41 g, 35.1 mmol, 1.0 eq.). The reaction mixture was cooled to 0°C and thoroughly stirred for 25 minutes, followed by addition of a solution of compound **10** (9.97 g, 35.2 mmol, 1 eq.) in DCM (8.0 mL). The reaction mixture was stirred at 5°C overnight, after which TLC analysis showed complete conversion of the starting material and it was quenched by the addition of sodium thiosulfate pentahydrate (53.3 g, 215 mmol, 7.0 eq.). After 30 minutes vigorously stirring, the suspension was diluted with H_2O and extracted with EtOAc (3x). The combined organic layers were washed with 2 M KOH (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (5→10% EtOAc in pentane) afforded the title compound (10.1 g, 31.9 mmol, 91%). R_f: 0.54 (1/1 pentane/EtOAc); $[\alpha]_D^{25}$ +4.4° (*c* = 0.85, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 3.96 (d, 1H, *J* = 2.3 Hz, CH), 3.82 (td, 1H, *J* = 6.8, 2.1 Hz, CH), 1.63 – 1.54 (m, 2H, CH_2), 1.53 – 1.43 (m, 11H, CH_2, 3x CH_3 *t*Bu), 1.37 – 1.18 (m, 16H, 8x CH_2), 0.91 – 0.83 (m, 3H, CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.1 (C=O), 83.3 (C_q *t*Bu), 73.3 (CH), 72.9 (CH), 34.1, 32.1, 29.8, 29.8, 29.7 29.7, 29.5 (CH_2), 28.2 (CH_3 *t*Bu), 25.9, 22.8 (CH_2), 14.3 (CH_3); FT-IR (neat, cm^{-1}): 3457, 2924, 2854, 1732, 1459, 1369, 1256, 1162, 1135, 849; HRMS: $[M+Na]^+$ calcd. for $C_{18}H_{36}O_4Na$: 339.2506, found 339.2511.

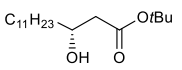
tert-Butyl (4*S*, 5*R*)-5-undecyl-1,3,2-dioxathiolane-4-carboxylate-2,2-dioxide (12)



Diol **11** (118 g, 373 mmol, 1 eq.) was dissolved in EtOAc (0.92 mL) and cooled to 0°C. Pyridine (75 mL, 0.93 mol, 2.5 eq.) was added, followed by slow addition of thionyl chloride (30 mL, 0.41 mol, 1.1 eq.) resulting in a white suspension. After 45 minutes, TLC analysis showed complete conversion of the starting material and the reaction was quenched by the addition of H_2O. The organic layer was washed with H_2O (2x) and the combined water layers were extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered, concentrated *in vacuo* and co-evaporated with toluene (2x). The obtained cyclic sulfite was used without further purification by dissolving in a mixture of CCl_4/CH_3CN (1/1 v/v, 1.3 L). The solution was cooled to 0°C, followed by the addition of $RuCl_3 \cdot H_2O$ (7.74 g, 37.3 mmol, 0.1 eq.), $NaIO_4$ (202 g, 935 mmol, 2.5 eq.) and H_2O (1.3 L) subsequently. The black suspension was

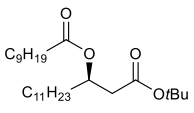
allowed to warm-up to room temperature and after stirring for 1.5 hours, the dark brown mixture was filtered twice over celite and a Whatmann-filter. The residu was washed with DCM and the combined filtrates were diluted with H_2O and brine. The aqueous layers were extracted with DCM (3x) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (2→40% Et_2O in pentane) gave the title compound (127 g, 336 mmol, 90%). R_f: 0.48 (9/1 pentane/Et_2O); $[\alpha]_D^{25}$ +35.3° (*c* = 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.92 – 4.85 (m, 1H, CH), 4.74 (d, 1H, *J* = 7.4 Hz, CH), 2.03 – 1.87 (m, 2H, CH_2), 1.59 – 1.40 (m, 12H, CH_2, CH_3 *t*Bu), 1.40 – 1.18 (m, 16H, 8x CH_2), 0.87 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 163.9 (C=O), 85.5 (C_q *t*Bu), 84.4 (CH), 80.3 (CH), 33.2, 32.0, 29.7, 29.5, 29.4, 29.3, 29.0 (CH_2), 28.0 (CH_3 *t*Bu), 24.9, 22.8 (CH_2), 14.2 (CH_3); FT-IR (neat, cm^{-1}): 2925, 2855, 1764, 1737, 1459, 1396, 1372, 1257, 1210, 1154, 1047, 951, 904, 835, 724, 650, 530; HRMS: $[M+Na]^+$ calcd. for $C_{18}H_{34}O_6SNa$: 401.1968, found 401.1974.

tert-Butyl (*R*)-3-hydroxytetradecanoate (13)



A solution of cyclic sulfate **12** (127 g, 336 mmol, 1.0 eq.) in DMF (0.84 L) was cooled to 0°C, followed by the addition of $NaBH_4$ (14.9 g, 394 mmol, 1.17 eq.). The reaction mixture was allowed to warm-up to room temperature and after 1.5 hours the reaction was quenched with acetone, concentrated *in vacuo* and co-evaporated with toluene. The resulting sulfate was dissolved THF (0.84 mL) and cooled to 0°C. H_2O (12 mL, 0.67 mol, 2.0 eq.) and concentrated H_2SO_4 (36 mL, 0.67 mol, 2.0 eq.) were added to the solution. After the reaction mixture was vigorously stirred for 2 hours, the reaction was neutralized by the addition of Et_3N and sat. aq. $NaHCO_3$. The reaction mixture was further diluted with brine and extracted with Et_2O (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (2→10% EtOAc in pentane) yielded compound **13** (101 g, 335 mmol, Quant.). R_f: 0.26 (9/1 pentane/Et_2O); $[\alpha]_D^{25}$ -14.7° (*c* = 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 3.98 – 3.88 (m, 1H, CH), 3.07 (s, 1H, OH), 2.46 – 2.24 (m, 2H, 2x CH_2), 1.56 – 1.34 (m, 13H, 2x CH_2, 3x CH_3 *t*Bu), 1.34 – 1.15 (m, 16H, 8x CH_2), 0.86 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 172.8 (C=O), 81.3 (C_q *t*Bu), 68.2 (CH), 42.4, 36.6, 32.0, 29.8, 29.7, 29.5 (CH_2), 28.2 (CH_3 *t*Bu), 25.6, 22.8 (CH_2), 14.3 (CH_3); FT-IR (neat, cm^{-1}): 3455, 2924, 2854, 1730, 1458, 1393, 1368, 1256, 1153, 954, 844; HRMS: $[M+Na]^+$ calcd. for $C_{18}H_{36}O_3Na$: 323.2557, found 323.2561.

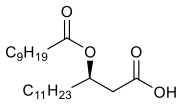
tert-Butyl (*R*)-3-(decanoyloxy)tetradecanoate (14)



A solution of compound **13** (10.2 g, 34.1 mmol, 1.0 eq.) in pyridine (85 mL) was cooled to 0°C under an argon atmosphere. Decanoyl chloride (10.8 mL, 51.0 mmol, 1.5 eq.) and DMAP (0.42 g, 3.4 mmol, 0.1 eq.) were added and after 45 minutes the resulting yellow suspension was allowed warm-up to room temperature. After 30 minutes, TLC analysis showed complete conversion of the starting material and the mixture was concentrated *in vacuo*. After purification by column chromatography (0→5% Et_2O in pentane), the title compound (14.5 g, 31.9 mmol, 94%) was obtained as a transparent oil. R_f: 0.78 (9/1

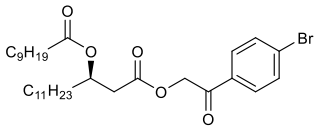
pentane/Et_2O); $[\alpha]_D^{25}$ +1.4° (*c* = 0.91, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.23 – 5.14 (m, 1H, CH), 2.53 – 2.34 (m, 2H, CH_2), 2.24 (t, 2H, *J* = 7.5 Hz, CH_2), 1.65 – 1.48 (m, 4H, 2x CH_2), 1.41 (s, 9H, 3x CH_3 *t*Bu), 1.35 – 1.14 (m, 30H, 15x CH_2), 0.85 (t, 6H, *J* = 6.8 Hz, 2x CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.2 (C=O), 169.8 (C=O), 80.8 (C_q *t*Bu), 70.6 (CH), 40.7, 34.6, 34.1, 32.0, 32.0, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3 (CH_2), 28.1 (CH_3 *t*Bu), 25.2, 25.1, 22.8 (CH_2), 14.2 (CH_3); FT-IR (neat, cm^{-1}): 2925, 2855, 1738, 1466, 1368, 1153; HRMS: $[M+Na]^+$ calcd. for $C_{28}H_{54}O_4Na$: 477.3914, found 477.3924.

(*R*)-3-(decanoyloxy)tetradecanoic acid (15)



Compound **14** (117.7 g, 258.9 mmol, 1 eq.) was dissolved in DCM (0.43 L) and cooled to 0°C. TFA (0.12 L, 1.57 mol, 6.0 eq.) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was subsequently concentrated *in vacuo* and co-evaporated several times with toluene. Purification by column chromatography (0→10% MeOH in DCM) gave acid **15** (96.5 g, 242 mmol, 93%). R_f: 0.63 (9/1 DCM/MeOH); $[\alpha]_D^{20}$ +4.0° (*c* = 2.0, DCM); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.20 (p, 1H, *J* = 7.0, 6.4 Hz, CH), 2.67 – 2.51 (m, 2H, CH_2), 2.27 (t, 2H, *J* = 7.5 Hz, CH_2), 1.70 – 1.51 (m, 4H, 2x CH_2), 1.39 – 1.16 (m, 30H, 15x CH_2), 0.87 (t, 6H, *J* = 6.8 Hz, 2x (CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 176.9 (C=O), 173.4 (C=O), 70.1 (CH), 39.0, 34.6, 34.1, 32.0, 32.0, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 25.2, 25.1, 22.8, 22.8 (CH_2), 14.2 (CH_3); FT-IR (neat, cm^{-1}): 2923, 2854, 1740, 1714, 1466, 1378, 1163, 1109, 722; HRMS: $[M+Na]^+$ calcd. for $C_{24}H_{46}O_4Na$: 421.3388, found 421.3289.

2-(4-Bromophenyl)-2-oxoethyl-(*R*)-3-(decanoyloxy)tetradecanoate (16)



Acid **15** (0.29 g, 0.98 mmol, 1 eq.) was dissolved in EtOAc (5.0 mL), followed by the addition of 2,4'-dibromoacetophenone (0.31 g, 1.1 mmol, 1.1 eq.) and Et_3N (0.15 mL, 1.1 mmol, 1.1 eq.). The reaction mixture was stirred overnight, after which it was diluted with H_2O and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (5→30% EtOAc in pentane) afforded the title compound (0.28 g, 0.47 mmol, 48%, ee = 98.55%). R_f: 0.17 (9/1 pentane/Et_2O); $[\alpha]_D^{25}$ -1.4° (*c* = 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.73 (d, 2H, *J* = 8.5 Hz, Ar), 7.59 (d, 2H, *J* = 8.5 Hz, Ar), 5.32 – 5.22 (m, 3H, CH_2, CH), 2.77 – 2.64 (m, 2H, CH_2), 2.28 (t, 2H, *J* = 7.5 Hz, CH_2), 1.69 – 1.53 (m, 4H, 2x CH_2), 1.37 – 1.13 (m, 30H, 15x CH_2), 0.90 – 0.79 (m, 6H, 2x CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 190.9, 173.3, 169.9 (C=O), 132.9 (C_q Ar), 132.2, 129.3 (Ar), 129.2 (C_q Ar), 70.1 (CH), 66.0, 38.9, 34.5, 34.1, 32.0, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.4, 29.4, 29.2, 25.2, 25.0, 22.7 (CH_2), 14.2 (CH_3); FT-IR (neat, cm^{-1}): 2925, 2854, 1739, 1708, 1588, 1164, 1072, 972; HRMS: $[M+Na]^+$ calcd. for $C_{32}H_{51}O_5BrNa$: 617.2812, found 617.2824.

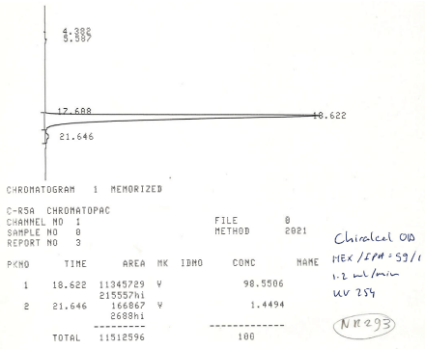
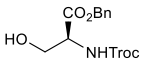


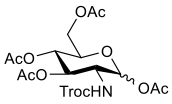
Figure 5. Analysis of *ee* of compound **16**.

Benzyl *N*-trichloroethoxycarbonyl-L-serinate (17)



L-Serine (49.6 g, 472 mmol, 1.0 eq.) was dissolved in a mixture of CCl_4/benzyl alcohol (1/1 v/v, 0.46 L). *p*-Toluenesulfonic acid (96.6 g, 508 mmol, 1.1 eq.) was added and the white suspension was heated to 100°C using a Dean-Stark apparatus. After stirring overnight, a clear solution was obtained, which was cooled down to room temperature before concentrating *in vacuo*. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$ (3x). The organic layer was extracted with 1 M HCl (3x) and the combined aqueous layers were concentrated *in vacuo*. Co-evaporation with toluene yielded the intermediate as a white solid (46.6 g, 201 mmol), which was dissolved in DCM (1.0 L) under an argon atmosphere. Succinimidyl-2,2,2-trichloroethyl carbonate[48] (61.5 g, 212 mmol, 1.05 eq.) was added to the reaction mixture, followed by the addition of Et_3N (42 mL, 0.30 mol, 1.5 eq.) under a flow of argon. After 1 hour, TLC analysis showed complete conversion of the starting material and the reaction mixture was washed with 1 M HCl (1x) and H_2O (1x). The aqueous layers were extracted with DCM (1x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (20→100% EtOAc in pentane) yielded the title compound (69.4 g, 187 mmol, 40% over two steps). HRMS: $[M+H]^+$ calcd. for $C_{13}H_{15}O_5NCl_3$: 370.00103, found 370.00105. Analytic data were in agreement with reported data.[48]

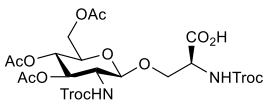
Acetyl 3,4,6-tri-*O*-acetyl-2-*N*-trichloroethoxycarbonyl-α/β-D-glucopyranoside (18)



$NaHCO_3$ (144 g, 1.65 mol, 3.0 eq.) and 2,2,2-trichloroethoxycarbonyl chloride (93 mL, 0.68 mol, 1.2 eq.) were added to a solution of D-glucosamine·HCl (0.12 kg, 0.55 mol, 1.0 eq.) in H_2O (1.1 L). The reaction was stirred vigorously at room temperature overnight, after which the resulting white suspension was filtered and the residue was washed with cold H_2O. The white solid was co-evaporated with toluene (3x) before dissolving in pyridine (0.60 L). The reaction mixture was cooled to 0°C and Ac_2O (0.30 L, 3.2 mol, 5.8 eq.) was added. The reaction mixture was allowed to warm-up to room temperature and stirred overnight. The reaction mixture was cooled to 0°C, quenched by the addition of H_2O and subsequently diluted with EtOAc. The organic layer was washed several times with 1 M HCl, dried over $MgSO_4$, filtered and concentrated *in vacuo*. TLC analysis showed no

full conversion, therefore the oil was dissolved in pyridine (0.60 L) and cooled to 0°C. Ac_2O (0.45 L, 4.8 mol, 8.7 eq.) was added and after 30 minutes the mixture was allowed to warm-up to room temperature. After 2.5 hours TLC analysis showed full conversion. The reaction was quenched by the addition of MeOH and concentrated *in vacuo*. Co-evaporation with toluene (3x) gave compound **18** (189 g, 362 mmol, 66%), which was used without further purification. R_f: 0.20 (7/3 pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 6.16 (d, 1H, *J* = 3.8 Hz, H-1), 5.43 (d, 1H, *J* = 9.5 Hz, NH), 5.27 – 5.17 (m, 1H, H-3), 5.13 (t, 1H, *J* = 9.9 Hz, H-4), 4.76 (d, 1H, *J* = 12.1 Hz, C*H*H Troc), 4.56 (d, 1H, *J* = 12.1 Hz, C*H*H Troc), 4.23 – 4.11 (m, 2H, H-2, C*H*H-6), 4.02 – 3.94 (m, 2H, H-5, C*H*H-6), 2.13 (s, 3H, CH_3 Ac), 2.02 (s, 3H, CH_3 Ac), 1.98 – 1.96 (m, 6H, 2x CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 171.2, 170.7, 169.2, 168.7 (C=O Ac), 154.1 (C=O Troc), 95.3 (C_q Troc), 90.4 (C-1), 74.6 (CH_2 Troc), 70.3 (C-3), 69.6 (C-5), 67.6 (C-4), 61.5 (CH_2-6), 53.1 (C-2), 20.9, 20.7, 20.6, 20.5 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3329, 2958, 2258, 2126, 1742, 1536, 1432, 1368, 1212, 1172, 1141, 1123, 1095, 1080, 1031, 1012, 952, 910, 820, 728, 681, 648, 599, 568, 526, 475; HRMS: $[M+Na]^+$ calcd. for $C_{17}H_{22}Cl_3NO_{11}Na$: 544.0151, found 544.0159.

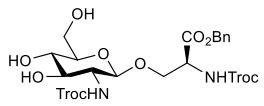
_N_-trichloroethoxycarbonyl-_O_-[3,4,6-tri-_O_-acetyl-2-_N_-trichloroethoxycarbonyl-β-D-glucopyranosyl]-L-serine (19)



Compounds **18** (88.9 g, 170 mol, 1.0 eq.) and **17** (69.3 g, 187 mmol, 1.1 eq.) were co-evaporated with toluene (2x) under an argon atmosphere and dissolved in DCM (0.28 L). The mixture was cooled to 0°C, followed by the slow addition of $BF_3{\cdot}OEt_2$ (42 mL, 0.34 mol, 2.0 eq.). The mixture was allowed to warm-up to room temperature and stirred for an additional 48 hours. The mixture was quenched with Et_3N and washed with sat. aq. $NaHCO_3$ (1x). The aqueous layer was extracted with DCM (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (20→100% EtOAc in pentane) gave an oil (117 g), which was a mixture of unreacted donor **18** and benzyl *N*-trichloroethoxycarbonyl-*O*-[3,4,6-tri-*O*-acetyl-2-*N*-trichloroethoxycarbonyl-β-D-glucopyranosyl]-L-serinate. After co-evaporating with toluene (3x) under an argon atmosphere, the oil was dissolved in THF (1.2 L), followed by the addition of Pd/C (10%, 11.7 g). The black suspension was purged with argon for 15 minutes, followed by purging with $H_{2(g)}$ and after 15 minutes a $H_{2(g)}$-filled balloon was applied. After stirring at room temperature overnight, the mixture was filtered over a Whatmann-filter and concentrated *in vacuo*. Purification by column chromatography (10→100% acetone in pentane) yielded the title compound (79.7 g, 107 mmol, 63% yield over two steps) and unreacted donor **18** (18.5 g, 35.4 mmol). R_f: 0.13 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +11.3° (*c* = 0.23, $CHCl_3$); ^{1}H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 5.31 – 5.21 (m, 1H, H-3), 4.98 (t, 1H, *J* = 9.7 Hz, H-4), 4.86 (d, 1H, *J* = 5.1 Hz, C*H*H Troc), 4.83 – 4.74 (m, 2H, 2x C*H*H Troc), 4.74 – 4.68 (m, 2H, H-1, C*H*H Troc), 4.41 (d, 1H, *J* = 5.0 Hz, CH serine), 4.31 (dd, 1H, *J* = 12.4, 4.6 Hz, C*H*H-6), 4.25 – 4.07 (m, 2H, C*H*H-6, C*H*H serine), 3.95 (dd, 1H, *J* = 10.5, 3.9 Hz, C*H*H serine), 3.85 – 3.73 (m, 1H, H-5), 3.66 – 3.52 (m, 1H, H-2), 2.07 (s, 3H, CH_3 Ac), 2.00 (s, 3H, CH_3 Ac), 1.97 (s, 3H, CH_3 Ac); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 172.5, 172.4, 171.7 (C=O Ac), 171.3 (C=O serine), 156.7, 156.5 (C=O Troc), 101.9 (C-

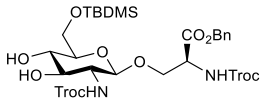
1), 97.1 (C_q Troc), 75.7, 75.4 (CH_2 Troc), 73.7 (C-3), 73.0 (C-5), 70.2 (C-4), 70.1 (CH_2 serine), 63.1 (CH_2-6), 57.1 (C-2), 55.7 (CH serine), 20.6 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3340, 2958, 1744, 1532, 1369, 1232, 1170, 1102, 1048, 819, 769, 734, 569; HRMS: $[M+Na]^+$ calcd. for $C_{21}H_{26}Cl_6N_2O_{14}Na$: 762.9407, found 762.9416.

Benzyl *N*-trichloroethoxycarbonyl-*O*-[2-*N*-trichloroethoxycarbonyl-β-D-glucopyranosyl]-L-serinate (20)



Compound **19** (79.6 g, 107 mmol, 1.0 eq.) was dissolved in MeOH (1.1 L) and NH_4OH (13.4 M, 73.5 mL, 985 mmol, 9.2 eq.) was added. After two days stirring at room temperature, TLC analysis showed complete conversion of the starting material. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The obtained oil was dissolved in a DCM/sat. aq. $NaHCO_3$ mixture (1/1 v/v, 2.6 L), after which tetrabutylammonium bromide (34.9 g, 108 mmol, 1.0 eq.) and benzyl bromide (64 mL, 0.54 mol, 5.0 eq.) were added. The reaction mixture was stirred overnight. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (2x) and DCM (1x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (20→100% EtOAc in pentane, then 20% MeOH in EtOAc) afforded compound **20** (44.2 g, 65.5 mmol, 79% yield over two steps). R_f: 0.49 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -15.2° (*c* = 0.48, MeOH); ^{1}H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.41 – 7.28 (m, 5H, Ar), 5.25 – 5.12 (m, 2H, CH_2 Bn), 4.85 (d, 1H, *J* = 12.2 Hz, C*H*H Troc), 4.79 – 4.69 (m, 3H, C*H*H Troc, CH_2 Troc), 4.49 (t, 1H, *J* = 4.4 Hz, CH serine), 4.45 (d, 1H, *J* = 8.2 Hz, H-1), 4.24 (dd, 1H, *J* = 10.2, 5.2 Hz, C*H*H serine), 3.93 – 3.83 (m, 2H, C*H*H serine, C*H*H-6), 3.67 (dd, 1H, *J* = 11.8, 5.5 Hz, C*H*H-6), 3.45 (dd, 1H, *J* = 10.2, 8.2 Hz, H-3), 3.41 – 3.33 (m, 1H, H-2), 3.31 – 3.21 (m, 2H, H-4, H-5); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 171.2 (C=O serine), 157.1, 156.5 (C=O Troc), 137.0 (C_q Ar), 129.6, 129.3, 129.1 (Ar), 102.8 (C-1), 96.8 (C_q Troc), 78.0 (C-5), 75.6 (CH_2 Troc), 75.5 (C-3), 72.0 (C-4), 69.6 (CH_2 serine), 68.2 (CH_2 Bn), 62.7 (CH_2-6), 58.9 (C-2), 56.2 (CH serine); FT-IR (neat, cm^{-1}): 3423, 2955, 2487, 1729, 1431, 1332, 1293, 1173, 1060, 820, 731, 569; HRMS: $[M+Na]^+$ calcd. for $C_{22}H_{26}Cl_6N_2O_{11}Na$: 726.9560, found 726.9576.

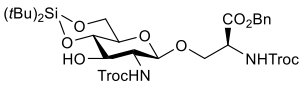
Benzyl *N*-trichloroethoxycarbonyl-*O*-[6-*O*-*tert*-butyldimethylsilyl-2-*N*-trichloroethoxycarbonyl-β-D-glucopyranosyl]-L-serinate (21)



Compound **20** (44.2 g, 62.5 mmol, 1.0 eq.) was dissolved in pyridine (0.30 L) and *tert*-butyldimethylsilyl chloride (14.6 g, 96.9 mmol, 1.5 eq.) was added. After 3 hours, TLC analysis showed complete conversion of the starting material and the reaction mixture was diluted with EtOAc. The organic layer was washed with 1 M HCl (2x), sat. aq. $NaHCO_3$ (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Coevaporation with toluene (2x) and purification by column chromatography (20→100% EtOAc in pentane, then 20% MeOH in EtOAc) yielded the title compound (42.3 g, 51.4 mmol, 82%) as a white foam. R_f: 0.72 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -18.2° (*c* = 0.71, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.41 – 7.30 (m, 5H, Ar), 6.15 (d, 1H, *J* = 7.8 Hz, NH serine), 5.63 (d, 1H, *J* = 7.0 Hz, NH GlcN), 5.20 (s, 2H, CH_2 Bn), 4.83

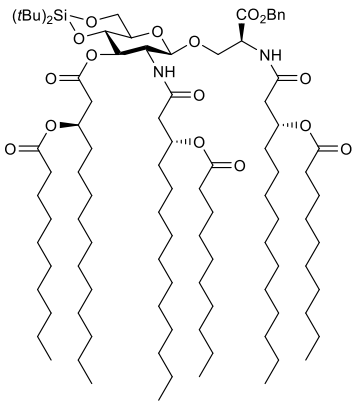
– 4.68 (m, 4H, 2x CH_2 Troc), 4.59 – 4.50 (m, 2H, H-1, CH serine), 4.24 (dd, 1H, *J* = 10.5, 4.2 Hz, C*H*H serine), 3.92 – 3.84 (m, 2H, C*H*H-6, C*H*H serine), 3.81 (dd, 1H, *J* = 10.5, 5.7 Hz, C*H*H-6), 3.75 – 3.59 (m, 3H, H-3, 2x OH), 3.59 – 3.49 (m, 1H, H-4), 3.37 – 3.25 (m, 2H, H-2, H-5), 0.89 (s, 9H, 3x CH_3 TBDMS), 0.12 – 0.05 (m, 6H, 2x CH_3 TBDMS); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 169.4 (C=O serine), 155.2, 154.7 (C=O Troc), 135.2 (C_q Ar), 128.7, 128.6, 128.3 (Ar), 100.6 (C-1), 95.5, 95.4 (C_q Troc), 74.9, 74.8 (CH_2 Troc), 74.3 (C-3), 74.3 (C-5), 73.6 (C-4), 68.7 (CH_2 serine), 67.8 (CH_2 CO_2Bn), 64.5 (CH_2-6), 57.6 (C-2), 54.5 (CH serine), 25.9, 18.3, -5.3 (CH_3 TBDMS); FT-IR (neat, cm^{-1}): 3341, 2954, 2930, 2857, 1733, 1531, 1462, 1389, 1253, 1203, 1165, 1062, 950, 836, 778, 733, 698, 569; HRMS: $[M+Na]^+$ calcd. for $C_{28}H_{40}Cl_6N_2O_{11}SiNa$: 841.0425, found 841.0437.

Benzyl *N*-trichloroethoxycarbonyl-*O*-[4,6-*O*-di-*tert*-butylsilylidene-2-*N*-trichloroethoxycarbonyl-β-D-glucopyranosyl]-L-serinate (24)



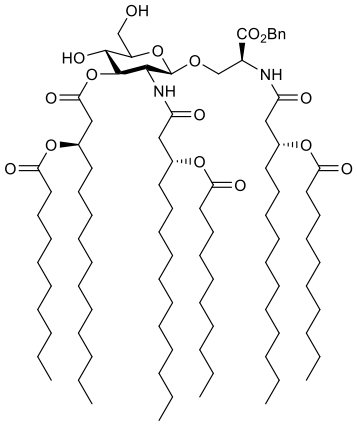
A solution of compound **20** (2.01 g, 2.84 mmol, 1.0 eq.) in DMF (14 mL) was cooled to -40°C. Di-*tert*-butylsilanediyl-bistriflate (0.92 mL, 3.1 mmol, 1.1 eq.) was added drop-wise. After one hour, the reaction was allowed to warm-up to room temperature and stirred overnight. The reaction mixture was quenched by the addition of pyridine (1.6 mL, 19.9 mmol, 7.0 eq.). The mixture was diluted with Et_2O and the organic layer was washed with H_2O (1x) and sat. aq. $NaHCO_3$ (3x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. After purification by column chromatography (2→3% acetone in DCM), the title compound (2.07 g, 2.44 mmol, 86%) was obtained as a white foam. R_f: 0.60 (1/1 pentane/Et_2O); $[\alpha]_D^{25}$ -24.0° (*c* = 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.38 – 7.28 (m, 5H, Ar), 6.30 (s, 1H, NH serine), 5.80 (d, 1H, *J* = 7.7 Hz, NH GlcN), 5.23 – 5.12 (m, 2H, CH_2 Bn), 4.81 – 4.65 (m, 5H, H-1, 2x CH_2 Troc), 4.53 (dt, 1H, *J* = 7.8, 3.4 Hz, CH serine), 4.25 (dd, 1H, *J* = 10.3, 3.3 Hz, C*H*H serine), 4.14 (dd, 1H, *J* = 10.1, 5.0 Hz, C*H*H-6), 3.89 – 3.80 (m, 2H, C*H*H-6, C*H*H serine), 3.80 – 3.72 (m, 1H, H-3), 3.67 (t, 1H, *J* = 8.9 Hz, H-4), 3.42 – 3.34 (m, 1H, H-5), 3.34 – 3.25 (m, 1H, H-2), 3.22 (br, 1H, OH), 1.04 (s, 9H, 3x CH_3 *t*Bu), 0.97 (s, 9H, 3x CH_3 *t*Bu); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 169.3 (C=O serine), 154.6, 154.5 (C=O Troc), 135.1 (C_q Ar), 128.6, 128.5, 128.2 (Ar), 100.8 (C-1), 95.5, 95.4 (C_q Troc), 77.4 (C-4), 74.6 (CH_2 Troc), 73.5 (C-3), 70.3 (C-5), 68.9 (CH_2 serine), 67.6 (CH_2 Bn), 66.0 (CH_2-6), 57.4 (H-2), 54.5 (CH serine), 27.4, 27.0 (CH_3 *t*Bu), 22.6, 19.9 (C_q *t*Bu); FT-IR (neat, cm^{-1}): 3340, 2935, 2886, 2860, 1730, 1523, 1473, 1387, 1365, 1336, 1243, 1201, 1160, 1076, 1009, 943, 909, 826, 765, 730, 697, 653, 618, 569, 476; HRMS: $[M+Na]^+$ calcd. for $C_{30}H_{42}Cl_6N_2O_{11}SiNa$: 867.0581, found 867.0599.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4,6-*O*-di-*tert*-butylsilylidene-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (25)



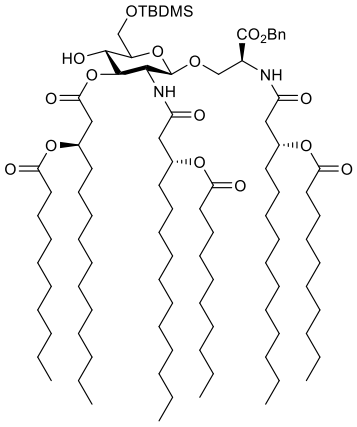
To a solution of compound **24 (**2.55 g, 3.00 mmol, 1.0 eq.) in THF (30 mL) was added activated zinc (4.0 g, 61 mmol, 20 eq.) and AcOH (0.69 mL, 12 mmol, 4.0 eq.) under an argon atmosphere. The suspension was stirred for 25 minutes and the mixture was subsequently sonicated for 5 min. The mixture was stirred again for 25 min, followed by sonicating for 5 minutes. TLC and LC-MS analysis showed complete conversion of the starting material. The suspension was filtered over a Whatmann filter and the residue was washed with DCM and EtOAc. The combined filtrates were concentrated *in vacuo*, co-evaporated with toluene (3x) and the obtained solid was dissolved in EtOAc. The solution was subsequently washed with 0.1 M HCl (1x), sat. aq. $NaHCO_3$ (1x) and brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. A mixture of the obtained yellow oil and acid **15** (5.39 g, 13.5 mmol, 4.5 eq.) was co-evaporated with toluene (1x) and dissolved in DCM (30 mL) under an argon atmosphere. EDC·MeI (4.01 g, 13.5 mmol, 4.5 eq.) and DMAP (11 mg, 90 µmol, 0.03 eq.) were added and the reaction mixture was stirred 4 hours, after which the mixture was concentrated *in vacuo*. Several purifications by column chromatography (2→20% EtOAc in DCM + 0.1% Et_3N and 0→10% acetone in DCM + 0.1% Et_3N) gave compound **25** (3.07 g, 1.87 mmol, 62% over two steps) as a white foam. R_f: 0.58 (95/5 DCM/acetone); $[\alpha]_D^{25}$ -15.4° (*c* = 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, HH-COSY, HSQC): δ 7.37 – 7.27 (m, 5H, Ar), 7.01 (d, 1H, *J* = 7.8 Hz, NH serine), 6.27 (d, 1H, *J* = 8.3 Hz, NH GlcN), 5.22 – 5.05 (m, 5H, 3x CH FA, CH_2 Bn), 5.06 – 4.98 (m, 1H, H-3), 4.72 – 4.66 (m, 2H, H-1, CH serine), 4.21 (dd, 1H, *J* = 10.7, 3.0 Hz, C*H*H serine), 4.14 (dd, 1H, *J* = 10.2, 5.0 Hz, C*H*H serine), 3.88 – 3.77 (m, 3H, H-4, C*H*H-6, C*H*H serine), 3.73 – 3.65 (m, 1H, H-2), 3.44 – 3.37 (m, 1H, H-5), 2.67 – 2.20 (m, 12H, 6x CH_2 FA), 1.71 – 1.50 (m, 12H, 6x CH_2 FA), 1.40 – 1.17 (m, 90H, 45x CH_2 FA), 1.02 (s, 9H, 3x CH_3 *t*Bu), 0.94 (s, 9H, 3x CH_3 *t*Bu), 0.87 (t, 18H, *J* = 6.7 Hz, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 126 MHz, HSQC): δ 173.9, 173.8, 173.3, 170.6, 170.3, 170.2 (C=O FA), 169.5 (C=O serine), 135.5 (C_q Ar), 128.6, 128.4, 128.1 (Ar), 101.6 (C-1), 75.1 (C-4), 74.4 (C-3), 71.5, 71.5 (CH FA), 70.8 (C-5), 70.1 (CH FA), 68.8 (CH_2 serine), 67.3 (CH_2 Bn), 66.3 (CH_2-6), 54.7 (C-2), 52.8 (CH serine), 42.2, 41.3, 39.2, 34.7, 34.6, 34.6, 34.5, 34.0, 32.1, 32.0, 32.0, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3 (CH_2 FA), 27.5, 27.0 (CH_3 *t*Bu), 25.5, 25.4, 25.2, 25.2, 25.1, 22.8 (CH_2 FA), 22.7, 20.0 (C_q *t*Bu), 14.2 (CH_3 FA); FT-IR (neat, cm^{-1}): 3285, 3068, 2956, 2923, 2854, 1734, 1652, 1540, 1450, 1466, 1378, 1364, 1246, 1173, 1075, 1030, 1011, 837, 827, 769, 723, 696, 652, 581, 463; HRMS: $[M+H]^+$ calcd. for $C_{96}H_{173}N_2O_{16}Si$: 1638,2549, found 1638.2493.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (26)



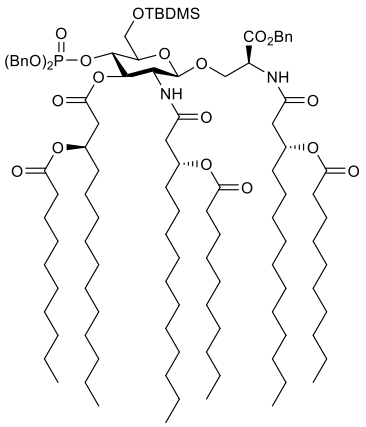
Compound **25** (1.92 g, 1.17 mmol, 1.0 eq.) was dissolved in THF (12 mL) under an argon atmosphere and cooled to 0°C. HF·Et_3N (0.58 mL, 3.6 mmol, 3.0 eq.) was added and the reaction mixture was stirred for 1.5 h, after which TLC analysis showed complete conversion of the starting material. The reaction was quenched with sat. aq. $NaHCO_3$, diluted with EtOAc and washed with brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0→4% MeOH in DCM) yielded the title compound (1.61 g, 1.08 mmol, 92%). R_f: 0.64 (8/2 DCM/acetone); $[\alpha]_D^{25}$ -11.0° (*c* = 0.71, $CHCl_3$/MeOH 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.41 – 7.24 (m, 5H, Ar), 7.04 (d, 1H, *J* = 7.8 Hz, NH serine), 6.38 (d, 1H, *J* = 8.5 Hz, NH GlcN), 5.23 – 5.03 (m, 5H, 3x CH FA, CH_2 Bn), 4.96 (t, 1H, *J* = 8.0 Hz, H-3), 4.74 – 4.64 (m, 1H, CH serine), 4.57 (d, 1H, *J* = 8.2 Hz, H-1), 4.24 – 4.12 (m, 1H, C*H*H serine), 3.93 – 3.80 (m, 2H, C*H*H serine, C*H*H-6), 3.80 – 3.67 (m, 2H, H-2, C*H*H-6), 3.67 – 3.52 (m, 2H, H-4, OH), 3.39 – 3.26 (m, 1H, H-5), 2.76 (br, 1H, OH), 2.67 – 2.35 (m, 6H, 3x CH_2 FA), 2.35 – 2.18 (m, 6H, 3x CH_2 FA), 1.72 – 1.46 (m, 12H, 6x CH_2 FA), 1.46 – 0.95 (m, 90H, 45x CH_2 FA), 0.86 (t, 18H, *J* = 6.7 Hz, 6x CH_3 FA).^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.6, 174.0, 173.8, 171.6, 170.4, 170.2 (C=O FA), 169.8 (C=O serine), 135.4 (C_q Ar), 128.6, 128.4, 128.1 (Ar), 101.3 (C-1), 76.0 (C-3), 75.8 (C-5), 71.5, 71.3, 71.2 (CH FA), 69.3 (C-4), 68.7 (CH_2 serine), 67.3 (CH_2 Bn), 62.2 (CH_2-6), 54.0 (C-2), 52.9 (CH serine), 42.0, 41.3, 40.3, 34.8, 34.7, 34.6, 34.5, 34.5, 32.0, 32.0, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 25.4, 25.4, 25.3, 25.1, 25.1, 25.1, 22.8 (CH_2 FA), 14.2 (CH_3 FA); FT-IR (neat, cm^{-1}): 3509, 3285, 3092, 2956, 2922, 2853, 1733, 1708, 1647, 1553, 1499, 1467, 1419, 1378, 1307, 1250, 1176, 1130, 1102, 1046, 1003, 964, 906, 722, 696, 673, 597, 510, 478; HRMS: $[M+H]^+$ calcd. for $C_{88}H_{157}N_2O_{16}$: 1498.15276, found 1498.15332.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[6-*O*-*tert*-butyldimethylsilyl-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (27)



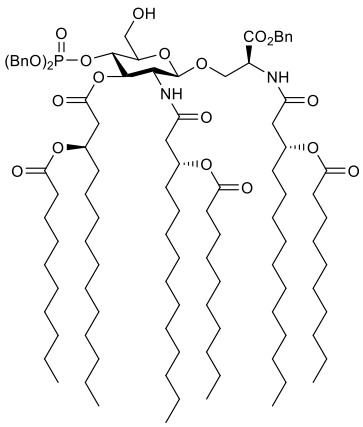
TBDMSCl (290 mg, 1.92 mmol, 1.5 eq.) was added to a solution of compound **26** (1.81 g, 1.21 mmol, 1.0 eq) in pyridine (8.0 mL). After stirring at room temperature for 3 hours, TLC analysis showed complete conversion of the starting material. The reaction mixture was diluted with EtOAc, washed with 1 M HCl (2x), sat. aq. $NaHCO_3$ (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (5→20% EtOAc in toluene) afforded the title compound (1.71 g, 1.06 mmol, 88%). R_f: 0.43 (6/3 toluene/EtOAc); $[\alpha]_D^{25}$ -10.0° (*c* = 0.47, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.34 – 7.23 (m, 5H, Ar), 7.09 (d, 1H, *J* = 7.7 Hz, NH serine), 6.40 (d, 1H, *J* = 8.3 Hz, NH GlcN), 5.19 – 5.04 (m, 5H, 3x CH FA, CH_2 Bn), 5.01 – 4.92 (m, 1H, H-3), 4.73 – 4.64 (m, 1H, CH serine), 4.58 (d, 1H, *J* = 8.0 Hz, H-1), 4.19 (dd, 1H, *J* = 10.8, 3.1 Hz, C*H*H serine), 3.90 – 3.75 (m, 3H, C*H*H serine, CH_2-6), 3.75 – 3.65 (m, 1H, H-2), 3.65 – 3.56 (m, 1H, H-4), 3.53 (d, 1H, *J* = 2.6 Hz, OH), 3.37 – 3.27 (m, 1H, H-5), 2.69 – 2.18 (m, 12H, 6x CH_2 FA), 1.73 – 1.45 (m, 12H, 6x CH_2 FA), 1.45 – 1.02 (m, 90H, 45x CH_2 FA), 0.93 – 0.74 (m, 27H, 6x CH_3 FA, 3x CH_3 TBDMS), 0.03 (s, 6H, 2x CH_3 TBDMS); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.0, 173.7, 173.7, 171.2, 170.3, 170.2 (C=O FA), 169.5 (C=O serine), 135.5 (C_q Ar), 128.5, 128.2, 127.9 (Ar), 101.2 (C-1), 75.7 (C-3), 74.8 (C-5), 71.5, 71.3, 70.8 (CH FA), 70.6 (C-4), 68.4 (CH_2 serine), 67.1 (CH_2 Bn), 63.8 (CH_2-6), 54.0 (C-2), 52.8 (CH serine), 41.9, 41.2, 39.9, 34.6, 34.5, 34.5, 31.9, 31.9, 31.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.2, 29.2 (CH_2 FA), 25.8 (CH_3 TBDMS), 25.4, 25.2, 25.0, 25.0, 22.7, 18.2 (CH_2 FA)), 14.1 (CH_3 FA), -5.4, -5.5 (CH_3 TBDMS); FT-IR (neat, cm^{-1}): 3284, 3094, 2955, 2920, 2852, 1729, 1645, 1538, 1498, 1467, 1419, 1378, 1322, 1250, 1211, 1179, 1139, 1067, 1006, 965, 909, 837, 816, 778, 749, 722, 695, 668, 560, 555, 498, 480, 463; HRMS: $[M+H]^{2+}$ calcd. for $C_{94}H_{171}N_2O_{16}Si$: 806.11962, found 806.34730.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-bis(benzyloxy)phosphoryl-6-*O*-*tert*-butyldimethylsilyl-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (28)



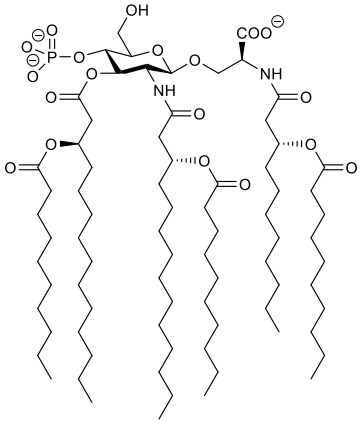
Compound **27** (1.71 g, 1.06 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry DCM (18 mL). Dibenzyl diisopropylaminephosphoramidite (0.70 mL, 1.9 mmol, 1.8 eq.) and tetrazole (186 mg, 2.65 mmol, 2.5 eq.) were added. After stirring for 35 minutes, the reaction mixture was cooled to 0°C, followed by the addition of m-CPBA (0.74 g, 3.0 mmol, 2.8 eq.). After 40 minutes, TLC analysis showed complete conversion into the phosphate. The reaction was diluted with aq. sat. $NaHCO_3$ and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (0→20% EtOAc in toluene) and several size exclusions (DCM/MeOH: 1/1) gave compound **28** in quantitative yield (2.00 g). R_f: 0.77 (2/1 toluene/EtOAc); $[\alpha]_D^{25}$ +3.6° (*c* = 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.39 – 7.23 (m, 15H, Ar), 7.13 (d, 1H, *J* = 8.0 Hz, NH serine), 6.41 (d, 1H, *J* = 7.4 Hz, NH GlcN), 5.32 (dd, 1H, *J* = 10.5, 9.0 Hz, H-3), 5.22 – 5.11 (m, 5H, 3x CH FA, CH_2 CO_2Bn), 4.97 (d, 3H, *J* = 7.8 Hz, H-1, 2x C*H*H dibenzyl phosphate), 4.91 (d, 2H, *J* = 7.7 Hz, 2x C*H*H dibenzyl phosphate), 4.77 – 4.70 (m, 1H, CH serine), 4.37 (q, 1H, *J* = 9.0 Hz, H-4), 4.26 (dd, 1H, *J* = 11.2, 3.2 Hz, C*H*H serine), 3.94 – 3.85 (m, 1H, C*H*H-6), 3.81 (dd, 1H, *J* = 11.2, 2.6 Hz, C*H*H serine), 3.72 (dd, 1H, *J* = 11.9, 5.1 Hz, C*H*H-6), 3.51 – 3.36 (m, 2H, H-2, H-5), 2.68 (dd, 1H, *J* = 14.8, 6.1 Hz, C*H*H FA), 2.59 – 2.46 (m, 2H, 2x C*H*H FA), 2.46 – 2.18 (m, 9H, 1x C*H*H FA, 4x CH_2 FA), 1.74 – 1.43 (m, 12H, 6x CH_2 FA), 1.43 – 1.14 (m, 90H, 45x CH_2 FA), 0.95 – 0.77 (m, 27H, 6x CH_3 FA, 3x CH_3 TBDMS), 0.03 – -0.06 (m, 6H, 2x CH_3 TBDMS); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.9, 173.7, 173.7, 171.0, 170.3, 170.3 (C=O FA), 169.6 (C=O), 135.6 (C_q Ar), 128.7, 128.7, 128.7, 128.6, 128.4, 128.1, 128.1, 128.0 (Ar), 100.4 (C-1), 75.4, 75.4 (C-5), 74.0, 73.9 (C-4), 73.1, 73.0 (C-3), 71.4, 71.1, 70.3 (CH FA), 69.7, 69.6, 69.6, 69.5 (CH_2 dibenzyl phosphate), 69.0 (CH_2 serine), 67.3 (CH_2 CO_2Bn), 62.0 (CH_2-6), 55.9 (C-2), 52.9 (CH serine), 41.7, 41.2, 39.8, 34.8, 34.7, 34.6, 34.6, 32.1, 32.0, 29.9, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4 (CH_2 FA), 25.9 (CH_3 TBDMS), 25.5, 25.4, 25.3, 25.2, 25.2, 25.1, 22.8, 18.4 (CH_2 FA), 14.2 (CH_3 FA), -5.0, -5.2 (CH_3 TBDMS); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -1.64; FT-IR (neat, cm^{-1}): 3306, 2924, 2854, 1735, 1660, 1541, 1499, 1465, 1379, 1251, 1164, 1108, 1013, 903, 837, 779, 733, 696, 600, 502; HRMS: $[M+H]^+$ calcd. for $C_{108}H_{184}N_2O_{19}PSi$: 1872.29947, found 1872.30474.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-bis(benzyloxy)phosphoryl-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (29)



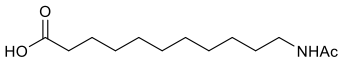
TFA (0.81 mL, 11 mmol, 10 eq.) was added to a solution of phosphate **28** (2.00 g, 1.06 mmol, 1.0 eq.) in DCM (21 mL) at 0°C. After 20 minutes, the resulting yellow solution was allowed to warm-up to room temperature and stirred for an additional 3 hours. TLC analysis showed complete conversion and the reaction was quenched with aq. sat. $NaHCO_3$ at 0°C. The reaction mixture was further diluted with H_2O and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. After purification by column chromatography (10→50% EtOAc in toluene), compound **29** (1.57 g, 0.893 mmol, 84%) was obtained as a white foam. R_f: 0.70 (1/1 pentane/EtOAc); $[\alpha]_D^{25}$ +2.2° (*c* = 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.38 – 7.24 (m, 15H, Ar), 7.19 – 7.08 (m, 1H, NH serine), 6.57 (d, 1H, *J* = 7.7 Hz, NH GlcN), 5.36 (t, 1H, *J* = 9.7 Hz, H-3), 5.23 – 5.07 (m, 5H, 3x CH FA, CH_2 CO_2Bn), 5.05 – 4.88 (m, 5H, H-1, 2x CH_2 dibenzyl phosphate), 4.76 – 4.67 (m, 1H, CH serine), 4.44 (q, 1H, *J* = 9.3 Hz, H-4), 4.28 – 4.18 (m, 1H, C*H*H serine), 3.93 – 3.84 (m, 1H, C*H*H serine), 3.82 – 3.71 (m, 3H, CH_2-6, OH), 3.60 – 3.51 (m, 1H, H-2), 3.37 (d, 1H, *J* = 9.7 Hz, H-5), 2.69 (dd, 1H, *J* = 14.7, 6.1 Hz, C*H*H FA), 2.54 (dd, 1H, *J* = 14.7, 5.8 Hz, C*H*H FA), 2.43 – 2.23 (m, 8H, 4x CH_2 FA), 2.20 (t, 2H, *J* = 7.5 Hz, CH_2 FA), 1.74 – 1.48 (m, 12H, 6x CH_2 FA), 1.48 – 1.06 (m, 90H, 45x CH_2 FA), 0.94 – 0.80 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.5, 173.4, 173.1, 170.6, 170.1, 169.7 (C=O FA), 169.4 (C=O serine), 135.3, 135.2, 135.2, 135.1, 135.1 (C_q Bn), 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8 (Ar), 100.4 (C-1), 74.7, 74.7 (C-5), 73.1, 73.0 (C-4), 72.3, 72.2 (C-3), 71.2, 70.8 (CH FA), 69.9, 69.9 (CH_2 dibenzyl phosphate), 69.8 (CH FA), 68.7 (CH_2 serine), 67.0 (CH_2 CO_2Bn), 60.3 (CH_2-6), 55.1 (C-2), 52.8 (CH serine), 41.4, 41.0, 39.0, 34.4, 34.3, 34.2, 31.8, 31.8, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 25.2, 25.2, 25.0, 24.9, 24.9, 22.6, 22.6 (CH_2 FA), 14.0 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -0.05; FT-IR (neat, cm^{-1}): 3317, 3066, 2956, 2923, 2853, 1733, 1654, 1640, 1541, 1499, 1466, 1456, 1379, 1238, 1166, 1128, 1106, 1080, 1034, 1016, 914, 736, 696, 602, 531, 498; HRMS: $[M+H]^+$ calcd. for $C_{102}H_{170}N_2O_{19}P$: 1758,2130, found 1758.2065.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (1)



After co-evaporating with toluene (3x) under an argon atmosphere, compound **29** (21.7 mg, 12.3 µmol, 1.0 eq.) was dissolved in THF (1.0 mL), followed by the addition of Pd/C (10%, 21 mg). A $H_{2(g)}$-filled balloon was applied on the obtained black suspension. After stirring at room temperature for 3 hours, the mixture was filtered over a Whatmann filter. The filter was washed with DCM, followed by the addition of Et_3N (3.4 µL, 24 µmol, 2.0 eq.). After mixing for 5 minutes, the clear solution was concentrated *in vacuo* and purified by size exclusion (DCM/MeOH: 1/1). Lyophilization gave compound **1** (18.2 mg, 12.2 µmol, 99%) as a white solid. ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC) δ 5.20 – 5.15 (m, 1H, CH FA), 5.14 – 5.07 (m, 3H, H-3, 2x CH FA), 4.54 – 4.49 (m, 2H, H-1, CH serine), 4.19 – 4.08 (m, 2H, H-4, C*H*H serine), 3.88 (d, *J* = 13.1 Hz, 1H, C*H*H-6), 3.84 – 3.78 (m, 1H, C*H*H serine), 3.72 – 3.66 (m, 2H, H-2, C*H*H-6), 3.28 (d, *J* = 9.8 Hz, 1H, H-5), 2.63 – 2.45 (m, 4H, 2x CH_2 FA), 2.40 (dd, *J* = 14.7, 7.3 Hz, 1H, C*H*H FA), 2.29 (dd, *J* = 14.7, 5.7 Hz, 1H, C*H*H FA), 2.28 – 2.20 (m, 6H, 3x CH_2 FA), 1.60 – 1.47 (m, 12H, 6x CH_2 FA), 1.30 – 1.15 (m, 90H, 45x CH_2 FA), 0.85 – 0.81 (m, 18H, 6x CH_3 FA); ^{13}C NMR ($CDCl_3$, 214 MHz, HSQC) δ 173.8, 173.8, 173.7, 170.8, 170.6, 170.5 (C=O), 100.7 (C-1), 75.3 (C-5), 73.5 (C-3), 71.1, 70.8 (CH FA), 70.3 (C-4), 70.1 (CH FA), 69.2 (CH serine), 59.9 (CH_2-6), 54.1 (C-2), 52.6 (CH serine), 41.0, 40.5, 38.8, 34.4, 34.4, 34.2, 34.1, 34.0, 31.8, 31.8, 31.8, 31.8, 29.6, 29.6, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.4, 29.3, 29.3, 29.3, 29.3, 29.2, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 25.2, 25.1, 25.1, 24.9, 24.9, 22.6, 22.5, 22.5 (CH_2 FA), 13.9 (CH_3 FA); ^{31}P NMR ($CDCl_3$, 202 MHz, HMBC) δ 2.40; HRMS: $[M+H]^+$ calcd. for $C_{81}H_{152}N_2O_{19}P$: 1488.0721, found 1488.0725.

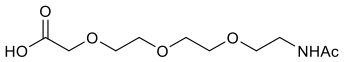
11-Acetamidoundecanoic acid (30)



11-Bromo undecanoic acid (2.87 g, 10.8 mmol, 1.0 eq.) was dissolved in aq. ammonium hydroxide (30%, 80 mL). After stirring overnight, the mixture was concentrated *in vacuo*. Co-evaporation with toluene afforded the amine as a white solid (3.16 g, quant.). The amine (0.61 g, 3.0 mmol, 1.0 eq.) was dissolved in pyridine (4.3 mL) and cooled to 0°C. Ac_2O (0.85 mL, 9.0 mmol, 3.0 eq.) and DMAP (cat.) were added and the solution was allowed to warm-up to room temperature and stirred for 1 hour. The reaction mixture was quenched with H_2O, concentrated *in vacuo* and co-evaporated with toluene (4x). The residue was dissolved in DCM and washed with 1 M HCl. The aqueous layer was extracted with DCM (3x) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crystallization in DCM/pentane yielded the title compound (0.41 g, 1.7 mmol, 56%) as a white solid. R_f: 0.43 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -1.9° (*c* = 0.47, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC):

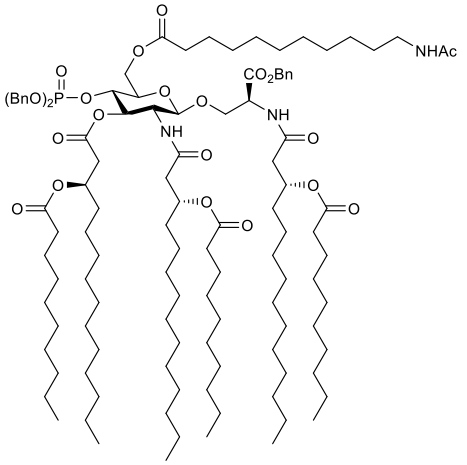
δ 5.65 (s, 1H, NH), 3.23 (q, 2H, *J* = 6.9 Hz, C*H*$_2$NHAc), 2.34 (t, 2H, *J* = 7.5 Hz, CH_2), 1.99 (s, 3H, CH_3 Ac), 1.68 – 1.58 (m, 2H, CH_2), 1.53 – 1.44 (m, 2H, CH_2), 1.35 – 1.23 (m, 12H, 6x CH_2); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 178.5 (C=O), 170.6 (C=O Ac), 39.9 (*C*H_2NHAc, 34.1, 29.6, 29.4, 29.3, 29.2, 29.2, 29.0, 26.9, 24.8 (CH_2), 23.4 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3289, 3086, 2916, 2850, 1695, 1641, 1543, 1470, 1434, 1372, 1298, 1245, 1217, 1192, 927, 721, 610; HRMS: $[M+Na]^+$ calcd. for $C_{13}H_{25}NO_3Na$: 266.1727, found 266.1731.

2-Oxo-6,9,12-trioxa-3-azatetradecan-14-oic acid (31)



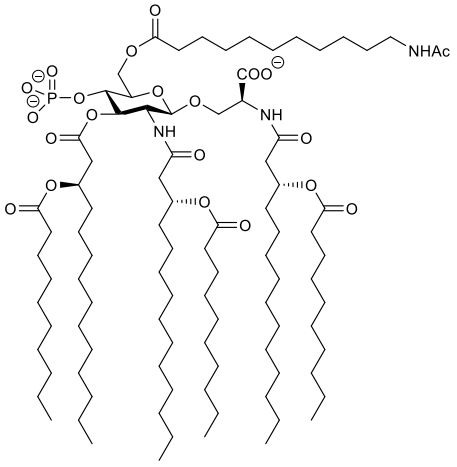
To a solution of *tert*-butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (0.58 g, 2.0 mmol, 1.0 eq.) in THF (15 mL) was added PPh_3 (0.68 g, 2.6 mmol, 1.3 eq.). After stirring for 24 hours, H_2O (0.1 mL, 5.2 mmol, 2.6 eq.) were added and the mixture was continued to stir for two more days. The reaction mixture was diluted with H_2O, washed with toluene (2x) and the aqueous layer was concentrated *in vacuo*. The obtained amine was dissolved in pyridine (3.0 mL), cooled to 0°C, followed by the addition of Ac_2O (1.3 mL, 14 mmol, 7.0 eq.) and DMAP (27 mg, 0.22 mmol, 0.11 eq.). The reaction mixture was allowed to warm-up to room temperature and stirred for 1 hour, after which the reaction was quenched with H_2O at 0°C and concentrated *in vacuo*. This gave acetyl (0.54 g, 1.77 mmol, 89% over two steps) as an oil. The intermediate (0.22 g, 0.70 mmol, 1.0 eq.) was dissolved in DCM (3.5 mL) and cooled to 0°C. TFA (1.1 mL, 14 mmol, 20 eq.) was added and after 30 minutes the mixture was allowed to warm-up to room temperature. R_f: 0.46 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -1.7° (*c* = 0.81, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz, HH-COSY, HSQC): δ 10.98 (s, 1H, OH), 6.80 (s, 1H, NH), 4.09 (s, 2H, CH_2), 3.68 (dd, 2H, *J* = 5.6, 3.1 Hz, CH_2), 3.66 – 3.51 (m, 6H, 3x CH_2), 3.49 (t, 2H, *J* = 5.1 Hz, CH_2), 3.36 (q, 2H, *J* = 5.2 Hz, C*H*$_2$NHAc), 1.95 (s, 3H, CH_3); ^{13}C-APT NMR ($CDCl_3$, 126 MHz, HSQC): δ 172.3, 171.7 (C=O), 70.9, 70.4, 70.3, 69.9, 69.6, 68.5 (CH_2), 39.4 (*C*H_2NHAc), 22.7 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3330, 2874, 1731, 1620, 1552, 1433, 1375, 1351, 1293, 1221, 1099, 939, 882, 723, 678, 560, 543; HRMS: $[M+Na]^+$ calcd. for $C_{10}H_{19}NO_6Na$: 272.1105, found 272.1112.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-[bis(benzyloxy)phosphoryl]-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-6-*O*-(11-acetamidoundecanoyl)-β-D-glucopyranosyl]-L-serinate (32)



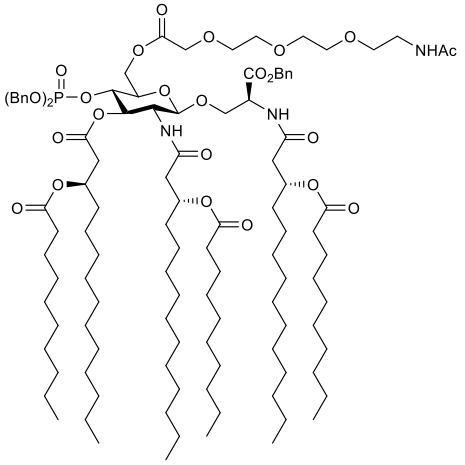
Compound **29** (57.6 mg, 32.8 µmol, 1.0 eq) and acid **30** (20.8 mg, 85.5 µmol, 2.6 eq.) were co-evaporated twice with toluene under an argon atmosphere before being dissolved in dry DCE (0.5 ml). The solution was cooled to 0°C, followed by the addition of EDC·MeI (20.8 mg, 68.6 µmol, 2.1 eq.) and DMAP (5.3 mg, 43 µmol, 1.3 eq.). The obtained yellow suspension was allowed to warm-up to room temperature and was stirred overnight. The white suspension was diluted with aq. sat. $NaHCO_3$ and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10→60% EtOAc in pentane) yielded the title compound (57.4 mg, 28.9 µmol, 88%). R_f: 0.34 (1/1 pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz, HH-COSY, HSQC): δ 7.37 – 7.21 (m, 15H, Ar), 7.10 (d, 1H, *J* = 7.9 Hz, NH serine), 6.44 (d, 1H, *J* = 7.1 Hz, NH GlcN), 5.67 (br, 1H, NHAc), 5.33 (d, 1H, *J* = 9.4 Hz, H-3), 5.22 – 5.10 (m, 5H, 3x CH FA, CH_2 CO_2Bn), 5.00 – 4.93 (m, 3H, H-1, 2x C*H*H dibenzyl phosphate), 4.89 (d, 2H, *J* = 7.7 Hz, 2x C*H*H dibenzyl phosphate), 4.72 (d, 1H, *J* = 7.9 Hz, CH serine), 4.43 – 4.32 (m, 2H, H-4, C*H*H-6), 4.29 – 4.23 (m, 1H, C*H*H serine), 4.11 (dd, 1H, *J* = 12.3, 5.1 Hz, C*H*H-6), 3.82 (d, 1H, *J* = 9.5 Hz, C*H*H serine), 3.57 (dd, 1H, *J* = 8.9, 4.1 Hz, H-5), 3.46 – 3.37 (m, 1H, H-2), 3.25 – 3.17 (m, 2H, CH_2NHAc), 2.67 (dd, 1H, *J* = 14.8, 6.1 Hz, C*H*H FA), 2.57 – 2.46 (m, 2H, 2x C*H*H FA), 2.41 – 2.18 (m, 11H, CH_2 linker, 4x CH_2 FA, C*H*H FA), 1.96 (s, 3H, CH_3 Ac), 1.71 – 1.41 (m, 16H, 2x CH_2 linker, 6x CH_2 FA), 1.39 – 1.11 (m, 102H, 6x CH_2 linker, 45x CH_2 FA), 0.92 – 0.81 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 126 MHz, HSQC): δ 173.8, 173.7, 173.7, 173.5, 171.1, 170.4, 170.2, 170.1 (C=O FA, linker, Ac), 169.5 (C=O serine), 135.5, 135.5, 135.5, 135.5, 135.5 (C_q Ar), 128.8, 128.7, 128.7, 128.6, 128.5, 128.2, 128.1, 128.1 (Ar), 100.5 (C-1), 74.0, 73.9 (C-4), 72.6, 72.6 (C-5), 72.5 (C-3), 71.4, 71.0, 70.4 (CH FA), 69.8, 69.8 (CH_2 dibenzyl phosphate), 69.2 (CH_2 serine), 67.3 (CH_2 CO_2Bn), 62.2 (CH_2-6), 55.8 (C-2), 52.8 (CH serine), 41.7, 41.2 (CH_2 FA), 39.8 (*C*H_2NHAc), 34.7, 34.6, 34.6 (CH_2 FA), 34.0 (CH_2 linker), 32.0, 32.0 (CH_2 FA), 29.9 (CH_2 linker), 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.4, 29.3, 29.3, 29.1 (CH_2 FA), 27.0 (CH_2 linker), 25.5, 25.4, 25.2, 25.2, 25.1 (CH_2 FA), 24.8 (CH_2 linker), 23.5 (CH_3 Ac), 22.8, 22.8 (CH_2 FA), 14.2 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ -1.32; FT-IR (neat, cm^{-1}): 3313, 3066, 2955, 2921, 2852, 1731, 1649, 1541, 1499, 1466, 1456, 1378, 1274, 1212, 1169, 1110, 1013, 906, 796, 732, 696, 603, 527, 501, 460; HRMS: $[M+H]^{2+}$ calcd. for $C_{115}H_{193}N_3O_{21}P$: 992.19657, found 992.19715.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-6-_O_-(11-acetamidoundecanoyl)-β-D-glucopyranosyl]-L-serine (2)



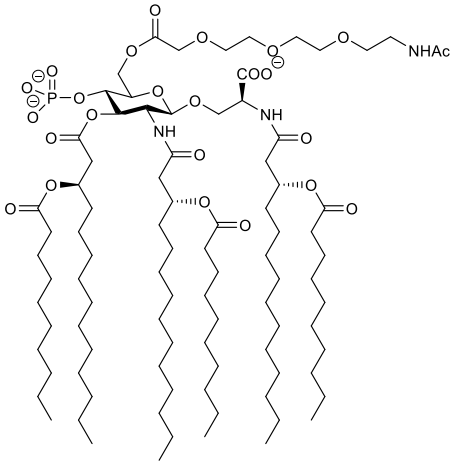
Compound **32** (26.7 mg, 13.5 µmol, 1 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in THF (1.0 mL). Pd/C (10%, 20.8 mg) was added and the reaction mixture was stirred for 2.5 hours at room temperature under a blanket of $H_{2(g)}$. The black suspension was filtered over a Whatmann filter and the filter was washed with $CHCl_3$. Et_3N (4.0 µL, 28.6 µmol, 2.1 eq.) was added to the combined filtrates, mixed for 5 minutes and the solution was concentrated *in vacuo*. After purification by size exclusion (DCM/MeOH: 1/1) and lyophilization, compound **2** (12.0 mg, 7.00 µmol, 52%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 5.21 – 5.15 (m, 2H, CH FA), 5.15 – 5.06 (m, 2H, H-3, CH FA), 4.60 (d, 1H, *J* = 7.0 Hz, H-1), 4.57 – 4.53 (m, 1H, CH serine), 4.49 (d, 1H, *J* = 11.0 Hz, C*H*H-6), 4.22 – 4.07 (m, 3H, H-4, C*H*H-6, C*H*H serine), 3.71 – 3.62 (m, 3H, H-2, H-5, C*H*H serine), 3.11 (t, 2H, *J* = 7.2 Hz, CH_2NHAc), 2.62 – 2.54 (m, 3H, CH_2 FA, C*H*H FA), 2.50 (dd, 1H, *J* = 14.6, 5.8 Hz, C*H*H FA), 2.40 (dd, 1H, *J* = 14.6, 7.3 Hz, C*H*H FA), 2.34 – 2.21 (m, 9H, C*H*H FA, 3x CH_2 FA, CH_2 linker), 1.90 (s, 3H, CH_3 Ac), 1.62 – 1.48 (m, 14H, 6x CH_2 FA, CH_2 linker), 1.46 – 1.41 (m, 2H, CH_2 linker), 1.33 – 1.15 (m, 102H, 45x CH_2 FA, 6x CH_2 linker), 0.83 (t, 18H, *J* = 6.9 Hz, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 151 MHz, HSQC): δ 174.5, 174.2, 174.0, 171.7, 171.0, 170.9, 170.7 (C=O), 100.1 (C-1), 73.5 (C-3), 73.3, 73.3 (C-5), 71.8, 71.7 (C-4), 71.3, 71.2, 70.5 (CH FA), 68.7 (CH_2 serine), 63.9 (CH_2-6), 54.3 (C-2), 52.6 (CH serine), 41.2, 40.7 (CH_2 FA), 39.8 (*C*H_2NHAc), 39.3, 34.7, 34.6, 34.6, 34.5, 34.5, 34.4, 34.2, 32.1, 32.1, 32.1, 32.0, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 27.0, 25.5, 25.4, 25.4, 25.2, 25.2, 25.0, 22.8, 22.8 (CH_2 FA, CH_2 linker), 22.6 (CH_3 Ac), 14.2 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 0.59; HRMS: $[M+H]^+$ calcd. for $C_{94}H_{175}N_3O_{21}P$: 1713.2450, found 1713.2458.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-[bis(benzyloxy)phosphoryl]-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-6-*O*-(13-acetamido-3-oxo-2,5,8,11-tetraoxatridecyl)-β-D-glucopyranosyl]-L-serinate (33)



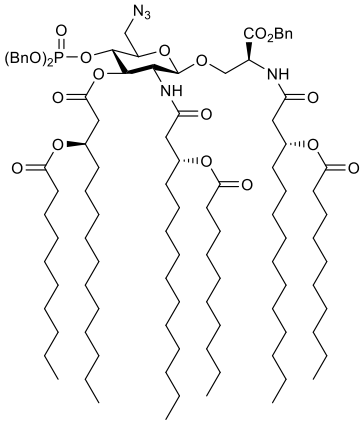
Compound **29** (49.6 mg, 28.2 µmol, 1.0 eq) and acid **31** (22.5 mg, 90.3 µmol, 3.2 eq.) were co-evaporated twice with toluene under an argon atmosphere before being dissolved in dry DCE (0.43 ml). The solution was cooled to 0°C, followed by the addition of EDC·MeI (17.7 mg, 59.6 µmol, 2.1 eq.) and DMAP (2.2 mg, 18 µmol, 0.6 eq.). The obtained yellow suspension was allowed to warm-up to room temperature and was stirred overnight. The resulting white suspension was diluted DCM (0.6 mL) and stirred for an additional 4 hours. The reaction mixture was subsequently diluted with aq. sat. $NaHCO_3$ and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10→30% acetone in DCM) yielded the title compound (41.3 mg, 20.8 µmol, 74%). R_f: 0.65 (6/4 DCM/acetone); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.40 – 7.21 (m, 15H, Ar), 7.10 (d, 1H, *J* = 7.9 Hz, NH serine), 6.46 (d, 1H, *J* = 7.4 Hz, NH GlcN), 6.30 (br, 1H, NHAc), 5.33 (dd, 1H, *J* = 10.3, 9.0 Hz, H-3), 5.22 – 5.09 (m, 5H, 3x CH FA, CH_2 CO_2 Bn), 4.99 (d, 2H, *J* = 8.1 Hz), H-1, 4.95 (d, 3H, *J* = 8.6 Hz, 2x C*H*H dibenzyl phosphate), 4.88 (d, 2H, *J* = 7.8 Hz, 2x C*H*H dibenzyl phosphate), 4.76 – 4.68 (m, 1H, CH serine), 4.44 – 4.31 (m, 2H, H-4, C*H*H-6), 4.29 – 4.18 (m, 2H, C*H*H-6, C*H*H serine), 4.12 (s, 2H, CH_2 linker), 3.83 (dd, 1H, *J* = 11.3, 2.6 Hz, C*H*H serine), 3.70 – 3.57 (m, 9H, H-5, 4x CH_2 linker), 3.53 (t, 2H, *J* = 5.0 Hz, CH_2 linker), 3.47 – 3.37 (m, 3H, H-2, CH_2NHAc), 2.68 (dd, 1H, *J* = 14.9, 5.9 Hz, C*H*H FA), 2.57 – 2.42 (m, 2H, 2x C*H*H FA), 2.40 – 2.18 (m, 9H, C*H*H FA, 4x CH_2 FA), 1.96 (s, 3H, CH_3 Ac), 1.71 – 1.50 (m, 12H, 6x CH_2 FA), 1.50 – 1.15 (m, 90H, 45x CH_2 FA), 0.90 – 0.83 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.9, 173.9, 173.7, 171.1, 170.4, 170.2, 170.0 (C=O FA, linker, Ac), 169.5 (C=O serine), 135.5, 135.5, 135.4, 135.4, 135.4 (C_q Ar), 128.9, 128.8, 128.8, 128.7, 128.7, 128.5, 128.2, 128.2 (Ar), 100.6 (C-1), 73.7, 73.7 (C-4), 72.4, 72.4, 72.3 (C-3, C-5), 71.5, 71.1 (CH FA), 70.9, 70.6, 70.6 (CH_2 linker), 70.3 (CH FA), 70.3, 69.9 (CH_2 linker), 69.9, 69.9, 69.8, 69.8 (CH_2 dibenzyl phosphate), 69.2 (CH_2 serine), 68.4 (CH_2 linker), 67.4 (CH_2 CO_2Bn), 62.5 (CH_2-6), 55.7 (C-2), 52.9 (CH serine), 41.7, 41.3, 39.7 (CH_2 FA), 39.4 (*C*H_2NHAc), 34.8, 34.8, 34.7, 34.6, 34.6, 32.1, 32.0, 29.9, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 25.5, 25.4, 25.3, 25.2, 25.1, 23.3, 22.8, 22.8 (CH_2 FA), 14.2 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -1.15; FT-IR (neat, cm^{-1}): 3301, 2923, 2853, 1734, 1654, 1541, 1457, 1378, 1265, 1152, 1111, 1012, 905, 736, 697, 601, 501; HRMS: $[M+H]^+$ calcd. for $C_{112}H_{181}N_3O_{24}P$: 1989.32367, found 1989.32201.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-6-_O_-(13-acetamido-3-oxo-2,5,8,11-tetraoxatridecyl)-β-D-glucopyranosyl]-L-serine (3)



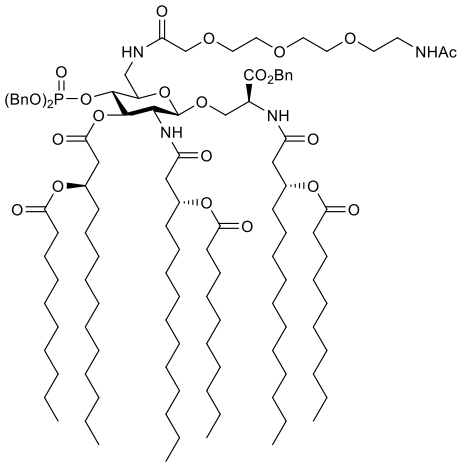
Compound **31** (19.7 mg, 9.90 µmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in THF (1.0 mL). Pd/C (10%, 19.8 mg) was added and the reaction mixture was stirred for 3 hours at room temperature under a blanket of $H_{2(g)}$. The black suspension was filtered over a Whatmann filter. The filter was washed with $CHCl_3$ and Et_3N (3.0 µL, 22 µmol, 2.2 eq.) was added to the combined filtrates. The solution was mixed for 5 minutes and concentrated *in vacuo*. After purification by size exclusion (DCM/MeOH: 1/1) and lyophilization, compound **3** (6.7 mg, 3.9 µmol, 39%) was obtained as a white solid. ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 5.22 – 5.15 (m, 2H, 2x CH FA), 5.15 – 5.04 (m, 2H, H-3, CH FA), 4.60 – 4.47 (m, 3H, H-1, CH serine, C*H*H-6), 4.33 – 4.12 (m, 5H, H-4, C*H*H-6, C*H*H serine, CH_2 linker), 3.78 – 3.71 (m, 3H, H-2, C*H*H serine, C*H*H linker), 3.69 – 3.53 (m, 10H, H-5, 4x CH_2 linker, C*H*H linker), 3.44 –3.32 (m, 2H, C*H*$_2$NHAc), 2.68 – 2.46 (m, 4H, 2x CH_2 FA), 2.41 (dd, 1H, *J* = 14.5, 7.2 Hz, C*H*H FA), 2.30 (dd, 1H, *J* = 14.5, 5.7 Hz, C*H*H FA), 2.28 – 2.19 (m, 6H, 3x CH_2 FA), 1.95 (s, 3H, CH_3 Ac), 1.63 – 1.46 (m, 12H, 6x CH_2 FA), 1.33 – 1.15 (m, 90H, 45x CH_2), 0.83 (t, 18H, *J* = 7.0 Hz, 6x CH_3); ^{13}C-APT NMR ($CDCl_3$, 151 MHz, HSQC): δ 174.1, 174.0, 173.8, 172.6, 171.2, 170.8, 170.7 (C=O), 100.6 (C-1), 73.9 (C-3), 72.7, 72.7 (C-5), 71.3 (CH FA), 71.1 (C-4, CH FA), 70.4 (CH_2 linker), 70.3 (CH FA), 70.1, 69.9 (CH_2 linker), 69.4 (CH_2 serine, CH_2 linker), 68.5 (CH_2 linker), 62.5 (CH_2-6), 53.8 (C-2), 52.4 (CH serine), 41.4, 40.7, 39.3 (CH_2 FA), 39.1 (*C*H_2NHAc), 34.7, 34.6, 34.6, 34.5, 34.5, 34.3, 32.1, 32.1, 32.0, 32.0, 29.9, 29.9, 29.9, 29.9, 29.8, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 25.5, 25.4, 25.3, 25.2, 25.2, 25.2, 22.8, 22.8 (CH_2 FA), 22.6 (CH_3 Ac), 14.1 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 1.52; HRMS: $[M+H]^+$ calcd. for $C_{91}H_{169}N_3O_{24}P$: 1719.18282, found 1719.18284.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[6-azide-4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (34)



After co-evaporating with toluene (2x), compound **29** (82 mg, 47 µmol, 1.0 eq.) was dissolved in THF under an argon atmosphere. PPh_3 (48 mg, 0.18 mmol, 3.9 eq.) was added and the reaction mixture was cooled to -20 °C. DEAD (15 µL, 96 µmol, 2.0 eq.) and DPPA (20.5 µL, 96 µmol, 2.0 eq.) were added subsequently and the stirring was continued for 1 hour, followed by the addition of DEAD (15 µL, 96 µmol, 2.0 eq.) and DPPA (20.5 µL, 96 µmol, 2.0 eq.). After stirring for 1 hour at -20°C, the reaction mixture was slowly warmed-up to room temperature overnight. The mixture was concentrated *in vacuo*. Purification by column chromatography (10→30% EtOAc in pentane) afforded the title compound (56 mg, 31 µmol, 66%). R_f: 0.52 (7/3 pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.38 – 7.24 (m, 15H, Ar), 7.02 (d, 1H, *J* = 8.1 Hz, NH serine), 6.44 (d, 1H, *J* = 7.4 Hz, NH GlcN), 5.34 (dd, 1H, *J* = 10.5, 8.9 Hz, H-3), 5.21 – 5.12 (m, 5H, 3x CH FA, CH_2 CO_2Bn), 5.03 (d, 1H, *J* = 8.2 Hz, H-1), 5.00 – 4.94 (m, 2H, 2x C*H*H Bn), 4.91 (dd, 2H, *J* = 7.9, 3.2 Hz, 2x C*H*H Bn), 4.78 – 4.70 (m, 1H, CH serine), 4.32 – 4.21 (m, 2H, H-4, C*H*H serine), 3.85 (dd, 1H, *J* = 11.2, 2.8 Hz, C*H*H serine), 3.55 – 3.48 (m, 1H, H-5), 3.46 – 3.37 (m, 2H, H-2, C*H*H-6), 3.28 (dd, 1H, *J* = 13.4, 6.2 Hz, C*H*H-6), 2.67 (dd, 1H, *J* = 14.9, 6.2 Hz, C*H*H FA), 2.55 – 2.44 (m, 2H, 2x C*H*H FA), 2.41 – 2.19 (m, 9H, C*H*H FA, 4x CH_2 FA), 1.71 – 1.43 (m, 12H, 6x CH_2 FA), 1.37 – 1.14 (m, 90H, 45x CH_2 FA), 0.93 – 0.81 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.8, 173.7, 171.1, 170.3, 170.0 (C=O FA), 169.5 (C=O serine), 135.5, 135.5, 135.4 (C_q Bn), 128.9, 128.8, 128.8, 128.7, 128.6, 128.4, 128.3, 128.3, 128.2 (Ar), 100.3 (C-1), 74.5, 74.5 (C-5), 74.0, 73.9 (C-4), 72.3, 72.3 (C-3), 71.4, 71.0, 70.3 (CH FA), 70.0, 70.0, 69.9, 69.9 (CH_2 dibenzyl phosphate), 69.2 (CH_2 serine), 67.3 (CH_2 CO_2Bn), 55.8 (C-2), 52.8 (CH serine), 50.7 (CH_2N_3), 41.6, 41.2, 39.8, 34.7, 34.6, 34.6, 34.6, 32.0, 32.0, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 25.5, 25.4, 25.3, 25.2, 25.2, 25.1, 22.8, 22.8 (CH_2 FA), 14.2 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -1.03; FT-IR (neat, cm^{-1}): 3276, 2992, 2853, 2361, 2102, 1733, 1654, 1543, 1498, 1457, 1274, 1248, 1171, 1108, 1010, 904, 734, 696, 601, 506; HRMS: $[M+H]^+$ calcd. for $C_{102}H_{169}N_5O_{18}P$: 1783.22059, found 1783.22059.

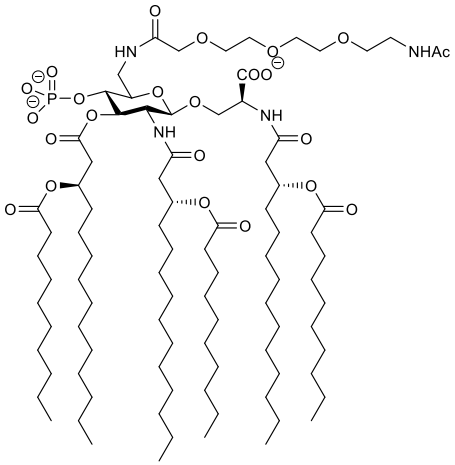
Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-[bis(benzyloxy)phosphoryl]-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-6-*N*-(13-acetamido-3-oxo-5,8,11-trioxa-2-azatridecyl)-β-D-glucopyranosyl]-L-serinate (35)



Compound **34** (23.6 mg, 13.2 µmol, 1.0 eq.) was dissolved in a mixture of DCM/MeOH/H_2O (1,1,0.1 v/v/v, 1.2 mL). Activated zinc powder (9.1 mg, 0.15 mmol, 11.6 eq.) and NH_4Cl (7.9 mg, 0.15 mmol, 11.2 eq.) were added and the reaction mixture was stirred for 6 hours. The reaction mixture was subsequently diluted with DCM and washed with aq. sat. $NaHCO_3$ (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The obtained amine (13.2 µmol, 1.0 eq.) and acid **31** (10.6 mg, 42.5 µmol, 3.2 eq.) were co-evaporated with toluene (2x) under an argon atmosphere before being dissolved in dry DCE (0.4 ml). The solution was cooled to 0°C, followed by the addition of EDC·MeI (8.5 mg, 29 µmol, 2.2 eq.) and DMAP (0.7 mg, 6 µmol, 0.4 eq.). The resulting yellow suspension was allowed to warm-up to room temperature and was stirred overnight. The obtained white suspension was diluted with aq. sat. $NaHCO_3$ and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10→40% acetone in DCM) and size exclusion (DCM/MeOH: 1/1) afforded the title compound (10.6 mg, 5.33 µmol, 40% over two steps). R_f: 0.44 (6/4 DCM/acetone); ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 7.41 – 7.37 (m, 1H, NH-6), 7.37 – 7.23 (m, 16H, NH serine, Ar), 6.60 (t, 1H, *J* = 4.9 Hz, NHAc), 6.41 (d, 1H, *J* = 7.8 Hz, NH GlcN), 5.26 – 5.09 (m, 6H, H-3, 3x CH-FA, CH_2 CO_2Bn), 5.02 (d, 2H, *J* = 8.1 Hz, 2x C*H*H dibenzyl phosphate), 4.98 – 4.89 (m, 2H, 2x C*H*H dibenzyl phosphate), 4.82 (d, 1H, *J* = 8.2 Hz, H-1), 4.77 – 4.72 (m, 1H, CH serine), 4.26 – 4.13 (m, 2H, H-4, C*H*H serine), 4.07 – 3.97 (m, 3H, C*H*H-6, CH_2 linker), 3.85 (dd, 1H, *J* = 11.2, 3.1 Hz, C*H*H serine), 3.69 – 3.47 (m, 11H, H-2, 5x CH_2 linker), 3.42 – 3.35 (m, 3H, H-5, CH_2NHAc), 3.16 – 3.10 (m, 1H, C*H*H-6), 2.68 (dd, 1H, *J* = 14.8, 6.1 Hz, C*H*H FA), 2.56 (dd, 1H, *J* = 14.8, 6.1 Hz, C*H*H FA), 2.40 – 2.19 (m, 10H, 5x CH_2 FA), 1.94 (s, 3H, Ac), 1.79 – 1.49 (m, 12H, 6x CH_2 FA), 1.48 – 1.10 (m, 90H, 45x CH_2 FA), 0.90 – 0.85 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 151 MHz, HSQC): δ 173.9, 173.8, 173.7, 170.9, 170.6, 170.5, 170.5, 170.2 (C=O FA, linker), 169.7 (C=O serine), 135.6, 135.5, 35.5, 135.5, 135.5 (C_q Ar), 128.9, 128.8, 128.8, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2 (Ar), 100.6 (C-1), 75.1, 75.1 (C-4), 73.2, 73.2 (C-5), 72.4, 72.4 (C-3), 71.5, 71.2 (CH FA), 70.9, 70.8, 70.6, 70.4 (CH_2 linker), 70.3 (CH FA), 70.2 (CH_2 linker), 70.0, 70.0, 70.0 (CH_2 dibenzyl phosphate), 69.1 (CH_2 serine), 67.4 (CH_2 CO_2Bn), 55.4 (C-2), 52.9 (CH serine), 41.8, 41.1, 39.6, 39.4 (CH_2 FA), 39.3 (CH_2-6), 34.8, 34.8, 34.6, 34.6, 32.1, 32.0, 32.0, 29.9, 29.9, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 25.5, 25.4, 25.3, 25.2, 25.2, 25.2 (CH_2 FA), 23.2 (CH_3 Ac), 22.8, 22.8 (CH_2 FA), 14.3 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -0.81; FT-IR (neat, cm^{-1}): 2924, 2854,

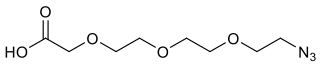
2016, 1734, 1663, 1547, 1466, 1163, 1016, 743, 698, 604, 595; HRMS: $[M+H]^{2+}$ calcd. for $C_{112}H_{188}N_4O_{23}P$: 994.1699, found 994.1779.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-6-_N_-(13-acetamido-3-oxo-5,8,11-trioxa-2-azatridecyl)-β-D-glucopyranosyl]-L-serine (4)



Compound **35** (10.1 mg, 5.08 µmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in THF (1.0 mL). Pd/C (10%, 21 mg) was added and the reaction mixture was stirred for 3.5 hours at room temperature under a blanket of $H_{2(g)}$. The black suspension was filtered over a Whatmann filter and the filter was washed with $CHCl_3$. Et_3N (1.5 µL, 10.8 µmol, 2.1 eq.) was added to the combined filtrates. The solution was mixed for 5 minutes and concentrated *in vacuo*. After purification by size exclusion (DCM/MeOH: 1/1) and lyophilization, the title compound (5.8 mg, 3.4 µmol, 67%) was obtained as a white solid; ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC) δ 5.11 – 5.06 (m, 1H, CH FA), 5.07 – 5.03 (m, 1H, CH FA), 5.03 – 4.97 (m, 1H, CH FA), 4.97 – 4.93 (m, 1H, H-3), 4.45 (s, 1H, CH serine), 4.39 (d, *J* = 7.6 Hz, 1H, H-1), 4.02 (d, *J* = 9.9 Hz, 1H, C*H*H serine), 4.00 – 3.87 (m, 3H, H-4, CH_2 linker), 3.84 – 3.78 (m, 1H, C*H*H-6), 3.69 (d, *J* = 8.3 Hz, 1H, C*H*H serine), 3.64 – 3.47 (m, 9H, H-2, 4x CH_2 linker), 3.45 – 3.39 (m, 2H, CH_2 linker), 3.36 (s, 1H, H-5), 3.27 – 3.24 (m, 2H, CH_2NHAc), 3.23 – 3.20 (m, 1H, C*H*H-6), 2.54 – 2.49 (m, 1H, C*H*H FA), 2.49 – 2.42 (m, 2H, 2x C*H*H FA), 2.42 – 2.38 (m, 1H, C*H*H FA), 2.29 (dd, *J* = 14.6, 7.2 Hz, 1H, C*H*H FA), 2.20 (dd, *J* = 14.6, 5.6 Hz, 1H, C*H*H FA), 2.18 – 2.09 (m, 6H, 3x CH_2 FA), 1.84 (s, 3H, CH_3 Ac), 1.51 – 1.37 (m, 12H, 6x CH_2 FA), 1.21 – 1.06 (m, 90H, 45x CH_2 FA), 0.74 (t, *J* = 7.2 Hz, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 214 MHz, HSQC): δ 173.7, 173.7, 170.9, 170.7 (C=O), 100.6 (C-1), 73.2, 73.2 (C-3/4/5), 71.1, 71.0 (CH FA), 70.9, 70.9 (CH FA), 70.6 (CH_2 linker), 70.1, 70.1 (CH FA), 69.9, 69.9, 69.8, 69.7 (CH_2 linker), 69.1 (CH_2 serine), 53.8 (C-2), 52.6 (CH serine), 41.1, 40.4 (CH_2 FA), 39.0 (*C*H_2NHAc), 39.0 (CH_2-6), 34.4, 34.4, 34.3, 34.2, 34.1, 31.8, 31.8, 29.6, 29.6, 29.6, 29.5, 29.4, 29.4, 29.4, 29.3, 29.2, 29.2, 29.1, 29.1, 25.2, 25.1, 25.1, 24.9, 22.5 (CH_2 FA), 13.9 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 1.19; HRMS: $[M+H]^+$ calcd. for $C_{91}H_{170}N_4O_{23}P$: 1718.19880, found 1718.19982.

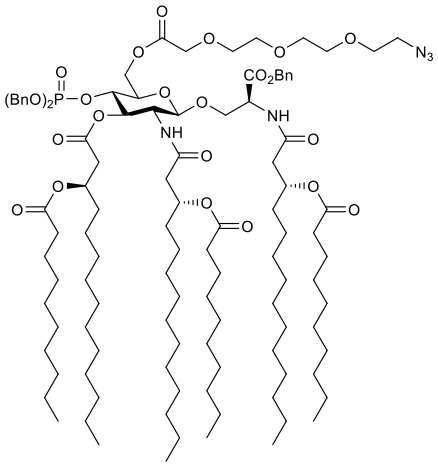
2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)acetic acid (36)



A mixture of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (4.39 g, 26.1 mmol, 1.0 eq.) and sodium azide (3.39 g, 52.1, 2.0 eq.) in DMF (35 mL) was heated to 90°C and stirred overnight. The suspension was cooled down to room temperature and concentrated *in vacuo*. The residue was dissolved in H_2O and extracted with EtOAc (3x).

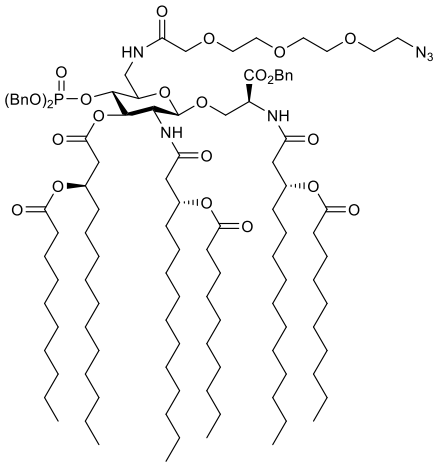
The combined organic layers were washed with brine (1x), dried over $MgSO_4$, filtered and concentred *in vacuo*, which yielded the azide in quantitative yield. After co-evaporation with toluene (2x) under an argon atmosphere, the oil was dissolved in THF (0.13 L) and cooled to 0°C. NaH (2.1 g, 52 mmol, 2.0 eq.) was added under an argon flow. The reaction mixture was stirred for 30 minutes, followed by the addition of *tert*-butyl bromoacetate (9.6 mL, 66 mmol, 2.5 eq.). After the reaction mixture was stirred at room temperature overnight, the reaction was quenched with MeOH and concentrated *in vacuo*. The residue was dissolved in DCM, filtered over celite and concentrated *in vacuo*. Purification by column chromatography (20→50% EtOAc in pentane) yielded methyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (3.90 g, 15.8 mmol, 61% over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.07 (s, 2H, CH_2), 3.68 – 3.53 (m, 13H, 5x CH_2, CH_3), 3.29 (t, 2H, *J* = 4.8 Hz, CH_2N_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 170.7 (C=O), 70.7, 70.5, 70.5, 69.9, 68.4 (CH_2), 51.6 (CH_3), 50.5 (CH_2N_3). Methyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (3.90 g, 15.8 mmol, 1.0 eq.) was dissolved in a mixture of THF/MeOH/H_2O (7/2/1 v/v/v, 50 mL). LiOH (0.97 g, 41 mmol, 2.6 eq.) was added and the suspension was heated to 50°C for 2 hours. The reaction mixture was cooled to room temperature, acidified with 1 M HCl to pH = 2/3, diluted with H_2O and extracted several times with EtOAc and DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Co-evaporation with toluene (1x) and purification by column chromatography (50→100% EtOAc in pentane, then 20% MeOH in EtOAc) yielded the title compound (3.90 g, 15.8 mmol, 61%). R_f: 0.72 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -0.29° (*c* = 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY, HSQC): δ 10.67 (br, 1H, OH), 4.15 (s, 2H, CH_2), 3.77 – 3.57 (m, 10H, 5x CH_2), 3.40 – 3.31 (m, 2H, CH_2N_3); ^{13}C-APT NMR ($CDCl_3$, 75 MHz, HSQC): δ 174.0 (C=O), 71.2, 70.6, 70.6, 70.4, 70.4, 70.0, 68.5 (CH_2), 50.6 (CH_2N_3); FT-IR (neat, cm^{-1}): 2873, 2102, 1744, 1286, 1120, 935, 855; HRMS: $[M+Na]^+$ calcd. for $C_8H_{15}N_3O_5Na$: 256,09039, found 256.09016.

Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[6-*O*-(11-azido-3,6,9-trioxaundecanoyl)-4-*O*-bis(benzyloxy)phosphoryl-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serinate (37)



A solution of alcohol **29** (0.57 g, 0.32 mmol, 1.0 eq.) and acid **36** (0.19 g, 0.81 mmol, 2.5 eq.) was cooled to 0 °C under an argon atmosphere. EDC·MeI (0.20 g, 0.67 mmol, 2.0 eq.) and DMAP (2.0 mg, 16 µmol, 0.05 eq.) were added. After stirring for 15 minutes, the reaction mixture was allowed to warm-up to room temperature and stirring continued for 3 hours. Silica was added and the suspension was concentrated *in vacuo*. Purification by column chromatography (20→50% EtOAc in Toluene + 0.1% Et_3N) gave the title compound (0.51 g, 0.26 mmol, 80%). R_f: 0.68 (1/1 pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.36 – 7.17 (m, 15H, Ar), 7.09 (d, 1H, *J* = 7.9 Hz, NH serine), 6.45 (d, 1H, *J* = 7.4 Hz, NH GlcN), 5.36 – 5.27 (m, 1H, H-3), 5.20 – 5.06 (m, 5H, 3x CH FA, CH_2 CO_2Bn), 4.97 (d, 1H, *J* = 8.2 Hz, H-1), 4.92 (d, 2H, *J* = 8.7 Hz, 2x C*H*H dibenzyl phosphate), 4.85 (d, 2H, *J* = 7.8 Hz, 2x C*H*H dibenzyl phosphate), 4.73 – 4.66 (m, 1H, CH serine), 4.41 – 4.29 (m, 2H, H-4, C*H*H-6), 4.26 – 4.16 (m, 2H, C*H*H-6, C*H*H serine), 4.09 (s, 2H, CH_2 linker), 3.85 – 3.78 (m, 1H, C*H*H serine), 3.68 – 3.54 (m, 13H, H-5, 5x CH_2 linker), 3.44 – 3.36 (m, 1H, H-2), 3.33 – 3.28 (m, 2H, CH_2N_3), 2.66 (dd, 1H, *J* = 14.9, 6.0 Hz, C*H*H FA), 2.55 – 2.42 (m, 2H, 2x C*H*H FA), 2.38 – 2.17 (m, 9H, CHH FA, 4x CH_2 FA), 1.69 – 1.39 (m, 12H, 6x CH_2 FA), 1.37 – 1.10 (m, 90H, 45x CH_2 FA), 0.90 – 0.78 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.7, 173.6, 173.4, 170.9, 170.2, 170.0, 169.8 (C=O FA, C=O linker), 169.3 (C=O serine), 135.4, 135.3, 135.3, 135.2, 135.2 (C_q Ar), 128.6, 128.6, 128.5, 128.4, 128.3, 128.0, 128.0 (Ar), 100.4 (C-1), 73.5, 73.5 (C-4), 72.2, 72.2, 72.1 (C-3, C-5), 71.3, 70.8 (CH FA), 70.7, 70.5 (CH_2 linker), 70.1 (CH FA), 69.9 (CH_2 linker), 69.7, 69.6, 69.6 (CH_2 dibenzyl phosphate), 69.0 (CH_2 serine), 68.2 (CH_2 linker), 67.1 (CH_2 CO_2Bn), 62.2 (CH_2-6), 55.5 (C-2), 52.7 (CH serine), 50.5 (CH_2N_3), 41.5, 41.1, 39.6, 34.5, 34.4, 34.4, 31.9, 31.8, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.2, 29.1, 25.3, 25.2, 25.0, 25.0, 24.9, 22.6, 22.6 (CH_2 Fa), 14.0 (CH_3 Fa); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ -1.34; FT-IR (neat, cm^{-1}): 3312, 2923, 2854, 2103, 1734, 1658, 1538, 1457, 1378, 1273, 1154, 1114, 1014, 736, 697, 601, 498; HRMS: $[M+H]^+$ calcd. for $C_{110}H_{183}N_5O_{23}P$: 1973.3036, found 1973.30465.

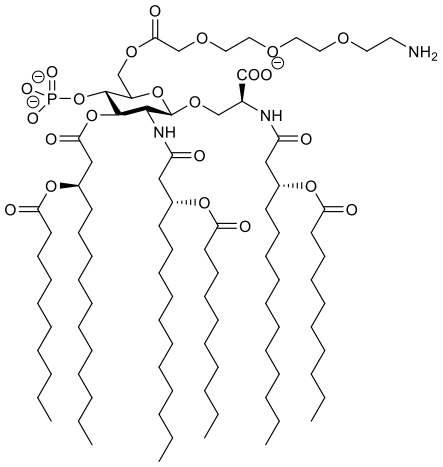
Benzyl *N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-*O*-[4-*O*-[bis(benzyloxy)phosphoryl]-2-*N*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-3-*O*-[(*R*)-3-(decanoyloxy)tetradecanoyl]-6-*N*-(13-acetamido-3-oxo-5,8,11-trioxa-2-azatridecyl)-β-D-glucopyranosyl]-L-serinate (38)



Azide **34** (76 mg, 43 µmol, 1.0 eq.) was dissolved in a mixture of DCM/MeOH/H_2O (1/1/0.1 v/v/v, 3.8 mL). Freshly prepared activated zinc powder (30 mg, 0.47 mmol, 11 eq.) and NH_4Cl (23.1 mg, 0.43 mmol, 11 eq.) were added and the suspension was stirred vigorously for 5.5 hours. The reaction mixture was subsequently diluted with DCM and washed with sat. aq. $NaHCO_3$ (1x). The aqueous layer was extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The obtained amine and acid **36** (27 mg, 0.12 mmol, 2.8 eq.) were dissolved in DCE (1.3 mL) under an argon atmosphere and cooled to 0°C. EDC·MeI (26 mg, 87 µmol, 2.0 eq.) and DMAP (2.8 mg, 23 µmol, 0.53 eq.) were added and the reaction mixture was allowed to warm-up to room temperature overnight. The white suspension was diluted with DCM and washed with sat. aq. $NaHCO_3$ (1x). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (30→70% EtOAc in pentane + 0.1% Et_3N) and size exclusion (DCM/MeOH: 1/1) afforded compound **38** (48 mg, 24 µmol, 56% over two steps). R_f: 0.63 (9/1 DCM/aceton). ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC): δ 7.39 – 7.36 (m, 1H, NH-6), 7.36 – 7.26 (m, 16H, NH serine, Ar), 6.36 (d, 1H, *J* = 7.8 Hz, NH GlcN), 5.23 – 5.19 (m, 2H, H-3, C*H*H CO_2Bn), 5.19 – 5.16 (m, 1H, CH FA), 5.15 – 5.10 (m, 3H, 2x CH FA, C*H*H CO_2Bn), 5.02 (d, 2H, *J* = 8.1 Hz, 2x C*H*H dibenzyl phosphate), 4.96 (dd, 1H, *J* = 11.6, 8.4 Hz, C*H*H dibenzyl phosphate), 4.91 (dd, 1H, *J* = 11.6, 7.2 Hz, C*H*H dibenzyl phosphate), 4.81 (d, 1H, *J* = 8.2 Hz, H-1), 4.76 – 4.73 (m, 1H, CH serine), 4.23 (dd, 1H, *J* = 11.4, 2.9 Hz, C*H*H serine), 4.16 (q, 1H, *J* = 9.2 Hz, H-4), 4.01 (d, 1H, *J* = 15.6 Hz, C*H*H linker), 3.99 – 3.96 (m, 1H, C*H*H-6), 3.95 (d, 1H, *J* = 15.5 Hz, C*H*H linker), 3.86 (dd, 1H, *J* = 11.3, 3.3 Hz, C*H*H serine), 3.68 – 3.57 (m, 10H, 5x CH_2 linker), 3.52 – 3.47 (m, 1H, H-2), 3.33 – 3.30 (m, 3H, H-5, CH_2N_3), 3.12 – 3.08 (m, 1H, C*H*H-6), 2.68 (dd, 1H, *J* = 14.8, 6.3 Hz, C*H*H FA), 2.57 (dd, 1H, *J* = 14.7, 6.1 Hz, C*H*H FA), 2.38 – 2.25 (m, 8H, 4x CH_2 FA), 2.21 (t, 2H, *J* = 7.6 Hz, CH_2 FA), 1.68 –1.37 (m, 12H, 6x CH_2 FA), 1.37 – 1.14 (m, 90H, 45x CH_2 FA), 0.90 – 0.83 (m, 18H, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 214 MHz, HSQC): δ 173.9, 173.8, 173.6, 170.8, 170.5, 170.3, 170.1 (C=O FA, linker), 169.7 (C=O serine), 135.6, 135.5, 135.5, 135.5, 135.5 (C_q Ar), 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.4, 128.3, 128.2 (Ar), 100.5 (C-1), 74.9, 74.9 (C-4), 73.2, 73.2 (C-5), 72.3, 72.3 (C-3), 71.5, 71.1 (CH FA), 71.1, 70.7, 70.7, 70.5 (CH_2 linker), 70.2 (CH FA), 70.1 (CH_2 linker), 70.0, 69.9 (CH_2 dibenzyl phosphate), 69.2 (CH_2 serine), 67.3 (CH_2 CO_2Bn), 55.4 (C-2), 52.9 (CH serine), 50.7 (CH_2N_3), 41.8, 41.0, 39.5 (CH_2 FA), 39.1 (CH_2-6), 34.7, 34.7, 34.6, 34.6, 34.6, 32.1, 32.0, 32.0, 29.9, 29.8, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 25.5, 25.4, 25.3, 25.2, 25.2, 25.1, 22.8, 22.8

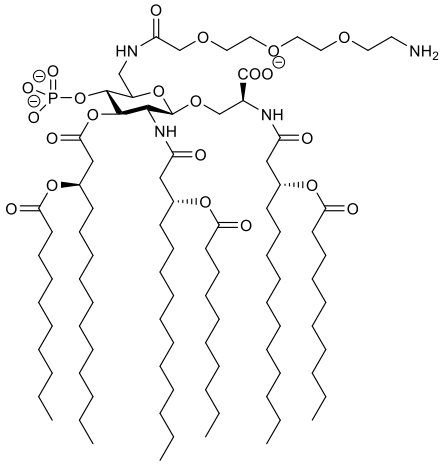
(CH_2 FA), 14.3, 14.2, 14.2 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ -0.78; FT-IR (neat, cm^{-1}): 3303, 2924, 2854, 2101, 1735, 1664, 1536, 1457, 1272, 1163, 1112, 1014, 735, 697; $[M+H]^+$ calcd. for $C_{110}H_{184}N_6O_{22}P$: 1972.31958, found 1972.31915.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[6-_O_-(11-amino-3,6,9-trioxaundecanoyl)-4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (39)



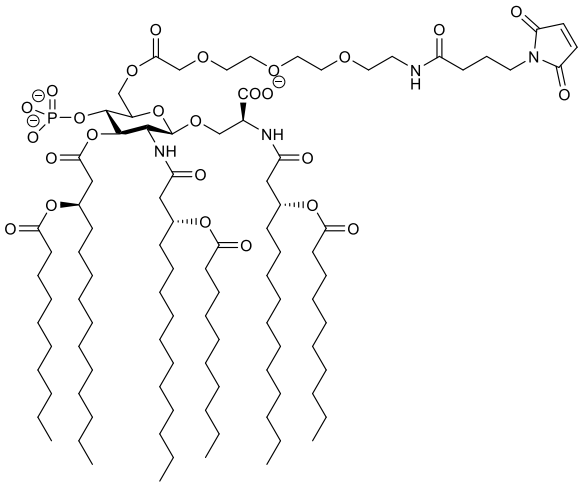
After co-evaporating with toluene (3x) under an argon atmosphere, compound **37** (0.51 g, 0.26 mmol, 1.0 eq.) was dissolved in dry THF (2.6 mL), followed by the addition of Pd/C (10%, 53 mg). A $H_{2(g)}$-filled balloon was applied on the obtained black suspension. After stirring at room temperature overnight, the black suspension was filtered over washed silica. The silica was washed $CHCl_3$, followed by the addition of Et_3N (0.14 mL, 1.0 mmol, 4 eq.). After mixing for 10 minutes, the clear solution was concentrated *in vacuo*. Lyophilization gave compound **39** (0.34 g, 0.20 mmol, 77%) as a white solid. ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC): δ 5.08 – 5.02 (m, 3H, 3x CH FA), 4.99 (t, 1H, *J* = 9.4 Hz, H-3), 4.53 (d, 1H, *J* = 9.6 Hz, C*H*H-6), 4.45 (d, 1H, *J* = 7.8 Hz, H-1), 4.33 (s, 1H, CH serine), 4.22 – 4.14 (m, 2H, C*H*H-6, C*H*H serine), 4.11 (q, 1H, *J* = 9.5 Hz, H-4), 4.07 – 3.92 (m, 3H, CHH serine, CH_2 linker), 3.74 – 3.69 (m, 1H, C*H*H linker), 3.68 – 3.48 (m, 11H, H-2, H-5, C*H*H linker, 4x CH_2 linker), 2.97 (s, 2H, CH_2NH_2), 2.56 (dd, 1H, *J* = 16.4, 6.7 Hz, C*H*H FA), 2.48 – 2.41 (m, 2H, 2x C*H*H FA), 2.38 – 2.30 (m, 2H, 2x C*H*H FA), 2.20 (dd, 1H, *J* = 14.7, 5.6 Hz, C*H*H FA), 2.18 – 2.11 (m, 6H, 3x CH_2 FA), 1.53 – 1.37 (m, 12H, 6x CH_2 FA), 1.25 – 1.03 (m, 90H, 45x CH_2 FA), 0.74 (t, 18H, *J* = 7.2 Hz, 6x CH_2 FA); ^{13}C-APT NMR ($CDCl_3$, 214 MHz, HSQC): δ 173.7, 173.6, 173.5, 170.9, 170.5, 170.1 (C=O), 100.3 (C-1), 73.8 (C-3), 72.9 (C-5), 71.1 (CH FA), 70.8, 70.8 (C-4), 70.8 (CH FA), 70.5, 70.2, 70.1, 70.1 (CH_2 linker), 70.1 (CH FA), 69.8, 69.8, 69.4 (CH_2 linker), 68.0 (CH_2 serine), 66.9 (CH_2 linker), 62.4 (CH_2-6), 53.7 (C-2), 53.1 (CH serine), 40.9, 40.8 (CH_2 FA), 38.9 (CH_2NH_2), 38.9, 34.4, 34.3, 34.2, 34.1, 34.0, 31.8, 31.8, 31.8, 31.8, 29.6, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 25.2, 25.2, 25.1, 25.0, 24.9, 22.5, 22.5 (CH_2 FA), 13.9 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 0.40; MALDI-FT-ICR MS (m/z): $[M+H]^+$ calcd. for $C_{89}H_{167}N_3O_{23}P$: 1677.1723, found 1677.1561.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[6-_N_-(11-amino-3,6,9-trioxaundecanoyl)-4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (40)



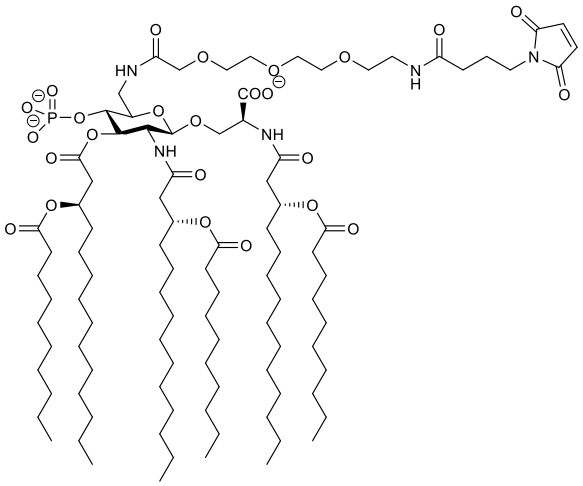
Compound **38** (21.4 mg, 10.8 µmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in THF (1.0 mL). Pd/C (10%, 21.1 mg) was added and the reaction mixture was stirred for 5.5 hours at room temperature under a blanket of $H_{2(g)}$. The black suspension was filtered over a Whatmann filter. The filter was washed with $CHCl_3$ and Et_3N (3.0 µL, 22 µmol, 2.0 eq.) was added to the combined filtrates. The solution was mixed for 5 minutes and concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH: 1/1) afforded compound **40** (15.1 mg, 9.0 µmol, 83%). ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC): δ 5.17 – 5.07 (m, 2H, 2x CH FA), 5.07 – 4.96 (m, 2H, H-3, CH FA), 4.54 (s, 1H, CH serine), 4.27 (d, 1H, *J* = 7.8 Hz, H-1), 4.10 – 3.98 (m, 2H, C*H*H serine, C*H*H linker) 3.98 – 3.91 (m, 2H, H-4, C*H*H linker), 3.83 (d, 1H, *J* = 15.4 Hz, C*H*H-6), 3.77 (dd, 1H, *J* = 11.8, 4.2 Hz, C*H*H serine), 3.74 – 3.69 (m, 1H, H-2), 3.69 – 3.51 (m, 10H, 5x CH_2 linker), 3.45 – 3.40 (m, 1H, H-5), 3.09 – 3.03 (m, 1H, C*H*H-6), 3.02 – 2.99 (m, 2H, C*H*$_2$NH$_2$), 2.60 – 2.49 (m, 4H, 2x CH_2 FA), 2.34 (dd, 1H, *J* = 14.4, 7.1 Hz, C*H*H FA), 2.22 – 2.10 (m, 7H, C*H*H FA, 3x CH_2 FA), 1.51 – 1.38 (m, 12H, 6x CH_2 FA), 1.21 – 1.05 (m, 90H, 45x CH_2 FA), 0.74 (t, 18H, *J* = 7.2 Hz, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 214 MHz, HSQC): δ 174.0, 173.7, 173.3, 170.9 (C=O), 100.6 (C-1), 73.8 (C-3), 72.9 (C-5), 71.7 (C-4), 71.1 (CH FA), 70.9, 70.8 (CH_2 linker), 70.7 (CH FA), 69.8 (CH_2 serine), 69.7 (CH FA), 69.6, 69.5, 69.1 (CH_2 linker), 66.1, 56.4, 53.1 (C-2), 52.3 (CH serine), 46.1, 46.1, 41.1, 40.0 (CH_2 FA), 39.1 (*C*H$_2$NH$_2$), 38.9 (CH_2-6), 34.4, 34.4, 34.3, 34.2, 34.0, 31.8, 31.8, 31.8, 31.8, 31.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.3, 29.3, 29.3, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 29.0, 25.3, 25.2, 25.1, 25.1, 25.0, 25.0, 24.9, 22.6 (CH_2 FA), 13.9 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 162 MHz, HMBC): δ 1.91; MALDI-FT-ICR MS (m/z): $[M+H]^+$ calcd. for $C_{89}H_{168}N_4O_{22}P$: 1676.1882, found 1676.1736.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[6-_O_-(11-_N_-(4-maleimidobutanoyl)-11-amino-3,6,9-trioxa-undecanoyl)-4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (41)



Amine **39** (32.9 mg, 19.6 µmol, 1.0 eq.) was dissolved in DCM (1.6 mL), followed by the addition of sulfo-*N*-succinimidyl 4-maleimidobutyrate sodium salt (9.1 mg, 23.8 µmol, 1.2 eq.) and Et_3N (16.4 µL, 0.12 mmol, 6.0 eq.). After stirring for 4 hours, DCM (0.8 mL) was added to the obtained white suspension and the mixture was stirred overnight. The reaction mixture was diluted with brine and extracted with DCM (3x). The combined organic layers were concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH: 1/1) afforded compound **41** (30.3 mg, 16.4 µmol, 84%). ^{1}H NMR ($CDCl_3$, 600 MHz, HH-COSY, HSQC): δ 6.64 (s, 2H, *H*C=C*H*), 5.11 – 5.01 (m, 3H, CH FA), 4.98 (t, 1H, *J* = 9.0 Hz, H-3), 4.63 – 4.54 (m, 1H, C*H*H-6), 4.45 – 4.32 (m, 2H, H-1, C*H*H-6), 4.28 (s, 1H, CH serine), 4.20 – 3.91 (m, 5H, H-4, CH_2 serine, CH_2 linker), 3.71 – 3.33 (m, 14H, H-2, H-5, 6x CH_2 linker), 3.24 – 3.17 (m, 2H, CH_2 linker), 2.56 (dd, 1H, *J* = 16.1, 7.0 Hz, C*H*H FA), 2.51 – 2.38 (m, 2H, 2x C*H*H FA), 2.38 – 2.28 (m, 2H, 2x C*H*H FA), 2.21 (dd, 1H, *J* = 14.6, 5.3 Hz, C*H*H FA), 2.17 – 2.11 (m, 6H, 3x CH_2 FA), 2.08 (t, 2H, *J* = 7.1 Hz, CH_2 linker), 1.82 – 1.72 (m, 2H, CH_2 linker), 1.51 – 1.37 (m, 12H, 6x CH_2 FA), 1.26 – 1.02 (m, 90H, 45x CH_2 FA), 0.73 (t, 18H, *J* = 7.0 Hz, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 151 MHz, HSQC): δ 173.8, 173.7, 173.6, 171.3, 171.1, 171.0, 170.9, 170.2 (C=O), 134.2 (C=C), 100.7 (C-1), 73.5 (C-3), 72.9 (C-5), 71.0 (CH FA), 70.9 (C-4), 70.8 (CH FA), 70.6, 70.3, 70.3, 70.2 (CH_2 linker), 70.1 (CH FA), 69.9, 69.7, 69.5, 69.5, 69.1, 69.0, 68.3 (CH_2 linker), 68.0 (CH_2 serine), 62.7 (CH_2-6) 53.6 (C-2), 53.3 (CH serine), 41.0, 40.7, 39.1, 39.0, 39.0, 38.7, 37.1, 36.9, 34.4, 34.3, 34.2, 34.2, 34.1, 32.6, 31.8, 31.8, 31.8, 31.8, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.4, 29.3, 29.3, 29.3, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 25.2, 25.2, 25.1, 25.0, 24.9, 24.2, 22.6, 22.5 (CH_2 FA, CH_2 linker), 13.9 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 0.82; HRMS: $[M+H]^+$ calcd. for $C_{97}H_{174}N_4O_{26}P$: 1842.21484, found 1842.21478.

_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-_O_-[6-_N_-(11-_N_-(4-maleimidobutanoyl)-11-amino-3,6,9-trioxa-undecanoyl)-4-_O_-phosphoryl-2-_N_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-3-_O_-[(_R_)-3-(decanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-L-serine (42)



A solution of amine **40** in DCE (22.4 mM, 375 µL, 1.0 eq.) to an eppendorf tube containing sulfo-*N*-succinimidyl 4-maleimidobutyrate sodium salt (4.1 mg, 10.7 µmol, 1.2 eq.) and Et_3N (7.0 µL, 50 µmol, 6.0 eq.). After mixing overnight at 850 rpm, the mixture was diluted with brine and extracted with DCM (3x). The combined organic layers were concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH: 1/1) afforded the title compound (12.5 mg, 6.8 µmol, 81%). ^{1}H NMR ($CDCl_3$, 850 MHz, HH-COSY, HSQC): δ 6.64 (s, 2H, *H*C=C*H*), 5.13 – 5.00 (m, 3H, 3x CH FA), 4.97 – 4.89 (m, 1H, H-3), 4.35 (d, 1H, *J* = 7.7 Hz, H-1), 4.23 (s, 1H, CH serine), 3.99 – 3.88 (m, 3H, H-4, C*H*H serine, CH_2 linker), 3.76 – 3.69 (m, 1H, C*H*H-6), 3.69 – 3.61 (m, 2H, H-2, C*H*H serine), 3.61 – 3.48 (m, 7H, 3x CH_2 linker, C*H*H linker), 3.48 – 3.39 (m, 4H, 2x CH_2 linker), 3.39 – 3.33 (m, 2H, H-5, C*H*H linker), 3.26 – 3.19 (m, 1H, C*H*H-6), 2.54 (dd, 1H, *J* = 16.2, 7.2 Hz, C*H*H FA), 2.49 – 2.39 (m, 2H, 2x C*H*H FA), 2.39 – 2.34 (m, 1H, C*H*H FA), 2.30 (dd, 1H, *J* = 14.6, 7.4 Hz, C*H*H FA), 2.20 (dd, 1H, *J* = 14.6, 5.4 Hz, C*H*H FA), 2.18 – 2.10 (m, 6H, 3x CH_2 FA), 2.08 (t, 1H, *J* = 7.2 Hz, CH_2 linker), 1.78 – 1.73 (m, 2H, CH_2 linker), 1.51 – 1.37 (m, 12H, 6x CH_2 FA), 1.26 – 1.05 (m, 90H, 45x CH_2 FA), 0.73 (t, 18H, *J* = 7.2 Hz, 6x CH_3 FA); ^{13}C-APT NMR ($CDCl_3$, 214 MHz, HSQC): δ 173.9, 173.9, 173.8, 173.6, 171.2, 171.1, 170.9, 170.4 (C=O), 134.2 (C=C), 100.9 (C-1), 73.9 (C-5), 73.5 (C-3), 72.0 (C-4), 71.0, 70.8 (CH FA), 70.3 (CH_2 linker), 70.1 (CH FA), 70.0, 69.5, 69.3, 69.3 (CH_2 serine, CH_2 linker), 53.6 (C-2, CH serine), 41.0, 40.7, 39.0, 39.0 (CH_2 FA), 38.7 (CH_2-6), 34.4, 34.3, 34.2, 32.7, 31.8, 31.8, 31.8, 31.8, 30.7, 29.6, 29.6, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.3, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 25.2, 25.2, 25.1, 25.0, 25.0, 24.9, 24.3, 22.6, 22.5 (CH_2 FA, CH_2 linker), 13.9 (CH_3 FA); ^{31}P-APT NMR ($CDCl_3$, 202 MHz, HMBC): δ 1.24; HRMS: $[M+H]^+$ calcd. for $C_{97}H_{175}N_5O_{25}P$: 1841.23083, found 1841.23006.

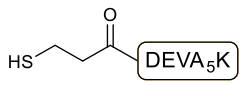
Automated solid phase synthesis general experimental information

The automated solid-phase peptide synthesis was performed on a 250 µmol scale on a Protein Technologies Tribute-UV IR Peptide Synthesizer applying Fmoc based protocol starting from Tentagel S RAM resin (loading 0.22 mmol/g). The synthesis was continued with Fmoc-amino acids specific for each peptide. The consecutive steps performed in each cycle for HCTU chemistry on 250 µmol scale: 1) Deprotection of the Fmoc-group with 20% piperidine in DMF for 10 min; 2) DMF wash; 3) Coupling of the appropriate

amino acid using a four-fold excess. Generally, the Fmoc amino acid (1.0 mmol) was dissolved in 0.2 M HCTU in DMF (5 mL), the resulting solution was transferred to the reaction vessel followed by 0.5 mL of 0.5 M DIPEA in DMF to initiate the coupling. The reaction vessel was then shaken for 30 min at 50°C; 4) DMF wash; 5) capping with 10% Ac_2O in 0.1 M DIPEA in DMF; 6) DMF wash; 7) DCM wash. Aliquots of resin of the obtained sequences were checked on an analytical Agilent Technologies 1260 Infinity system with a Gemini 3 µm, C18, 110 Å, 50 x 4.6 mm column or a Vydac 219TP 5 µm Diphenyl, 150 x 4.6 mm column with a 1 ml/min flow. The Fmoc amino acids applied in the synthesis were: Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(MMT)-OH, Fmoc-Phe-OH, Fmoc-Ser(O*t*Bu)-OH Fmoc-Val-OH.

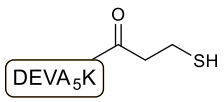
General procedure for cleavage from the resin, deprotection and purification
30 µmol resin was washed with DMF, DCM and dried after the last synthesis step followed by a treatment for 180 minutes with 0.6 mL cleavage cocktail of 95% TFA, 2.5% TIS and 2.5% H_2O. The suspension was filtered, the resin was washed with 0.6 mL of the cleavage cocktail, and the combined TFA solutions were added dropwise to cold Et_2O and stored at -20°C overnight. The obtained suspension of the product in Et_2O was centrifuged, Et_2O was removed and the precipitant was dissolved in $CH_3CN/H_2O/t$BuOH (1/1/1 v/v/v) or DMSO/$CH_3CN/H_2O/t$BuOH (3/1/1/1 v/v/v/v). Purification was performed on a Gilson GX-281 preparative RP-HPLC with a Gemini-NX 5u, C18, 110 Å, 250 x 10.0 mm column or a Vydac 219TP 5 µm Diphenyl, 250 x 10 mm column.

3-Mercaptoproponamide-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (43)



Tentagel S Ram resin loaded with H-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Ser(*t*Bu)-Gly-Leu-Glu(O*t*Bu)-Gln(Trt)-Leu-Glu(O*t*Bu)-Ser(*t*Bu)-Ile-Ile-Asn(Trt)-Phe-Glu(O*t*Bu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT) on 70 µmol scale was washed with DMF (5x), followed by the addition of a solution of 3-(tritylthio)propionic acid (52 mg, 150 µmol, 2.1 eq.) and HCTU (58 mg, 140 µmol, 2.0 eq.) in DMF (1.4 mL) and DIPEA (49 µL, 280 µmol, 4.0 eq.). The reaction vessel was shaken overnight at 850 rpm, after which it was washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H_2O/1,2-ethanedithiol (94/2.5/2.5/1 v/v/v/v, 2.8 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (2.8 mL). The product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, compound **43** (14 mg, 5.3 µmol, 8%) was obtained as a white solid. LC-MS: Rt = 5.15 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1317.7 $[M+H]^{2+}$; HRMS: $[M+H]^{2+}$ calcd. for $C_{115}H_{191}N_{29}O_{39}S$: 1317.17819, found 1317.17784.

Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-(N_ε-(3-mercaptoproponamide))-Lys-NH_2 (44)



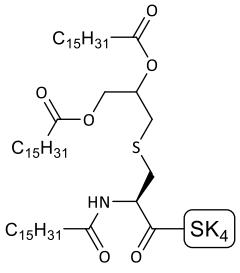
Tentagel S Ram resin loaded with H-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Ser(*t*Bu)-Gly-Leu-Glu(O*t*Bu)-Gln(Trt)-Leu-Glu(O*t*Bu)-Ser(*t*Bu)-Ile-Ile-Asn(Trt)-Phe-Glu(O*t*Bu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT) on 50 µmol scale was washed with DMF (5x), treated with a mixture of Boc_2O (0.11 g, 0.50 mmol, 10 eq.) in 0.1 M DIPEA in DMF (0.5 mL) for one hour, and washed with DMF (3x) and DCM (3x). The peptide was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 15 mL) over 5 minutes. The resin was washed subsequently with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL), DCM (3x) and DMF (3x). A solution of 3-(tritylthio)propionic acid (49 mg, 140 µmol, 2.0 eq.) and HCTU (58 mg, 140 µmol, 2.0 eq.) in DMF (1.4 mL) and DIPEA (49 µL, 280 µmol, 4.0 eq.) were added. The reaction vessel was shaken overnight at 850 rpm, after which it was washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H_2O/1,2-ethanedithiol (95/2/2/1 v/v/v/v, 2.8 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (2.8 mL). The product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, compound **44** (7.9 mg, 3.0 µmol, 6%) was obtained as a white solid. LC-MS: Rt = 5.31 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1317.2 $[M+H]^{2+}$; HRMS: $[M+H]^{2+}$ calcd. for $C_{115}H_{191}N_{29}O_{39}S$: 1317.17819, found 1317.17916.

Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (45)

DEVA$_5$K

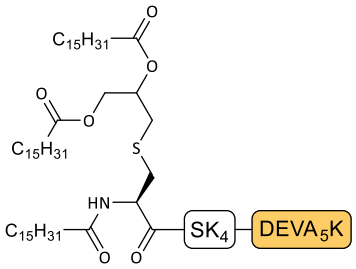
Tentagel S Ram resin loaded with H-Asp(O*t*Bu)-Glu(O*t*Bu)-Val-Ser(*t*Bu)-Gly-Leu-Glu(O*t*Bu)-Gln(Trt)-Leu-Glu(O*t*Bu)-Ser(*t*Bu)-Ile-Ile-Asn(Trt)-Phe-Glu(O*t*Bu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT) on 30 µmol scale was washed with DCM (5x). The peptide was cleaved from the resin after treatment with TFA/TIS/H_2O (95/2/2/1 v/v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, compound **45** (12 mg, 4.7 µmol, 16%) was obtained as a white solid. LC-MS: Rt = 4.82 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1273.7 $[M+H]^{2+}$; HRMS: $[M+H]^{2+}$ calcd. for $C_{112}H_{187}N_{29}O_{38}$: 1273.17904, found 1273.17779.

Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys-NH_2 (46)



Reference compound was synthesized according to literature.[53] LC-MS: Rt = 13.02 min (Diphenyl Vydac, 10 - 90% CH_3CN, 21 min); ESI-MS: m/z 1509.1 $[M+H]^+$; HRMS: $[M+H]^+$ calcd. for $C_{81}H_{158}N_{11}O_{12}S$: 1509.18067, found 1509.18052.

Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (47)

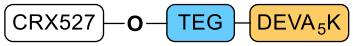


Tentagel S Ram resin loaded with H-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT) on 50 µmol scale was washed with DMF (5x), followed by the addition of a solution of 3-(palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl) (91 mg, 100 µmol, 2.0 eq.) and HCTU (41 mg, 100 µmol, 2.0 eq.) in DMF/DCM (1/1 v/v, 1.0 mL) and DIPEA (35 µL, 200 µmol, 4.0 eq.). The reaction vessel was shaken overnight at 850 rpm, after which it was washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H_2O (95/2.5/2.5 v/v/v/v) (2.0 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (2.0 mL). The product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, compound **47** (9.7 mg, 2.4 µmol, 5%) was obtained as a white solid. LC-MS: Rt = 12.31 min (Diphenyl Vydac, 10 - 90% CH_3CN, 21 min); ESI-MS: m/z 1346.7 $[M+H]^{3+}$; HRMS: $[M+H]^{3+}$ calcd. for $C_{193}H_{342}N_{39}O_{50}S$: 1346.17073, found 1346.17063.

General purification method for CRX-527-*O*-conjugates.
A C18 column was washed subsequently with CH_3CN, MeOH, DCM/MeOH (1/1 v/v), MeOH, CH_3CN, CH_3CN/H_2O, H_2O. The reaction mixture was added on the column and the Eppendorf was rinsed with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.50 mL), which was also added on the C18 column. The column was subsequently flushed with 6 mL of the follow solvent systems: H_2O, CH_3CN/H_2O (1/1 v/v), CH_3CN, DMSO, CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v) and collected in Eppendorfs containing 1.0 mL of each solvent system. The column was then flush with MeOH (6.0 mL), followed by DCM/MeOH (1/1 v/v, 6.0 mL), which were collected in separate flasks, concentrated *in vacuo* at 35°C and lyophilized by dissolving in CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v), yielding the conjugate as a white solid.

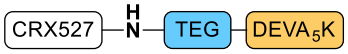
General purification method for CRX-527-*NH*-conjugates.
A C18 column was washed subsequently with CH_3CN, MeOH, DCM/MeOH (1/1 v/v), MeOH, CH_3CN + 0.1% TFA, CH_3CN/H_2O (1/1 v/v) + 0.1% TFA, H_2O + 0.1% TFA. The reaction mixture was added on the column and the Eppendorf was rinsed with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.50 mL), which was also added on the C18 column. The column was subsequently flushed with 6 mL of the follow solvent systems: H_2O + 0.1% TFA, CH_3CN/H_2O (1/1 v/v) + 0.1% TFA, CH_3CN + 0.1% TFA, DMSO, CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v) and collected in Eppendorfs containing 1.0 mL of each solvent system. The column was then flush with MeOH (6.0 mL), followed by DCM/MeOH (1/1 v/v, 6.0 mL), which were collected in separate flasks, concentrated *in vacuo* at 35°C and lyophilized by dissolving in CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v), yielding the conjugate as a white solid.

***N-Terminus 6-O*-DEVA$_5$K conjugate (5)**



Thiol-peptide **43** (1.4 mg, 0.53 µmol, 1.5 eq.) was dissolved in a mixture of DMF/MilliQ H_2O (4/1 v/v, 114 µl) in an Eppendorf tube. A solution of compound **41** (5.0 mM, 114 µL, 0.36 µmol, 1.0 eq.) was added and the mixture was shaken at 850 rpm for 48 hours. LC-MS analysis showed complete conversion of the starting material. The reaction mixture was diluted with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.80 mL) and sonicated for 5 minutes. After purification using a C18 column, LC-MS analysis showed that the DCM/MeOH (1/1 v/v) flush contained pure conjugate. After lyophilization, conjugate **5** (0.86 mg, 0.19 µmol, 52%) was obtained as a white solid. LC-MS: Rt = 14.27 min (Diphenyl Vydac, 10 - 90% IPA, 25 min run, 0.8 mL/min); ESI-MS: m/z 1500.2 $[M+Na]^{3+}$; MALDI-TOF MS (m/z): $[M+2Na]^+$ calcd. for $C_{212}H_{362}N_{33}O_{65}SPNa_2$: 4522.406, found 4531.383.

***N*-Terminus 6-*NH*-DEVA$_5$K conjugate (6)**

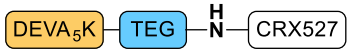


Thiol-peptide **43** (3.65 mg, 1.39 µmol, 1.5 eq.) was dissolved in a mixture of DMF/MilliQ H_2O (4/0.5 v/v, 277 µl) in an Eppendorf tube. A solution of compound **42** (5.0 mM, 182 µL, 0.91 µmol, 1.0 eq.) was added and the mixture was shaken at 850 rpm for 48 hours. LC-MS analysis showed complete conversion of the starting material. The reaction mixture was diluted with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.59 mL) and sonicated for 5 minutes. After purification using a C18 column, LC-MS analysis showed that the MeOH and DCM/MeOH (1/1 v/v) flush contained pure conjugate. After lyophilization, conjugate **6** (2.19 mg, 0.49 µmol, 54%) was obtained as a white solid. LC-MS: Rt = 16.85 min (Diphenyl Vydac, 10 - 90% IPA, 25 min run, 0.7 mL/min); ESI-MS: m/z 1499.9 $[M+Na]^{3+}$; MALDI-TOF MS (m/z): $[M+2Na]^+$ calcd. for $C_{212}H_{363}N_{34}O_{64}SPNa_2$: 4521.422, found 4530.340.

***C*-Terminus 6-*O*-DEVA$_5$K conjugate (7)**

Thiol-peptide **44** (2.1 mg, 0.80 µmol, 1.5 eq.) was dissolved in a mixture of DMF/MilliQ H_2O (4/0.5 v/v, 161 µl) in an Eppendorf tube. A solution of compound **41** (5.0 mM, 107 µL, 0.53 µmol, 1.0 eq.) was added and the mixture was shaken at 850 rpm for 48 hours. LC-MS analysis showed complete conversion of the starting material. The reaction mixture was diluted with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.80 mL) and sonicated for 5 minutes. After purification using a C18 column, LC-MS analysis showed that the MeOH and DCM/MeOH (1/1 v/v) flush contained pure conjugate. After lyophilization, conjugate **7** (1.37 mg, 0.30 µmol 57%) was obtained as a white solid. LC-MS: Rt = 14.24 min (Diphenyl Vydac, 10 - 90% IPA, 25 min run, 0.8 mL/min); ESI-MS: m/z 1508.6 $[M+2Na]^{3+}$; MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{212}H_{362}N_{33}O_{65}SPNa$: 4499.417, found 4497.786.

_C_-Terminus 6-_NH_-DEVA$_5$K conjugate (8)



Thiol-peptide **44** (2.0 mg, 0.76 µmol, 1.5 eq.) was dissolved in a mixture of DMF/MilliQ H_2O (4/0.5 v/v, 152 µl) in an Eppendorf tube. A solution of compound **42** (5.0 mM, 101 µL, 505 nmol, 1.0 eq.) was added and the mixture was shaken at 850 rpm for 48 hours. LC-MS analysis showed complete conversion of the starting material. The reaction mixture was diluted with a mixture of CH_3CN/*t*BuOH/MilliQ H_2O (1/1/1 v/v/v, 0.80 mL) and sonicated for 5 minutes. After purification using a C18 column, LC-MS analysis showed that the DCM/MeOH (1/1 v/v) flush contained pure conjugate. After lyophilization, conjugate **8** (0.94 mg, 0.21 µmol, 42%) was obtained as a white solid. LC-MS: Rt = 16.90 min (Diphenyl Vydac, 10 - 90% IPA, 25 min run, 0.7 mL/min); ESI-MS: m/z 1507.4 $[M+2Na]^{3+}$; MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{212}H_{363}N_{34}O_{64}SPNa$: 4498.433, found 4508.766.

Cell culture

The D1 cell line is a growth factor-dependent immature spleen-derived DC cell line from C57BL/6 (H-2^b) mice. D1 cells were cultured as described elsewhere.[54] B3Z is a $CD8^+$ T cell hybridoma specific for the H-2Kb CTL-epitope SIINFEKL of ovalbumin and was cultured in IMDM medium (Lonza, Basel, Switzerland) supplemented with 8% FCS (Greiner, Kremsmünster, Austria), penicillin and streptomycin, glutamine (Gibco, Carlsbad, CA, USA), β-mercaptoethanol (Merck, Kenilworth, NJ USA), and hygromycin B (AG Scientific Inc, San Diego, CA, USA) to maintain expression of the beta-galactosidase reporter gene.

In vitro DC maturation assay

50.000 D1 cell were seeded in 96-well round bottom plates (Corning, Amsterdam, The Netherlands) in 100 µl of R1 supplemented IMDM medium and 100 µl of 2 times concentrated test compounds were added. After 24 hours of incubation at 37°C, supernatant was taken from the wells for ELISA analysis (BioLegend, San Diego, USA) to measure the amount of produced IL-12p40.

In vitro antigen presentation assay

50.000 D1 cells were seeded in 96-well flat bottom plates and pulsed for 2 hours with the indicated test compounds. After 2 hours, cells were washed once with fresh medium and 50.000 B3Z were added per well and incubated with the pulsed D1 cells overnight. The following day, TCR activation triggered by recognition of the SIINFEKL epitope was detected by measurement of absorbance at 595 nm upon color conversion of chlorophenol red-β-D-galactopyranoside (Calbiochem®, Merck, Bullington MA, USA).

Footnotes and References

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