Synthetic carbohydrate ligands for immune receptors

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Chapter 5

*Synthesis of C-rhamnoside–antigen conjugates to recruit anti-rhamnose antibodies for vaccine delivery**

Introduction

Tumor-associated carbohydrate antigens or cancer neo-epitopes are used in cancer vaccination strategies to trigger T helper cell responses and induce cytotoxic effector T cells. Vaccination with short peptides, that can be presented by major histocompatibility complex (MHC) mole

cules, can lead to immunological tolerance instead of immunity.[1,2] Therefore, longer peptide sequences that cannot bind directly to MHC and require intracellular processing by antigen presenting cells are generally used in peptide vaccine constructs.[3] Several methods have been developed to enhance the immunogenicity of synthetic long peptide (SLPs). To activate the immune system and up-regulate the production of inflammatory cytokines, the antigens are administered with an adjuvant[4–6], for example Toll-like receptor ligands. This can be achieved either by mixing the antigen and adjuvant or by generating (covalent) antigen-adjuvant conjugates. Enhanced immune activity can also be achieved by stimulating antigen uptake by antigen presenting cells, such as dendritic cells (DCs) by targeting uptake receptors on DCs for example by using

*The data presented in this Chapter were gathered in collaboration with Nick Zilverschoon, Robert A. Cordfunke, Jan Wouter Drijfhout, Herman S. Overkleeft, Dmitri V. Filippov, Gijsbert A. van der Marel and Jeroen D. C. Codée.

C-type lectin ligands.[7] The uptake of antigens can also be improved using antibody-recruiting molecules (ARM). This strategy is depicted in Figure 1 and builds on the formation of an immune complex of the antigen conjugate with pre-existing circulating antibodies, instead of binding directly to a DC surface receptor. The formed complex can then bind to Fcγ receptors[8] on the DCs leading to enhanced uptake. After internalization, the antigens are processed and the epitope is presented to T cells resulting in T cell mediated immune response.

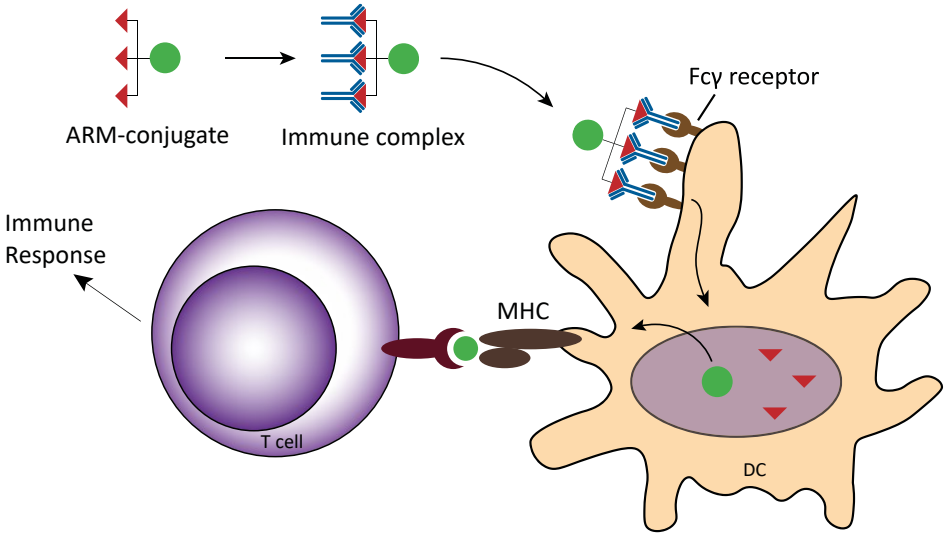


Figure 1. Mechanism of action of ARM-conjugates.

The α-Gal epitope (Figure 2) plays a crucial role in organ xenotransplantation, as it represents a highly immunogenic trisaccharide structure, against which most individuals have a naturally acquired high antibody titer. Therefore it has been explored in model ARM-conjugate systems for vaccination against for example the influenza virus and the HIV gp120 protein.[9–11] This epitope was even used to make cancer cells susceptible to lysis.[12] Other ARM-based strategies use 2,4-dinitroaniline analogues[13–15] (Figure 2) or tetanus toxoid epitopes[16,17] to enhance the immunogenicity of vaccines. Screening of human serum against broad carbohydrate antigen microarrays has shown that anti-L-rhamnose antibodies are amongst the most abundant circulating antibodies in human blood.[18,19] Several studies have exploited this abundance and used rhamnose-functionalized peptides[20], proteins[21] and liposomes[22–24] to be used in cancer immunotherapy. These studies have demonstrated that the xenoantigen L-rhamnose is an excellent alternative to the α-Gal epitope in model vaccination studies, especially as wild-type mice can be used instead of KO mice.[25]

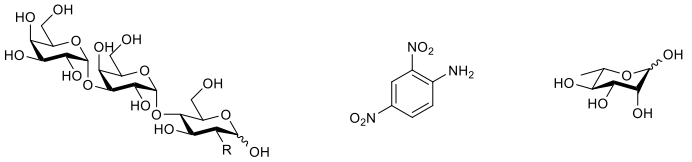


Figure 2. Structure of ARM-molecules, α-Gal-epitope, 2,4-dinitroaninine and L-rhamnose.

This Chapter describes the design and synthesis of conjugates **1**-**7** consisting of rhamnose and an ovalbumin derived peptide LEQLESIINFEKLAAAAAK, harboring the MHC-I epitope SIINFEKL to be used as model antigen (Figure 3). Functionalization of the peptide with one, two, three or six rhamnose monosaccharides will allow one to investigate the effect of multivalent binding to anti-rhamnose antibodies and the effect thereof on the immunogenicity of the vaccine. To generate these constructs two lysine building blocks **8** and **9**, equipped with an L-rhamnose-*C*-glycoside are designed to allow for application in an online solid phase peptide synthesis (SPPS) protocol. The *C*-rhamnosidic linkage in the building blocks is stable against the acidic conditions used in SPPS, while the *p*-methoxybenzyl protecting groups were chosen for their acid lability to obtain a fully deprotected conjugate after cleaving the peptides from the resin after SPPS. The building blocks **8** and **9** only differ in the length of the spacer bridging the *C*-rhamnoside and the lysine moiety.

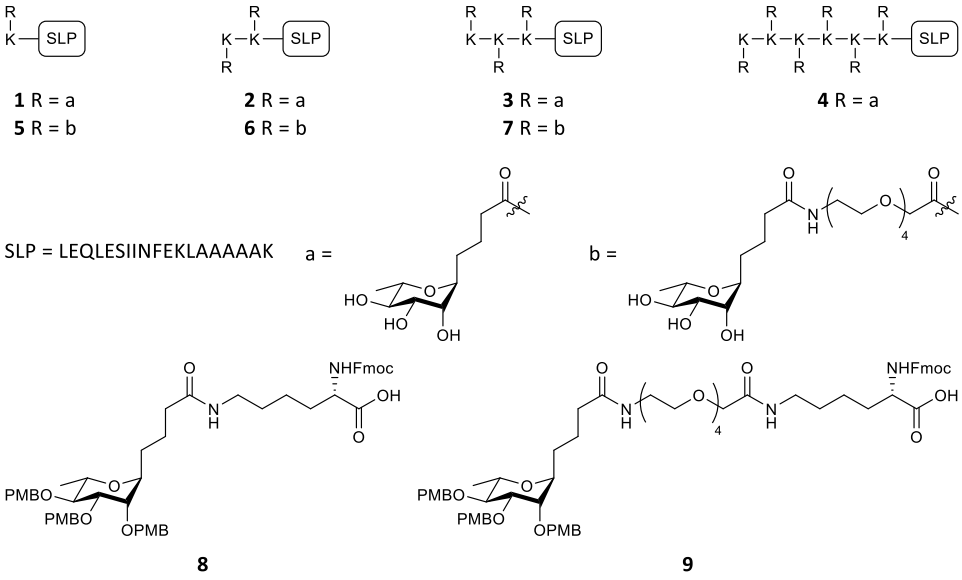


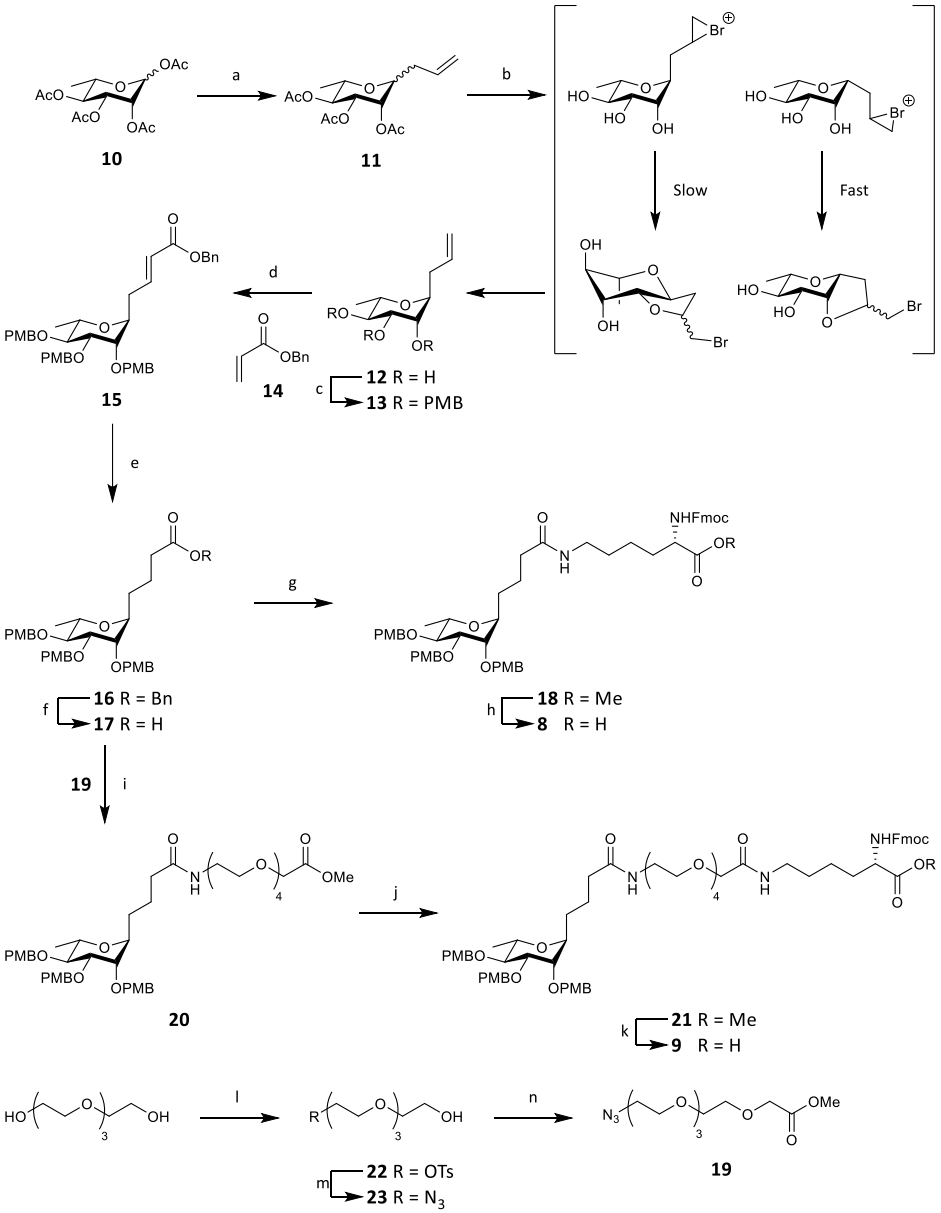
Figure 3. Structures of *C*-rhamnose conjugates **1-7** and SPPS building blocks **8** and **9**.

Results & Discussion

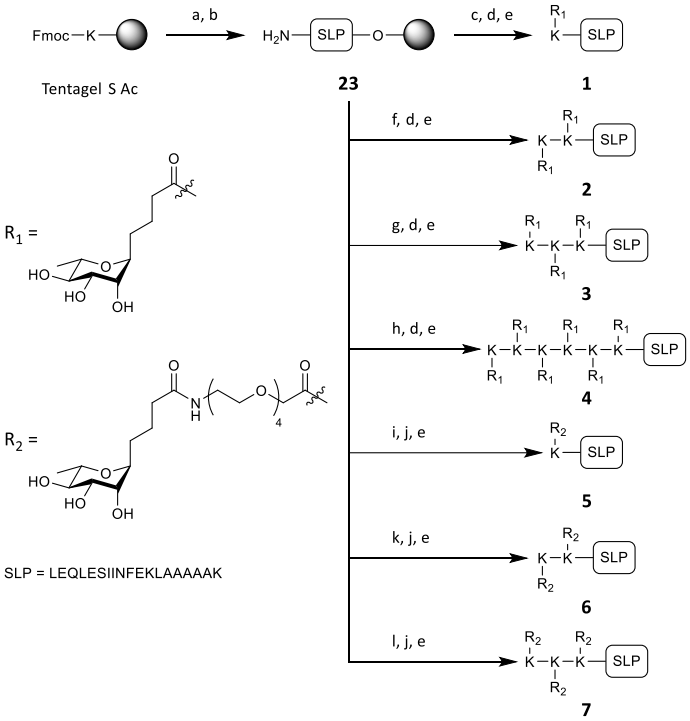
Synthesis of SPPS building blocks **8** and **9** started with the preparation of *C*-rhamnose **12** (Scheme 1). Treatment of acetylated rhamnose **10** with allyltrimethylsilane and *in situ* generated $BF_2OTf{\cdot}OEt_2$ as Lewis acid[26] afforded the desired allyl rhamnoside **11** as an inseparable 5/1 α/β-mixture. Therefore compound **11** was deacetylated using sodium methoxide and an intramolecular cyclization was induced by the addition of *N*-bromosuccinimide. A nucleophilic attack of the *C*-2-OH on the formed bromonium ion of the β-rhamnose occurred fast, while the α-rhamnose reacted slowly due to energetically unfavorable $^1C_4$ conformation and the formation of the *trans*-fused 5,6-bicyclic ring system, which is necessary for α-cyclization as shown in Scheme 1. The cyclized product and the unreacted α-rhamnose could be readily separated via column chromatography giving pure α-compound **12** in 86% over three steps. Close monitoring of the reaction progress was required as too short reaction times led to incomplete conversion of the β-rhamnose while longer reaction times decreased the yield due to cyclization of the α-rhamnose. Next, triol **12** was alkylated with *p*-methoxybenzyl chloride in the presence of sodium hydride to provide the fully protected *C*-rhamnoside **13**. Installation of the required acid functionality was achieved by a cross-metathesis with benzyl acrylate **14**, which was followed by reduction of the obtained alkene (**15**) with $NaBH_4$ and ruthenium trichloride and saponification of the so formed benzyl ester **16**.[27] To acquire an orthogonal protected SPPS building block, acid **17** and Fmoc-L-lysine-OMe[28] were coupled under the influence of HCTU and DIPEA. Subsequent careful hydrolysis of the methyl ester **18** with LiOH at 0°C gave Fmoc-protected *C*-rhamnose-functionalized lysine building block **8**. As the multivalent binding of the anti-rhamnose antibodies may be dependent on the length of the spacers between the *C*-rhamnosides, the lysine building block **9** was prepared. Spacer **19** was synthesized by successively subjecting tetraethylene glycol to mono-tosylation, azide substitution and alkylation with methyl bromoacetate. After reduction of the azide in **19** by hydrogenation, the produced amine was directly condensed with acid **17** to give rhamnoside **20** in 82%. Scale-up (5 mmol) of this coupling decreased the yield dramatically to 11% due to the formation of side-products and the difficult separation via column chromatography. After saponification of methyl ester **20**, the formed acid was coupled with Fmoc-L-lysine-OMe to give **21** and the methyl ester was carefully hydrolyzed with LiOH yielding SPPS rhamnose-functionalized lysine building block **9**.

With rhamnose-functionalized lysine building blocks **8** and **9** in hand, the SPPS of conjugates **1**-**7** was undertaken (Scheme 2). For the automated synthesis of LEQLESIINFEKLAAAAAK, Tentagel S Ac resin was used. The obtained immobilized

peptide **23** was elongated at the *N*-terminus with one, two, three or six rhamnose-functionalized lysines to obtain conjugates **1**-**4**. The coupling was performed with three equivalents of **8** in the presence of PyBOP as condensing agent and NMM as base at room temperature for two hours. After RP-HPLC purification conjugates **1**-**4** were obtained in respectively 19% (4.5 mg), 16% (4.5 mg), 10% (3.2 mg) and 6% (2.6 mg) yield. The high yields show that **8** is an excellent SPPS building block and the compatibility of the designed protecting group strategy with the SPPS approach. Unfortunately, elongation of peptide **23** with **9** proved to be more difficult and the PyBOP mediated couplings did not go to completion, not even by increasing the reaction time and temperature. Replacing PyBOP with the more reactive reagent HATU and performing the reaction at 39°C overnight did provide full conversion of the starting peptide. Coupling of **9** once, twice or thrice to immobilized peptide **23** afforded conjugates **5**-**7** in respectively 15% (2.0 mg), 2% (0.9 mg) and 4% (0.4 mg). The low yields of **6** and **7** can be caused by reaction of HATU with the *N*-terminal amine, preventing further elongation of the immobilized peptide. This side reaction was confirmed with MALDI analysis.



Scheme 1. Synthesis of SPPS building blocks **8** and **9**. *Reagents and conditions*: a) allyltrimethylsilane, $BF_3{\cdot}OEt_2$, TMSOTf, MeCN, 86%; b) *i*. NaOMe, MeOH; *ii*. *N*-bromosuccinimide, THF, 3 h, then $Na_2S_2O_3$, 79% over two steps; c) *p*-methoxybenzyl chloride, NaH, TBAI, DMF, 80%; d) benzyl acrylate (**14**), Grubbs 2nd gen. catalyst, DCM, 50°C, 92%; e) $NaBH_4$, ruthenium trichloride, MeOH, DCE, 40°C, 93%; f) LiOH, THF/MeOH/H_2O, 40°C, 96%; g) Fmoc-L-Lys-OMe, HCTU, DIPEA, DMF, 80%; h) LiOH, THF/H_2O, 0°C, 71%; i) *i*. **19**, Pd/C, H_2, THF; *ii*. HCTU, DIPEA, DMF, 82% over two steps; j) *i*. LiOH, THF/H_2O; *ii*. Fmoc-L-Lys-OMe, HCTU, DIPEA, DMF, 89% over two steps; k) LiOH, THF/H_2O, 0°C, 66%; l) TsCl, Et_3N, DCM, 93%; m) NaN_3, DMF, 90°C, 96%; n) methyl bromoacetate, NaH, THF, 83%.



Scheme 2. Synthesis of conjugates **1**-**7**. *Reagents and conditions*: a) Fmoc SPPS cycle for LEQLESIINFEKLAAAAA; b) 20% piperidine, NMP; (c) *i*. **8**, PyBOP, NMM, NMP; *ii*. 20% piperidine, NMP; d) TFA/TIS/H_2O (93/2/5 v/v/v); e) RP-HPLC; f) repeat conditions c two times; g) repeat conditions c three times; h) repeat conditions c six times; i) *i*. **9**, HATU, NMM, NMP; *ii*. 20% piperidine, NMP; j) TFA/H_2O (95/5 v/v/v); k) repeat conditions i two times; l) repeat conditions i three times. Yield conjugates: **1**) 4.5 mg, 19%; **2**) 4.5 mg, 16%; **3**) 3.2 mg, 10%; **4**) 2.6 mg, 6%; **5**) 2.0 mg, 15%; **6**) 0.9 mg, 2%; **7**) 0.4 mg, 4%.

Conclusion

This Chapter describes the synthesis of seven novel rhamnose-peptide conjugates using an SPPS approach in which the rhamnosides were incorporated by an online assembly process. In these rhamnose constructs, designed as model vaccines, one, two, three or six *C*-rhamnose-functionalized lysines were linked to the *N*-terminus end of an antigenic peptide containing the MHC-I epitope, SIINFEKL. To enable the online SPPS two building blocks **8** and **9**, differing in spacer length were prepared using α-*C*-rhamnose intermediate for which an efficient synthesis has been developed. While conjugates **1**-**4** could be obtained in high yield using building block **8**, the condensation reactions using **9** proceeded less efficiently. By the use of the condensing agent HATU together with relatively long reaction times and an increased reaction temperature conjugates **5**-**7** were obtained, albeit in relatively low yields. The immunological evaluation of the conjugates is ongoing.

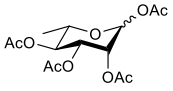
Experimental

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 and ^{13}C spectra were recorded on a Bruker AV-400 (400/100 MHz) spectrometer 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. Absorbance was measured at 214 nm and 256 nm and an Agilent Technologies 6120 Quadrupole mass spectrometer was used as detector. 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. Infrared spectra were recorded on a Perkin Elmer Spectrum 2 FT-IR.

Automated solid phase synthesis general experimental information

The synthesis of LEQLESIINFEKLAAAAAK was performed as has been described before by Hiemstra *et al.*[29] In short, the peptides were synthesized using solid-phase peptide synthesis on a Tentagel S Ac resin (Rapp, Tübingen) using a Syro II peptide synthesizer (MultiSyntech, Witten, Germany). Normal couplings (1.5 h - 2 h) were performed using Fmoc amino acids carrying acid labile side chain protection groups (were required). Activation of Fmoc amino acids was performed with PyBOP and NMM unless stated otherwise. Fmoc deprotection was performed with 20% piperidine in NMP. Washings were performed with NMP. Cleavage from the resin and side chain deprotection was performed with TFA/TIS/H_2O (93/2/5 v.v.v) unless stated otherwise. Purification was performed with RP-HPLC (C18). Analysis of the purified peptide was performed with UPLC-MS (Acquity, Waters) and showed the expected molecular masses. Building bock

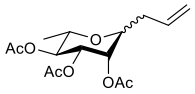
Acetyl 2,3,4-tri-*O*-acetyl-α/β-L-rhamnopyranoside (10)



A solution of L-rhamnose monohydrate (8.3 g, 51 mmol, 1.0 eq.) in pyridine (70 mL) was cooled to 0°C, followed by the addition of Ac_2O (31 mL, 0.35 mol, 6.9 eq.). The reaction was allowed to warm-up to room temperature overnight, after which it was quenched with methanol at 0°C and diluted with EtOAc. The organic layer was washed with 1 M HCl (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Co-evaporation with toluene gave the title compound (16 g, 48 mmol, 94%, α/β ratio: 8/1) as a transparent sticky oil. R_f: 0.40 (7/3 pentane/EtOAc); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.99 (d, 1H, *J* = 1.9 Hz,

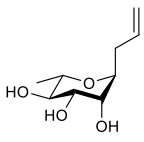
H-1), 5.28 (dd, 1H, *J* = 10.1, 3.5 Hz, H-3), 5.24 – 5.21 (m, 1H, H-4), 5.10 (t, 1H, *J* = 9.9 Hz, H-2), 3.96 – 3.87 (m, 1H, H-5), 2.14 (m, 6H, *J* = 4.6 Hz, 2x CH_3 Ac), 2.04 (s, 3H, CH_3 Ac), 1.98 (s, 3H, CH_3 Ac), 1.21 (d, 3H, *J* = 6.2 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 170.2, 169.9, 169.9, 168.5 (C=O), 90.7 (C-1), 70.5 (C-2), 68.8 (C-3), 68.8 (C-5), 68.7 (C-4), 21.0, 20.9, 20.9, 20.8 (CH_3 Ac), 17.5 (CH_3-6); FT-IR (neat, cm^{-1}): 2987, 1743, 1433, 1369, 1209, 1181, 1147, 1086, 1052, 1025, 969, 947, 909, 888, 840, 783, 736, 698, 601, 563, 533, 511, 499, 480; $[M+Na]^+$ calcd. for $C_{14}H_{20}O_9Na$: 355.1005, found 355.1010. *NMR analysis only given for the α-anomer.

3-(2,3,4-tri-*O*-acetyl-α/β-L-rhamnosyl)-1-propene (11)



After co-evaporating with toluene (4x), compound **10** (44.7 g, 134 mmol, 1.0 eq.) was dissolved in dry MeCN (0.25 L) under an argon atmosphere, followed by the addition of allyltrimethylsilane (44 mL, 0.28 mol, 2.0 eq.). After cooling the mixture to 0°C, $BF_3{\cdot}OEt_2$ (35 mL, 0.28 mmol, 2.0 eq.) and TMSOTf (2.3 mL, 13 mmol, 0.10 eq.) were added and the reaction was allowed to warm-up to room temperature overnight. Upon completion determined by TLC analysis, the reaction was cooled to 0°C and slowly quenched with Et_3N. The mixture was diluted with sat. aq. $NaHCO_3$ and extracted with EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10%→16% EtOAc in pentane) gave compound **11** (36.2 g, 115 mmol, 86%, α/β ratio: 5/1) as a sticky yellow oil. R_f: 0.40 (7/2 pentane/EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.75 – 5.58 (m, 1H, CH_2-C*H*=CH_2), 5.13 – 5.07 (m, 2H, H-2, H-3), 5.06 – 5.00 (m, 1H, CH_2-CH=C*H*H), 4.97 – 4.85 (m, 2H, H-4, CH_2-CH=C*H*H), 3.88 – 3.81 (m, 1H, H-1), 3.70 – 3.61 (m, 1H, H-5), 2.48 – 2.38 (m, 1H, C*H*H-CH=CH_2), 2.35 – 2.23 (m, 1H, C*H*H-CH=CH_2), 2.01 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.12 (d, 3H, *J* = 6.3 Hz, CH_3-6).^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 170.5, 170.3, 170.0 (C=O), 133.0 (CH_2-*C*H=CH_2), 118.3 (CH_2-CH=*C*H_2), 74.5 (C-1), 71.6 (C-4), 70.5 (C-3), 69.2 (C-2), 68.3 (C-5), 33.8 (*C*H_2-CH=CH_2), 21.1, 21.0, 20.8 (CH_3 Ac), 17.7 (CH_3-6); FT-IR (neat, cm^{-1}): 2983, 2361, 1745, 1371, 1222, 1051, 668; HRMS: $[M+Na]^+$ calcd. for $C_{15}H_{22}O_7Na$: 337.1263, found 337.1264. *Only data given for the α-anomer.

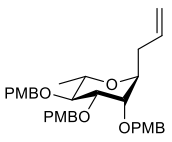
3-(α-L-rhamnosyl)-1-propene (12)



Compound **11** (36.3 g, 115 mmol, 1.0 eq., α/β ratio: 5/1) was co-evaporated with toluene (3x) under argon atmosphere and dissolved in MeOH (0.58 L). Sodium methoxide (5.4 M in MeOH, 2.2 mL, 12 mmol, 0.1 eq.) was added and the solution was stirred for two hours, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was acidified by the addition of amberlite H^+ resin, filtered and concentrated *in vacuo*. The obtained residue was co-evaporated with toluene (1x) under argon atmosphere and dissolved in THF (1.2 L). *N*-bromosuccinimide (10 g, 55 mmol, 0.48 eq.) was added and the reaction was allowed to stir for 3 hours, after which the reaction was quenched with an aqueous solution of $Na_2S_2O_3$ (4.4 M, 40 mL). The mixture was further diluted with toluene and concentrated *in vacuo*. The crude product was imbedded on silica and purified by column chromatography (2→8% MeOH in DCM) yielded compound **12** (14.2 g, 75.4 mmol, 79%) as a white solid. R_f: 0.24 (9/1

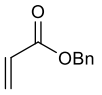
DCM/MeOH); $[\alpha]_D^{20}$ +18.0° (*c* = 1.0, MeOH); ^{1}H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 5.89 – 5.75 (m, 1H, CH_2-C*H*=CH_2), 5.17 – 5.03 (m, 2H, CH_2-CH=C*H*$_2$), 3.91 – 3.82 (m, 1H, H-1), 3.81 – 3.75 (m, 1H, H-2), 3.65 (dd, 1H, *J* = 8.9, 3.4 Hz, H-3), 3.54 – 3.45 (m, 1H, H-5), 3.40 (t, 1H, *J* = 8.9 Hz, H-4), 2.54 – 2.42 (m, 1H, C*H*H-CH=CH_2), 2.38 – 2.26 (m, 1H, C*H*H-CH=CH_2), 1.24 (d, 3H, *J* = 6.1 Hz, CH_3-6); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 135.9 (CH_2-*C*H=CH_2), 117.4 (CH_2-CH=*C*H$_2$), 78.5 (C-1), 74.3 (C-4), 72.4 (C-3), 72.3 (C-2), 71.0 (C-5), 34.7 (*C*H$_2$-CH=CH_2), 18.3 (CH_3-6); FT-IR (neat, cm^{-1}): 3371, 2977, 2934, 2361, 1644, 1418, 1253, 1140, 1057, 981, 916, 825, 779, 668, 550; HRMS: $[M+Na]^+$ calcd. for $C_9H_{16}O_4Na$: 211.0946, found 211.0944.

3-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-1-propene (13)



Compound **12** (1.92 g, 10.2 mmol, 1.0 eq.) was co-evaporated with toluene (1x) under argon atmosphere and dissolved in DMF (0.10 L). Sodium hydride (60% dispersion in mineral oil, 1.47 g, 36.5 mmol, 3.6 eq.) was added at 0°C. After 20 minutes, *p*-methoxybenzyl chloride (5.0 mL, 37 mmol, 3.6 eq.) and TBAI (0.38 g, 1.0 mmol, 0.1 eq.) were added. The reaction was allowed to warm-up to room temperature. After 6 hours, another portion of sodium hydride (60% dispersion in mineral oil, 0.40 g, 10 mmol, 1.0 eq.) was added and the reaction was allowed to stir overnight. The reaction mixture was quenched with MeOH at 0°C, diluted with H_2O and extracted with DCM. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10→20% Et_2O in pentane) gave compound **13** (4.4 g, 8.0 mmol, 79%) as a white solid. R_f: 0.84 (8/2 pentane/EtOAc); $[\alpha]_D^{20}$ +20.0° (*c* = 2.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.32 (d, 2H, Ar), 7.27 (d, 4H, Ar), 6.93 – 6.84 (m, 6H, Ar), 5.79 – 5.66 (m, 1H, CH_2-C*H*=CH_2), 5.08 – 4.98 (m, 2H, CH_2-CH=C*H*$_2$), 4.78 (d, 1H, *J* = 10.8 Hz, C*H*H PMB), 4.65 – 4.49 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.05 – 3.97 (m, 1H, H-1), 3.79 (s, 9H, 3x CH_3 PMB), 3.73 (dd, 1H, *J* = 7.9, 3.1 Hz, H-3), 3.70 – 3.65 (m, 1H, H-4), 3.62 (t, 1H, *J* = 3.3 Hz, H-5), 3.60 – 3.54 (m, 1H, H-2), 2.42 – 2.32 (m, 1H, C*H*H-CH=CH_2), 2.30 – 2.20 (m, 1H, C*H*H-CH=CH_2), 1.34 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 159.1 (C_q Ar), 134.2 (CH_2-*C*H=CH_2), 130.5, 130.3, 130.2 (C_q Ar), 129.5, 129.5, 129.3, 128.3 (Ar), 116.9 (CH_2-CH=*C*H$_2$), 113.6, 113.6, 113.5 (Ar), 79.5 (C-2), 77.3 (C-3), 74.5 (C-5), 74.0 (CH_2 PMB), 72.7 (C-1), 71.4, 71.1 (CH_2 PMB), 69.5 (C-4), 64.4 (CH_2 PMB), 55.0 (CH_3 PMB), 34.2 (*C*H$_2$-CH=CH_2), 17.9 (C-6); FT-IR (neat, cm^{-1}): 2934, 2836, 2360, 1641, 1612, 1586, 1512, 1464, 1421, 1358, 1302, 1245, 1173, 1079, 1033, 917, 820, 783, 755, 710, 668, 637, 587, 517; HRMS: $[M+Na]^+$ calcd. For $C_{33}H_{40}O_7Na$: 571.2672, found 571.2670.

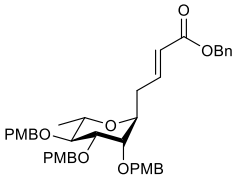
Benzyl acrylate (14)



Acrylic acid (19 mL, 0.28 mol, 1.0 eq.) was dissolved in DMF (0.56 L), followed by the addition of benzyl bromide (37 mL, 0.30 mol, 1.1 eq.) and K_2CO_3 (78 g, 0.56 mol, 2.0 eq.). The suspension was heated to 45°C overnight. The mixture was cooled to room temperature, diluted with brine and extracted with EtOAc (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (0→10% Et_2O in pentane) afforded the title compound (32 g, 0.20 mol, 71%) as a transparent oil. R_f: 0.88 (9/1 pentane/EtOAc);

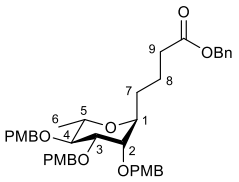
$[\alpha]_D^{20}$ +1.3° (*c* = 2.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.43 – 7.31 (m, 5H, Ar), 6.47 (dd, 1H, *J* = 17.3, 1.5 Hz, C*H*=CH), 6.23 – 6.13 (m, 1H, CH_2=C*H*), 5.86 (dd, 1H, *J* = 10.4, 1.4 Hz, C*H*=CH), 5.22 (s, 2H, CH_2 Bn); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 166.1 (C=O), 135.9 (C_q Ar), 131.2 (*C*H_2=CH), 128.7, 128.4, 128.4, 128.3 (CH_2=*C*H , Ar) 66.4 (CH_2 Bn); FT-IR (neat, cm^{-1}): 3035, 1724, 1635, 1498, 1456, 1407, 1372, 1296, 1269, 1176, 1049, 984, 809, 751, 698.

Benzyl but-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-cis/trans-2-enoate (15)



Compound **13** (25.1 g, 45.8 mmol, 1.0 eq.) and benzyl acrylate **14** (19.3 mL, 128 mmol, 2.8 eq.) were co-evaporated with toluene (1x) under argon atmosphere. The mixture was dissolved in DCM (0.23 L) and the flask was shielded from light with aluminum foil. Grubbs 2nd gen. catalyst (0.78 g, 0.92 mmol, 0.02 eq.) was added and the reaction was continued to reflux overnight at 50°C. Upon completion determined by TLC analysis, the reaction mixture was filtered over Celite® and concentrated *in vacuo*. Purification by column chromatography (10→25% EtOAc in pentane) gave compound 1**5** (28.9 g, 42.2 mmol, 92%) as a white solid. R_f: 0.22 (7/3 pentane/EtOAc); $[\alpha]_D^{20}$ -12.4° (*c* = 2.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.45 – 7.31 (m, 5H, Ar), 7.31 – 7.17 (m, 7H, CH_2-C*H*=CH, Ar), 7.00 – 6.82 (m, 6H, Ar), 5.87 (d, 1H, CH_2-CH=C*H*), 5.20 (s, 2H, CH_2 Bn), 4.68 (d, 1H, *J* = 11.1 Hz, C*H*H PMB), 4.61 – 4.47 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.11 – 4.01 (m, 1H, H-1), 3.81 (s, 9H, 3x CH_3 PMB), 3.77 – 3.68 (m, 2H, H-3, H-5), 3.58 – 3.49 (m, 2H, H-2, H-4), 2.53 – 2.38 (m, 2H C*H*$_2$-CH=CH), 1.34 (d, 3H, *J* = 6.5 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 166.0 (C=O), 159.3 (C_q Ar), 145.6 (CH_2-*C*H=CH), 136.1, 130.5, 130.2, 130.0 (C_q Ar), 129.8, 129.6, 129.5, 128.6, 128.2 (Ar), 123.0 (CH_2-CH=*C*H), 113.8 (Ar), 78.8 (C-2), 76.1 (C-3), 75.0 (C-4), 73.5, 71.7, 71.2 (CH_2 PMB), 70.9 (C-5), 70.3 (C-1), 66.1 (CH_2 Bn), 55.2 (CH_3 PMB), 33.3 (*C*H_2-CH=CH), 17.7 (CH_3-6); FT-IR (neat, cm^{-1}): 2934, 2836, 1717, 1655, 1612, 1586, 1512, 1456, 1376, 1302, 1247, 1211, 1172, 1111, 1080, 1033, 820, 753, 698, 589, 518; HRMS: $[M+Na]^+$ calcd. for $C_{41}H_{46}O_9Na$: 705.3040, found 705.3052.

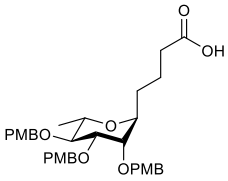
Benzyl 4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-butanoate (16)



Compound **15** (15.4 g, 22.6 mmol, 1.0 eq.) was co-evaporated with toluene (1x) under argon atmosphere and dissolved in DCE (90 mL). Ruthenium trichloride (0.89 g, 4.2 mmol, 0.19 eq.) was added and the argon balloon was replaced with an empty balloon. The reaction was cooled to 0°C, $NaBH_4$ (2.7 g, 72.3 mmol, 3.2eq.) was added after which MeOH (9.15 mL) was carefully added. The mixture was heated to 40°C for 4.5 hours, subsequently quenched with MeOH at 0°C. The reaction mixture was diluted with DCM and washed with brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (1→4% acetone in DCM) yielded the title compound (13.3 g, 22.6 mmol, 86%) as a transparent sticky oil. R_f: 0.22 (7/3 pentane/EtOAc); $[\alpha]_D^{20}$ +24.0° (*c* = 2.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.39 – 7.18 (m, 11H, Ar), 6.92 – 6.80 (m, 6H, Ar), 5.11 (s, 2H, CH_2 Bn), 4.74 (d,

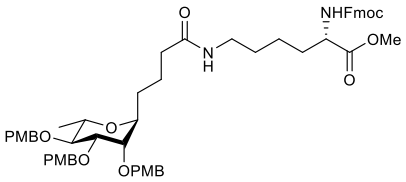
1H, *J* = 10.7 Hz, C*H*H PMB), 4.63 – 4.47 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 3.93 – 3.84 (m, 1H, H-1), 3.80 (s, 9H, 3x CH_3 PMB), 3.65 (dd, 1H, *J* = 7.8, 3.1 Hz, H-3), 3.60 – 3.47 (m, 3H, H-2, H-4, H-5), 2.34 (t, 2H, *J* = 7.1 Hz CH_2-9), 1.81 – 1.66 (m, 1H, C*H*H-8), 1.66 – 1.53 (m, 2H, C*H*H-8, C*H*H-7), 1.42 – 1.33 (m, 1H, C*H*H-7), 1.30 (d, 3H, CH_3-6, *J* = 6.1 Hz); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.3 (C=O), 159.3, 159.3, 136.1, 130.5 (C_q Ar), 129.7, 129.5, 128.7, 128.3, 128.3, 113.9, 113.8, 113.8 (Ar), 79.9 (C-2), 78.2 (C-3), 75.6 (C-4), 74.3 (CH_2 PMB), 73.2 (C-1), 71.8, 71.4 (CH_2 PMB), 69.5 (C-5), 66.3 (CH_2 Bn), 55.4 (CH_3 PMB), 33.9 (CH_2-9), 28.7 (CH_2-7), 21.5 (CH_2-8), 18.2 (CH_3-6); FT-IR (neat, cm^{-1}): 2934, 2836, 1733, 1611, 1586, 1512, 1456, 1421, 1567, 1301, 1245, 1172, 1109, 1079, 1032, 820, 752, 699, 637, 581, 516; HRMS: $[M+Na]^+$ calcd. for $C_{41}H_{48}O_9Na$: 707.3196, found 707.3216.

3-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-butanoic acid (17)



Compound **16** (22.7 g, 33.2 mmol, 1.0 eq.) was dissolved in a mixture of THF/MeOH/H_2O (7/2/1 v/v/v, 0.11 L). The reaction was cooled to 0°C and $LiOH{\cdot}H_2O$ (3.48 g, 83 mmol, 2.5 eq.) was added. The reaction was heated to 40°C for 4 hours, after which TLC analysis showed full conversion of the starting material. The reaction mixture was acidified with 1 M HCl to pH = 4-5 and extracted with DCM (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (1→20% acetone in DCM + 0.1% AcOH) addorded the title compound (18.3 g, 31 mmol, 96%) as a sticky yellow oil. R_f: 0.32 (4/1 DCM/acetone); $[\alpha]_D^{20}$ +37.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30 – 7.18 (m, 6H, Ar), 6.90 – 6.81 (m, 6H, Ar), 4.72 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.58 – 4.48 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 3.92 – 3.85 (m, 1H, H-1), 3.80 (s, 9H, 3x CH_3 PMB), 3.67 (dd, 1H, *J* = 7.7, 3.1 Hz, H-3), 3.63 – 3.56 (m, 1H, H-5), 3.54 – 3.48 (m, 2H, H-2, H-4), 2.32 (t, 2H, *J* = 7.1 Hz, CH_2-9), 1.77 – 1.63 (m, 1H, C*H*H-8), 1.63 – 1.51 (m, 2H, C*H*H-8, C*H*H-7), 1.47 – 1.36 (m, 1H, C*H*H-7), 1.30 (d, 3H, *J* = 6.3 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 178.9 (C=O), 159.3, 130.7, 130.6, 130.4 (C_q PMB), 129.8, 129.7, 129.5, 113.9, 113.9 (Ar), 79.7 (C-2), 77.9 (C-3), 75.6 (C-4), 74.2 (CH_2 PMB), 73.0 (C-1), 71.8, 71.4 (CH_2 PMB), 69.6 (C-5), 55.4 (CH_3 PMB), 33.5 (CH_2-9), 28.7 (CH_2-7), 21.2 (CH_2-8), 18.1 (CH_3-6); FT-IR (neat, cm^{-1}): 2934, 2836, 1721, 1707, 1611, 1586, 1512, 1463, 1359, 1302, 1245, 1173, 1108, 1077, 1032, 819, 756, 710, 637, 584, 516; HRMS: $[M+Na]^+$ calcd. for $C_{34}H_{42}O_9Na$: 617.2727, found 617.2736.

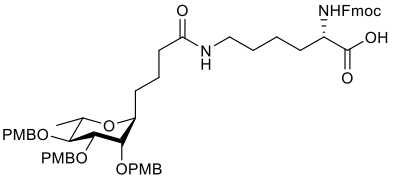
N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-L-lysine-methyl ester (18)



Compound **17** (3.0 g, 5.0 mmol, 1.0 eq.) and compound Fmoc-L-Lys-OMe·HCl (2.4 g, 6.3 mmol, 1.3 eq.) were co-evaporated with toluene (2x) under argon atmosphere. The mixture was dissolved in DMF (25 mL). HCTU (2.49 g, 6.0 mmol, 1.2 eq.) and DIPEA (2.6 mL, 15 mmol, 3.0 eq.) were subsequently added at 0°C. The reaction was allowed to warm-up to room temperature and stirred for 5 hours. The reaction mixture was quenched

with H_2O and diluted with EtOAc. The organic layer was subsequently washed with 1 M HCl (2x), sat. aq. $NaHCO_3$ (1x) and brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10→70% acetone in DCM) gave compound **18** (3.8 g, 4.0 mmol, 80%) as a white solid. R_f: 0.69 (4/1 DCM/acetone); $[\alpha]_D^{20}$ -33.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.78 – 7.72 (m, 2H, Ar), 7.64 – 7.57 (m, 2H, Ar), 7.43 – 7.36 (m, 2H, Ar), 7.34 – 7.18 (m, 9H, Ar, Ar), 6.89 – 6.81 (m, 6H, Ar), 5.63 (t, 1H, *J* = 5.8 Hz, NH), 5.53 (d, 1H, *J* = 8.3 Hz, N*H*Fmoc), 4.72 (d, 1H, *J* = 10.8 Hz, C*H*H PMB), 4.60 – 4.45 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.45 – 4.31 (m, 3H, 3H, CH_2 Fmoc, CH L-Lys), 4.22 (t, 1H, *J* = 7.1 Hz, CH Fmoc), 3.94 – 3.84 (m, 1H, H-1), 3.79 (s, 9H, 3x CH_3 PMB), 3.74 (s, 3H, OCH_3), 3.66 (dd, 1H, *J* = 7.7, 3.1 Hz, H-3), 3.63 – 3.55 (m, 1H, H-5), 3.54 – 3.49 (m, 2H, H-2, H-4), 3.21 (q, 2H, *J* = 6.7 Hz, CH_2 ε-L-Lys), 2.10 (t, 2H, *J* = 7.0 Hz, CH_2-9), 1.92 – 1.78 (m, 1H, C*H*H-8), 1.76 – 1.33 (m, 9H, C*H*H-8, CH_2-7, 3x CH_2 β/γ/δ-L-Lys), 1.30 (d, 3H, *J* = 6.2 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.0, 172.8 (C=O), 159.3, 159.2, 156.1, 143.9, 143.8, 141.3, 130.7, 130.5, 130.5 (C_q Ar), 129.6, 129.4, 127.8, 127.1, 125.1, 120.0, 113.8, 113.7 (Ar), 79.7 (C-2), 77.9 (C-3), 75.6 (C-4), 74.1 (CH_2 PMB), 73.1 (C-1), 71.7, 71.3 (CH_2 PMB), 69.5 (C-5), 67.0 (CH_2 Fmoc), 55.3 (CH_3 PMB), 53.7 (CH L-Lys), 52.5 (OCH_3), 47.2 (CH Fmoc), 39.0 (CH_2 ε-L-Lys), 36.1 (CH_2-9), 32.1 (CH_2-7), 29.1, 28.7, 22.5, 22.1 (CH_2-8, CH_2 β/γ/δ-L-Lys), 17.9 (CH_3-6); FT-IR (neat, cm^{-1}): 3331, 2935, 1752, 1650, 1612, 1513, 1451, 1302, 1248, 1174, 1081, 1034, 847, 760, 742, 563; HRMS: $[M+Na]^+$ calcd. for $C_{56}H_{66}N_2O_{12}Na$: 981.4513, found 981.4545; LC-MS: Rt = 6.35 min (Gemini C_{18}, 10-90% MeCN, 12.5 min run).

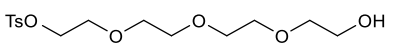
N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-L-lysine (8)



Compound **18** (2.8 g, 2.9 mmol, 1.0 eq.) was dissolved in THF (40 mL) and cooled to 0°C. A solution of LiOH in H_2O (0.30 M, 19 mL, 5.7 mmol, 2.0 eq.) was slowly added. After 40 minutes, the solution was diluted with EtOAc and acidified with 1 M HCl. The aqueous layer was extracted with EtOAc (2x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (15→25% acetone in DCM + 0.1% AcOH) afforded the title compound (1.94 g, 2.06 mmol, 71%) as a white solid. R_f: 0.26 (4/1 DCM/acetone + 0.1% AcOH); $[\alpha]_D^{20}$ +39.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.76 (d, 2H, *J* = 7.6 Hz, Ar), 7.62 (t, 2H, *J* = 7.3 Hz, Ar), 7.43 – 7.36 (m, 2H, Ar), 7.35 – 7.18 (m, 9H, Ar, Ar), 6.91 – 6.82 (m, 6H, Ar), 5.89 (t, 1H, *J* = 5.8 Hz, NH), 5.78 (d, 1H, *J* = 7.9 Hz, N*H*Fmoc), 4.71 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.61 – 4.46 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.44 – 4.34 (m, 3H, CH_2 Fmoc, CH L-Lys), 4.22 (t, 1H, *J* = 7.0 Hz CH Fmoc), 3.96 – 3.88 (m, 1H, H-1), 3.81 (s, 9H, 3x CH_3 PMB), 3.73 – 3.62 (m, 2H, H-3, H-5), 3.59 – 3.50 (m, 2H, H-2, H-4), 3.31 – 3.17 (m, 2H, CH_2 ε-L-Lys), 2.16 (t, 2H, CH_2-9), 1.95 – 1.84 (m, 1H, C*H*H-8), 1.83 – 1.75 (m, 1H, C*H*H-8), 1.74 – 1.64 (m, 1H, C*H*H-7), 1.64 – 1.35 (m, 7H, C*H*H-7, 3x CH_2 β/γ/δ-L-Lys), 1.32 (d, 3H, *J* = 6.4 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.2, 173.7 (C=O), 159.4, 159.3, 156.2, 144.0, 143.9, 141.4, 130.5, 130.4, 129.8 (C_q Ar), 129.6, 127.8, 127.2, 125.3, 120.1,

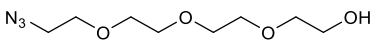
113.9 (Ar), 79.3 (C-2), 77.3 (C-3), 75.6 (C-4), 74.0 (CH_2 PMB), 72.7 (C-1), 71.8, 71.5 (CH_2 PMB), 70.0 (C-5), 67.1 (CH_2 Fmoc), 55.4 (CH_3 PMB), 53.6 (CH L-Lys), 47.2 (CH Fmoc), 39.1 (CH_2 ε-L-Lys), 36.1 (CH_2-9), 31.8 (CH_2-7), 29.0, 28.7, 22.3, 22.0 (CH_2-8, CH_2 β/γ/δ-L-Lys), 17.9 (CH_3-6); FT-IR (neat, cm^{-1}): 2930, 1719, 1612, 1512, 1451, 1302, 1247, 1174, 1080, 1034, 821, 741; HRMS: $[M+Na]^+$ calcd. for $C_{55}H_{64}N_2O_{12}Na$: 967.4357, found 967.4385; LC-MS: Rt = 9.38 min (Gemini C_{18}, 10-90% MeCN, 12.5 min run).

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (22)



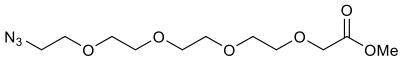
Triethylamine (10 mL, 74 mmol, 1.5 eq.) and *p*-toluenesulfonyl chloride (9.5 g, 50 mmol, 1.0 eq.) were added to a solution of tetraethyleneglycol (86 mL, 0.50 mol, 10 eq.) in DCM (62 mL) under an argon atmosphere. After stirring overnight, the reaction mixture was washed with H_2O (1x) and the aqueous layer was extracted with DCM (1x). The combined organic layers were washed three times with an aqueous solution of citric acid (0.28 M, 0.28 L). After concentration of the organic layer *in vacuo*, the title compound (16 g, 46 mmol, 93%) was obtained as a yellow oil. R_f: 0.76 (1/9 pentane/EtOAc); $[\alpha]_D^{20}$ +19.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.61 (d, 2H, *J* = 7.8 Hz, Ar), 7.19 (d, 2H, *J* = 7.9 Hz, Ar), 3.99 (t, 2H, *J* = 4.7 Hz, CH_2), 3.54 – 3.48 (m, 4H, 2x CH_2), 3.45 (s, 4H, 2x CH_2), 3.43 – 3.35 (m, 6H, 3x CH_2), 3.16 (s, 1H, OH), 2.27 (s, 3H, CH_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 144.5, 132.4 (C_q Ar), 129.5, 127.5 (Ar), 72.1, 70.1, 69.9, 69.8, 69.0, 68.1, 61.1 (CH_2), 21.2 (CH_3); FT-IR (neat, cm^{-1}): 2876, 1598, 1453, 1354, 1189, 1176, 1096, 1009, 922, 817, 776, 664, 555; HRMS: $[M+Na]^+$ calcd. for $C_{15}H_{23}N_3O_6SNa$: 373.1308, found 373.1132.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (23)



NaN_3 (6.0 g, 92 mmol, 2.0 eq.) was added to a solution of compound **22** (16 g, 46 mmol, 1.0 eq.) in DMF (62 mL) and the obtained suspension was heated to 90°C overnight. The reaction mixture was diluted with H_2O and extracted with DCM (2x) and EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo,* which gave compound **23** (10 g, 44 mmol, 96%) as a yellow oil. R_f: 0.22 (1/9 pentane/EtOAc); $[\alpha]_D^{20}$ +7.5° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 3.59 – 3.48 (m, 12H, 6x CH_2), 3.44 (dd, 2H, *J* = 5.4, 3.9 Hz, C*H*$_2$OH), 3.24 (t, 2H, CH_2N_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 72.3 (CH_2OH), 70.3, 70.2, 70.2, 69.9, 69.7, 61.2 (CH_2), 50.3 (CH_2N_3); FT-IR (neat, cm^{-1}): 2988, 1748, 1434, 1371, 1217, 1182, 1149, 1055, 1027, 973, 889, 601, 563, 501; HRMS: $[M+Na]^+$ calcd. for $C_8H_{17}N_3O_4Na$: 242.1117, found 242.1118.

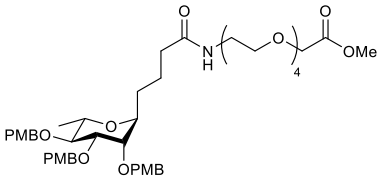
Methyl 14-azido-3,6,9,12-tetraoxatetradecanoate (19)



Compound **23** (2.2 g, 9.7 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under argon atmosphere and dissolved in THF (50 mL). The solution was cooled to 0°C and sodium hydride (60% dispersion in mineral oil, 0.78 g, 19 mmol, 2.0 eq.) was added. After stirring for 15 minutes, methyl bromoacetate (2.4 mL, 25 mmol, 2.6 eq.) was added and the mixture was allowed to warm-up to room temperature overnight. The reaction

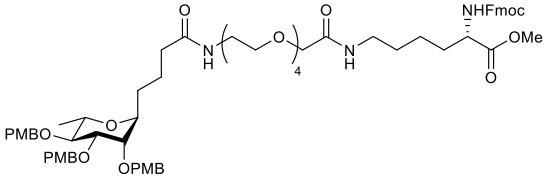
mixture was quenched with MeOH at 0°C and concentrated *in vacuo*. Purification by column chromatography (20→80% EtOAc in pentane) yielded compound **19** (2.3 g, 8.0 mmol, 83%) as a yellow oil. R_f: 0.51 (1/9 pentane/EtOAc); $[\alpha]_D^{20}$ +14.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 4.17 (s, 2H, CH_2), 3.77 – 3.71 (m, 5H, CH_2, OCH_3), 3.71 – 3.63 (m, 12H, 6x CH_2), 3.38 (t, 2H, *J* = 5.6, 4.5 Hz, CH_2N_3); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 171.0 (C=O), 71.0, 70.8, 70.8, 70.7, 70.2, 68.8 (CH_2), 51.9 (CH_3), 50.8 (CH_2N_3); FT-IR (neat, cm^{-1}): 2870, 2103, 1755, 1439, 1349, 1285, 1211, 1121, 942, 853, 706, 558; HRMS: $[M+Na]^+$ calcd. For $C_{11}H_{21}N_3O_6Na$: 314.1328, found 314.1331.

Methyl 3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate (20)



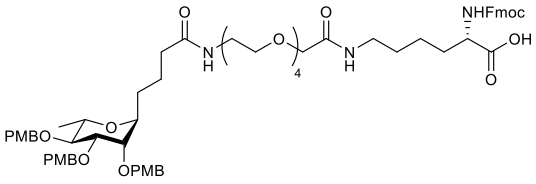
Compound **19** (0.18 g, 0.67 mmol, 3.0 eq.) was co-evaporated with toluene (3x) under argon atmosphere and dissolved in dry THF (6.5 mL). Pd/C (10%, 18 mg) was added and a $H_{2(g)}$-filled balloon replaced the argon balloon. The reaction was allowed to stir for 5 hours. The mixture was filtered over a Whatmann-filter and concentrated *in vacuo*. The obtained amine and was co-evaporated with toluene (2x) under argon atmosphere. Compound **17** (0.14 g, 0.22 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under argon atmosphere and dissolved in DMF (1.0 mL), followed by the addition of HCTU (0.11 g, 0.27 mmol, 1.2 eq.). After 15 minutes a solution of the obtained amine in DMF (0.20 mL) and DIPEA (0.11 mL, 0.69 mmol, 3.0 eq.) were added and the reaction mixture was stirred for 75 minutes. The reaction was quenched with 1 M HCl at 0°C and diluted with EtOAc. The organic layer was subsequently washed with 1 M HCl (1x) and brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (10→40% acetone in DCM) yielded the title compound (0.15 g, 0.18 mmol, 82% over two steps) as a sticky oil. R_f: 0.25 (4/1 DCM/acetone); $[\alpha]_D^{20}$ +44.3° (*c* = 2.0, DCM); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.31 – 7.25 (m, 4H, Ar), 7.25 – 7.19 (m, 2H, Ar), 6.92 – 6.84 (m, 6H, Ar), 6.53 (t, 1H, NH), 4.66 (d, 1H, *J* = 10.6 Hz, C*H*H PMB), 4.55 – 4.45 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.09 (s, 2H, CH_2 spacer), 3.89 – 3.82 (m, 1H, H-1), 3.77 (s, 9H, 3x CH_3 PMB), 3.70 (dd, 1H, *J* = 8.0, 3.2 Hz, H-3), 3.67 (s, 3H, OCH_3), 3.63 – 3.59 (m, 3H, H-4, CH_2 spacer), 3.58 – 3.55 (m, 2H, CH_2 spacer), 3.55 – 3.50 (m, 9H, H-5, 4x CH_2 spacer), 3.45 (t, 2H, *J* = 5.6 Hz, CH_2 spacer), 3.37 (t, 1H, *J* = 7.9 Hz, H-2), 3.29 (q, 2H, *J* = 5.6 Hz, CH_2 spacer), 2.12 (t, 2H, CH_2-9), 1.69 – 1.55 (m, 2H, C*H*H-8, C*H*H-7), 1.55 – 1.44 (m, 1H, C*H*H-8), 1.44 – 1.33 (m, 1H, C*H*H-7), 1.23 – 1.16 (m, 3H, CH_3-6); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ 173.6, 171.8 (C=O), 160.2, 132.1, 131.9, 131.9 (C_q Ar), 130.6, 130.5, 130.5, 114.6, 114.6, 114.5 (Ar), 80.7 (C-2), 79.2 (C-3), 77.3 (C-4), 74.6 (CH_2 PMB), 74.1 (C-1), 72.0, 71.9 (CH_2 PMB), 71.4, 71.1, 71.0, 70.9, 70.4 (CH_2 spacer), 69.9 (C-5), 68.9 (CH_2 spacer), 55.9 (CH_3 PMB), 52.2 (OCH_3), 39.8 (CH_2 spacer), 36.2 (CH_2-9), 29.3 (CH_2-7), 22.9 (CH_2-8), 18.7 (CH_3-6); FT-IR (neat, cm^{-1}): 3331, 2872, 1754, 1716, 1648, 1607, 1585, 1512, 1460, 1351, 1301, 1250, 1170, 1101, 1031, 827, 770, 698, 582; HRMS: $[M+Na]^+$ calcd. for $C_{45}H_{63}O_{14}Na$: 864,4146, found 864.4169; LC-MS: Rt = 4.14 min (Gemini C_{18}, 50-90% MeCN, 11 min run).

N_α-Fmoc-N_ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate]-L-lysine-methyl ester (21)



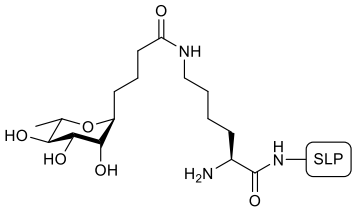
Compound **20** (0.48 g, 0.57 mmol, 1.0 eq.) was dissolved in a mixture of THF/H_2O (4/1 v/v, 5.7 mL) mixture and cooled to 0°C. LiOH·H_2O (73 mg, 1.7 mmol, 3.0 eq.) was added and the reaction was allowed to warm-up to room temperature and stirred for 40 minutes. The reaction mixture was diluted with EtOAc, acidified with 1 M HCl and subsequently washed with brine (1x). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The obtained intermediate was co-evaporated with toluene (1x) under argon atmosphere and dissolved in DMF (2.9 mL). Fmoc-L-Lys-OMe·HCl (0.29 g, 0.69 mmol, 1.2eq.), HCTU (0.28 g, 0.68 mmol, 1.2eq.) and DIPEA (0.30 mL, 1.7 mmol, 3.0 eq.) were subsequently added. After 2 hours, the reaction mixture was diluted with EtOAc and quenched with 1 M HCl. The organic layer was washed with 1 M HCl (1x), sat. aq. $NaHCO_3$ (1x) and brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (20→70% acetone in DCM) gave compound **21** (0.61 g, 0.51 mmol, 89%) as a sticky oil: R_f: 0.40 (8/1 DCM/acetone); $[\alpha]_D^{20}$ +24.3° (*c* = 2.0, DCM); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.85 – 7.78 (m, 2H, Ar), 7.71 – 7.63 (m, 2H, Ar), 7.45 – 7.37 (m, 2H, Ar), 7.36 – 7.29 (m, 2H, Ar), 7.29 – 7.23 (m, 4H, Ar), 7.23 – 7.18 (m, 2H, Ar), 7.16 (d, 1H, *J* = 7.8 Hz, NH), 6.91 – 6.82 (m, 6H, Ar), 6.54 (t, 1H, *J* = 5.8 Hz, NH), 6.27 (d, 1H, *J* = 7.9 Hz, N*H*Fmoc), 4.65 (d, 1H, *J* = 10.7 Hz, C*H*H PMB), 4.54 – 4.43 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.36 – 4.30 (m, 2H, CH_2 Fmoc), 4.24 – 4.18 (m, 1H, CH Fmoc), 4.15 – 4.06 (m, 1H, CH L-Lys), 3.90 – 3.81 (m, 3H, H-1, CH_2 spacer), 3.80 – 3.72 (m, 9H, 3x CH_3 PMB), 3.72 – 3.67 (m, 1H, H-3), 3.65 (s, 3H, OCH_3), 3.60 (t, 1H, *J* = 3.3 Hz, H-4), 3.59 – 3.47 (m, 13H, H-5, 6x CH_2 spacer), 3.46 (d, 2H, *J* = 5.2 Hz, CH_2 spacer), 3.37 (t, 1H, *J* = 7.9 Hz, H-2), 3.31 – 3.25 (m, 2H, CH_2 spacer), 3.24 – 3.12 (m, 2H, CH_2 ε-L-Lys), 2.15 – 2.10 (m, 2H, CH_2-9), 1.81 – 1.71 (m, 1H, C*H*H-8), 1.69 – 1.28 (m, 9H, C*H*H-8, CH_2-7, CH_2 β/γ δ-L-Lys), 1.19 (d, 3H, *J* = 3.1 Hz, CH_3-6); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ 173.9, 173.5, 170.7 (C=O), 160.1, 157.1, 145.0, 142.1, 132.0, 131.8 (C_q Ar), 130.4, 128.6, 128.0, 126.1, 120.9, 114.5 (Ar), 80.6 (C-2), 79.2 (C-3), 77.3 (C-4), 74.5 (CH_2 PMB), 74.0 (C-1), 72.0, 71.8, 71.6, 71.1 (CH_2 spacer), 71.0, 70.9, 70.8 (CH_2 PMB), 70.7 (CH_2 spacer), 70.3 (CH_2 Fmoc), 69.8 (C-5), 67.1 (CH_2 Fmoc), 55.8 (CH_3 PMB), 55.0 (CH L-Lys), 52.7 (OCH_3), 47.9 (CH Fmoc), 39.7 (CH_2 spacer), 38.7, (CH_2 ε-L-Lys), 36.2 (CH_2-9), 31.6 (CH_2-7), 29.8, 29.7, 29.2, 23.5 (CH_2 β/γ/δ-L-Lys), 22.9 (CH_2-8), 18.6 (CH_3-6); FT-IR (neat, cm^{-1}): 3333, 2934, 1719, 1662, 1611, 1514, 1451, 1249, 1173, 1107, 1034, 822, 742; HRMS: $[M+Na]^+$ calcd. for $C_{66}H_{85}N_3O_{17}Na$: 1214.5777, found 1214.5812; LC-MS: Rt = 9.23 min (Gemini C_{18}, 10-90% MeCN, 12.5 min run).

N_α-Fmoc-N_ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate]-L-lysine (9)



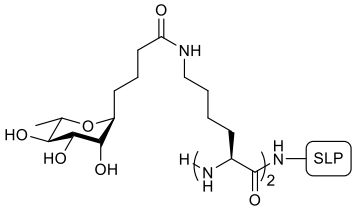
Compound **21** (0.34 g, 0.28 mmol, 1.0 eq.) was dissolved in THF (4.0 mL) and cooled to 0°C. A solution of LiOH in H_2O (0.30 M, 1.9 mL, 0.57 mmol, 2.0 eq.) was slowly added. After 45 minutes, the reaction mixture was diluted with EtOAc and acidified 1 M HCl to pH = 4/5. The organic layer was washed with brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (2→20% methanol in DCM) gave compound **9** (0.19 g, 0.16 mmol, 66%) as a sticky oil. R_f: 0.77 (4/1 DCM/MeOH); $[\alpha]_D^{20}$ +30.3° (*c* = 2.0, DCM); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.80 (d, 2H, *J* = 7.5 Hz, Ar), 7.71 – 7.59 (m, 2H, Ar), 7.46 – 7.11 (m, 10H, Ar,), 6.92 – 6.78 (m, 6H, Ar), 6.66 (s, 1H, NH), 6.19 (d, 1H, *J* = 7.7 Hz, N*H*Fmoc), 4.64 (d, 1H, *J* = 10.7 Hz, C*H*H PMB), 4.53 – 4.40 (m, 5H, 2x CH_2 PMB, C*H*H PMB), 4.31 (d, 2H, *J* = 7.0 Hz, CH_2 Fmoc), 4.20 (t, 1H, *J* = 7.1 Hz, CH Fmoc), 4.14 – 4.04 (m, 1H, CH L-Lys), 3.85 (s, 3H, H-1, CH_2 spacer), 3.80 – 3.72 (m, 9H, 3x CH_3 PMB), 3.70 – 3.66 (m, 1H, H-3), 3.60 (t, 1H, *J* = 3.2 Hz, H-5), 3.55 – 3.48 (m, 10H, 5x CH_2 spacer), 3.43 (t, 2H, *J* = 5.6 Hz, CH_2 spacer), 3.39 – 3.32 (m, 2H, H-2, H-4), 3.31 – 3.24 (m, 2H, CH_2 spacer), 3.22 – 3.13 (m, 2H, CH_2 ε-L-Lys), 2.16 – 2.07 (m, 2H, CH_2-9), 1.85 – 1.73 (m, 1H, C*H*H-8), 1.73 – 1.30 (m, 9H, C*H*H-8, CH_2-7, CH_2 β/γ/δ-L-Lys), 1.18 (d, 3H, *J* = 6.3 Hz, CH_3-6); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ 159.9, 141.8, 131.7, 131.6, 131.5 (C_q Ar), 130.3, 130.2, 129.6, 128.9, 128.4, 127.8, 125.9, 120.7, 114.3, 114.2 (Ar), 80.4 (C-2), 78.9 (C-3), 77.0 (C-4), 74.2 (CH_2 PMB), 73.8 (C-1), 71.7, 71.6, 71.3 (CH_2 spacer), 70.8, 70.7 (CH_2 PMB), 70.5, 70.4, 70.1 (CH_2 spacer), 69.6 (C-5), 66.9 (CH_2 Fmoc), 55.6 (CH_3 PMB), 54.0 (CH L-lys), 47.7 (CH Fmoc), 39.5 CH_2 spacer, 38.6 (CH_2 ε-L-Lys), 36.0 (CH_2-9), 31.5 (CH_2-7), 29.5, 28.9, 23.2 (CH_2 β/γ/δ-L-Lys), 22.7 (CH_2-8), 18.3 (CH_3-6); FT-IR (neat, cm^{-1}): 3321, 2932, 1715, 1657, 1611, 1585, 1512, 1451, 1301, 1246, 1173, 1081, 1036, 821, 760, 733, 701, 662, 621, 583, 543, 515; HRMS: $[M+Na]^+$ calcd. for $C_{65}H_{83}N_3O_{17}Na$: 1200,5620, found 1200.5673; LC-MS: Rt = 8.79 min (Gemini C_{18}, 10-90% MeCN, 12.5 min run).

Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (1)



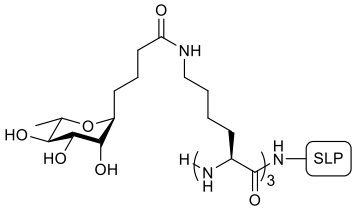
Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 10 µmol scale was elongated with **8** (3.0 eq., two hours coupling time). After cleaving from the resin, the peptide was purified by RP-HPLC. After lyophilisation, conjugate **1** (4.5 mg, 1.9 µmol, 19%) was obtained as a white solid. UPLC-MS: Rt = 3.56 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{109}H_{184}N_{25}O_{35}Na$: 2403.3, found 2403.6; HRMS: $[M+H]^{3+}$ calcd. for $C_{109}H_{186}O_{35}N_{25}$: 801.78422, found 801.78483.

Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (2)



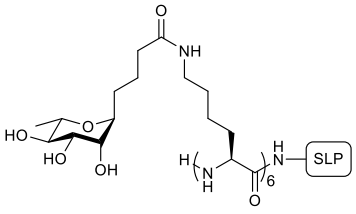
Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 10 µmol scale was elongated two times with **8** (3.0 eq., two hours coupling time). After cleaving from the resin, the peptide was purified by RP-HPLC. After lyophilisation, conjugate **2** (4.5 mg, 1.6 µmol, 16%) was obtained as a white solid. UPLC-MS: Rt = 3.49 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{125}H_{222}N_{27}O_{41}Na$: 2747.5, found 2749.5; HRMS: $[M+H]^{3+}$ calcd. for $C_{125}H_{214}O_{41}N_{27}$: 916.51580, found 916.51645.

Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (3)



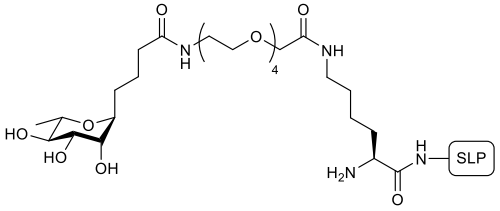
Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 10 µmol scale was elongated three times with **8** (3.0 eq., two hours coupling time). After cleaving from the resin, the peptide was purified by RP-HPLC. After lyophilisation, conjugate **3** (3.2 mg, 1.0 µmol, 10%) was obtained as a white solid. UPLC-MS: Rt = 3.47 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{141}H_{240}N_{29}O_{47}Na$: 3091.7, found 3095.5; HRMS: $[M+H]^{3+}$ calcd. for $C_{141}H_{242}O_{47}N_{29}$: 1031.24738, found 1031.25004.

Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(*N*ε-[butan-4-(α-L-rhamnosyl)-amide])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (4)



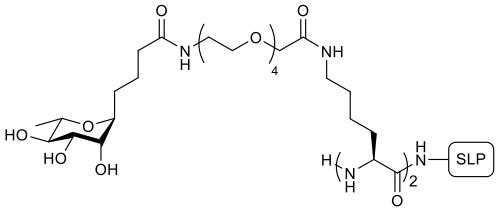
Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 10 µmol scale was elongated six times with **8** (3.0 eq., two hours coupling time). After cleaving from the resin, the peptide was purified by RP-HPLC. After lyophilisation, conjugate **4** (2.6 mg, 0.63 µmol, 6%) was obtained as a white solid. UPLC-MS: Rt = 3.34 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{189}H_{324}N_{35}O_{65}Na$: 4124.9, found 4128.7; HRMS: $[M+H]^{3+}$ calcd. for $C_{189}H_{326}O_{65}N_{35}$: 1375.44212, found 1375.44806.

Lys(*N*ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (5)



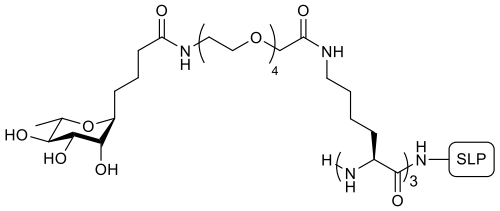
Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 5 µmol scale was elongated with **9** (3.0 eq.). Compound **9** (50 µL, 0.3 M in NMP) was preactivated by the addition of a solution of HATU (45µL, 0.67 M in NMP) and NMM (7 µL). The mixture was added to the resin and heated overnight to 39°C. MALDI analysis showed complete conversion of the starting peptide. Fmoc was cleaved using 3x 20% piperidine in NMP (400 µL) for 2, 5 and 10 min at RT. The resin was washed with NMP and DCM. The peptide was cleaved from the resin using TFA/H_2O (19/1 v/v, 1.0 mL, 3 h) and the peptide was precipitated in pentane/Et_2O (1/1 v/v, 12 mL). The precipitate was purified by RP-HPLC. After lyophilisation, conjugate **5** (2.0 mg, 0.76 µmol, 15%) was obtained as a white solid. UPLC-MS: Rt = 3.55 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{119}H_{203}N_{26}O_{40}Na$: 2636.5, found 2638.5; HRMS: $[M+H]^{3+}$ calcd. for $C_{119}H_{205}O_{40}N_{26}$: 879.49300, found 879.49355.

Lys(*N*ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Lys(*N*ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (6)



Two times elongation with **9** was performed using the conditions described for compound **5**. The synthesis was performed three times on 5 µmol scale with Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK. The combined precipitation was purified over RP-HPLC. After lyophilisation, conjugate **6** (0.9 mg, 0.28 µmol, 2%) was obtained as a white solid. UPLC-MS: Rt = 3.49 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{145}H_{251}N_{29}O_{51}Na$: 3214.8, found 3215.3; HRMS: $[M+H]^{3+}$ calcd. for $C_{145}H_{252}O_{51}N_{29}$: 1071.93335, found 1071.93421.

Lys(N_ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Lys(N_ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Lys(N_ε-[3,6,9,12-tetraoxatetra-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-decanoate])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-OH (7)



Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 2.5 µmol scale was elongated three times with **9** (3.0 eq.). Compound **9** (25 µL, 0.6 M in NMP) was preactivated by the addition of a solution of HATU (22.5 µL, 0.67 M in NMP) and NMM (1.8 µL). The mixture was added to the resin and heated overnight to 39°C. MALDI analysis showed still starting peptide and the mixture was heated for an additional 4 hours at 43°C. Fmoc was cleaved using 3x 20% piperidine in NMP (300 µL) for 2, 5 and 10 min at RT. For the second coupling, compound **9** (25 µL, 0.6 M in NMP) was preactivated by the addition of a solution of HATU (22.5 µL, 0.67 M in NMP) and NMM (1.8 µL). The mixture was added to the resin and heated overnight to 39°C. Fmoc was cleaved using 3x 20% piperidine in NMP (300 µL) for 2, 5 and 10 min at RT. For the third coupling, compound **9** (27 µL, 0.6 M in NMP) was preactivated by the addition of a solution of HATU (20 µL, 0.67 M in NMP) and NMM (1.8 µL). The mixture was added to the resin and heated overnight to 39°C. Fmoc was cleaved using 3x 20% piperidine in NMP (300 µL) for 2, 5 and 10 min at RT. The resin was washed with NMP, DCM and Et_2O. The peptide was cleaved from the resin using TFA/H_2O (19/1 v/v, 0.5 mL, 4 h) and the peptide was precipitated in pentane/Et_2O (1/1 v/v, 10 mL). The precipitation was purified over RP-HPLC. After lyophilisation, conjugate **7** (0.4 mg, 0.11 µmol, 4%) was obtained as a white solid. UPLC-MS: Rt = 3.47 min (ACQUITY UPLC BEH C18, 5 - 100% MeCN, 10 min run); MALDI-TOF MS (m/z): $[M+Na]^+$ calcd. for $C_{171}H_{297}N_{32}O_{62}Na$: 3791.1, found 3793.7; HRMS: $[M+H]^{3+}$ calcd. for $C_{171}H_{299}O_{62}N_{32}$: 1264.37370, found 1264.37536.

Footnotes and References

(1) Toes, R. E.; Blom, R. J.; Offringa, R.; Kast, W. M.; Melief, C. J. *J. Immunol.* **1996**, *156* (10), 3911–3918.
(2) Bijker, M. S.; van den Eeden, S. J. F.; Franken, K. L.; Melief, C. J. M.; van der Burg, S. H.; Offringa, R. *Eur. J. Immunol.* **2008**, *38* (4), 1033–1042.
(3) Zwaveling, S.; Mota, S. C. F.; Nouta, J.; Johnson, M.; Lipford, G. B.; Offringa, R.; van der Burg, S. H.; Melief, C. J. M. *J. Immunol.* **2002**, *169* (1), 350–358.
(4) Vermaelen, K. *Front. Immunol.* **2019**, *10*.
(5) Temizoz, B.; Kuroda, E.; Ishii, K. J. *Int. Immunol.* **2016**, *28* (7), 329–338.
(6) Ignacio, B. J.; Albin, T. J.; Esser-Kahn, A. P.; Verdoes, M. *Bioconjug. Chem.* **2018**, *29* (3), 587–603.
(7) van Dinther, D.; Stolk, D. A.; van de Ven, R.; van Kooyk, Y.; de Gruijl, T. D.; den Haan, J. M. M. *J. Leukoc. Biol.* **2017**, *102* (4), 1017–1034.
(8) Takai, T. *Nat. Rev. Immunol.* **2002**, *2* (8), 580–592.
(9) Abdel-Motal, U.; Wang, S.; Lu, S.; Wigglesworth, K.; Galili, U. *J. Virol.* **2006**, *80* (14), 6943–6951.
(10) Abdel-Motal, U. M.; Guay, H. M.; Wigglesworth, K.; Welsh, R. M.; Galili, U. *J. Virol.* **2007**, *81* (17), 9131–9141.
(11) Perdomo, M. F.; Levi, M.; Sallberg, M.; Vahlne, A. *Proc. Natl. Acad. Sci.* **2008**, *105* (34), 12515–12520.
(12) Carlson, C. B.; Mowery, P.; Owen, R. M.; Dykhuizen, E. C.; Kiessling, L. L. *ACS Chem. Biol.* **2007**, *2* (2),

119–127.
(13) McEnaney, P. J.; Parker, C. G.; Zhang, A. X.; Spiegel, D. A. *ACS Chem. Biol.* **2012**, *7* (7), 1139–1151.
(14) Jakobsche, C. E.; Parker, C. G.; Tao, R. N.; Kolesnikova, M. D.; Douglass, E. F.; Spiegel, D. A. *ACS Chem. Biol.* **2013**, *8* (11), 2404–2411.
(15) Parker, C. G.; Domaoal, R. A.; Anderson, K. S.; Spiegel, D. A. *J. Am. Chem. Soc.* **2009**, *131* (45), 16392–16394.
(16) Fletcher, E. A. K.; van Maren, W.; Cordfunke, R.; Dinkelaar, J.; Codee, J. D. C.; van der Marel, G.; Melief, C. J. M.; Ossendorp, F.; Drijfhout, J. W.; Mangsbo, S. M. *J. Immunol.* **2018**, *201* (1), 87–97.
(17) Mangsbo, S. M.; Fletcher, E. A. K.; van Maren, W. W. C.; Redeker, A.; Cordfunke, R. A.; Dillmann, I.; Dinkelaar, J.; Ouchaou, K.; Codee, J. D. C.; van der Marel, G. A.; *et al*. *Mol. Immunol.* **2018**, *93*, 115–124.
(18) Huflejt, M. E.; Vuskovic, M.; Vasiliu, D.; Xu, H.; Obukhova, P.; Shilova, N.; Tuzikov, A.; Galanina, O.; Arun, B.; Lu, K.; *et al*. *Mol. Immunol.* **2009**, *46* (15), 3037–3049.
(19) Oyelaran, O.; McShane, L. M.; Dodd, L.; Gildersleeve, J. C. *J. Proteome Res.* **2009**, *8* (9), 4301–4310.
(20) Sarkar, S.; Lombardo, S. A.; Herner, D. N.; Talan, R. S.; Wall, K. A.; Sucheck, S. J. *J. Am. Chem. Soc.* **2010**, *132* (48), 17236–17246.
(21) Zhang, H.; Wang, B.; Ma, Z.; Wei, M.; Liu, J.; Li, D.; Zhang, H.; Wang, P. G.; Chen, M. *Bioconjug. Chem.* **2016**, *27* (4), 1112–1118.
(22) Sarkar, S.; Salyer, A. C. D.; Wall, K. A.; Sucheck, S. J. *Bioconjug. Chem.* **2013**, *24* (3), 363–375.
(23) Li, X.; Rao, X.; Cai, L.; Liu, X.; Wang, H.; Wu, W.; Zhu, C.; Chen, M.; Wang, P. G.; Yi, W. *ACS Chem. Biol.* **2016**, *11* (5), 1205–1209.
(24) Hossain, M. K.; Vartak, A.; Karmakar, P.; Sucheck, S. J.; Wall, K. A. *ACS Chem. Biol.* **2018**, *13* (8), 2130–2142.
(25) Chen, W.; Gu, L.; Zhang, W.; Motari, E.; Cai, L.; Styslinger, T. J.; Wang, P. G. *ACS Chem. Biol.* **2011**, *6* (2), 185–191.
(26) Myers, E. L.; Butts, C. P.; Aggarwal, V. K. *Chem. Commun.* **2006**, No. 42, 4434–4436.
(27) Sharma, P. K.; Kumar, S.; Kumar, P.; Nielsen, P. *Tetrahedron Lett.* **2007**, *48* (49), 8704–8708.
(28) See compound **35** of Chapter 4.
(29) Hiemstra, H. S.; Duinkerken, G.; Benckhuijsen, W. E.; Amons, R.; de Vries, R. R. P.; Roep, B. O.; Drijfhout, J. W. *Proc. Natl. Acad. Sci.* **1997**, *94* (19), 10313.