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Synthetic carbohydrate ligands for immune receptors

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Chapter 3

*Synthesis of O- and C-muramyl dipeptide–antigen conjugates**

Introduction

Currently, much effort is directed to improve and develop therapeutic cancer vaccines.¹ Cancer specific epitopes, such as neoantigens² or tumor-associated carbohydrate antigens³ are not actively targeted to and taken up by antigen presenting cells or elicit poor immunological responses. Therefore these are aided by adjuvants to enhance the immune response. Among the first was Freund's adjuvant: a water-in-oil emulsion of heat-killed mycobacteria resulting in a mixture of bacterial components, which turned out too toxic for human use. So far, only alum salts, oil-in-water emulsions, virosomes and a mixture of alum and monophosphoryl lipid A (AS04) have been licensed for human use.⁴ Although alum has proven its ability to enhance the potency of bacterial vaccines (requiring a humoral response), it cannot be used in cancer vaccines as it is unable to induce a cell-mediated immune response.⁵ One of the strategies to enhance the immunogenicity of cancer vaccines is the employment of conjugates in which the antigen is covalently bound to an adjuvant.^{6–8} In the search for suitable adjuvants, pathogen-associated molecular patterns have been extensively explored, as they bind

*The data presented in this Chapter were gathered in collaboration with Tony S. Koemans, Nick Zilverschoon, Nico J. Meeuwenoord, Stefan van der Vorm, Herman S. Overkleef, Dmitri V. Filippov, Gijsbert A. van der Marel and Jeroen D. C. Codée.

to pattern recognition receptors (PRRs), for example Toll-like receptors^{9,10}, which play an important role in activating our immune system. The Nucleotide binding Oligomerization Domain (NOD)-like receptors represent an intracellular PRR family recognizing specific parts of the bacterial cell wall peptidoglycan (PG).¹¹ Freund's adjuvant lends its adjuvant activity from many components of the PG of the cell wall of bacteria present in the mixture.¹² The PG polymer consists of repeating disaccharide units of β -(1,4)-linked *N*-acetylglucosamine and *N*-acetylmuramic acid, where the muramic acid is elongated with a peptide (Figure 1). NOD-1 is able to recognize and bind to *D*-glutamyl-meso-diaminopimelic acid (*iE*-DAP) and muramyl dipeptide (MDP) is the minimal structure of a NOD-2 ligand (Figure 1). MDP generally contains an *N*-acetyl group at the C-2 of the muramic acid residue (MDP(Ac)), but the PG of mycobacteria and actinobacteria contains MDP bearing a *N*-glycolyl moiety, MDP(Gly).

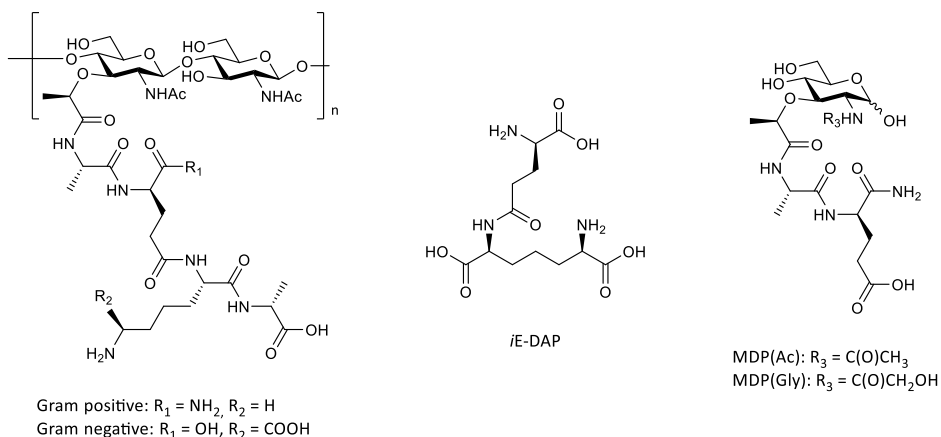


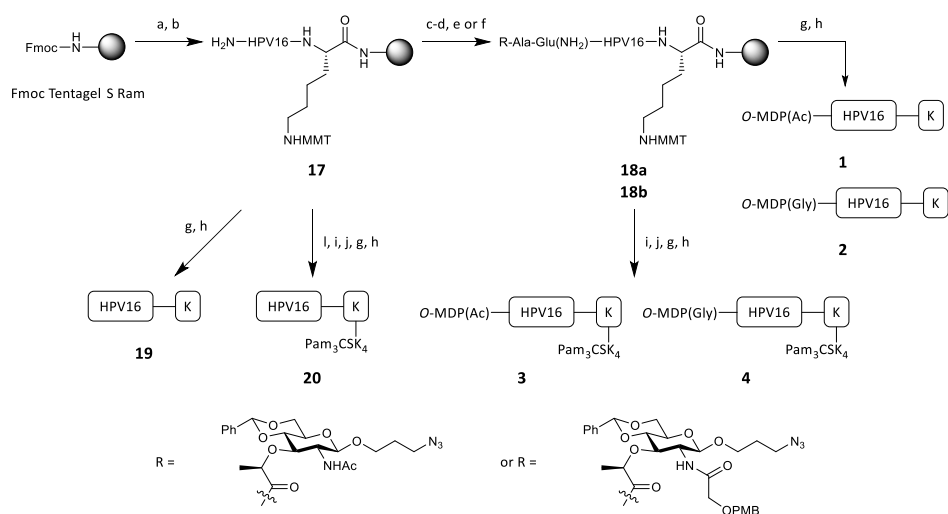
Figure 1. PG structures of Gram-positive or Gram-negative bacteria, NOD-1 ligand *iE*-DAP and NOD-2 ligands MDP(Ac) and MDP(Gly).

Willems *et al.* synthesized a set of conjugates, wherein the NOD-2 ligand, MDP(Ac), was covalently linked to an ovalbumin-derived peptide, harboring the MHC-I epitope SIINFEKL.¹³ Immunological evaluation of these conjugates indicated that the conjugates were internalized and processed, but they were unable to effectively induce maturation of dendritic cells (DCs). Incubation with a combination of PRR ligands can act synergistically^{14–16} to produce an enhanced immune response and synergy between NOD-2 and TLR2 ligands have been reported by several groups.^{17–19} Therefore, several bis-conjugates containing both an MDP(Ac) and a TLR2-ligand (Pam₃CSK₄) were synthesized.²⁰ These bis-conjugates improved the maturation of DCs leading to the proper activation of antigen-specific T cells.

In this Chapter, a set of MDP-human papillomavirus (HPV) conjugates is explored. HPV16 is one of the two types of HPV that are responsible for cervical cancer and the HPV16-derived peptide, GQAEPDRAHYNIVTFBKBKBDSTLRLBV, contains both a MHC-I and a MHC-II epitope. To prevent disulfide formation, the cysteine residues in this sequence are replaced for (S)-2-aminobutyric acid residues (B). Besides the MDP(Ac) ligand, the MDP(Gly) is also used for conjugation as it has been shown to be more potent than MDP(Ac).^{12,21,22} In the first part of this Chapter, the work of Zom *et al.*²⁰ is extended by conjugation of MDP(Ac) and MDP(Gly) to HPV16 via the carboxylic acid function of the *D*-isoglutamine of the MDP moiety using solid phase peptide synthesis (SPPS). Bis-conjugates carrying the TLR2-ligand, Pam₃CSK₄, in addition to the NOD2-ligand and the peptide antigen, have shown that good immunostimulatory properties are obtained using this conjugation site.²⁰ This led to the design of the first generation mono- and bis-conjugates **1-4**. Herein, a MDP building block **9a** or **9b** with a 3-azidopropanol linker at the anomeric position (*O*-MDP) was coupled to the peptide at the *N*-terminus and Pam₃CSK₄ via the *C*-terminal lysine (Figure 2). The anomeric 3-azidopropanol can be used for conjugation of MDP to additional peptides, fluorophores and other moieties at a later stage.

Previous work has shown that the glycosidic linkage of the *O*-MDP in the previously described peptide conjugates is relatively labile and that hydrolysis of this linkage can take place during acidic cleavage of the conjugates from the solid phase resin.¹³ The second part of this Chapter therefore describes the synthesis of a *C*-glycoside analogue of MDP, *C*-MDP, of which the anomeric linkage is stable against the acidic conditions used in SPPS as the exocyclic oxygen is replaced with a CH₂. Two lysine building blocks provided with a *C*-MDP were designed for application in SPPS, thereby facilitating the incorporation of MDP into peptides. One of the opportunities of these building blocks is the conjugation of MDP via the anomeric position as this was previously shown to be an ideal conjugation site.^{13,22} This resulted in the design of the second generation mono- and bis-conjugates **5-8** depicted in Figure 2. Both the *O*-MDP building blocks (**9a** and **9b**) and the *C*-MDP building blocks (**10a** and **10b**) are protected with acid-labile benzylidene, *p*-methoxybenzyl and *tert*-butyl groups to ensure the simultaneous deprotection and cleavage of the conjugate from the resin in the final stage of the SPPS.

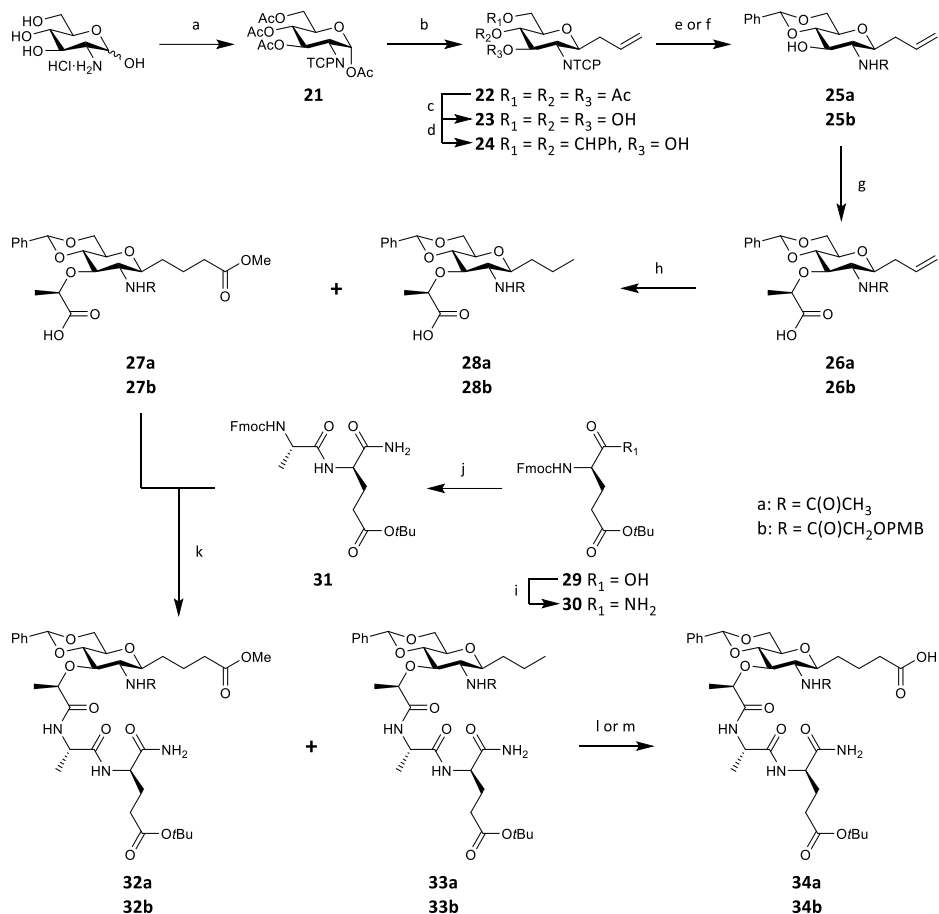
prepared with standard SPPS HCTU/Fmoc chemistry on a Tentagel S Ram solid support. Peptide **17** was elongated with Fmoc-Ala-OH, Fmoc-Glu(NH₂)-OH and **9a** or **9b**. The obtained peptides **18a** and **18b** were cleaved from the resin by treatment with a cocktail of TFA/TIS/H₂O (95/2.5/2.5 v/v/v) for 60 minutes. Longer reaction times lead to substantial hydrolysis of MDP-azidopropyl spacer, a side reaction previously also observed by Willems *et al.*¹³ The mono-conjugates were precipitated with Et₂O and purified by RP-HPLC yielding 5.4 mg **1** and 14.7 mg **2** in 3% and 8% respectively. To obtain bis-conjugates **3** and **4**, the MMT protecting group at the C-terminal lysine of immobilized peptides **18a** and **18b** was selectively removed with a cocktail of TFA/TIS/DCM (2/2/96 v/v/v). The obtained amino groups were extended with SK₄ using the automated peptide synthesizer, followed by manual coupling with palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH overnight. The peptides were then cleaved from the solid support and purification by RP-HPLC lead to **3** (5.2 mg, 1% yield) and **4** (3.3 mg, 1% yield).²⁴ Treatment of immobilized peptide **17** with a cocktail of TFA/TIS/H₂O (95/2.5/2.5 v/v/v) gave reference peptide **19** (9.4 mg, 10%). Besides, capping of immobilized peptide **17** with an acetyl, followed by MMT removal and elongation with Pam₃CSK₄ gave reference TLR2L-conjugate **20** (5.5 mg, 2%).



Scheme 2. Solid phase peptide synthesis of O-MDP mono- and bis-conjugates **1-4** and reference compound **19**. *Reagents and conditions:* a) 20% piperidine, DMF; b) Fmoc SPPS cycle for GQAEPDRAHYNIIVTFBKBDBSTLRLBVK; c) Fmoc-*i*-D-Glu(NH₂)-OH, HCTU, DIPEA, DMF; d) Fmoc-L-Ala-OH, HCTU, DIPEA, DMF; e) **9**, HCTU, DIPEA, DMF; f) **9b**, HCTU, DIPEA, DMF; g) TFA/TIS/H₂O (95/2.5/2.5 v/v/v), 1h; i) RP-HPLC; j) TFA/TIS/DCM (2/2/96 v/v/v); k) Fmoc SPPS cycle for SK₄; l) palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH, HCTU, DIPEA, DMF/DCM; Yield conjugates: **1**) 5.4 mg, 3%; **2**) 14.7 mg, 8%; **3**) 5.2 mg, 1%; **4**) 3.3 mg, 1%; **19**) 9.4 mg, 10%; **20**) 5.5 mg, 2%.

2nd generation: C-MDP conjugates

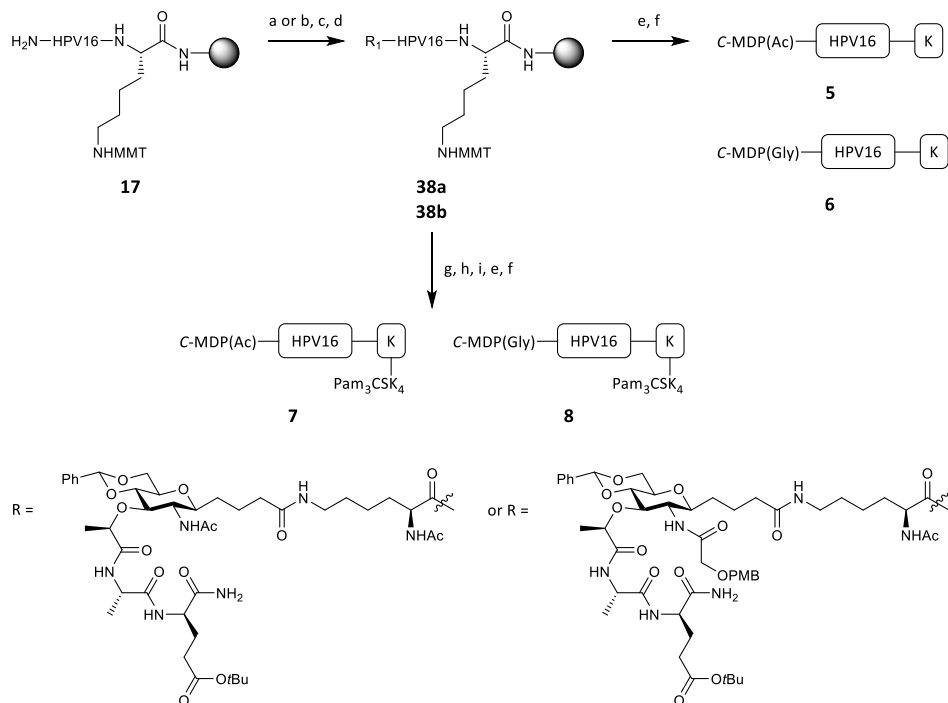
Synthesis of the 2nd generation MDP-conjugates **5-8**, required the SPPS building blocks **10a** and **10b**. Their synthesis starts with the installation of a TCP protecting group on commercially available glucosamine, followed by acetylation giving donor **21** (Scheme 3). Fuchss *et al.* reported a synthesis of **22** in which they first transformed acetyl donor **21** into the corresponding α -fluoride, which was then used to stereoselectively install the C-allyl group.²⁵ To shorten the synthesis of **22**, donor **21** was used directly for the C-glycosylation. Sonication of **21** with allyltrimethylsilane (5.0 eq.), and $\text{BF}_3 \cdot \text{OEt}_2$ (5.0 eq.) and TMSOTf (1.0 eq.), generating the strong Lewis acid $\text{BF}_2\text{OTf} \cdot \text{OEt}_2$ *in situ*,²⁶ delivered the C-glycoside **22** in 58% yield on 40 mmol scale. Deacetylation with *in situ* generated HCl (0.8 eq.) gave triol **23** in 94%. The use of more equivalents of HCl, or the use of sodium methoxide resulted in lower yields as ring opening of the TCP protecting group was observed. Subsequent installation of the benzylidene protecting group gave alcohol **24** in 87%. Removal of the TCP protecting group with ethylene diamine, followed by selective acetylation or glycolylation gave **25a** and **25b** in 83% and 98% respectively. Alkylation of **25a** and **25b** with (S)-(-)-2-chloropropionic acid provided the acids **26a** and **26b**. The next step entailed cross metathesis with methyl acrylate and subsequent reduction of the double bond to obtain **27a** and **27b**. Initial metatheses in DCM or DCE proceeded very sluggishly due to the poor solubility of the starting materials. Switching to THF as reaction solvent and the addition of CuI with heating to 60°C increased the conversion as indicated by NMR analysis. Voigtritter *et al.* have shown that the addition of CuI increases the reaction rate by stabilization of the catalyst by the iodine ion and simultaneous scavenging of the phosphine ligand.²⁷ However, even under these forcing conditions the cross-metatheses did not go to full completion, and the starting alkenes and α,β -unsaturated ester products could not be separated with column chromatography. Reduction of the double bonds in the metatheses product mixture was carried out with NaBH_4 and ruthenium trichloride²⁸ to give compound **27a**, contaminated with reduced starting material **28a**. Also reduction of the corresponding glycolyl derivative gave a mixture of target **27b** and side-product **28b**. Because purification was impossible, both mixtures (**27a/ 28a** and **27b/ 28b**) were condensed with dipeptide **31**, to generate the protected C-MDP building blocks **32a** and **32b**. The required dipeptide **31** was obtained by treatment of Fmoc protected *tert*-butyl glutamic acid **29** with di-*tert*-butyl dicarbonate, followed by NH_4HCO_3 mediated conversion of the intermediate anhydride to give amide **30** in 96% yield over two steps. Removal of the Fmoc-group in amino acid **30** with DBU, quenching with HOBt and coupling of the resulting free amine with Fmoc protected alanine afforded dipeptide **31** in 73% yield after crystallization.



Scheme 3. Synthesis of compounds **34a** and **34b**. *Reagents and conditions:* a) *i.* tetrachlorophthalic anhydride, NaOMe, MeOH, 50°C; *ii.* Ac₂O, pyridine, 51% over two steps; b) allyltrimethylsilane, BF₃·OEt₂, TMSOTf, MeCN, 58%; c) AcCl, MeOH, 94%; d) benzaldehyde dimethyl acetal, *p*-toluenesulfonic acid, DMF/MeCN, 60°C, 87%; e) *i.* ethylene diamine, EtOH, 90°C; *ii.* Ac₂O, NaHCO₃, THF/H₂O, **25a**: 83% over two steps; f) *i.* ethylene diamine, EtOH, 90°C; *ii.* *N*-succinimidyl-(*p*-methoxybenzyloxy)acetate (**16**), Et₃N, DCM, **25b**: 98% over two steps; g) (S)-(-)-2-chloropropionic acid, NaH, DMF, **26a**: 95%, **26b**: 91%; h) *i.* methyl acrylate, CuI, Grubbs 2nd gen. catalyst, THF, 40°C; *ii.* NaBH₄, RuCl₃, MeOH, THF, 40°C, **27a**: 64% over two steps, **27b**: 69% over two steps; i) Boc₂O, NH₄HCO₃, pyridine, dioxane, 96%; j) *i.* DBU, DCM; *ii.* HOBT, Fmoc-L-Ala-OH, EDC·HCl, DIPEA, DCM, 73%; k) *i.* DBU, DMF; *ii.* HOBT, **27a** or **27b**, HCTU, DIPEA, **32a**: quant. over two steps, **32b**: 89% over two steps; l) LiOH, H₂O₂, MeOH, room temperature, 5 h, **34a**: 73%; m) LiOH, H₂O₂, THF/H₂O, 0°C, 8 h, **34b**: 92%.

The same one-pot procedure was used for the coupling of dipeptide **31** to the acids **27a/28a** and **27b/28b** resulting in **32a** and **32b**, still inseparable from the corresponding side products **33a** and **33b**, respectively.²⁹ To obtain acids **34a** and **34b**, the methyl esters in **32a** and **32b** were carefully hydrolysed to prevent hydrolysis of the *tert*-butyl ester. To this end **32a** was treated with a mixture of LiOH and H₂O₂ in MeOH, yielding

with SK₄ using the automated synthesizer. After manual coupling with palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH overnight, the peptides were cleaved from the solid support, after which purification by RP-HPLC led to **7** (2.3 mg, 0.6% yield) and **8** (1.4 mg, 0.4% yield) respectively.



Scheme 5. Solid phase peptide synthesis of C-MDP mono- and bis-conjugates **5-8**. *Reagents and conditions:* a) **10a**, HCTU, DIPEA, DMF; b) **10b**, HCTU, DIPEA, DMF; c) 20% piperidine, DMF, d) Ac₂O, DIPEA, DMF; e) TFA/TIS/H₂O (95/2.5/2.5 v/v/v), 3h; f) RP-HPLC; g) TFA/TIS/DCM (2/2/96 v/v/v); h) Fmoc SPPS cycle for SK₄; i) palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH, HCTU, DIPEA, DMF/DCM. Yield conjugates: **5**) 7.2 mg, 6%; **6**) 9.2 mg, 6%; **7**) 2.3 mg, 0.6%; **8**) 1.4 mg, 0.4%.

Conclusion

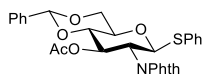
The synthesis of O-MDP and C-MDP building blocks, carrying either an N-acetyl or an N-glycolyl group and their incorporation in novel HPV16-conjugates is described. A crucial step in the synthesis of the C-MDP building blocks entailed the Grubbs cross metathesis, to functionalize the anomeric C-allyl moiety. Due to the poor solubility of the compounds this turned out to be a challenging transformation, giving rise to side products that could only be separated from the target compounds at a late stage of the synthesis.

The applicability of the novel C-MDP building blocks has been demonstrated in the assembly of four peptide-antigen conjugates. The acid stability of the C-MDP enables conjugation via the anomeric position of the MDP building block and its use in online solid phase syntheses of MDP functionalized oligopeptides. The ease of incorporation of the building block will allow the future generation of conjugates carrying multiple MDP moieties. As the building block can be incorporated in the peptide sequences through standard automated SPPS, all other types of conjugation chemistry remain available for the attachment of additional PRR-ligands, targeting entities and or fluorophores. The immunological properties of the prepared conjugates are presently under investigation.

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 3 Å 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: (NH₄)₆Mo₇O₂₄·H₂O (25 g/L), (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) and 10% H₂SO₄ in H₂O; bromocresol (0.4 g/L) in EtOH; KMnO₄ (7.5 g/L), K₂CO₃ (50 g/L) in H₂O. Staining was followed by charring at ~150°C. ¹H and ¹³C spectra were recorded on a Bruker AV-400 (400/100 MHz) spectrometer or a Bruker AV-500 Ultrashield (500/126 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 CDCl₃ 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. 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, a Vydac 219TP 5 µm Diphenyl, 250 x 10 mm column or a Cosmosil 5C4-MS 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. Infrared spectra were recorded on a Perkin Elmer Spectrum 2 FT-IR.

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside (**12**)

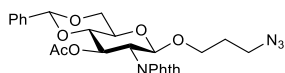


To a solution of phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside²³ (46 g, 93 mmol, 1.0 eq.) in DCM (0.37 L) was added DMAP (2.4 g, 19 mmol, 0.2 eq.), pyridine (23 mL, 0.29 mol, 3.0 eq.) and Ac₂O (13 mL, 0.14 mol, 1.5 eq.). The reaction was stirred for 5.5 hours, after which TLC analysis showed full conversion of the starting material. The mixture was diluted with EtOAc and subsequently washed with 1 M HCl (2x), sat. aq. NaHCO₃ (1x) and brine (1x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. Crystallization in pentane/Et₂O gave compound **12** in quantitative yield (49 g) as white crystals. R_f: 0.70 (1/1 pentane/EtOAc); [α]_D²⁰ +29.5° (*c* = 2.0, DCM); ¹H NMR (CDCl₃, 500 MHz, HH-COSY, HSQC): δ 7.90 – 7.86 (m, 2H, Ar), 7.78 – 7.73 (m, 2H, Ar), 7.47 – 7.43 (m, 2H, Ar), 7.41 – 7.37 (m, 2H, Ar), 7.37 – 7.34 (m, 3H, Ar), 7.30 – 7.25 (m, 3H, Ar), 5.90 (t, 1H, *J* = 9.5, 9.0, 0.9 Hz, H-3), 5.83 (d, 1H, *J* = 10.6, 0.9 Hz, H-1), 5.54 (s, 1H, CH benzylidene), 4.46 – 4.41 (m, 1H, CHH-6), 4.39 – 4.33 (m, 1H, H-2), 3.87 – 3.73 (m, 3H, H-4, H-5, CHH-6), 1.88 (s, 3H, CH₃ Ac); ¹³C-APT NMR (CDCl₃, 126 MHz, HSQC): δ 170.3, 168.0, 167.4 (C=O), 137.0 (C_q Ar), 134.6, 134.4, 133.2 (Ar), 131.8, 131.3 (C_q Ar), 131.3, 129.3, 129.2, 128.5, 128.4, 126.4, 123.9, 123.8 (Ar), 101.8 (CH benzylidene), 84.0 (C-1), 79.2 (C-4), 70.7, 70.7 (C-3, C-5), 68.7 (CH₂-6), 54.4 (C-2), 20.7 (CH₃ Ac); FT-IR (neat, cm⁻¹): 2877, 1776, 1742, 1716, 1584, 1479, 1440, 1382, 1294, 1220, 1094, 1033, 1013, 995, 965, 917, 893, 872, 827, 794, 749, 720, 699, 659, 643, 610, 530, 477; HRMS: [M+Na]⁺ calcd. for C₂₉H₂₅NO₇SNa: 554.1249, found 554.1251.

3-Azidopropanol (**13**)

HO-CH₂-CH₂-CH₂-N₃ NaN₃ (40 g, 0.60 mol, 2.0 eq.) was added to a solution of 3-bromopropanol (28 mL, 0.30 mol, 1.0 eq.) in DMF (0.50 L) under argon atmosphere. The reaction mixture was heated to 70°C. After stirring for 72 hours, the reaction was cooled to 0°C and diluted with H₂O. The mixture was extracted with Et₂O (4x) and the combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (20→50% Et₂O in pentane) yielded the title compound (19 g, 0.19 mol, 64%) as a transparent liquid. R_f: 0.69 (pentane/EtOAc: 3/7); [α]_D²⁰ -0.5° (*c* = 1.0, DCM). ¹H NMR (CDCl₃, 500 MHz, HH-COSY, HSQC): δ 3.91 (s, 1H, OH), 3.46 (t, 2H, *J* = 6.3 Hz, CH₂OH), 3.20 (t, 2H, *J* = 6.8 Hz, CH₂, CH₂N₃), 1.68 – 1.50 (m, 2H, CH₂); ¹³C-APT NMR (CDCl₃, 126 MHz, HSQC): δ 58.6 (CH₂, CH₂OH), 47.7 (CH₂N₃), 30.9 (CH₂); FT-IR (neat, cm⁻¹): 3349, 2946, 2880, 2092, 1456, 1344, 1260, 1049, 967, 902, 639, 557, 513.

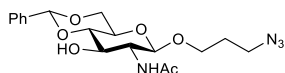
3-Azidopropyl-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranoside (**14**)



A mixture of compound **12** (20.7 g, 38.4 mmol, 1.0 eq.) and alcohol **13** (5.4 mL, 59 mmol, 1.5 eq.) was co-evaporated with toluene (3x) under argon atmosphere. The mixture was dissolved in dry DCM (0.40 L), followed by the addition of 3 Å flame dried molecular sieves and NIS (10.8 g, 49.1 mmol, 1.2 eq.). After 1 hour, TMSOTf (0.70 mL, 3.9 mmol, 0.10 eq.) was added and the reaction was continued to stir for 2.5 hours, after which TLC analysis showed full conversion of the starting material. The reaction was cooled to

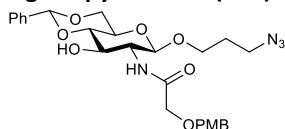
0°C, quenched with sat. aq. NaHCO₃, diluted with EtOAc and washed with sat. aq. NaHCO₃ (2x) and sat. aq. Na₂SO₄ (2x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (20→100% DCM in pentane, followed by 0→2% EtOAc in DCM) gave the title compound **14** (13.8 g, 26.4 mmol, 69%) as a white solid. *R*_f: 0.50 (2/98 EtOAc/DCM); [α]_D²⁰ -9.8° (*c* = 2.0, DCM); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.93 – 7.79 (m, 2H, Ar), 7.78 – 7.67 (m, 2H, Ar), 7.49 – 7.42 (m, 2H, Ar), 7.40 – 7.30 (m, 3H, Ar), 5.90 (dd, 1H, *J* = 10.4, 8.8 Hz, H-3), 5.54 (s, 1H, CH benzylidene), 5.45 (d, 1H, *J* = 8.4 Hz, H-1), 4.41 (dd, 1H, *J* = 10.3, 4.3 Hz, CHH-6), 4.30 (dd, 1H, *J* = 10.4, 8.4 Hz, H-2), 3.94 – 3.69 (m, 4H, H-4, H-5, CHH-6, CHH C₃H₆N₃), 3.59 – 3.48 (m, 1H, CHH C₃H₆N₃), 3.24 – 3.07 (m, 2H, CH₂, C₃H₆N₃), 1.88 (s, 3H, CH₃ Ac), 1.81 – 1.58 (m, 2H, CH₂, C₃H₆N₃); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 170.1 (C=O), 136.9 (C_q Ar), 129.1, 128.2, 126.2, 123.6 (Ar), 101.6 (CH benzylidene), 98.7 (C-1), 79.2 (C-4), 69.7 (C-3), 68.6 (CH₂-6), 66.6 (CH₂ C₃H₆N₃), 66.2 (C-5), 55.3 (C-2), 47.8, 28.8 (CH₂ C₃H₆N₃), 20.5 (CH₃ Ac); FT-IR (neat, cm⁻¹): 2883, 2098, 1776, 1742, 1716, 1469, 1386, 1225, 1104, 1084, 1033, 999, 970, 872, 764, 722, 700, 665, 530; HRMS: [M+Na]⁺ calcd. for C₂₆H₂₆N₄O₈Na: 545.1648, found 545.1646.

3-Azidopropyl-2-N-acetyl-4,6-O-benzylidene-2-deoxy- β -D-glucopyranoside (15a)



Compound **33** (2.6 g, 5.0 mmol, 1.0 eq.) was suspended in EtOH (50 mL). Ethylene diamine (17 mL, 0.25 mol, 50 eq.) was added and the reaction was heated to 90°C for 100 minutes, after which the mixture was concentrated *in vacuo*. The residue was purified by column chromatography (1→10% MeOH in DCM). The obtained free amine was co-evaporated with dioxane (2x) and dissolved in a mixture of H₂O/THF (1/1 v/v, 40 mL). The mixture was cooled to 0°C, followed by the addition of Ac₂O (2.4 mL, 25 mmol, 5.0 eq.) and NaHCO₃ (4.2 g, 50 mmol, 10 eq.). The suspension was further diluted with THF (4.0 mL) and after stirring at room temperature for 72 hours, TLC analysis showed full conversion of the intermediate. The reaction mixture was diluted with EtOAc and washed with H₂O (1x), 1 M HCl (1x) and brine (1x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by crystallization from DCM/MeOH/pentane, yielding compound **15a** (1.6 g, 4.1 mmol, 81%) as a white solid. *R*_f: 0.68 (1/9 MeOH/DCM); [α]_D²⁰ -74.0° (*c* = 1.0, MeOH); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.52 – 7.46 (m, 2H, Ar), 7.37 – 7.31 (m, 3H, Ar), 5.60 (s, 1H, CH benzylidene), 4.53 – 4.49 (m, 1H, H-1), 4.29 (dd, 1H, *J* = 10.3, 4.9 Hz, CHH-6), 3.95 – 3.87 (m, 1H, CHH C₃H₆N₃), 3.85 – 3.73 (m, 3H, CHH-6, H-5, H-2), 3.62 – 3.49 (m, 2H, H-3, CHH C₃H₆N₃), 3.47 – 3.36 (m, 3H, H-4, CH₂ C₃H₆N₃), 1.99 (s, 3H, CH₃ Ac), 1.85 – 1.75 (m, 2H, CH₂-C₃H₆N₃); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 173.7 (C=O), 139.1 (C_q Ar), 129.9, 129.0, 127.5 (Ar), 103.4 (C-1), 102.9 (CH benzylidene), 82.9 (C-4), 72.5 (C-3), 69.7 (CH₂-6), 67.7 (CH₂ C₃H₆N₃), 67.4 (C-5), 58.0 (C-2), 49.1, 30.1 (CH₂ C₃H₆N₃), 23.0 (CH₃ Ac); FT-IR (neat, cm⁻¹): 3266, 2871, 2103, 1659, 1627, 1555, 1034, 756, 698, 473; HRMS: [M+Na]⁺ calcd. for C₁₈H₂₄N₄O₆Na: 415.1588, found 415.15873.

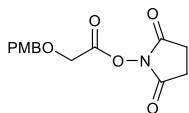
3-Azidopropyl-4,6-O-benzylidene-2-deoxy-2-N-(*p*-methoxybenzyl)oxy)acetamide- β -D-glucopyranoside (**15b**)



Compound **14** (4.2 g, 8.1 mmol, 1.0 eq.) was suspended in EtOH (80 mL). Ethylene diamine (27 mL, 0.40 mol, 50 eq.) was added and the reaction was heated to 90°C for 2 hours, after which the mixture was concentrated *in vacuo*.

Purification by column chromatography (1 \rightarrow 10% MeOH in DCM) yielded the desired free amine, which was co-evaporated with dioxane (2x) under argon atmosphere and dissolved in DCM (40 mL). Compound **16** (3.28 g, 11.2 mmol, 1.4 eq.) and Et₃N (1.7 mL, 12 mmol, 1.5 eq.) were added. After stirring overnight, the reaction was washed with sat. aq. NaHCO₃ (1x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by crystallization from DCM/pentane, yielding the title compound **15b** (3.3 g, 6.3 mmol, 78%) as a white solid. R_f: 0.80 (1/9 MeOH/DCM); [α]_D²⁰ -38.0° (c = 1.0, MeOH); ¹H NMR (MeOD, 500 MHz, HH-COSY, HSQC): δ 7.52 – 7.47 (m, 2H, Ar), 7.37 – 7.30 (m, 5H, Ar), 6.95 – 6.90 (m, 2H, Ar), 5.60 (s, 1H, CH benzylidene), 4.63 – 4.60 (m, 1H, H-1), 4.58 – 4.52 (m, 2H, CH₂ glycol), 4.29 (dd, 1H, J = 10.3, 5.0 Hz, CHH-6), 4.00 – 3.91 (m, 2H, CH₂ PMB), 3.91 – 3.84 (m, 3H, H-2, H-3, CHH C₃H₆N₃), 3.84 – 3.77 (m, 4H, CHH-6, CH₃ PMB), 3.61 – 3.51 (m, 2H, H-4, CHH C₃H₆N₃), 3.49 – 3.42 (m, 1H, H-5), 3.39 – 3.32 (m, 2H, CH₂, C₃H₆N₃), 1.82 – 1.75 (m, 2H, CH₂, C₃H₆N₃); ¹³C-APT NMR (MeOD, 126 MHz, HSQC): δ 173.1 (C=O), 161.2, 139.1 (C_q Ar), 131.0, 130.5, 129.9, 129.0, 127.5, 114.9 (Ar), 103.1 (C-1), 102.9 (CH benzylidene), 83.0 (C-4), 74.0 (CH₂ glycol), 72.2 (C-3), 69.8 (CH₂ PMB), 69.7 (CH₂-6), 67.6 (C-5), 67.5 (CH₂ C₃H₆N₃), 57.6 (C-2), 55.7 (CH₃ PMB), 49.1, 30.1 (CH₂ C₃H₆N₃); FT-IR (neat, cm⁻¹): 3676, 2972, 2097, 1660, 1514, 1454, 1381, 1250, 1175, 1089, 1033, 754, 700; HRMS: [M+Na]⁺ calcd. for C₂₆H₃₂N₄O₈Na: 551.2112, found 551.21124.

N-succinimidyl-(*p*-methoxybenzyloxy)acetate (**16**)

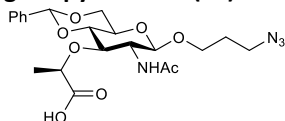


Methyl glycolate (5.0 g, 55 mmol, 1.0 eq.) was dissolved in DMF (0.50 L) and cooled to 0°C after which sodium hydride (60% dispersion in mineral oil, 3.3 g, 83 mmol, 1.5 eq.) was added. After 20 minutes, *p*-methoxybenzyl chloride (11 mL, 83 mmol, 1.5 eq.)

was added and the reaction mixture was allowed to warm-up to room temperature overnight. The reaction was cooled to 0°C, quenched with MeOH/H₂O and diluted with Et₂O. The obtained mixture was washed with H₂O (3x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was dissolved in a mixture of ethanol/H₂O (7/1 v/v, 160 mL), followed by the addition of LiOH·H₂O (5.8 g, 0.14 mol, 2.5 eq.) at 0°C. After stirring overnight, the solution was diluted with H₂O. The mixture was acidified with 1 M HCl to pH = 5 and extracted with DCM (2x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (2 \rightarrow 10% methanol in DCM) afforded (*p*-Methoxybenzyloxy) acetic acid (6.4 g, 32 mmol, 59%) as a yellow oil. R_f: 0.4 (1/9 MeOH/DCM); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.29 (d, 2H, Ar), 6.90 (d, 2H, Ar), 4.58 (s, 2H, CH₂ Glycolyl), 4.11 (s, 2H, CH₂ PMB), 3.81 (s, 3H, CH₃ PMB); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 175.5 (C=O), 159.7 (C_q Ar), 130.6, 129.8 (Ar), 128.7 (C_q Ar), 114.1 (Ar), 73.2 (CH₂ Glycolyl), 66.3 (CH₂ PMB), 55.4 (CH₃ PMB); FT-IR (neat, cm⁻¹): 2937, 2838, 1726,

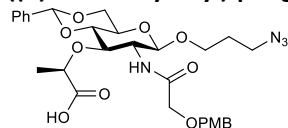
1611, 1586, 1513, 1464, 1441, 1301, 924, 817, 759, 735, 669, 637, 580, 518; HRMS: $[M+Na]^+$ calcd. for $C_{10}H_{12}O_4Na$: 219.0634, found 219.0632. (*p*-Methoxybenzyloxy) acetic acid (4.7 g, 24 mmol, 1.0 eq.) was dissolved in MeCN (0.24 L), followed by the addition of DCC (3.7 mL, 24 mmol, 1.0 eq.) and *N*-hydroxysuccinimide (4.1 g, 36 mmol, 1.5 eq.). After 16 hours, TLC analysis showed full conversion of the starting material and the reaction mixture was filtered over celite and concentrated *in vacuo*. Purification by column chromatography (20→50% EtOAc in pentane) gave the title compound (5.90 g, 20.1 mmol, 85%) as a white solid. R_f : 0.28 (3/2 pentane/EtOAc); $[\alpha]_D^{20} +5.8^\circ$ ($c = 2.0$, DCM); 1H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.30 (d, 2H, Ar), 6.90 (d, 2H, Ar), 4.61 (s, 2H, 2H, CH₂ Glycolyl), 4.40 (s, 2H, CH₂ PMB), 3.81 (s, 3H, CH₃ PMB), 2.85 (s, 4H, CH₂ succinimide); ^{13}C -APT NMR (CDCl₃, 101 MHz, HSQC): δ 168.9, 166.1 (C=O), 159.8, 130.8 (C_q Ar), 130.1, 130.0, 128.4, 114.1, 113.9 (Ar), 73.3 (CH₂ Glycolyl), 64.5 (CH₂ PMB), 55.4 (CH₃ PMB), 25.7 (CH₂ Succinimide) FT-IR (neat, cm⁻¹): 2939, 1706, 1612, 1586, 1514, 1465, 1429, 1303, 1247, 1213, 1176, 1109, 1031, 818, 761, 715, 656, 579, 521; HRMS: $[M+Na]^+$ calcd. for $C_{14}H_{15}NO_6Na$: 316.0797, found 316.0802.

3-Azidopropyl-2-*N*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-*O*-((*R*)-1-carboxyethyl)- β -D-glucopyranoside (**9a**)



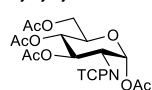
Compound **15a** (1.5 g, 3.8 mmol, 1.0 eq.) was co-evaporated with dioxane (3x) under argon atmosphere and dissolved in DMF (19 mL). The mixture was cooled to 0°C and sodium hydride (60% dispersion in mineral oil, 0.75 g, 19 mmol, 5.0 eq.) was added. After stirring for 1 hour, (*S*)-(-)-2-chloropropionic acid (0.71 mL, 8.3 mmol, 2.2 eq.) was slowly added. After 2 hours, sodium hydride (60% dispersion in mineral oil, 0.76 g, 19 mmol, 5.0 eq.) was added and the mixture was allowed to warm-up to room temperature overnight, after which TLC analysis showed full conversion of the starting material. The reaction mixture was cooled to 0°C, slowly quenched with H₂O, acidified with 1 M HCl to pH = 4 and extracted with DCM (3x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by crystallization in DCM/MeOH/pentane, gave compound **9a** (1.47 g, 3.16 mmol, 83%) as white crystals. R_f : 0.57 (1/9 MeOH/DCM); $[\alpha]_D^{20} -46.5^\circ$ ($c = 1.0$, MeOH); 1H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.49 – 7.44 (m, 2H, Ar), 7.40 – 7.34 (m, 3H, Ar), 5.63 (s, 1H, CH benzylidene), 4.56 (d, 1H, $J = 7.7$ Hz, H-1), 4.39 (q, 1H, $J = 6.9$ Hz, CH lactic acid), 4.29 (dd, 1H, $J = 10.3, 5.0$ Hz, CHH-6), 3.94 – 3.86 (m, 1H, CHH C₃H₆N₃), 3.85 – 3.70 (m, 3H, H-2, H-3, CHH-6), 3.70 – 3.64 (m, 1H, H-4), 3.61 – 3.54 (m, 1H, CHH C₃H₆N₃), 3.50 – 3.41 (m, 1H, H-5), 3.38 (t, 2H, $J = 6.6$ Hz, C₃H₆N₃), 1.99 (s, 3H, CH₃ Ac), 1.86 – 1.73 (m, 2H, CH₂, C₃H₆N₃), 1.33 (d, 3H, $J = 6.9$ Hz, CH₃ lactic acid); ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 176.7, 173.9 (C=O), 139.1 (C_q Ar), 130.0, 129.2, 127.2 (Ar), 103.4 (C-1), 102.5 (CH₂ benzylidene), 83.6 (C-4), 79.6 (C-3), 77.0 (CH lactic acid), 69.6 (CH₂-6), 67.5 (CH₂ C₃H₆N₃), 67.3 (C-5), 56.6 (C-2), 49.1, 30.1 (CH₂ C₃H₆N₃), 23.2 (CH₃ Ac), 19.4 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 3269, 2876, 2104, 1712, 1657, 1562, 1452, 1374, 1308, 1177, 1120, 1095, 1013, 966, 748, 695; HRMS: $[M+Na]^+$ calcd. for $C_{21}H_{19}N_4O_8Na$: 465.1980, found 465.19795; LC-MS: Rt = 6.36 min (Gemini C₁₈, 10-90% MeCN, 12.5 min run).

3-Azidopropyl-4,6-O-benzylidene-2-deoxy-2-N-((p-methoxybenzyl)oxy)acetamide-O-((R)-1-carboxyethyl)-β-D-glucopyranoside (9b)



Compound **15b** (2.6 g, 5.0 mmol, 1.0 eq.) was co-evaporated with dioxane (3x) under argon atmosphere and dissolved in DMF (20 mL). The mixture was cooled to 0°C and sodium hydride (60% dispersion in mineral oil, 1.0 g, 25 mmol, 5.0 eq.) was added. After stirring for 1 hour, (S)-(-)-2-chloropropionic acid (0.94 mL, 11 mmol, 2.2 eq.) was slowly added. After 1 hour, sodium hydride (60% dispersion in mineral oil, 1.0 g, 25 mmol, 5.0 eq.) was added and the mixture was allowed to warm-up to room temperature overnight, after which TLC analysis showed full conversion of the starting material. The reaction mixture was cooled to 0°C, slowly quenched with H₂O, acidified with 1 M HCl to pH = 4 and extracted with DCM (3x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by crystallization in DCM/MeOH/Pentane afforded compound **9b** (2.6 g, 4.3 mmol, 86%) as white crystals. R_f: 0.57 (1/9 DCM/MeOH); [α]_D²⁰ -34.5° (c = 1.0, MeOH); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.48 – 7.42 (m, 2H, Ar), 7.41 – 7.35 (m, 3H, Ar), 7.29 – 7.24 (m, 3H, Ar), 7.06 (d, 1H, J = 7.9 Hz, NH), 6.89 (d, 2H, Ar), 5.55 (s, 1H, CH benzylidene), 4.83 (d, 1H, J = 8.3 Hz, H-1), 4.58 – 4.47 (m, 2H, CH₂ Glycol), 4.47 – 4.38 (m, 1H, CH lactic acid), 4.35 (dd, 1H, J = 10.5, 5.0 Hz, CHH-6), 4.16 – 3.87 (m, 4H, H-3 CH₂ PMB, CHH C₃H₆N₃), 3.83 – 3.74 (m, 4H, CHH-6, CH₃ PMB), 3.68 – 3.53 (m, 3H, H-2, H-4, CHH C₃H₆N₃), 3.53 – 3.43 (m, 1H, H-5), 3.34 (t, 2H, J = 6.6 Hz, C₃H₆N₃), 1.91 – 1.74 (m, 2H, CH₂, C₃H₆N₃), 1.42 (d, 3H, J = 7.0 Hz, CH₃ lactic acid); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 174.6, 171.8 (C=O), 159.8, 137.1 (C_q Ar), 130.0, 129.3 (Ar), 129.0 (C_q Ar), 128.5, 126.0, 120.3, 114.2 (Ar), 101.5 (CH₂ benzylidene), 100.8 (C-1), 82.4 (C-4), 78.1 (C-3), 76.2 (CH lactic acid), 73.4 (CH₂ glycol), 69.3 (CH₂ PMB), 68.8 (CH₂-6), 66.7 (CH₂ C₃H₆N₃), 66.0 (C-5), 56.6 (C-2), 56.6 (CH₃ PMB), 48.1, 29.1 (CH₂ C₃H₆N₃), 19.1, (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 2973, 2099, 1659, 1514, 1454, 1381, 1250, 1177, 1091, 1033, 751, 699; HRMS: [M+Na]⁺ calcd. for C₂₉H₃₇N₄O₁₀Na: 601.2504, found 601.25021; LC-MS: Rt = 7.78 min (Gemini C₁₈, 10-90% MeCN, 12.5 min run).

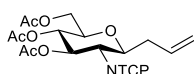
1,3,4,6-tetra-O-acetyl-2-deoxy-2-tetrachlorophthalimido-α-D-glucopyranoside (21)



Glucosamine hydrochloride (21.6 g, 100 mmol, 1.0 eq.) was added to a solution of sodium methoxide (1.0 M in MeOH, 0.10 L, 1.0 eq.) at room temperature and the obtained solution was stirred for 10 minutes, followed by the addition of tetrachlorophthalic anhydride (14.3 g, 50.0 mmol, 0.5 eq.). After 20 minutes, additional tetrachlorophthalic anhydride (14.3 g, 50.0 mmol, 0.5 eq.) and Et₃N (10 mL, 0.10 mol, 1.0 eq.) were added and the reaction was stirred at 50°C for 20 minutes. The mixture was concentrated *in vacuo*. The residue was dissolved in pyridine (98 mL), followed by slow addition of Ac₂O (0.15 L, 1.6 mol, 16.0 eq.). The resulting mixture was stirred for 16 hours at room temperature, after which it was poured into ice water (0.15 L) and extracted with DCM (3x). The combined organic layers were subsequently washed with a 1 M HCl (2x), sat. aq. NaHCO₃ (2x) and brine (1x). The organic layer was dried over MgSO₄, filtered, concentrated *in vacuo* and co-evaporated with toluene (1x). Recrystallization in MeOH yielded the title compound (31.4 g, 51.0 mmol, 51%) as a white solid. R_f: 0.6 (3/2 pentane/EtOAc); [α]_D²⁰ = +96.6° (c

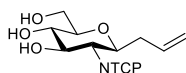
= 1.0, DCM); ^1H NMR (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 6.48 (dd, 1H, J = 11.5, 9.1 Hz, H-3), 6.24 (d, 1H, J = 3.4 Hz, H-1), 5.15 (t, 1H, J = 10.1, 9.0 Hz, H-4), 4.70 (dd, 1H, J = 11.5, 3.4 Hz, H-2), 4.38 – 4.27 (m, 2H, H-5, CHH-6), 4.13 (dd, 1H, J = 12.2, 1.8 Hz, CHH-6), 2.11 (s, 3H, CH_3 Ac), 2.08 (s, 3H, CH_3 Ac), 2.05 (s, 3H, CH_3 Ac), 1.90 (s, 3H, CH_3 Ac); ^{13}C -APT NMR (CDCl_3 , 101 MHz, HSQC): δ 170.8, 169.9, 169.8, 169.6 (C=O), 140.9 (C_q Ar), 130.3, 126.8 (C-Cl), 90.6 (C-1), 70.4 (C-5), 69.3 (C-4), 67.0 (C-3), 61.5 (CH_2 -6), 53.5 (C-2), 21.1, 20.9, 20.8, 20.8 (CH_3 Ac); FT-IR (neat, cm^{-1}): 2965, 1750, 1731, 1385, 1370, 1219, 1154, 1081, 1040, 1015, 922, 794, 752, 740, 603, 540, 485; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{22}\text{H}_{19}\text{Cl}_4\text{NO}_{11}\text{Na}$ 635.9610, found 635.9617.

3-C-(3,4,6-tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido- β -D-glucopyranosyl)-1-propene (22)



Compound **21** (24.6 g, 40.0 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in acetonitrile (0.24 L) and cooled to 0°C. Allyltrimethylsilane (32 mL, 0.20 mol, 5.0 eq.) was added, followed by slow addition of TMSOTf (7.2 mL, 40 mmol, 1.0 eq.) and $\text{BF}_3\cdot\text{OEt}_2$ (25 mL, 0.20 mol, 5.0 eq.). The yellow suspension was sonicated for 90 minutes and stirred for an additional hour at room temperature. The resulting brown solution was cooled to 0°C and quenched with Et_3N to pH = 7. The reaction was diluted with EtOAc, washed with sat. aq. NaHCO_3 (1x) and brine (1x). The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Purification column chromatography (10 \rightarrow 50% Et_2O in pentane) yielded the title compound (13.9 g, 23.2 mmol, 58%) as a white foam. R_f : 0.5 (1/1 pentane/ Et_2O); $[\alpha]_D^{20} = +74.4^\circ$ (c = 1.0, DCM); ^1H NMR (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 5.78 – 5.65 (m, 2H, H-3, $\text{CH}_2\text{-CH}=\text{CH}_2$), 5.12 (t, 1H, J = 10.2 Hz, H-4), 5.00 – 4.87 (m, 2H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 4.47 – 4.34 (m, 1H, H-1), 4.27 (dd, 1H, J = 12.3, 4.9 Hz, CHH-6), 4.21 (t, 1H, J = 10.2 Hz, H-2), 4.10 (dd, 1H, J = 12.3, 2.3 Hz, CHH-6), 3.79 – 3.73 (m, 1H, H-5), 2.26 (t, 2H, J = 6.8 Hz, $\text{CH}_2\text{-CH}=\text{CH}_2$), 2.09 (s, 3H, CH_3 Ac), 2.01 (s, 3H, CH_3 Ac), 1.86 (s, 3H, CH_3 Ac); ^{13}C -APT NMR (CDCl_3 , 101 MHz, HSQC): δ 170.9, 170.8, 169.6, 163.5, 162.8 (C=O), 140.9, 140.6 (C_q Ar), 132.5 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 130.2, 130.0, 127.1, 126.8 (C-Cl), 118.1 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 75.8 (C-5), 74.0 (C-1), 71.9 (C-3), 69.0 (C-4), 62.4 (CH_2 -6), 55.3 (C-2), 36.8 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 20.9, 20.7, 20.6 (CH_3 Ac); FT-IR (neat, cm^{-1}): 2957, 1782, 1746, 1724, 1384, 1370, 1352, 1226, 1150, 1047, 908, 791, 753, 740, 603; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{23}\text{H}_{22}\text{Cl}_4\text{NO}_9$ 596.0043, found 596.0045.

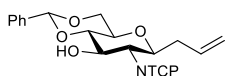
3-C-(2-deoxy-2-tetrachlorophthalimido- β -D-glucopyranosyl)-1-propene (23)



Acetyl chloride (1.6 mL, 23 mmol, 0.8 eq.) was added to a solution of compound **22** (17.1 g, 28.7 mmol, 1.0 eq.) in a mixture of DCM/MeOH (1:4 v/v, 0.29 L) at 0°C. After stirring for 1 hour, reaction mixture was allowed to warm-up to room temperature and stirred for 72 hours. The mixture was diluted with toluene (30 mL) and concentrated *in vacuo*. The residue was co-evaporated with toluene (2x) and purified by column chromatography (1 \rightarrow 10% MeOH in DCM) to obtain the title compound (12.7 g, 26.9 mmol, 94%) as a white solid. R_f : 0.5 (1/9 MeOH/DCM); $[\alpha]_D^{20} = +34.7^\circ$ (c = 1.0, DCM); ^1H NMR (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 5.76 – 5.63 (m, 1H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 4.88 (t, 2H, $\text{CH}_2\text{-CH}=\text{CH}_2$),

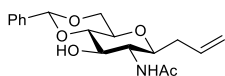
4.24 (t, 1H, $J = 10.5, 8.8$ Hz, H-3), 4.21 – 4.13 (m, 1H, H-1), 3.91 (t, 1H, $J = 10.3, 10.3$ Hz, H-2), 3.86 – 3.77 (m, 2H, CH₂-6), 3.56 (t, 1H, $J = 9.2, 9.2$ Hz, H-4), 3.52 – 3.43 (m, 3H, OH), 3.40 (dt, 1H, $J = 9.6, 3.2, 3.2$ Hz, H-5), 2.27 – 2.10 (m, 2H, CH₂-CH=CH₂); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 163.9, 163.8 (C=O), 140.4, 140.4 (C_q Ar), 133.4 (CH₂-CH=CH₂), 130.1, 129.7, 127.3, 127.3 (C-Cl), 117.5 (CH₂-CH=CH₂), 79.2 (C-5), 74.2 (C-1), 71.8 (C-3), 71.4 (C-4), 62.0 (CH₂-6), 57.5 (C-2), 37.2 (CH₂-CH=CH₂); FT-IR (neat, cm⁻¹): 3378, 2929, 1779, 1718, 1387, 1370, 1351, 1299, 1202, 1142, 1085, 1000, 919, 791, 753, 740, 643; HRMS: [M+Na]⁺ calcd. for C₁₇H₁₅Cl₄NO₆Na 491.9551, found 491.9551.

3-C-(4,6-di-O-benzylidene-2-deoxy-2-tetrachlorophthalimido- β -D-glucopyranosyl)-1-propene (24)



Compound **23** (10.6 g, 22.5 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in a mixture of DMF/acetonitrile (9:1 v/v, 113 mL). Benzaldehyde dimethyl acetal (6.9 mL, 45 mmol, 2.0 eq.) and *p*-toluenesulfonic acid (0.43 g, 2.3 mmol, 0.1 eq.) were added and the mixture was heated to 60°C. After stirring overnight, the mixture was cooled to 0°C and quenched with Et₃N to pH = 7. The solution was diluted with EtOAc and the organic layer was washed with H₂O (3x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→40% Et₂O in pentane) gave compound **24** (11.0 g, 19.7 mmol, 87%) as a white solid. R_f: 0.8 (1/1 pentane/Et₂O); [α]_D²⁰ = +17.5° ($c = 1.0$, DCM); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.39 – 7.27 (m, 5H, Ar), 5.78 – 5.64 (m, 1H, CH₂-CH=CH₂), 5.49 (s, 1H, CH benzylidene), 4.94 (t, 2H, $J = 9.4$ Hz, CH₂-CH=CH₂), 4.60 (t, 1H, $J = 10.2, 9.0$ Hz, H-3), 4.33 (dd, 1H, $J = 10.2, 4.7$ Hz, CHH-6), 4.30 – 4.22 (m, 1H, H-1), 4.05 (t, 1H, $J = 10.2$ Hz, H-2), 3.70 (t, 1H, $J = 10.1$ Hz, CHH-6), 3.64 – 3.55 (m, 1H, H-5), 3.48 (t, 1H, $J = 9.1$ Hz, H-4), 3.19 (s, 1H, OH), 2.30 – 2.17 (m, 2H, CH₂-CH=CH₂); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 163.7, 163.2 (C=O), 140.4, 140.3 (C-Cl), 136.9 (C_q Ar), 133.0 (CH₂-CH=CH₂), 130.1, 129.8 (C-Cl), 129.3, 128.3 (Ar), 127.1, 127.1 (C-Cl), 126.0 (Ar), 117.7 (CH₂-CH=CH₂), 101.7 (CH benzylidene), 82.5 (C-4), 75.0 (C-1), 70.1 (C-5), 68.8 (CH₂-6), 68.6 (C-3), 57.1 (C-2), 37.0 (CH₂-CH=CH₂); FT-IR (neat, cm⁻¹): 3485, 2864, 1779, 1720, 1371, 1351, 1300, 1203, 1124, 1096, 988, 918, 790, 753, 740, 699, 643; HRMS: [M+H]⁺ calcd. for C₂₄H₂₀Cl₄NO₆ 558.0039, found 558.0047.

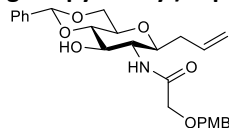
3-C-(2-deoxy-2-N-acetyl-4,6-O-di-benzylidene- β -D-glucopyranosyl)-1-propene (25a)



To a solution of compound **24** (3.8 g, 6.8 mmol, 1.0 eq.) in EtOH (70 mL) was added ethylenediamine (23 mL, 0.34 mol, 50 eq.) and the reaction was heated to 90°C. After 16 hours, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was co-evaporated with toluene (3x) and imbedded on silica gel. Purification by column chromatography (2→5% MeOH in DCM) gave 3-C-(4,6-di-O-benzylidene-2-deoxy-2-amine- β -D-glucopyranosyl)-1-propene (1.92 g, 6.59 mmol) as a yellow solid. R_f: 0.42 (1/9 MeOH/DCM). The obtained amine (1.92 g, 6.59 mmol, 1.0 eq.) was dissolved in a mixture of THF/H₂O (1/1 v/v, 50 mL). Sodium bicarbonate (5.6 g, 66 mmol, 10 eq.) and Ac₂O (3.1 mL, 33 mmol, 5.0 eq.) were added. The mixture was stirred at room temperature for 4 days, after which the reaction mixture was diluted with EtOAc. The

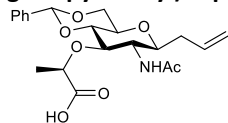
obtained suspension was filtered and the obtained pure title compound was collected as a white solid. The filtrate was washed with sat. aq. NaHCO_3 (1x) and brine (1x). The organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo*. The remaining crude product was crystallized using DCM/MeOH/pentane giving compound **25a**. The remaining residue was imbedded on silica and purified by column chromatography (2→6% MeOH in DCM). The combined title compound (1.87 g, 5.63 mmol, 83% over two steps) was collected as a white solid. R_f : 0.5 (1/9 MeOH/DCM); $[\alpha]_D^{20} = -38.5^\circ$ ($c = 0.3$, MeOH); $^1\text{H NMR}$ (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.53 – 7.46 (m, 2H, Ar), 7.38 – 7.30 (m, 3H, Ar), 5.93 – 5.80 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.59 (s, 1H, CH benzylidene), 5.03 (t, 2H, $J = 17.5$, 8.7 Hz, $\text{CH}_2\text{-CH=CH}_2$), 4.25 (dd, 1H, $J = 10.3$, 5.0 Hz, CHH-6), 3.75 (q, 2H, $J = 11.5$, 10.0 Hz, H-2, CHH-6), 3.66 (t, 1H, $J = 9.7$, 8.9 Hz, H-3), 3.50 (t, 1H, $J = 9.1$ Hz, H-4), 3.47 – 3.34 (m, 2H, H-1, H-5), 2.41 – 2.29 (m, 1H, CHH-CH=CH₂), 2.26 – 2.13 (m, 1H, CHH-CH=CH₂), 1.99 (s, 3H, CH₃ Ac); $^{13}\text{C-APT NMR}$ (MeOD, 101 MHz, HSQC): δ 173.7 (C=O), 139.2 (C_q Ar), 135.7 ($\text{CH}_2\text{-CH=CH}_2$), 129.9, 129.0, 127.5 (Ar), 117.2 ($\text{CH}_2\text{-CH=CH}_2$), 102.9 (CH benzylidene), 83.2 (C-4), 80.4 (C-1), 73.9 (C-3), 71.8 (C-5), 69.8 (CH_2 -6), 57.2 (C-2), 37.7 ($\text{CH}_2\text{-CH=CH}_2$), 22.9 (CH₃ Ac); FT-IR (neat, cm^{-1}): 3380, 2361, 1630, 1377, 1125, 1033, 999, 698; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{18}\text{H}_{24}\text{NO}_5$ 334.1655, found 334.1654.

3-C-(4,6-O-di-benzylidene-2-deoxy-2-N-((p-methoxybenzyl)oxy)acetamide- β -D-glucopyranosyl)-1-propene (**25b**)



A mixture of 3-C-(4,6-di-O-benzylidene-2-deoxy-2-amine- β -D-glucopyranosyl)-1-propene (see synthesis of **25a**) (6.13 g, 21.0 mmol, 1.0 eq.), compound **16** (7.13 g, 24.3 mmol, 1.2 eq.) and Et_3N (4.2 mL, 32 mmol, 1.5 eq.) in DCM (0.10 L) stirred for 16 hours under an argon atmosphere. The reaction was washed with sat. aq. NaHCO_3 (1x) and the organic layer was dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (40→100% EtOAc in pentane) yielded compound **25b** (9.66 g, 20.6 mmol, 98%) as a white solid. R_f : 0.4 (3/7 pentane/EtOAc); $[\alpha]_D^{20} = -43.3^\circ$ ($c = 1.0$, MeOH); $^1\text{H NMR}$ (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 7.52 – 7.46 (m, 2H, Ar), 7.40 – 7.31 (m, 3H, Ar), 7.28 – 7.22 (m, 2H, Ar), 6.93 – 6.87 (m, 2H, Ar), 6.53 (d, 1H, $J = 8.9$ Hz, NH), 5.89 – 5.76 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.53 (s, 1H, CH benzylidene), 5.11 – 5.00 (m, 2H, $\text{CH}_2\text{-CH=CH}_2$), 4.50 (q, 2H, $J = 11.1$, 3.6 Hz, CH_2 glycolyl), 4.31 (dd, 1H, $J = 10.4$, 5.0 Hz, CHH-6), 3.99 (q, 2H, $J = 15.3$, 14.2, 4.0 Hz, CH_2 PMB), 3.90 – 3.74 (m, 5H, H-2, H-4, CH₃ PMB), 3.70 (t, 1H, $J = 10.3$ Hz, CHH-6), 3.54 – 3.44 (m, 2H, H-1, H-3), 3.44 – 3.35 (m, 1H, H-5), 2.42 – 2.32 (m, 1H, CHH₂-CH=CH₂), 2.32 – 2.15 (m, 1H, CHH-CH=CH₂); $^{13}\text{C-APT NMR}$ (CDCl_3 , 101 MHz, HSQC): δ 170.9 (C=O), 159.9, 137.3 (C_q Ar), 133.9 ($\text{CH}_2\text{-CH=CH}_2$), 129.9, 129.3 (Ar), 128.8 (C_q Ar), 128.4, 126.5 (Ar), 117.6 ($\text{CH}_2\text{-CH=CH}_2$), 114.2 (Ar), 101.9 (CH benzylidene), 82.0 (C-4), 78.6 (C-1), 73.5 (CH_2 glycolyl), 73.4 (C-3), 70.3 (C-5), 69.2 (CH_2 PMB), 68.9 (CH_2 -6), 55.4 (CH₃ PMB), 55.4 (C-2), 36.5 ($\text{CH}_2\text{-CH=CH}_2$); FT-IR (neat, cm^{-1}): 3386, 2862, 2360, 1666, 1612, 1514, 1454, 1250, 1097, 1033, 822, 763, 700; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{26}\text{H}_{32}\text{NO}_7$ 470.2180, found 470.2177.

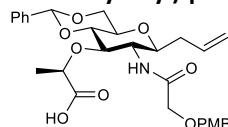
3-C-(2-deoxy-2-N-acetyl-4,6-O-di-benzylidene-3-O-((R)-1-carboxyethyl)-β-D-glucopyranosyl)-1-propene (26a)



Compound **25a** (2.76 g, 8.28 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere and dissolved in DMF (41 mL). The solution was cooled to 0°C and NaH (60% dispersion in mineral oil, 1.66 g, 42 mmol, 5.1 eq.) was added.

The mixture was stirred at 0°C for 30 minutes before dropwise addition of (S)-(-)-2-chloropropionic acid (1.6 mL, 18.7 mmol, 2.3 eq.). After stirring for an additional 30 minutes at 0°C, NaH (60% dispersion in mineral oil, 1.66 g, 42 mmol, 5.1 eq.) was added and the mixture was allowed to slowly warm-up to room temperature overnight. The reaction mixture was diluted with DCM, cooled to 0°C and quenched with H₂O. The suspension was acidified with 1 M HCl to pH = 1 and extracted with DCM (3x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Precipitation from DCM with pentane gave the title compound (3.20 g, 7.89 mmol, 95%) as a white solid. R_f: 0.3 (1/9 MeOH/DCM); [α]_D²⁰ = -56.6° (c = 1.0, MeOH); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.43 (m, 2H, Ar), 7.40 – 7.31 (m, 3H, Ar), 5.92 – 5.80 (m, 1H, CH₂-CH=CH₂), 5.62 (s, 1H, CH benzylidene), 5.02 (t, 2H, J = 17.4, 9.3 Hz, CH₂-CH=CH₂), 4.40 (q, 1H, J = 6.9 Hz, CH lactic acid), 4.25 (dd, 1H, J = 10.4, 5.0 Hz, CHH-6), 3.79 – 3.71 (m, 2H, H-2, H-3), 3.70 – 3.60 (m, 2H, H-4, CHH-6), 3.49 – 3.35 (m, 2H, H-1, H-5), 2.40 – 2.31 (m, 1H, CHH-CH=CH₂), 2.24 – 2.14 (m, 1H, CHH-CH=CH₂), 1.99 (s, 3H, CH₃ Ac), 1.33 (d, 3H, J = 6.9 Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 176.9 (C=O lactic acid), 174.1 (C=O Ac), 139.2 (C_q Ar), 135.7 (CH₂-CH=CH₂), 129.9, 129.2, 127.2 (Ar), 117.3 (CH₂-CH=CH₂), 102.5 (CH benzylidene), 84.1 (C-4), 81.1 (C-3), 80.6 (C-1), 76.9 (CH lactic acid), 71.5 (C-5), 69.7 (CH₂-6), 55.8 (C-2), 37.7 (CH₂-CH=CH₂), 23.1 (CH₃ Ac), 19.4 (CH₃ PMB); FT-IR (neat, cm⁻¹): 2871, 1654, 1552, 1103, 1033, 1011, 696; HRMS: [M+H]⁺ calcd. for C₂₁H₂₈NO₇ 406.1861, found 406.1872.

3-C-(4,6-O-di-benzylidene-2-deoxy-2-N-((p-methoxybenzyl)oxy)acetamide-3-O-((R)-1-carboxyethyl)-β-D-glucopyranosyl)-1-propene (26b)

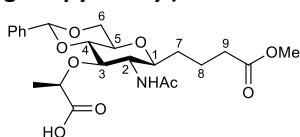


Compound **25b** (9.48 g, 20.2 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere and dissolved in DMF (0.10 L). The solution was cooled to 0°C, NaH (60% dispersion in mineral oil, 4.04 g, 0.10 mol, 5.0 eq.) was added

and the mixture was stirred at 0°C for 30 minutes. (S)-(-)-2-chloropropionic acid (3.8 mL, 44.4 mmol, 2.2 eq.) was added dropwise and stirring was continued for 30 minutes at 0°C. After addition of NaH (60% dispersion in mineral oil, 4.04 g, 0.10 mol, 5.0 eq.), the mixture was stirred for another 15 minutes at 0°C before being allowed to warm-up to room temperature. After stirring for 16 hours, TLC analysis showed full conversion of the starting material, the reaction mixture was diluted with DCM, cooled to 0°C and quenched with H₂O. The suspension was acidified with 1 M HCl to pH = 1 and extracted with DCM (3x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Precipitation from DCM with pentane gave the title compound as a white solid (10.0 g, 18.5 mmol, 91%). R_f: 0.6 (1/9 MeOH/DCM); [α]_D²⁰ = -33.0° (c = 1.0, MeOH); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.48 – 7.41 (m, 2H, Ar), 7.40 – 7.32 (m, 3H, Ar), 7.25 (d, 2H, J = 8.5 Hz, Ar), 6.98 (d, 1H, J = 8.0 Hz, NH), 6.88 (d, 2H, J

= 8.6 Hz, Ar), 5.87 – 5.74 (m, 1H, CH₂-CH=CH₂), 5.54 (s, 1H, CH benzylidene), 5.09 – 5.01 (m, 2H, CH₂-CH=CH₂), 4.51 (q, 2H, *J* = 11.2, 9.3, 3.8 Hz, CH₂ glycolyl), 4.45 (q, 1H, *J* = 6.8, 4.6 Hz, CH lactic acid), 4.31 (dd, 1H, *J* = 10.5, 5.0 Hz, CHH-6), 4.00 (s, 2H, CH₂ PMB), 3.84 – 3.74 (m, 5H, H-2, H-3, CH₃ PMB), 3.71 (t, 1H, *J* = 10.3 Hz, CHH-6), 3.64 – 3.54 (m, 2H, H-1, H-4), 3.44 – 3.36 (m, 1H, H-5), 2.40 – 2.31 (m, 1H, CHH-CH=CH₂), 2.28 – 2.18 (m, 1H, CHH-CH=CH₂), 1.41 (d, 3H, *J* = 6.9 Hz, CH₃ lactic acid); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 175.9 (C=O lactic acid), 171.6 (C=O glycolyl), 159.8, 137.2 (C_q Ar), 133.9 (CH₂-CH=CH₂), 129.9, 129.2 (Ar), 128.9 (C_q Ar), 128.5, 126.0 (Ar), 117.6 (CH₂-CH=CH₂), 114.2 (Ar), 101.3 (CH benzylidene), 82.8 (C-4), 79.7 (C-3), 78.8 (C-1), 75.7 (CH lactic acid), 73.4 (CH₂ glycolyl), 70.3 (C-5), 69.2 (CH₂ PMB), 68.9 (CH₂-6), 55.4 (CH₃ PMB), 54.6 (C-2), 36.6 (CH₂-CH=CH₂), 19.1 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 2938, 1514, 1250, 1105, 1055, 1033, 1011; HRMS: [M+H]⁺ calcd. for C₂₉H₃₆NO₉ 542.2385, found 542.2386.

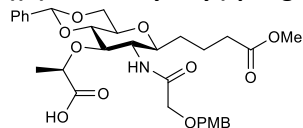
Methyl 4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-((*R*)-1-carboxyethyl)-β-*D*-glucopyranosyl)-butanoate (**27a**)



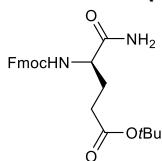
Compound **26a** (2.35 g, 5.79 mmol, 1.0 eq.) was co-evaporated with dioxane (2x) and THF (1x) under an argon atmosphere before being dissolved in THF (58 mL). Methyl acrylate (1.5 mL, 16.2 mmol, 2.8 eq.) and copper iodide (0.17 g, 0.87 mmol, 0.15 eq.) were added, followed by the addition of Grubbs 2nd generation catalyst (0.51 g, 0.58 mmol, 0.1 eq.). After shielding the flask from light with aluminium foil, the reaction was heated to 40°C for 48 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x) under an argon atmosphere and dissolved in THF (23 mL). The solution was purged with argon for 5 minutes. Ruthenium trichloride (0.26 g, 1.16 mmol, 0.2 eq.) and NaBH₄ (0.70 g, 18.5 mmol, 3.3 eq.) were added and an empty balloon was connected to the reaction. The mixture was cooled to 0°C before dropwise addition of MeOH (6.7 mL). The reaction was stirred at 40°C for 3 hours. After completion of the reaction determined by LC-MS, the reaction was cooled to 0°C, quenched with H₂O and diluted with DCM. The mixture was acidified with 1 M HCl to pH = 1, and the aqueous layer was extracted with DCM (3x). The combined organic layers were washed with brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (2 → 10% MeOH in DCM + 0.1% AcOH) and recrystallization (DCM/pentane) to obtain a mixture of compound **27a** (1.73 g, 3.71 mmol, 64%) and **28a** (0.50 g, 1.24 mmol). Analysis given for title compound only. R_f: 0.3 (1/9 MeOH/DCM); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.43 (m, 2H, Ar), 7.40 – 7.31 (m, 3H, Ar), 5.61 (s, 1H, CH benzylidene), 4.40 (q, 1H, *J* = 6.9 Hz, CH lactic acid), 4.25 (dd, 1H, *J* = 10.3, 5.0 Hz, CHH-6), 3.77 – 3.68 (m, 2H, H-2, CHH-6), 3.68 – 3.60 (m, 5H, H-3, H-4, OCH₃), 3.43 – 3.36 (m, 2H, H-1, H-2), 2.35 – 2.28 (m, 2H, CH₂-9), 1.99 (s, 3H, CH₃ Ac), 1.88 – 1.76 (m, 1H, CHH-8), 1.69 – 1.56 (m, 2H, CHH-7, CHH-8), 1.49 – 1.37 (m, 1H, CHH-7), 1.33 (d, 3H, *J* = 6.8 Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 177.0, 175.7, 174.1 (C=O), 139.2 (C_q Ar), 129.9, 129.1, 127.2 (Ar), 102.5 (CH benzylidene), 84.0 (C-4), 81.0 (C-3), 80.7 (C-1), 77.0 (CH lactic acid), 71.6 (C-5), 69.8 (CH₂-6), 56.0 (C-2), 52.0 (OCH₃), 34.5 (C-9), 32.5 (C-7), 23.1 (CH₃ Ac), 22.1 (C-8), 19.5 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 2950, 1737, 1651, 1552, 1372, 1103, 1055, 1033,

1012, 697; HRMS: $[M+H]^+$ calcd. for $C_{23}H_{32}NO_9$ 466.2072, found 466.2076; LC-MS: Rt = 5.81 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

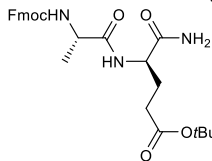
Methyl 4-(4,6-*O*-di-benzylidene-2-deoxy-2-*N*-((*p*-methoxybenzyl)oxy)acetamide-3-*O*-((*R*)-1-carboxyethyl)- β -D-glucopyranosyl)-butanoate (27b**)**



After co-evaporation with toluene (2x) and THF (1x) under an argon atmosphere, compound **26b** (0.27 g, 0.50 mmol, 1.0 eq.) was dissolved in THF (5.0 mL). Methyl acrylate (0.21 mL, 1.4 mmol, 2.8 eq.) and copper iodide (15 mg, 0.08 mmol, 0.15 eq.) were added, followed by the addition of Grubbs 2nd generation catalyst (43 mg, 0.05 mmol, 0.1 eq.). The flask was shielded from light with aluminium foil, heated to 40°C and stirred overnight. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in THF (1.9 mL) and the solution was purged with argon for 5 minutes. Ruthenium trichloride (33 mg, 0.16 mmol, 0.3 eq.) and NaBH₄ (61 mg, 1.6 mmol, 3.2 eq.) were added. An empty balloon was put on the reaction flask. After cooling to 0°C, MeOH (0.58 mL) was slowly added and the reaction was stirred at 40°C. After 3 hours, LC-MS analysis showed full conversion of the starting material. The reaction was quenched with H₂O and diluted with DCM. The mixture was acidified with 1 M HCl to pH = 1. The aqueous layer was extracted with DCM (3x). The combined organic layers were washed with brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (2→10% MeOH in DCM + 0.1% AcOH) gave a mixture of compound **27b** (0.21 g, 0.35 mmol, 69%) and compound **28b** (0.04 g, 0.08 mmol). Analysis given for title compound only. R_f: 0.5 (1/9 MeOH/DCM); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.48 – 7.41 (m, 2H, Ar), 7.38 – 7.29 (m, 5H, Ar), 6.93 – 6.87 (m, 2H, Ar), 5.60 (s, 1H, CH benzylidene), 4.54 (q, 2H, *J* = 14.2, 12.0, 11.5 Hz, CH₂ glycolyl), 4.33 (q, 1H, *J* = 6.8 Hz, CH lactic acid), 4.24 (dd, 1H, *J* = 10.3, 4.9 Hz, CHH-6), 3.97 (q, 2H, *J* = 15.2, 14.9, 6.2 Hz, CH₂ PMB), 3.89 – 3.70 (m, 6H, H-2, H-3, H-6, CH₃ PMB), 3.67 (t, 1H, *J* = 8.9 Hz, H-4), 3.62 (s, 3H, OCH₃), 3.52 – 3.44 (m, 1H, H-1), 3.44 – 3.36 (m, 1H, H-5), 2.30 (t, 2H, *J* = 7.2 Hz, CH₂-9), 1.87 – 1.74 (m, 1H, CHH-8), 1.68 – 1.51 (m, 2H, CHH-7, CHH-8), 1.51 – 1.37 (m, 1H, CHH-7), 1.33 (d, 3H, *J* = 6.9 Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 175.7 (C=O), 161.1, 139.2 (C_q Ar), 130.9 (Ar), 130.7 (C_q Ar), 129.9, 129.1, 127.2, 114.8 (Ar), 102.5 (CH benzylidene), 83.6 (C-4), 80.6 (C-3), 80.4 (C-1), 74.1 (CH₂ glycolyl), 71.8 (C-5), 69.8 (CH₂-6), 69.8 (CH₂ PMB), 55.9 (C-2), 55.7 (CH₃ PMB), 52.0 (OCH₃), 34.5 (C-9), 32.5 (C-7), 22.0 (C-8), 19.7 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 3676, 2988, 2901, 2361, 2342, 1735, 1654, 1514, 1455, 1394, 1250, 1175, 1077, 752, 699, 668; HRMS: $[M+H]^+$ calcd. for $C_{31}H_{40}NO_{11}$ 602.2596, found 602.2606; LC-MS: Rt = 7.55 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

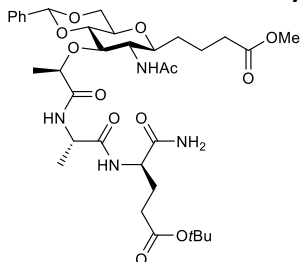
Fmoc-*i*-D-Gln(OtBu)-NH₂ (30)

Fmoc-D-Glu(OtBu)-OH (8.5 g, 20 mmol, 1.0 eq.) was dissolved in dioxane (0.20 L) followed by the addition of ammonium bicarbonate (7.2 g, 90 mmol, 4.5 eq.), di-*tert*-butyl dicarbonate (5.9 g, 27 mmol, 1.35 eq.) and pyridine (2.5 mL, 31 mmol, 1.55 eq.). After stirring at room temperature for 16 hours, the mixture was cooled to 0°C and quenched with H₂O. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with H₂O (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Recrystallization in MeOH gave compound **30** (8.6 g, 19 mmol, 96%) as a white solid. R_f: 0.3 (3/7 pentane/EtOAc); ¹H NMR (DMSO, 400 MHz, HH-COSY, HSQC): δ 7.89 (d, 2H, *J* = 7.8, 0.9 Hz, Ar), 7.73 (dd, 2H, *J* = 7.4, 4.9 Hz, Ar), 7.42 (t, 3H, *J* = 7.5, 1.2 Hz, Ar, NH), 7.32 (t, 3H, Ar, *NHH*), 6.14 (s, 1H, *NHH*), 4.35–4.13 (m, 3H, CH Fmoc, CH₂ Fmoc), 4.00–3.86 (m, 1H, CH *i*-D-Gln), 2.22 (t, 2H, *J* = 7.9 Hz, CH₂ γ-*i*-D-Gln), 1.98–1.81 (m, 1H, *CHH* β-*i*-D-Gln), 1.81–1.62 (m, 1H, *CHH* β-*i*-D-Gln), 1.39 (s, 9H, 3x CH₃ tBu), 1.36 (s, 4H); ¹³C-APT NMR (DMSO, 101 MHz, HSQC): δ 173.4, 171.7, 156.0 (C=O), 143.9, 143.8, 140.7 (C_q Ar), 127.7, 127.1, 125.4, 120.2 (Ar), 79.7 (C_q tBu), 65.6 (CH₂ Fmoc), 53.7 (CH *i*-D-Gln), 46.7 (CH Fmoc), 31.5 (CH₂ γ-*i*-D-Gln), 27.8 (CH₃ tBu), 27.3 (CH₂ β-*i*-D-Gln); FT-IR (neat, cm⁻¹): 2988, 2361, 2342, 1684, 1394, 1250, 1066, 668; HRMS: [M+H]⁺ calcd. for C₂₄H₂₉N₂O₅ 425.2071, found 425.2068.

Fmoc-L-Ala-*i*-D-Gln(OtBu)-NH₂ (31)

Compound **30** (8.12 g, 19.1 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere and dissolved in DCM (0.19 L). DBU (2.9 mL, 19.1 mmol, 1.0 eq.) was added and the mixture was stirred for 20 minutes. To quench the reaction, HOBt (12.9 g, 84.2 mmol, 4.4 eq.) was added and stirred for 20 minutes. Fmoc-L-Ala-OH (7.12 g, 23.0 mmol, 1.2 eq.), EDC-HCl (4.44 g, 23.0 mmol, 1.2 eq.) and DIPEA (19.3 mL, 111 mmol, 5.8 eq.) were added and stirring was continued for 16 hours. 1 M HCl was added and the resulting suspension was filtered. The filtrate was extracted with DCM (3x). The combined organic layers were washed with sat. aq. NaHCO₃ (3x), dried over MgSO₄, filtered and concentrated *in vacuo*. Recrystallization (EtOAc/pentane) gave the title compound (6.93 g, 14.0 mmol, 73%) as a white solid. R_f: 0.2 (3/7 pentane/EtOAc); ¹H NMR (DMSO, 400 MHz, HH-COSY, HSQC): δ 8.09–8.03 (m, 1H, NH), 7.88 (d, 2H, *J* = 7.5 Hz, Ar), 7.84 (dt, 1H, *J* = 7.6, 1.0 Hz, NH), 7.72 (t, 2H, *J* = 6.6 Hz, Ar), 7.62 (d, 1H, *J* = 7.1 Hz, NH), 7.41 (t, 2H, *J* = 7.4, 1.2 Hz, Ar), 7.37–7.29 (m, 2H, Ar), 7.27 (s, 1H, *NHH*), 7.14 (s, 1H, *NHH*), 4.33–4.10 (m, 3H, CH *i*-D-Gln, CH Fmoc, CH₂ Fmoc), 4.06 (p, 1H, *J* = 7.2 Hz, CH L-Ala), 2.23–2.11 (m, 2H, CH₂ γ-*i*-D-Gln), 2.05–1.87 (m, 1H, *CHH* β-*i*-D-Gln), 1.77–1.63 (m, 1H, *CHH* β-*i*-D-Gln), 1.36 (d, 9H, *J* = 10.7 Hz, 4x CH₃ tBu), 1.22 (d, 3H, *J* = 7.0 Hz, CH₃ L-Ala); ¹³C-APT NMR (DMSO, 101 MHz, HSQC): δ 173.1, 172.6, 171.7, 171.6, 155.9 (C=O), 143.9, 143.8, 142.6, 140.8, 139.5, 137.5 (C_q Ar), 129.0, 127.7, 127.3, 127.1, 125.3, 125.3, 121.4, 120.1, 120.1 (Ar), 79.7 (C_q tBu), 65.8 (CH₂ Fmoc), 51.4 (CH *i*-D-Gln), 50.3 (CH L-Ala), 46.6 (CH Fmoc), 31.2 (CH₂ γ-*i*-D-Gln), 27.7 (CH₃ tBu), 27.2 (CH₂ β-*i*-D-Gln), 18.0 (CH₃ L-Ala); FT-IR (neat, cm⁻¹): 3286, 2975, 1726, 1692, 1675, 1644, 1539, 1448, 1367, 1329, 1259, 1153, 1121, 1085, 1045, 981, 850, 756, 737, 621, 590, 550; HRMS: [M+H]⁺ calcd. for C₂₇H₃₄N₃O₆ 496.2442, found 496.2443.

Methyl 4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-5-*O*-*tert*-butoxy-D-isoglutaminyl)-β-D-glucopyranosyl)-butanoate (32a**)**

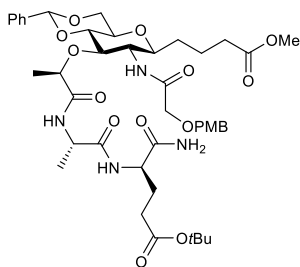


To a solution of compound **31** (3.72 g, 7.50 mmol, 1.5 eq.) in DMF (67 mL) was added DBU (1.2 mL, 8.0 mmol, 1.6 eq.) and the solution was stirred at room temperature for 1 hour. The reaction was quenched by addition HOBt (2.7 g, 17.6 mmol, 3.4 eq.) and the mixture was stirred for 20 minutes. A mixture of compound **27a** (1.79 g, 3.85 mmol, 0.75 eq.) and compound **28a** (0.52 g, 1.28 mmol, 0.25 eq.) was added, followed by the addition of HCTU (2.48 g, 6.0 mmol, 1.2 eq.) and DIPEA (3.5 mL, 20 mmol, 3.9 eq.). The

reaction mixture was stirred for overnight after which TLC analysis showed full conversion of the starting material. The reaction mixture was diluted with DCM and washed with brine (1x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was embedded on QuadraSil[®] aminopropyl and purification by column chromatography (2→10% MeOH in DCM) gave a mixture of compound **32a** and compound **33a** in quantitative yield (3.83 g) as a white solid. R_f: 0.4 (1/9 MeOH/DCM); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.51 – 7.44 (m, 2H, Ar), 7.39 – 7.31 (m, 3H, Ar), 5.63 (s, 1H, CH benzylidene), 4.35 (dd, 1H, *J* = 9.6, 4.6 Hz, CH *i*-D-Gln), 4.31 – 4.21 (m, 2H, *CHH*-6, CH L-Ala), 4.17 (q, 1H, *J* = 6.7 Hz, CH lactic acid), 3.87 (t, 1H, *J* = 9.6 Hz, H-2), 3.76 (t, 1H, *J* = 10.2 Hz, *CHH*-6), 3.71 – 3.58 (m, 5H, H-3, H-4, OCH₃), 3.47 – 3.34 (m, 2H, H-1, H-5), 2.39 – 2.23 (m, 4H, CH₂-9, CH₂ *γ*-*i*-D-Gln), 2.23 – 2.10 (m, 1H, *CHH* β-*i*-D-Gln), 1.96 (s, 3H, CH₃ Ac), 1.91 – 1.76 (m, 2H, *CHH*-8, *CHH* β-*i*-D-Gln), 1.70 – 1.57 (m, 2H, *CHH*-8, *CHH*-7), 1.57 – 1.41 (m, 10H, *CHH*-7, 3x CH₃ *t*Bu), 1.41 – 1.35 (m, 3H, CH₃ L-Ala), 1.35 – 1.28 (m, 3H, CH₃ lactic acid); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 175.7, 175.0, 173.7 (C=O), 139.1 (C_q Ar), 129.9, 129.1, 127.3 (Ar), 102.6 (CH benzylidene), 82.8 (C-4), 82.0 (C-3), 81.8 (C_q *t*Bu), 80.6 (C-1), 79.0 (CH lactic acid), 71.8 (C-5), 69.7 (CH₂-6), 56.1 (C-2), 53.5 (OCH₃), 52.0 (CH *i*-D-Gln), 50.7 (CH L-Ala), 34.5 (C-9), 32.6 (C-7), 32.2 (CH₂ *γ*-*i*-D-Gln), 28.3 (CH₃ *t*Bu), 28.3 (CH₂ β-*i*-D-Gln), 23.2 (CH₃ Ac), 22.0 (C-8), 19.7 (CH₃ lactic acid), 17.9 (CH₃ L-Ala); FT-IR (neat, cm⁻¹): 3280, 1731, 1643, 1544, 1369, 1155; HRMS: [M+H]⁺ calcd. for C₃₅H₅₃N₄O₁₂ 721.3655, found 721.3664.

*Data given for title compound only.

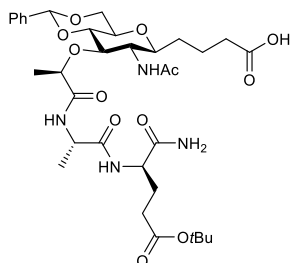
Methyl 4-(4,6-*O*-di-benzylidene-2-deoxy-2-*N*-((*p*-methoxybenzyl)oxy)acetamide-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-5-*O*-*tert*-butoxy-D-isoglutaminyl)- β -D-glucopyranosyl)-butanoate (32b**)**



Compound **31** (3.73 g, 7.52 mmol, 1.5 eq.) was dissolved in DMF (67 mL). DBU (1.2 mL, 8.0 mmol, 1.6 eq.) was added and the reaction was stirred at room temperature for 1 hour. After quenching with HOBt (0.18 g, 1.35 mmol, 3.5 eq.), the suspension was stirred for 20 minutes. A mixture of compound **27b** (2.43 g, 4.04 mmol, 0.80 eq.) and compound **28b** (0.58 g, 1.0 mmol, 0.20 eq.) was added, followed by the addition of HCTU (2.5 g, 6.0 mmol, 1.2 eq.) and DIPEA (3.5 mL, 20 mmol, 4.0 eq.). The

reaction mixture was stirred overnight. Upon completion of the reaction determined by TLC analysis, 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*. The residue was embedded on QuadraSil[®] aminopropyl and purification by column chromatography (2 \rightarrow 6% MeOH in DCM) gave a mixture of compound **32b** (3.08 g, 3.60 mmol, 89%) and compound **33b** (0.67 g, 0.84 mmol). R_f : 0.6 (1/9 MeOH/DCM); $^1\text{H NMR}$ (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.43 (m, 2H, Ar), 7.39 – 7.27 (m, 5H, Ar), 6.95 – 6.89 (m, 2H, Ar), 5.60 (s, 1H, CH benzylidene), 4.53 (q, 2H, $J = 11.9, 11.4, 3.9$ Hz, CH_2 glycolyl), 4.34 (dd, 1H, $J = 9.5, 4.7$ Hz, CH *i*-D-Gln), 4.30 – 4.20 (m, 2H, CHH-6, CH L-Ala), 4.16 (q, 1H, $J = 6.8$ Hz, CH lactic acid), 4.02 – 3.91 (m, 3H, H-2, CH_2 PMB), 3.82 – 3.69 (m, 5H, H-3, CHH-6, CH_3 PMB), 3.68 – 3.58 (m, 4H, H-4, OCH_3), 3.54 – 3.46 (m, 1H, H-1), 3.47 – 3.37 (m, 1H, H-5), 2.35 – 2.25 (m, 4H, CH_2 -9, CH_2 γ -*i*-D-Gln), 2.21 – 2.11 (m, 1H, CHH β -*i*-D-Gln), 1.89 – 1.75 (m, 2H, CHH-8, CHH β -*i*-D-Gln), 1.70 – 1.49 (m, 2H, CHH-7, CHH-8), 1.49 – 1.37 (m, 10H, CHH-7, 3x CH_3 *t*Bu), 1.37 – 1.26 (m, 6H, CH_3 lactic acid, CH_3 L-Ala); ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 176.1, 175.6, 175.5, 174.9, 173.7, 173.2 (C=O), 161.1, 139.1 (C_q Ar), 130.9 (Ar), 130.4 (C_q Ar), 129.9, 129.1, 127.2, 114.9 (Ar), 102.5 (CH benzylidene), 82.6 (C-4), 81.8 (C_q *t*Bu), 81.5 (C-3), 80.0 (C-1), 79.0 (CH lactic acid), 74.0 (CH_2 glycolyl), 71.7 (C-5), 69.7 (CH_2 PMB), 69.7 (CH_2 -6), 55.8 (C-2), 55.7 (CH_3 PMB), 53.4 (CH *i*-D-Gln), 52.0 (OCH_3), 50.6 (CH L-Ala), 34.4 (CH_2 γ -*i*-D-Gln), 32.7 (C-9), 32.2 (C-7), 28.3 (CH_3 *t*Bu), 28.2 (CH_2 β -*i*-D-Gln), 21.9 (C-8), 19.8 (CH_3 lactic acid), 18.0 (CH_3 L-Ala); FT-IR (neat, cm^{-1}): 2360, 1665, 1515, 1250, 1103, 1038; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{43}\text{H}_{61}\text{N}_4\text{O}_{14}$ 857.4179, found 857.4201. *Data given for title compound only.

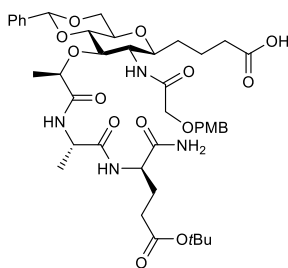
4-C-(2-deoxy-2-N-acetyl-4,6-O-di-benzylidene-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-5-O-tert-butoxy-D-isoglutaminyl)-β-D-glucopyranosyl)-butanoic acid (34a)



The previously obtained mixture of compound **32a** (0.37 g, 0.51 mmol, 0.72 eq.) and compound **33a** (0.13 g, 0.20 mmol, 0.28 eq) was dissolved in MeOH (23 mL). LiOH (91 mg, 2.2 mmol, 3.0 eq.) and a 35% H₂O₂ in H₂O solution (0.69 mL, 7.9 mmol, 11 eq.) were added. After 8 hours of stirring, the reaction mixture was acidified with acetic acid to pH = 1. Toluene (30 mL) was added and the solution was concentrated *in vacuo*. Recrystallization (MeOH/DCM/Et₂O) gave the title compound (0.26 g, 0.37

mmol, 73%) as a white solid. R_f: 0.6 (1/9 MeOH/DCM); [α]_D²⁰ = -21.2° (c = 1.0, MeOH); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.51 – 7.44 (m, 2H, Ar), 7.39 – 7.30 (m, 3H, Ar), 5.62 (s, 1H, CH benzylidene), 4.34 (dd, 1H, J = 9.7, 4.5 Hz, CH *i*-D-Gln), 4.31 – 4.21 (m, 2H, CHH-6, CH L-Ala), 4.15 (q, 1H, J = 6.6 Hz, CH lactic acid), 3.85 (t, 1H, J = 9.6 Hz, H-2), 3.75 (t, 1H, J = 10.2 Hz, CHH-6), 3.68 – 3.57 (m, 2H, H-3, H-4), 3.46 – 3.36 (m, 2H, H-1, H-5), 2.36 – 2.23 (m, 2H, CH₂ γ-*i*-D-Gln), 2.23 – 2.10 (m, 3H, CH₂-9, CHH β-*i*-D-Gln), 1.96 (s, 3H, CH₃ Ac), 1.88 – 1.75 (m, 2H, CHH-8, CHH β-*i*-D-Gln), 1.69 – 1.56 (m, 2H, CHH-8, CHH-7), 1.44 (s, 9H, 3x CH₃ tBu), 1.42 – 1.36 (m, 4H, CH₃ L-Ala, CHH-7), 1.32 (d, 3H, J = 6.7 Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 175.7, 175.1, 173.7, 173.7 (C=O), 139.2 (C_q Ar), 129.9, 129.1, 127.3 (Ar), 102.6 (CH benzylidene), 82.8 (C-4), 82.3 (C-3), 81.8 (C_q tBu), 80.5 (C-5), 79.1 (CH lactic acid), 71.8 (C-1), 69.8 (CH₂-6), 56.5 (C-2), 53.5 (CH *i*-D-Gln), 50.8 (CH L-Ala), 38.7 (C-9), 32.9 (C-7), 32.7 (CH₂ γ-*i*-D-Gln), 28.3 (CH₃ tBu), 28.2 (CH₂ β-*i*-D-Gln), 23.7 (C-8), 23.3 (CH₃ Ac), 19.7 (CH₃ lactic acid), 17.9 (CH₃ L-Ala); FT-IR (neat, cm⁻¹): 3274, 2360, 1643, 1562, 1423, 1369, 1153, 1105, 1038, 1028, 694; HRMS: [M+H]⁺ calcd. for C₃₄H₅₁N₄O₁₂ 707.3498, found 707.3515; LC-MS: Rt = 5.25 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

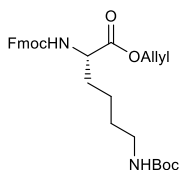
4-C-(4,6-O-di-benzylidene-2-deoxy-2-N-((p-methoxybenzyl)oxy)acetamide-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-5-O-tert-butoxy-D-isoglutaminy)-β-D-glucopyranosyl)-butanoic acid (34b)



LiOH (0.57 g, 13.6 mmol) and a 35% H₂O₂ in H₂O solution (4.35 mL, 50.6 mmol) were dissolved in H₂O (25.7 mL). A previously obtained mixture of compound **32b** (0.44 g, 0.51 mmol, 0.86 eq.) and compound **33b** (66 mg, 83 μmol, 0.14 eq) was dissolved in THF (5.1 mL) and cooled to 0°C, followed by the addition of prepared LiOH/H₂O₂ solution (3.4 mL). The reaction was stirred at 0°C for 11 hours and subsequently quenched with AcOH to pH = 1. The mixture was diluted with toluene, concentrated *in vacuo* and

recrystallization (MeOH/DCM/Et₂O) gave acid **34b** (0.40 g, 0.47 mmol, 92%) as a white solid. R_f: 0.6 (1/9 MeOH/DCM). [α]_D²⁰ = -15.5° (c = 1.0, MeOH); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.43 (m, 2H, Ar), 7.39 – 7.28 (m, 5H, Ar), 6.95 – 6.88 (m, 2H, Ar), 5.61 (s, 1H, CH benzylidene), 4.53 (s, 2H, CH₂ glycolyl), 4.33 (dd, 1H, J = 9.5, 4.6 Hz, CH *i*-D-Gln), 4.30 – 4.19 (m, 2H, CHH-6, CH L-Ala), 4.16 (q, 1H, J = 6.7 Hz, CH lactic acid), 3.98 (t, 1H, J = 9.9 Hz, H-2), 3.93 (s, 2H, CH₂ PMB), 3.82 – 3.71 (m, 5H, H-3, CHH-6, CH₃ PMB), 3.64 (t, 1H, J = 9.2 Hz, H-4), 3.55 – 3.47 (m, 1H, H-1), 3.47 – 3.38 (m, 1H, H-5), 2.33 – 2.23 (m, 4H, CH₂-9, CH₂ γ-*i*-D-Gln), 2.22 – 2.10 (m, 1H, CHH β-*i*-D-Gln), 1.89 – 1.75 (m, 2H, CHH-8, CHH β-*i*-D-Gln), 1.70 – 1.54 (m, 2H, CHH-8, CHH-7), 1.51 – 1.37 (m, 10H, CHH-7, 3x CH₃ tBu), 1.33 (dd, 6H, J = 7.0, 1.3 Hz, CH₃ lactic acid, CH₃ L-Ala); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 177.2, 176.2, 175.5, 174.9, 173.7, 173.2 (C=O), 161.1, 139.1 (C_q Ar), 130.9 (Ar), 130.4 (C_q Ar), 129.9, 129.1, 127.2, 114.9 (Ar), 102.6 (CH benzylidene), 82.6 (C-4), 81.8 (C_q tBu), 81.6 (C-3), 80.1 (C-1), 79.0 (CH lactic acid), 74.0 (CH₂ glycolyl), 71.7 (C-5), 69.8, 69.7 (CH₂-6, CH₂ PMB), 55.9 (C-2), 55.7 (CH₃ PMB), 53.5 (CH *i*-D-Gln), 50.6 (CH L-Ala), 34.6 (CH₂ γ-*i*-D-Gln), 32.7 (C-9), 32.3 (C-7), 28.3 (CH₃ tBu), 28.2 (CH₂ β-*i*-D-Gln), 22.0 (C-8), 19.8 (CH₃ lactic acid), 18.0 (CH₃ L-Ala); FT-IR (neat, cm⁻¹): 3319, 2974, 2360, 2342, 1663, 1515, 1454, 1394, 1250, 1154, 1076, 668; HRMS: [M+H]⁺ calcd. for C₄₂H₅₉N₄O₁₄ 843.4023, found 843.4047; LC-MS: Rt = 6.59 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

Fmoc-L-Lys(Boc)-OAllyl (35)

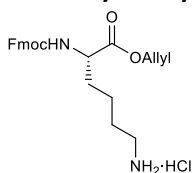


A solution of Fmoc-Lys(Boc)-OH (4.70 g, 10.0 mmol, 1.0 eq.) in DMF (40 mL) under an argon atmosphere was cooled to 0°C. Silver carbonate (3.59 g, 13.0 mmol, 1.3 eq.) was added and the mixture was stirred for 15 minutes at 0°C, followed by the addition of allyl bromide (3.98 mL, 46.0 mmol, 4.6 eq.). The reaction mixture was allowed to warm-up to room temperature and stirred for 2.5 hours,

before TLC analysis showed that the reaction was complete. The suspension was filtered, diluted with Et₂O, washed with a 10 wt% KHSO₄ solution (2x) and H₂O (2x). The organic layer was dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (20→60% EtOAc in pentane) gave the title compound in quantitative yield (5.52 g) as a yellow solid. R_f: 0.7 (3/2 pentane/EtOAc); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.80 (d, 2H, J = 7.6, 0.9 Hz, Ar), 7.67 (t, 2H, J = 6.4,

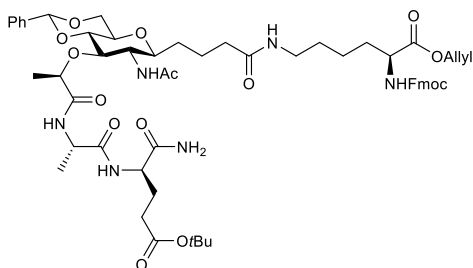
0.9 Hz, Ar), 7.39 (t, 2H, $J = 7.5$ Hz, Ar), 7.31 (t, 2H, $J = 7.5, 1.2$ Hz, Ar), 6.00 – 5.86 (m, 1H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 5.32 (dd, 1H, $J = 17.2, 1.6$ Hz, $\text{CH}_2\text{-CH}=\text{CHH}$), 5.21 (dd, 1H, $J = 10.5, 1.3$ Hz, $\text{CH}_2\text{-CH}=\text{CHH}$), 4.62 (dd, 2H, $J = 5.6, 1.4$ Hz, $\text{CH}_2\text{-CH}=\text{CH}_2$), 4.41 – 4.29 (m, 2H, CH_2 Fmoc), 4.25 – 4.13 (m, 2H, CH Fmoc, CH L-Lys), 3.03 (t, 2H, $J = 7.1$ Hz, CH_2 ϵ -Lys), 1.89 – 1.78 (m, 1H, CHH β -Lys), 1.75 – 1.63 (m, 1H, CHH β -Lys), 1.56 – 1.33 (m, 13H, CH_2 γ -Lys, CH_2 δ -Lys, 3x CH_3 t Bu); ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 173.8, 158.7 (C=O), 145.3, 145.1, 142.6 (C_q Ar), 133.4 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 128.8, 128.2, 128.2, 126.3, 126.2, 120.9 (Ar), 118.6 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 79.9 (C_q t Bu) 68.0 (CH_2 Fmoc), 66.7 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 55.5 (CH L-Lys), 48.4 (CH Fmoc), 41.0 (CH_2 ϵ -Lys), 32.2 (CH_2 β -Lys), 30.5 (CH_2 δ -Lys), 28.8 (CH_3 t Bu), 24.2 (CH_2 γ -Lys); FT-IR (neat, cm^{-1}): 3341, 2937, 1710, 1522, 1451, 1366, 1250, 1173, 760, 741; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{29}\text{H}_{36}\text{N}_2\text{O}_6\text{Na}$ 531.2471, found 531.2475.

Fmoc-L-Lys-OAllyl (36)



Compound **35** (5.01 g, 10 mmol, 1.0 eq.) was cooled to 0°C . 4 M HCl in dioxane (25 mL, 10 eq.) was added and the reaction was stirred at 0°C . After complete solvation of the starting material, the ice bath was removed and the clear solution was stirred for 1 hour at room temperature. The mixture was diluted with toluene (5 mL) and concentrated *in vacuo*. Co-evaporation with toluene (3x) and purification by column chromatography (4 \rightarrow 16% MeOH in DCM) gave the title compound (3.95 g, 9.70 mmol, 97%) as a white solid. R_f : 0.2 (1/9 MeOH/DCM); ^1H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.79 (d, 2H, $J = 7.6, 1.0$ Hz, Ar), 7.66 (t, 2H, $J = 15.4, 7.8$ Hz, Ar), 7.39 (t, 2H, Ar), 7.31 (t, 2H, $J = 14.9$ Hz, Ar), 6.00 – 5.85 (m, 1H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 5.32 (dq, 1H, $J = 17.2, 1.6$ Hz, $\text{CH}_2\text{-CH}=\text{CHH}$), 5.21 (dq, 1H, $J = 10.5, 1.4$ Hz, $\text{CH}_2\text{-CH}=\text{CHH}$), 4.62 (dt, 2H, $J = 5.6, 1.5$ Hz, $\text{CH}_2\text{-CH}=\text{CH}_2$), 4.45 – 4.29 (m, 2H, CH_2 Fmoc), 4.25 – 4.16 (m, 2H, CH Fmoc, CH L-Lys), 2.97 – 2.84 (m, 2H, CH_2 ϵ -Lys), 1.94 – 1.79 (m, 1H, CHH β -Lys), 1.79 – 1.55 (m, 3H, CHH β -Lys, CH_2 δ -Lys), 1.55 – 1.25 (m, 2H, CH_2 γ -Lys); ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 173.5, 158.7 (C=O), 145.3, 145.1, 142.6 (C_q Ar), 133.3 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 128.8, 128.2, 128.1, 126.2, 126.2, 120.9 (Ar), 118.7 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 67.9 (CH_2 Fmoc), 66.8 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 55.2 (CH L-Lys), 48.4 (CH Fmoc), 40.5 (CH_2 ϵ -Lys), 31.9 (CH_2 β -Lys), 28.0 (CH_2 δ -Lys), 23.9 (CH_2 γ -Lys); FT-IR (neat, cm^{-1}): 2944, 1716, 1648, 1609, 1520, 1478, 1450, 1412, 1331, 1248, 1195, 1170, 1121, 1047, 987, 936, 782, 760, 738, 621, 541; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{24}\text{H}_{29}\text{N}_2\text{O}_4$ 409.2122, found 409.2129.

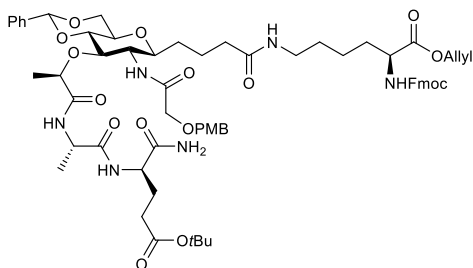
N_{α} -Fmoc- N_{ϵ} -[butan-4-C-(2-deoxy-2-N-acetyl-4,6-O-di-benzylidene-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-5-O-tert-butoxy-D-isoglutaminyl)- β -D-glucopyranosyl)-amide]-L-lysine-allyl ester (37a)



Compound **34a** (0.21 g, 0.30 mmol, 1.0 eq.) and compound **36** (0.18 g, 0.45 mmol, 1.5 eq.) were co-evaporated with toluene (3x) under an argon atmosphere and dissolved in DMF (12 mL). HCTU (0.15 g, 0.36 mmol, 1.2 eq.) and DIPEA (78 μ L, 0.45 mmol, 3.0 eq.) were added and the mixture was stirred for 3 hours.

The reaction was diluted with Et₂O and the precipitate was collected by filtration. Recrystallization (MeOH/DCM/Et₂O) gave the title compound (0.28 g, 0.26 mmol, 87%) as a white solid. *R*_f: 0.4 (1/9 MeOH/DCM); $[\alpha]_D^{20} = -18.6^{\circ}$ (*c* = 1.0, DCM/MeOH: 1/1); ¹H NMR (MeOD/CD₂Cl₂ 1/1, 400 MHz, HH-COSY, HSQC): δ 7.78 (d, 2H, *J* = 7.5 Hz, Ar), 7.65 (dd, 2H, *J* = 7.6, 4.1 Hz, Ar), 7.48 – 7.43 (m, 2H, Ar), 7.40 (t, 2H, *J* = 7.4 Hz, Ar), 7.37 – 7.28 (m, 5H, Ar), 5.98 – 5.85 (m, 1H, CH₂-CH=CH₂), 5.55 (s, 1H, CH benzylidene), 5.32 (d, 1H, *J* = 17.1 Hz, CH₂-CH=CHH), 5.22 (d, 1H, *J* = 10.5 Hz, CH₂-CH=CHH), 4.62 (d, 2H, *J* = 5.4 Hz, CH₂-CH=CH₂), 4.45 – 4.30 (m, 3H, CH *i*-D-Gln, CH₂ Fmoc), 4.30 – 4.16 (m, 4H, CHH-6, CH L-Lys, CH Fmoc, CH L-Ala), 4.13 (q, 1H, *J* = 6.7 Hz, CH lactic acid), 3.90 – 3.79 (m, 1H, H-2), 3.68 (t, 1H, *J* = 10.3 Hz, CHH-6), 3.63 – 3.54 (m, 2H, H-3, H-4), 3.43 – 3.33 (m, 2H, H-1, H-5), 3.15 (t, 2H, *J* = 7.0 Hz, CH₂ ϵ -L-Lys), 2.30 (t, 2H, *J* = 7.6 Hz, CH₂ γ -*i*-D-Gln), 2.21 – 2.10 (m, 3H, CH₂-9, CHH β -*i*-D-Gln), 1.93 (s, 3H, CH₃ Ac), 1.90 – 1.75 (m, 3H, CHH β -*i*-D-Gln, CHH β -L-Lys, CHH-8), 1.75 – 1.46 (m, 6H, CHH β -L-Lys, CHH-8, CH₂-7, CH₂ γ -L-Lys), 1.42 (s, 9H, 3x CH₃ *t*Bu), 1.37 (d, 5H, *J* = 7.0 Hz, CH₂ δ -L-Lys, CH₃ L-Ala), 1.32 (d, 3H, *J* = 6.8 Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD/CD₂Cl₂ 1/1, 101 MHz, HSQC): δ 175.4, 175.2, 175.0, 174.1, 173.4, 173.3, 173.0 (C=O), 157.8, 144.6, 142.0, 138.2 (C_q Ar), 132.6 (CH₂-CH=CH₂), 129.6, 128.8, 128.4, 127.8, 127.7, 126.7, 125.8, 125.8, 120.6 (Ar), 118.7 (CH₂-CH=CH₂), 102.0 (CH benzylidene), 82.2 (C-4), 81.6 (C_q *t*Bu), 81.4 (C-3), 80.1 (C-1), 78.5 (CH lactic acid), 71.1 (C-5), 69.4 (CH₂-6), 67.6 (CH₂ Fmoc), 66.5 (CH₂-CH=CH₂), 55.6 (C-2), 54.9 (CH L-Lys), 52.9 (CH *i*-D-Gln), 50.1 (CH L-Ala), 47.9 (CH Fmoc), 39.6 (CH₂ ϵ -L-Lys), 36.7 (C-9), 32.3 (CH₂ γ -*i*-D-Gln), 31.9 (C-7), 29.5 (CH₂ γ -L-Lys), 28.2 (CH₃ *t*Bu), 27.7 (CH₂ β -*i*-D-Gln), 23.7 (CH₂ δ -L-Lys), 23.2 (CH₃ Ac), 22.6 (C-8), 19.5 (CH₃ L-Ala), 17.6 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 3281, 1728, 1642, 1541, 1451, 1369, 1275, 1153, 1105, 1028, 741, 696; HRMS: $[M+H]^+$ calcd. for C₅₈H₇₇N₆O₁₅ 1097.5442, found 1097.5452; LC-MS: *R*_t = 7.30 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

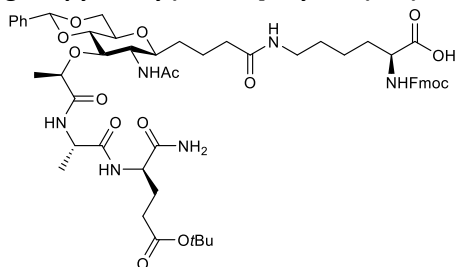
***N*_α-Fmoc-*N*_ε-[butan-4-(4,6-*O*-di-benzylidene-2-deoxy-2-*N*-((*p*-methoxybenzyl)oxy)acetamide-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-5-*O*-*tert*-butoxy-*D*-isoglutaminyl)-β-*D*-glucopyranosyl)-amide]-L-lysine-allyl ester (**37b**)**



Compound **34b** (1.19 g, 1.41 mmol, 1.0 eq.) and compound **36** (0.87 g, 2.12 mmol, 1.5 eq.) were co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in DMF (14 mL), followed by the addition of HCTU (0.71 g, 1.70 mmol, 1.2 eq.) and DIPEA (0.37 mL, 2.12 mmol, 3.0 eq.). The mixture was stirred 3 hours and

subsequently diluted with Et₂O to precipitate the product. The precipitate was filtered and purification by recrystallization (MeOH/DCM/Et₂O) and column chromatography (5→15% MeOH in DCM) gave the title compound (1.32 g, 1.07 mmol, 76%) as a white solid. *R*_f: 0.6 (1/9 MeOH/DCM); $[\alpha]_D^{20} = -13.6^\circ$ (*c* = 1.0, DCM/MeOH: 1/1); ¹H NMR (MeOD/CD₂Cl₂ 1/1, 400 MHz, HH-COSY, HSQC): δ 7.77 (d, 2H, *J* = 6.9 Hz, Ar), 7.65 (dd, 2H, *J* = 7.6, 4.4 Hz, Ar), 7.48 – 7.24 (m, 11H, Ar), 6.90 (d, 2H, *J* = 9.0 Hz, Ar), 5.97 – 5.84 (m, 1H, CH₂-CH=CH₂), 5.55 (s, 1H, CH benzylidene), 5.35 – 5.26 (m, 1H, CH₂-CH=CHH), 5.26 – 5.18 (m, 1H, CH₂-CH=CHH), 4.49 (d, 2H, *J* = 2.8 Hz, CH₂ glycolyl), 4.45 – 4.14 (m, 7H, CHH-6, CH *i*-D-Gln, CH L-Lys, CH Fmoc, CH₂ Fmoc, CH L-Ala), 4.11 (q, 1H, *J* = 6.6 Hz, CH lactic acid), 3.99 – 3.87 (m, 3H, H-2, CH₂ PMB), 3.78 (s, 3H, CH₃ PMB), 3.70 (q, 2H, *J* = 10.0, 9.4 Hz, H-3, H-4), 3.58 (t, 1H, *J* = 9.2 Hz, CHH-6), 3.50 – 3.35 (m, 2H, H-1, H-5), 3.18 – 3.09 (m, 2H, CH₂ ε-L-Lys), 2.29 (t, 2H, *J* = 8.0 Hz, CH₂ γ-*i*-D-Gln), 2.20 – 2.08 (m, 3H, CH₂-9, CHH β-*i*-D-Gln), 1.90 – 1.75 (m, 3H, CHH β-*i*-D-Gln, CHH β-L-Lys, CHH-8), 1.75 – 1.45 (m, 5H, CHH β-L-Lys, CHH-8, CHH-7, CH₂ γ-L-Lys), 1.45 – 1.35 (m, 12H, CHH-7, CH₂ δ-L-Lys, 3x CH₃ *t*Bu), 1.32 (t, 6H, *J* = 7.2 Hz, CH₃ lactic acid, CH₃ L-Ala); ¹³C-APT NMR (MeOD/CD₂Cl₂ 1/1, 126 MHz, HSQC): δ 175.4, 175.1, 174.9, 174.8, 174.1, 174.0, 173.5, 173.4, 172.3 (C=O), 160.5, 157.9, 144.8, 144.7, 142.1, 138.3 (C_q Ar), 132.7 (CH₂-CH=CH₂), 130.5 (Ar), 129.9 (C_q Ar), 129.6, 128.8, 128.4, 127.8, 127.8, 126.8, 125.9, 125.8, 120.6 (Ar), 118.7 (CH₂-CH=CH₂), 114.6 (Ar), 102.1 (CH benzylidene), 82.1 (C-4), 81.6 (C_q *t*Bu), 81.1 (C-3), 79.6 (C-1), 78.5 (CH L-Ala), 73.8 (CH₂ glycolyl), 71.2 (C-5), 69.6 (CH₂ PMB), 69.4 (CH₂-6), 67.6 (CH₂ Fmoc), 66.5 (CH₂-CH=CH₂), 55.7 (CH₃ PMB), 55.5 (C-2), 54.9 (CH L-Lys), 53.0 (CH *i*-D-Gln), 50.1 (CH lactic acid), 48.0 (CH Fmoc), 39.6 (CH₂ ε-L-Lys), 36.7 (C-9), 32.4 (CH₂ γ-*i*-D-Gln), 32.0 (CH₂ β-L-Lys), 32.0 (CH₂ γ-L-Lys), 29.5 (CH₂ δ-L-Lys), 28.2 (CH₃ *t*Bu), 27.8 (CH₂ β-*i*-D-Gln), 23.7 (C-7), 22.6 (C-8), 19.6 (CH₃ L-Ala), 17.7 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 3315, 2937, 1729, 1691, 1644, 1537, 1451, 1368, 1253, 1105, 1129, 845, 741, 696; HRMS: [M+H]⁺ calcd. for C₆₆H₈₅N₆O₁₇ 1233.5966, found 1233.5964; LC-MS: *R*_t = 8.80 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

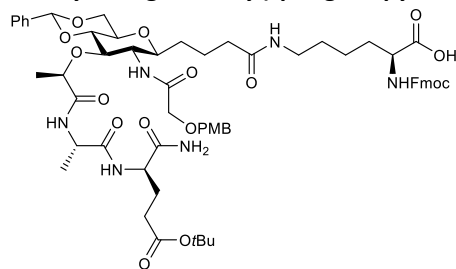
N_{α} -Fmoc- N_{ϵ} -[butan-4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-5-*O*-*tert*-butoxy-D-isoglutaminyl)- β -D-glucopyranosyl)-amide]-L-lysine (10a)



Compound **37a** (0.54 g, 0.49 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in DMF (20 mL) and cooled to 0°C. Phenylsilane (0.12 mL, 0.98 mmol, 2.0 eq.) and Pd(PPh₃)₄ (59 mg, 0.05 mmol, 0.1 eq.) were added and the reaction was stirred at 0°C for 30 minutes. Upon completion of the reaction determined by

LC-MS, Et₂O was added to precipitate the crude product. After filtration, the precipitate was purified by recrystallization (MeOH/DCM/Et₂O) to yield the title compound (0.42 g, 0.40 mmol, 82%) as a pale yellow solid. $[\alpha]_D^{20} = -10.2^{\circ}$ ($c = 1.0$, DCM/MeOH: 1/1); ¹H NMR (MeOD/CD₂Cl₂ 1/1, 500 MHz, HH-COSY, HSQC): δ 7.78 (d, 2H, $J = 7.6$ Hz, Ar), 7.65 (t, 2H, $J = 7.0$ Hz, Ar), 7.48–7.42 (m, 2H, Ar), 7.42–7.36 (m, 2H, Ar), 7.36–7.27 (m, 5H, Ar), 5.55 (s, 1H, CH benzylidene), 4.42–4.30 (m, 3H, CH L-Lys, CH *i*-D-Gln, CH Fmoc), 4.28–4.15 (m, 4H, CHH-6, CH₂ Fmoc, CH L-Ala), 4.13 (q, 1H, $J = 6.7$ Hz, CH lactic acid), 3.88–3.80 (m, 1H, H-2), 3.69 (t, 1H, $J = 10.3$ Hz, CHH-6), 3.62–3.55 (m, 2H, H-3, H-4), 3.37 (q, 2H, $J = 11.2, 9.2$ Hz, H-1, H-5), 3.16 (t, 2H, $J = 6.9$ Hz, CH₂ ϵ -L-Lys), 2.33–2.27 (m, 2H, CH₂ γ -*i*-D-Gln), 2.20–2.11 (m, 3H, CHH β -*i*-D-Gln, CH₂-9), 1.94 (s, 3H, CH₃ Ac), 1.90–1.76 (m, 3H, CHH β -*i*-D-Gln, CH₂ β -L-Lys), 1.76–1.46 (m, 6H, CH₂ γ -L-Lys, CH₂ δ -L-Lys, CH₂-8), 1.41 (s, 11H, CH₂-7, 3x CH₃ *t*Bu), 1.37 (d, 3H, $J = 7.1$ Hz, CH₃ L-Ala), 1.32 (d, 3H, $J = 6.7$ Hz, CH₃ lactic acid); ¹³C-APT NMR (MeOD/CD₂Cl₂ 1/1, 126 MHz, HSQC): δ 175.4, 175.2, 175.0, 174.1, 173.4, 173.0 (C=O), 144.8, 144.7, 142.0, 138.3 (C_q Ar), 129.6, 128.8, 128.4, 127.8, 126.7, 125.8, 120.6 (Ar), 102.1 (CH benzylidene), 82.2 (C-4), 81.6 (C_q *t*Bu), 81.5 (C-3), 80.0 (C-1), 78.5 (CH lactic acid), 71.2 (C-5), 69.4 (CH₂-6), 67.5 (CH₂ Fmoc), 55.7 (C-2), 54.9 (CH L-Lys), 53.0 (CH *i*-D-Gln), 50.2 (CH L-Ala), 47.9 (CH Fmoc), 39.7 (CH₂ ϵ -L-Lys), 36.7 (C-9), 32.4 (CH₂ γ -*i*-D-Gln), 31.9 (C-7), 29.5 (CH₂ γ -L-Lys), 28.2 (CH₃ *t*Bu), 27.8 (CH₂ β -*i*-D-Gln), 23.6 (CH₂ δ -L-Lys), 23.2 (CH₃ Ac), 22.6 (C-8), 19.5 (CH₃ L-Ala), 17.6 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 3300, 2934, 1656, 1537, 1451, 1369, 1252, 1154, 1104, 1029, 742, 698; HRMS: $[M+H]^+$ calcd. for C₅₅H₇₃N₆O₁₅ 1057.5129, found 1057.5153; LC-MS: Rt = 7.37 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

N_{α} -Fmoc- N_{ϵ} -[butan-4-(4,6-*O*-di-benzylidene-2-deoxy-2-*N*-((*p*-methoxybenzyl)oxy)acetamide-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-5-*O*-tert-butoxy-D-isoglutaminyl)- β -D-glucopyranosyl)-amide]-L-lysine (10b**)**



Compound **37b** (0.75 g, 0.60 mmol, 1.0 eq.) was co-evaporated with toluene (3x) under argon atmosphere. The residue was dissolved in DMF (12 mL) and cooled to 0°C. Phenylsilane (0.15 mL, 1.20 mmol, 2.0 eq.) and Pd(PPh₃)₄ (69.0 mg, 0.06 mmol, 0.1 eq.) were added and the reaction was stirred at 0°C. After 30 minutes, TLC showed full conversion of the starting

material. Et₂O was added to precipitate the crude product and after filtration, the precipitate was purified by recrystallization (MeOH/DCM/Et₂O) to give the title compound (0.62 g, 0.52 mmol, 87%) as a pale yellow solid. R_f: 0.2 (1/9 MeOH/DCM); [α]_D²⁵ = -18.7° (c = 0.30, DCM/MeOH: 1/1); ¹H NMR (MeOD/CD₂Cl₂ 1/1, 500 MHz, HH-COSY, HSQC): δ 7.77 (d, 2H, *J* = 7.6 Hz, Ar), 7.65 (t, 2H, *J* = 7.0 Hz, Ar), 7.50 – 7.42 (m, 3H, Ar), 7.42 – 7.36 (m, 3H, Ar), 7.36 – 7.30 (m, 3H, Ar), 7.30 – 7.25 (m, 3H, Ar), 6.90 (d, 2H, *J* = 8.6 Hz, Ar), 5.54 (s, 1H, CH benzylidene), 4.49 (q, 2H, *J* = 12.0, 11.6, 3.3, 2.8 Hz, CH₂ glycolyl), 4.42 – 4.29 (m, 3H, CH₂ Fmoc, CH *i*-D-Gln), 4.29 – 4.15 (m, 4H, *CHH* -6, CH Fmoc, CH L-Lys, CH L-Ala), 4.11 (q, 1H, *J* = 6.7 Hz, CH lactic acid), 3.98 – 3.86 (m, 3H, H-2, CH₂ PMB), 3.77 (s, 3H, CH₃ PMB), 3.69 (q, 2H, *J* = 10.9, 10.3 Hz, H-3, *CHH* -6), 3.58 (t, 1H, *J* = 9.2 Hz, H-4), 3.45 (t, 1H, *J* = 9.2 Hz, H-1), 3.42 – 3.36 (m, 1H, H-5), 3.19 – 3.10 (m, 2H, CH₂ ϵ -L-Lys), 2.33 – 2.23 (m, 2H, CH₂ γ -*i*-D-Gln), 2.18 – 2.09 (m, 3H, *CHH* β -*i*-D-Gln, CH₂-9), 1.89 – 1.75 (m, 3H, *CHH* β -*i*-D-Gln, CH₂-8), 1.74 – 1.45 (m, 5H, CH₂ β -L-Lys, *CHH* γ -L-Lys, CH₂ δ -L-Lys), 1.41 (s, 12H, CH₂-7, *CHH* γ -L-Lys, 3x CH₃ *t*Bu), 1.32 (dd, 6H, *J* = 9.7, 6.9 Hz, CH₃ lactic acid, CH₃ L-Ala); ¹³C-APT NMR (MeOD/CD₂Cl₂ 1/1, 126 MHz, HSQC): δ 175.4, 175.1, 174.9, 173.4, 172.3 (C=O), 160.5, 144.7, 142.0, 138.3 (C_q Ar), 130.5 (Ar), 129.8 (C_q Ar), 129.6, 128.8, 128.4, 127.8, 126.7, 125.8, 120.6, 114.6 (Ar), 102.1 (CH benzylidene), 82.0 (C-4), 81.6 (C_q *t*Bu), 81.1 (C-3), 79.6 (C-1), 78.5 (CH lactic acid), 73.8 (CH₂ glycol), 71.1 (C-5), 69.5 (CH₂ PMB), 69.4 (CH₂-6), 67.5 (CH₂ Fmoc), 55.7 (CH₃ PMB), 55.4 (C-2), 53.1 (CH L-Lys), 53.0 (CH *i*-D-Gln), 50.2 (CH L-Ala), 47.9 (CH Fmoc), 39.7 (CH₂ ϵ -L-Lys), 36.7 (C-9), 33.2 (CH₂ γ -*i*-D-Gln), 32.4 (CH₂ β -L-Lys), 32.0 (CH₂ γ -L-Lys), 29.5 (CH₂ δ -L-Lys), 28.2 (CH₃ *t*Bu), 27.7 (CH₂ β -*i*-D-Gln), 23.6 (C-7), 22.5 (C-8), 19.5 (CH₃ L-Ala), 17.7 (CH₃ lactic acid); FT-IR (neat, cm⁻¹): 33141, 2934, 1658, 1514, 1451, 1368, 1249, 1153, 1102, 1029, 847, 760, 742, 699, 621; HRMS: [M+H]⁺ calcd. for C₆₃H₈₁N₆O₁₇ 1193.5653, found 1193.5674; LC-MS: Rt = 8.03 min (Gemini C₁₈, 10 - 90% MeCN, 12.5 min run).

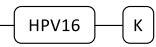
Automated solid phase synthesis general experimental information

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-Abu-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(MMT)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Val-OH, Fmoc-Val-Thr(ψMe, Mepro)-OH and Fmoc-Asp(OtBu)-Ser(ψMe, Mepro)-OH.

General procedure for cleavage from the resin, deprotection and purification

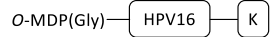
30 μmol resin was washed with DMF, DCM and dried after the last synthesis step followed by a treatment for 180 minutes with 0.6 mL cleavage cocktail of 95% TFA, 2.5% TIS and 2.5% H_2O . The suspension was filtered, the resin was washed with 0.6 mL of the cleavage cocktail, and the combined TFA solutions 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 $\text{CH}_3\text{CN}/\text{H}_2\text{O}/t\text{BuOH}$ (1/1/1 v/v/v) or $\text{DMSO}/\text{CH}_3\text{CN}/\text{H}_2\text{O}/t\text{BuOH}$ (3/1/1/1 v/v/v/v). Purification was performed on a Gilson GX-281 preparative RP-HPLC with a Gemini-NX 5 μ , C18, 110 Å, 250 x 10.0 mm column or a Vydac 219TP 5 μm Diphenyl, 250 x 10 mm column.

3-Azidopropyl-MDP(Ac)-Ala-*i*-D-Gln-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys-NH₂ (1)


O -MDP(Ac) —  Tentagel S Ram resin loaded with Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe, Mepro)-Phe-Abu-Abu-Lys(Boc)-Abu-Asp(OtBu)-Ser(ψMe, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 50 μmol scale was elongated with Fmoc-*i*-D-Gln-OH (74 mg, 0.20 mmol, 4.0 eq), Fmoc-L-Ala-OH (63 mg, 0.20 mmol, 4.0 eq) and compound **9a** (70 mg, 0.15 mmol, 3.0 eq.) with standard HCTU/Fmoc cycle. The resin was washed with DCM and treated with the standard cleavage cocktail for 60 minutes. The suspension was filtered and the product was precipitated with Et_2O . After purification by RP-HPLC and lyophilisation, conjugate **1** (5.4 mg, 1.5 μmol , 3%) was obtained as a white solid. LC-MS: Rt = 5.03 min (C18 Gemini, 10 - 90% MeCN, 15 min

run); ESI-MS: m/z 1829.0 $[M+H]^{2+}$; HRMS: $[M+H]^{4+}$ calcd. for $C_{160}H_{264}N_{48}O_{50}$: 914.48922, found 914.48996.

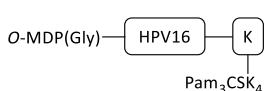
3-Azidopropyl-MDP(Gly)-Ala-*i*-D-Gln-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys-NH₂ (2)

 Tentagel S Ram resin loaded with Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe, Mepro)-Phe-Abu-Abu-Lys(Boc)-Abu-Asp(OtBu)-Ser(ψMe, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 50 μmol scale was elongated with Fmoc-*i*-D-Gln-OH (74 mg, 0.20 mmol, 4.0 eq), Fmoc-L-Ala-OH (63 mg, 0.20 mmol, 4.0 eq) and compound **9b** (91 mg, 0.20 mmol, 4.0 eq.) with standard HCTU/Fmoc cycle. The resin was washed with DCM and treated with the standard cleavage cocktail for 60 minutes. The suspension was filtered and the product waconjugate **2** (14.7 mg, 3.8 μmol, 8%) was obtained as a white solid. LC-MS: Rt = 7.33 min (C18 Gemini, 10 - 50% MeCN, 15 min run); ESI-MS: m/z 1836.8 $[M+H]^{2+}$; HRMS: $[M+H]^{4+}$ calcd. for $C_{160}H_{264}N_{48}O_{51}$: 918.48795, found 918.48729.

3-Azidopropyl-MDP(Ac)-Ala-*i*-D-Gln-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys(Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys)-NH₂ (3)

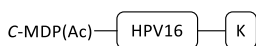
 50 μmol of crude [3-Azidopropyl-2-*N*-acetyl-4,6-*O*-benzylidene-2-deoxy-3-*O*-((*R*)-1-carboxyethyl)-β-*D*-glucopyranoside]-Ala-*i*-D-Gln-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe, Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψMe, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT)-Tentagel S Ram (see synthesis of compound **1**) was washed with DCM (3x) and 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 subsequently washed with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL) and DCM (5x). The peptide was elongated on 25 μmol scale with Ser(*t*Bu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) with standard HCTU/Fmoc cycle on the peptide synthesizer concluding with a final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was washed with DMF (5x) and treated with Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH (46 mg, 50 μmol, 2.0 eq.) in the presence of HCTU (21 mg, 50 μmol, 2.0 eq.) and DIPEA (18 μL, 0.10 mmol, 4.0 eq.) in DMF/DCM (1/1 v/v, 0.5 mL) overnight. The resin was washed with DMF (3x), DCM (3x) and treated with the standard cleavage cocktail for 60 minutes. The suspension was filtered and the product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **3** (5.2 mg, 1.0 μmol, 1%) was obtained as a white solid. LC-MS: Rt = 12.50 min (Vydac 219TP 5 μm Diphenyl, 10 - 90% MeCN, 21 min run); ESI-MS: m/z 1716.9 $[M+H]^{2+}$; HRMS: $[M+H]^{6+}$ calcd. for $C_{241}H_{420}N_{58}O_{62}S$: 858.51972, found 858.51999.

3-Azidopropyl-MDP(Gly)-Ala-*i*-D-Gln-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys(Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys)-NH₂ (4)



50 μ mol of crude [3-Azidopropyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-((*p*-methoxybenzyl)oxy)acetamide-*O*-((*R*)-1-carboxyethyl)- β -D-glucopyranoside]-Ala-*i*-D-Gln-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψ Me, Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψ Me, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT)-Tentagel S Ram (see synthesis of compound **2**) was washed with DCM (3x) and 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 subsequently washed with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL) and DCM (5x). The peptide was elongated with Ser(*t*Bu)-Lys(Boc)-Lys(Boc)-Lys(Boc) with standard HCTU/Fmoc cycle on the peptide synthesizer concluding with a final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was washed with DMF (5x) and treated with Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH (91 mg, 0.10 mmol, 2.0 eq.) in the presence of HCTU (41 mg, 0.10 mmol, 2.0 eq.) and DIPEA (35 μ L, 0.20 mmol, 4.0 eq.) in DMF/DCM (1/1 v/v, 1.0 mL) overnight. The resin was washed with DMF (3x), DCM (3x) and treated with the standard cleavage cocktail for 60 minutes. The suspension was filtered and the product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **4** (3.3 mg, 0.64 μ mol, 1%) was obtained as a white solid. LC-MS: Rt = 12.04 min (Vydac 219TP 5 μ m Diphenyl, 10 - 90% MeCN, 21 min run); ESI-MS: m/z 1722.2 [M+H]²⁺; HRMS: [M+H]⁶⁺ calcd. for C₂₄₁H₄₂₀N₅₈O₆₃S: 861.18553, found 861.18634.

N_α-Acetyl-N_ε-[butan-4-(2-deoxy-2-*N*-acetamide-3-*O*-((*R*)-1-carboxyethyl-L-alanyl-acetamide-D-isoglutaminy)- β -D-glucopyranosyl)-amide]-L-lysine-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys-NH₂ (5)



Tentagel S Ram resin loaded with H-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψ Me, Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψ Me, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 70 μ mol scale was washed with DMF (5x), followed by the addition of a solution of compound **10a** (148 mg, 140 μ mol, 2.0 eq.) and HCTU (58 mg, 140 μ mol, 2.0 eq.) in a mixture of DMF/DMSO (1.4 mL/0.4 mL) and DIPEA (49 μ L, 280 μ mol, 4.0 eq.). The reaction vessel was shaken overnight, after which it was washed with DMF (5x), treated with 20% piperidine in DMF (2x 1.4 mL, 10 min), washed with DMF (5x), treated with a mixture of 10% Ac₂O in 0.1 M DIPEA in DMF (2x 1.4 mL, 20 min) and washed with DMF (3x) and DCM (3x). The resin dried on air and split in two portions of which 30 μ mol of the crude was washed with DCM (3x). After treatment with a standard cleavage cocktail (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the standard cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **5** (7.2 mg, 1.9 μ mol, 6%) was obtained as a white

solid. LC-MS: Rt = 7.38 min (C18 Gemini, 10 - 50% MeCN, 15 min run); ESI-MS: m/z 1907.2 [M+H]²⁺; HRMS: [M+H]⁴⁺ calcd. for C₁₆₉H₂₇₉N₄₇O₅₃: 953.76399, found 953.76373.

N_α-Acetyl-N_ε-[butan-4-(2-deoxy-2-N-(2-hydroxyacetamide)-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-D-isoglutaminyl)-β-D-glucopyranosyl)-amide]-L-lysine-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys-NH₂ (6)

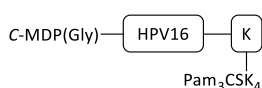
C-MDP(Gly) — HPV16 — K Tentagel S Ram resin loaded with H-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe, Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψMe, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 40 μmol scale was washed with DMF (5x), followed by the addition of a solution of compound **10b** (95 mg, 80 μmol, 2.0 eq.) and HCTU (34 mg, 82 μmol, 2.0 eq.) in DMF (0.8 mL) and DIPEA (28 μL, 160 μmol, 4.0 eq.). The reaction vessel was shaken overnight, after which it was washed with DMF (5x), treated with 20% piperidine in DMF (2x 0.8 mL, 10 min), washed with DMF (5x), treated with a mixture of 10% Ac₂O in 0.1 M DIPEA in DMF (2x 0.8 mL, 20 min) and washed with DMF (3x) and DCM (3x). After treatment with a standard cleavage cocktail (1.6 mL) for 3 hours the suspension was filtered and the residue was washed with the standard cleavage cocktail (1.6 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **6** (9.2 mg, 2.4 μmol, 6.1%) was obtained as a white solid. LC-MS: Rt = 7.89 min (C18 Gemini, 10 - 50% MeCN, 15 min run); ESI-MS: m/z 1914.9 [M+H]²⁺; HRMS: [M+H]⁴⁺ calcd. for C₁₆₉H₂₇₉N₄₇O₅₄: 957.76271, found 957.76246.

N_α-Acetyl-N_ε-[butan-4-(2-deoxy-2-N-acetamide-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-D-isoglutaminyl)-β-D-glucopyranosyl)-amide]-L-lysine-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys(Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys-Lys)-NH₂ (7)

C-MDP(Ac) — HPV16 — K 70 μmol of crude N_α-Ac-N_ε-[butan-4-(4,6-O-di-benzylidene-2-deoxy-2-N-acetamide-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-5-O-tert-butoxy-D-isoglutaminyl)-β-D-glucopyranosyl)-amide]-L-lysine-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe, Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψMe, Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT)-Tentagel S Ram (see synthesis of compound **5**) was washed with DCM (3x) and treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 20 mL) over 10 minutes. The resin was washed with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL) and DCM (5x). The peptide was elongated with Ser(tBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) with standard HCTU/Fmoc chemistry on the peptide synthesizer concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3 x 3 min). The resin was treated with Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH (128 mg, 0.14 mmol, 2.0 eq.) in the presence of HCTU (59 mg, 0.14 mmol, 2.0 eq.) and DIPEA (49 μL, 0.28 mmol, 4.0 eq.) in DMF/DCM (1/1 v/v, 1.4 mL) overnight. After treatment with a standard cleavage cocktail (2.8 mL) for 3 hours the suspension

was filtered and the residue was washed with the standard cleavage cocktail (2.8 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **7** (2.3 mg, 433 nmol, 0.6%) was obtained as a white solid. LC-MS: Rt = 12.31 min (Vydac 219TP 5 μm Diphenyl, 10 - 90% MeCN, 21 min run); ESI-MS: m/z 1769.4 [M+H]³⁺; HRMS: [M+H]⁴⁺ calcd. for C₂₅₀H₄₃₃N₅₇O₆₅S: 1326.55070, found 1326.55125.

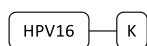
N_α-Acetyl-N_ε-[butan-4-(2-deoxy-2-N-(2-hydroxyacetamide)-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-D-isoglutaminyl)-β-D-glucopyranosyl)-amide]-L-lysine-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys(Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys)-NH₂ (8**)**



70 μmol of crude N_α-Ac-N_ε-[butan-4-(4,6-O-di-benzylidene-2-deoxy-2-N-((p-methoxybenzyl)oxy)acetamide-3-O-((R)-1-carboxyethyl-L-alanyl-acetamide-5-O-tert-butoxy-D-isoglutaminyl)-β-D-glucopyranosyl)-amide]-L-lysine-Gly-

Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(psiMe,Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(psiMe,Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT)-Tentagel S Ram (see synthesis of compound **6**) was washed with DMF (3x) and DCM (3x). The resin was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 30 mL) over 10 minutes. The resin was washed with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL) and DCM (5x). The peptide was elongated with Ser(tBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) with standard HCTU/Fmoc chemistry on the peptide synthesizer concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3 x 3 min). The resin was treated with Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH (128 mg, 0.14 mmol, 2.0 eq.) in the presence of HCTU (59 mg, 0.14 mmol, 2.0 eq.) and DIPEA (49 μL, 0.28 mmol, 4.0 eq.) in DMF/DCM (1/1 v/v, 1.4 mL) overnight. After treatment with a standard cleavage cocktail (2.8 mL) for 3 hours the suspension was filtered and the residue was washed with the standard cleavage cocktail (2.8 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, conjugate **8** (1.4 mg, 263 nmol, 0.4%) was obtained as a white solid. LC-MS: Rt = 12.21 min (Diphenyl Vydac, 10 - 90% CH₃CN, 21 min); ESI-MS: m/z 1774.7 [M+H]³⁺; HRMS: [M+H]⁴⁺ calcd. for C₂₅₀H₄₃₃N₅₇O₆₆S: 1330.54942, found 1330.54563.

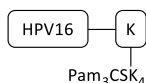
Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys-NH₂ (19**)**



Tentagel S Ram resin loaded with H-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(psiMe,Mepro)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(psiMe,Mepro)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 30 μmol scale was washed with DCM (5x). After treatment with a standard cleavage cocktail (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the standard cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and

lyophilisation, conjugate **19** (9.4 mg, 3.0 μmol , 10%) was obtained as a white solid. LC-MS: Rt = 4.85 min (C18 Gemini, 10 - 90% MeCN, 15 min run); ESI-MS: m/z 1549.8 $[\text{M}+\text{H}]^{2+}$; HRMS: $[\text{M}+\text{H}]^{4+}$ calcd. for $\text{C}_{138}\text{H}_{229}\text{N}_{41}\text{O}_{40}$ 775.17809, found 775.17790.

Acetyl-Gly-Gln-Ala-Glu-Pro-Asp-Arg-Ala-His-Tyr-Asn-Ile-Val-Thr-Phe-Abu-Abu-Lys-Abu-Asp-Ser-Thr-Leu-Arg-Leu-Abu-Val-Lys(Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ser-Lys-Lys-Lys)-NH₂ (20)



Tentagel S Ram resin loaded with H-Gly-Gln(Trt)-Ala-Glu(OtBu)-Pro-Asp(OtBu)-Arg(Pbf)-Ala-His(Trt)-Tyr(OtBu)-Asn(Trt)-Ile-Val-Thr(ψMe,MeprO)-Phe-Abu-Abu-Lys(OtBu)-Abu-Asp(OtBu)-Ser(ψMe,MeprO)-Thr(OtBu)-Leu-Arg(Pbf)-Leu-Abu-Val-Lys(MMT) on 50 μmol scale was washed with DMF (5x), treated with a mixture of 10% Ac_2O in 0.1 M DIPEA in DMF (2x 1.0 mL, 20 min) and washed with DMF (3x) and DCM (3x). The resin was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 20 mL) over 10 minutes. The resin was washed with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL) and DCM (5x). The peptide was elongated with Ser(tBu)-Lys(Boc)-Lys(Boc)-Lys(Boc)-Lys(Boc) with standard HCTU/Fmoc chemistry on the peptide synthesizer concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3 x 3 min). The resin was treated with Palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-OH (92 mg, 0.10 mmol, 2.0 eq.) in the presence of HCTU (42 mg, 0.10 mmol, 2.0 eq.) and DIPEA (35 μL , 0.20 mmol, 4.0 eq.) in DMF/DCM (1/1 v/v, 1.0 mL) overnight. After treatment with a standard cleavage cocktail (2.0 mL) for 3 hours the suspension was filtered and the residue was washed with the standard cleavage cocktail (2.0 mL). The product was precipitated with Et_2O . After purification by RP-HPLC and lyophilisation, conjugate **20** (5.5 mg, 1.2 μmol , 2%) was obtained as a white solid. LC-MS: Rt = 12.17 min (Diphenyl Vydac, 10 - 90% CH_3CN , 21 min); ESI-MS: m/z 1545.1 $[\text{M}+\text{H}]^{3+}$; HRMS: $[\text{M}+\text{H}]^{4+}$ calcd. for $\text{C}_{221}\text{H}_{385}\text{N}_{51}\text{O}_{53}\text{S}$ 1158.46744, found 1158.46863.

Footnotes and References

- (1) Vermaelen, K. *Front. Immunol.* **2019**, *10*.
- (2) Schumacher, T. N.; Schreiber, R. D. *Science*. **2015**, *348* (6230), 69–74.
- (3) Heimbürg-Molinaro, J.; Lum, M.; Vijay, G.; Jain, M.; Almogren, A.; Rittenhouse-Olson, K. *Vaccine* **2011**, *29* (48), 8802–8826.
- (4) Xu, Z.; Moyle, P. M. *Bioconjug. Chem.* **2018**, *29* (3), 572–586.
- (5) Kool, M.; Fierens, K.; Lambrecht, B. N. *J. Med. Microbiol.* **2012**, *61* (Pt 7), 927–934.
- (6) Liu, H.; Irvine, D. J. *Bioconjug. Chem.* **2015**, *26* (5), 791–801.
- (7) Zom, G. G. P.; Khan, S.; Filippov, D. V.; Ossendorp, F. In *Advances in Immunology*; **2012**; pp 177–201.
- (8) Ingale, S.; Wolfert, M. A.; Gaekwad, J.; Buskas, T.; Boons, G.-J. *Nat. Chem. Biol.* **2007**, *3* (10), 663–667.
- (9) Kawai, T.; Akira, S. *Nat. Immunol.* **2010**, *11* (5), 373–384.
- (10) Ignacio, B. J.; Albin, T. J.; Esser-Kahn, A. P.; Verdoes, M. *Bioconjug. Chem.* **2018**, *29* (3), 587–603.
- (11) Philpott, D. J.; Sorbara, M. T.; Robertson, S. J.; Croitoru, K.; Girardin, S. E. *Nat. Rev. Immunol.* **2014**, *14* (1), 9–23.
- (12) Behr, M. A.; Divangahi, M. *Curr. Opin. Microbiol.* **2015**, *23*, 126–132.
- (13) Willems, M. M. J. H. P.; Zom, G. G.; Meeuwenoord, N.; Ossendorp, F. A.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C.; Filippov, D. V. *Beilstein J. Org. Chem.* **2014**, *10*, 1445–1453.

- (14) Fritz, J. H.; Girardin, S. E.; Fitting, C.; Werts, C.; Mengin-Lecreux, D.; Caroff, M.; Cavaillon, J.-M.; Philpott, D. J.; Adib-Conquy, M. *Eur. J. Immunol.* **2005**, *35* (8), 2459–2470.
- (15) Mutwiri, G.; Gerdt, V.; van Drunen Littel-van den Hurk, S.; Auray, G.; Eng, N.; Garlapati, S.; Babiuk, L. A.; Potter, A. *Expert Rev. Vaccines* **2011**, *10* (1), 95–107.
- (16) Schwarz, H.; Posselt, G.; Wurm, P.; Ulbing, M.; Duschl, A.; Horejs-Hoeck, J. *Immunobiology* **2013**, *218* (4), 533–542.
- (17) Pavot, V.; Rochereau, N.; Rességuier, J.; Gutjahr, A.; Genin, C.; Tiraby, G.; Perouzel, E.; Lioux, T.; Vernejoul, F.; Verrier, B.; *et al. J. Immunol.* **2014**, *193* (12), 5781–5785.
- (18) Natsuka, M.; Uehara, A.; Shuhua Yang; Echigo, S.; Takada, H. *Innate Immun.* **2008**, *14* (5), 298–308.
- (19) Tada, H.; Aiba, S.; Shibata, K.-I.; Ohteki, T.; Takada, H. *Infect. Immun.* **2005**, *73* (12), 7967–7976.
- (20) Zom, G. G.; Willems, M. M. J. H. P.; Meeuwenoord, N. J.; Reintjens, N. R. M.; Tondini, E.; Khan, S.; Overkleef, H. S.; van der Marel, G. A.; Codee, J. D. C.; Ossendorp, F.; *et al. Bioconjug. Chem.* **2019**, *30* (4), 1150–1161.
- (21) Coulombe, F.; Divangahi, M.; Veyrier, F.; de Léséleuc, L.; Gleason, J. L.; Yang, Y.; Kelliher, M. A.; Pandey, A. K.; Sasseti, C. M.; Reed, M. B.; *et al. J. Exp. Med.* **2009**, *206* (8), 1709–1716.
- (22) Chen, K.-T.; Huang, D.-Y.; Chiu, C.-H.; Lin, W.-W.; Liang, P.-H.; Cheng, W.-C. *Chem. - A Eur. J.* **2015**, *21* (34), 11984–11988.
- (23) de la Fuente, J. M.; Penadés, S. *Synthesis of Le X-Neoglycoconjugate to Study Carbohydrate-Carbohydrate Associations and Its Intramolecular Interaction*; **2002**; Vol. 13.
- (24) Anomeric hydrolysis was observed for the conjugates 2-4 using LC-MS analysis, however it was not possible to determine the amount of hydrolyzed product as it was inseparable from the desired conjugate.
- (25) Fuchss, T.; Schmidt, R. R. *Synthesis (Stuttg.)*. **1998**, *1998* (05), 753–758.
- (26) Myers, E. L.; Butts, C. P.; Aggarwal, V. K. *Chem. Commun.* **2006**, No. 42, 4434–4436.
- (27) Voigtritter, K.; Ghorai, S.; Lipshutz, B. H. *J. Org. Chem.* **2011**, *76* (11), 4697–4702.
- (28) Sharma, P. K.; Kumar, S.; Kumar, P.; Nielsen, P. *Tetrahedron Lett.* **2007**, *48* (49), 8704–8708.
- (29) At this stage, the remaining ruthenium impurities were removed by embedding the compound on QuadraSil® aminopropyl, followed by column chromatography.

