Synthetic carbohydrate ligands for immune receptors

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Chapter 6

*Synthesis of C-glycosyl amino acid building blocks suitable for solid phase peptide synthesis**

Introduction

Carbohydrates are involved in various inter- and intracellular recognition events and can be recognized by lectins leading to a row of biological processes. Lectins can function as pattern recognition receptors playing a role in innate immunity by promoting the secretion of cytokines and in adaptive immunity by contributing to endocytosis.[1,2] Examples of these receptors are the C-type lectins DC-SIGN and the mannose receptor, which are both present on dendritic cells. Since the binding interactions between lectins and their carbohydrate binding partners are often relatively weak, strong interactions therefore depend on multivalent binding. A lot of research has been devoted to the design, synthesis and evaluation of multivalent carbohydrate structures such as polymers, glycoconjugates and dendrimers to effect strong lectin binding for example to develop new therapeutics and more efficient vaccine therapies.[2] For instance, mannosylated polymers and peptides have been used as therapeutics against HIV, SARS and influenza virus.[3,4] These multivalent conjugates can not only be tailored to effectively mimic complex glycan structures[5–7], also their

*The data presented in this Chapter were gathered in collaboration with Nico J. Meeuwenoord, Herman S. Overkleeft, Gijsbert A. van der Marel and Jeroen D. C. Codée.

physical properties can be changed and tuned.[4,8,9] In this respect, the development of an automated solid phase assembly approach to deliver a coherent row of multivalent glycoconjugates will be beneficial. Ponader *et al.* developed a solid phase method to obtain homo- and hetero-multivalent glycooligomers using alkyne-functionalized building block, that were functionalized by a Cu-catalyzed cycloaddition with mannose, galactose or glucose synthons equipped with an azide.[10] However, *O*-glycosides[11] can be degraded enzymatically and are generally not stable enough to withstand the acidic conditions used in solid phase peptide synthesis. Therefore several groups have worked on the synthesis of *C*-glycosides[12,13] and their incorporation into a *C*-glycosyl amino acid building blocks[14–17] allowing an online solid phase peptide synthesis (SPPS) of glycopeptides. This chapter expands the library of *C*-glycoside functionalized lysine building blocks described in Chapters 3, 4 and 5 of this Thesis (Figure 1A) with α-mannose **1**, β-*N*-acetylglucosamine **2**, β- and α-galactose **3** and **4** functionalized lysine synthons (Figure 1B). These building blocks are suitable for Fmoc SPPS chemistry, and can be used for the synthesis of homo- and heteromultivalent glycomimetics. Comparable with the *C*-glycosides described in earlier Chapters, the route of synthesis to building blocks **1**-**4** comprised the key reactions shown in Figure 1C: introduction of the anomeric *C*-allyl group, cross metathesis to install the carboxylic acid and condensation with a suitably protected lysine. The monosaccharides are protected with acid-labile trityl, *p*-methoxybenzyl, isopropylidene, and benzylidene groups to allow a one-step protocol in the final stage of the SPPS that simultaneously removes all protecting groups and releases the glycopeptides or glycoclusters from the resin.

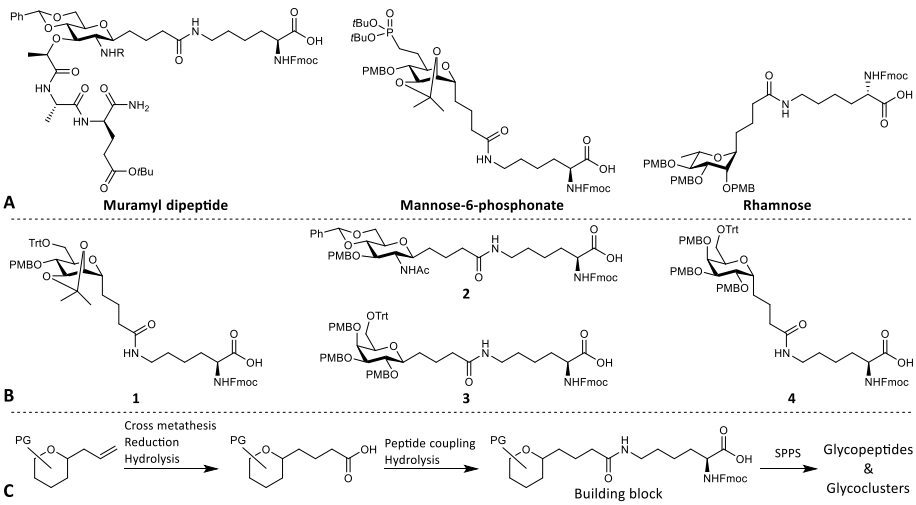


Figure 1. A) Structures of the *C*-glycoside SPPS building blocks described in previous Chapters of this Thesis; B) The SPPS building blocks **1**-**4** described in this Chapter; C) Key steps in the synthesis of the *C*-glycosidic SPPS building blocks; PG: protecting group.

Results & Discussion

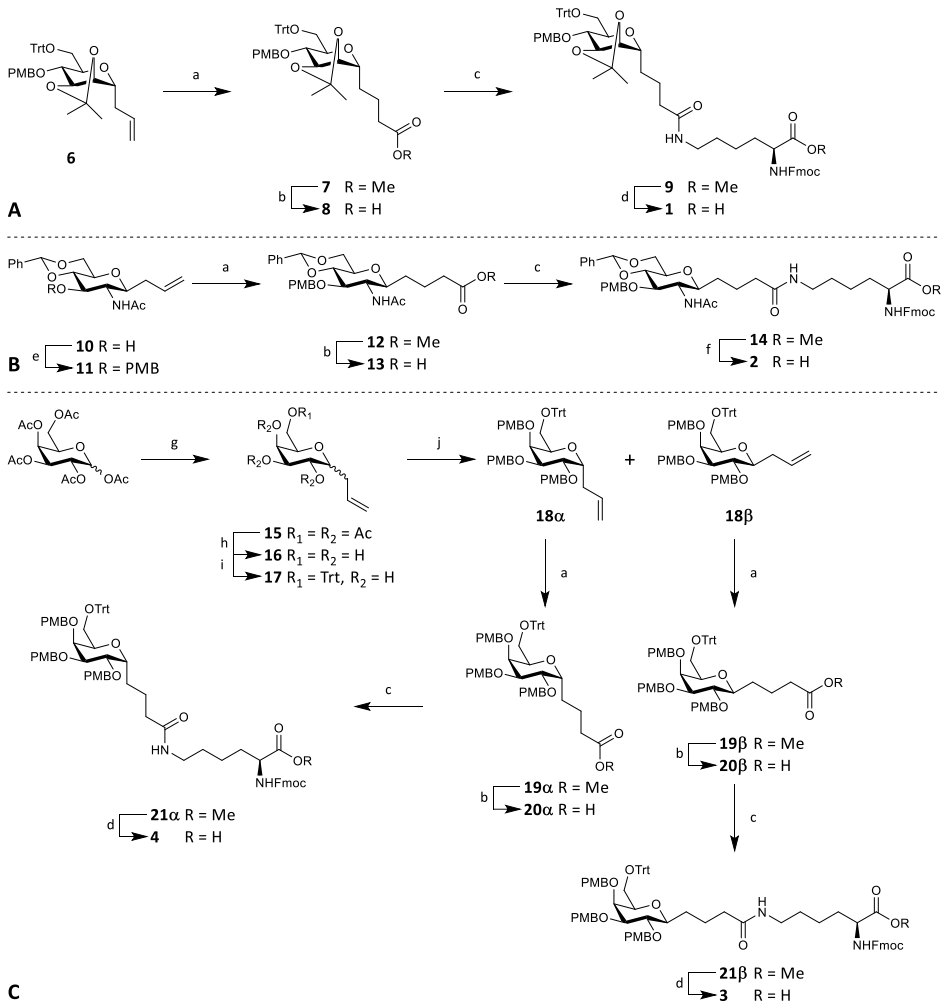
Mannose SPPS building block **1** was synthesized from fully protected allyl-*C*-mannose **6**[18], obtained as described in Chapter 4. Cross metathesis of **6** with methyl acrylate under influence of Grubbs 2nd generation catalyst was followed by reduction of the obtained α,β-unsaturated ester with $NaBH_4$ and ruthenium trichloride to give compound **7** in 73% yield over two steps (Scheme 1A).[19,20] Saponification of the methyl ester using LiOH yielded acid **8**, which was condensed with Fmoc-L-lysine-OMe[21] under the influence of HCTU and DIPEA to give **9** in 95% yield. Compound **9** was treated with LiOH at 0°C to obtain to SPPS building block **1**.

En route to GlcNAc SPPS building block **2**, the *C*-3-OH of *N*-acetyl *C*-allyl glucosamine **10**[22] (obtained as described in Chapter 3) was alkylated by treatment with *p*-methoxybenzyl-2,2,2-trichloroacetimidate and a catalytic amount of TfOH (Scheme 1B). The installation of the PMB-protecting group using sodium hydride and the alkyl chloride was low yielding, because of the presence of the *N*-acetyl function. Subsequently, a cross metathesis with methyl acrylate, reduction of the resulting double bond and hydrolysis of the obtained methyl ester led to acid **13** in 68% yield over three steps. Also, the coupling with Fmoc-L-lysine-OMe, as performed for the synthesis of **1**, went well and compound **14** was isolated in 78% yield after crystallization. However, selective hydrolysis of the methyl ester proved challenging due to the poor solubility of **14**. To solve this problem, the reaction was performed at room temperature, while closely monitoring the conversion with LC-MS. As partial Fmoc cleavage could not be prevented, the mixture was quenched with 1 M HCl and then treated with $NaHCO_3$, Fmoc *N*-hydroxysyccinimide ester to reinstall the Fmoc-protecting group. Subsequent precipitation with Et_2O and recrystallization from MeOH/DCM/Et_2O gave SPPS building block **2** in 91% yield.

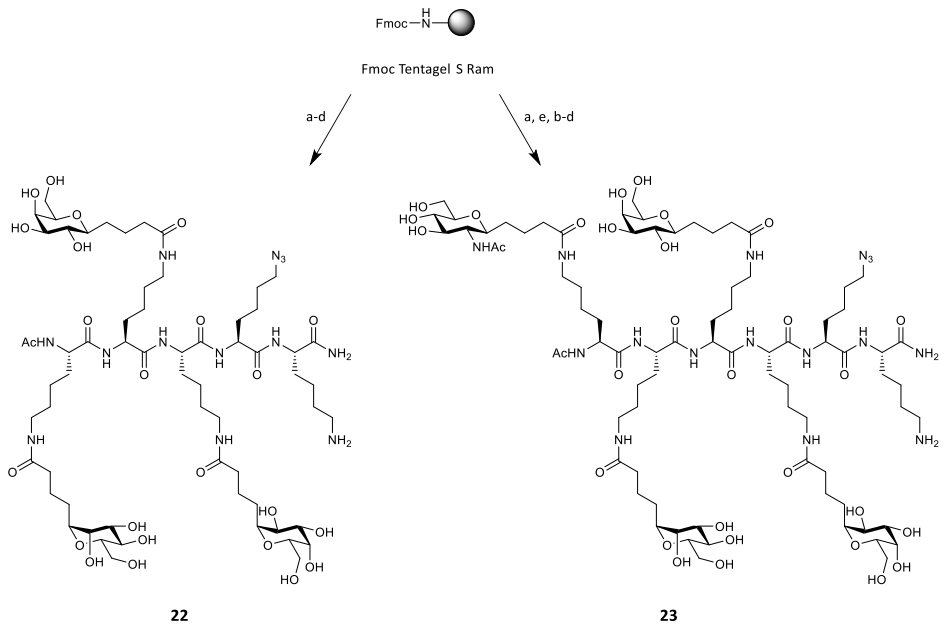
The synthesis of galactose SPPS building blocks **3** and **4** starts with the *C*-glycosylation of acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose with allyltrimethylsilane (Scheme 1C). Performing this reaction in MeCN gave **15** as an 6/1 α/β mixture (98% yield), while in nitromethane an 2/1 α/β mixture of (89% yield) was obtained. These reactions show, in line with literature precedent[23,24] that neighbouring group participation of the *C*-2-*O*-acetate is not a decisive factor in determining the stereochemical outcome of these reactions. The preference for the formation of the α-product, can be accounted for by the reactivity of the galactopyranosyl oxocarbenium ion.[25] Deacetylation of **15** with sodium methoxide and subsequent tritylation of the primary alcohol with TrtCl and Et_3N, produced compound **17** as an inseparable α/β mixture.[26] Alkylation of **17** with *p*-

methoxybenzyl chloride and separation of the anomers by chromatography yielded the individual α-anomer **18α** and β-anomer **18β**. Both anomers were subjected to the previously described cross metathesis, reduction of the double bond and saponification of the methyl ester to furnish acids **20α** and **20β**. The acids were condensed with Fmoc-L-lysine-OMe in the presence of HCTU and DIPEA to give **21α** and **21β**, which were carefully hydrolyzed with LiOH at 0°C to prevent Fmoc cleavage, providing galactose SPPS building blocks **3** and **4**.[27]

With the four *C*-glycoside SPPS building blocks (α-Man **1**, β-GlcNAc **2**, β-Gal **3**, α-Gal **4**) in hand the SPPS of glycopeptides **22** and **23**, which also feature an 6-azido lysine and a lysine to introduce further functionalities in the constructs, was undertaken (Scheme 2). Initial experiments showed that the condensations of building block **2** did not proceed well due to solubility issues. Therefore, pentamer **22** was synthesized first using the automated solid phase peptide synthesizer on Tentagel S Ram resin. The obtained immobilized peptide was then cleaved from the resin by treatment with a cocktail of TFA/TIS/H_2O (95/2.5/2.5 v/v/v) for 3 hours. Precipitation of the peptide from Et_2O, followed by RP-HPLC purification gave **22** (3.4 mg, 5% yield). For hexamer **23**, the immobilized peptide was treated with a mixture of **2**, HCTU and DIPEA in DMSO for two hours at 50°C. After a test cleavage, LC-MS showed that some unreacted pentamer was still present and therefore the resin was treated one more time with the previously mentioned mixture and shaken overnight. After cleaving from the resin and HPLC purification, **23** was obtained in 2% yield.



Scheme 1. Synthesis of mannose SPPS building blocks **1**-**4**. *Reagents and conditions*: a) *i*. methyl acrylate, CuI, Grubbs 2nd gen. catalyst, DCE, 50°C; *ii*. $NaBH_4$, $RuCl_3$, MeOH, DCE, 45°C, **7**: 73%, **12** 68%, **19α** 73%, **19β** 68% over two steps; b) LiOH, THF/H_2O/MeOH or THF/H_2O, 40°C, **8**: 96%, **13**: 100%, **20α**: 92%, **20β**: 96%; c) Fmoc-L-Lys-OMe, HCTU, DIPEA, DMF, **9**: 95%, **14**: 78%, **21α**: 90%, **21β**: 93%; d) LiOH, THF/H_2O, 0°C, **1**: 57%, **3**: 48%, **4**: 46%; e) *p*-methoxybenzyl-2,2,2-trichloroacetimidate, TfOH, THF, 78%; f) *i*. LiOH, THF/H_2O; *ii*. 1 M HCl; *iii*. $NaHCO_3$, Fmoc *N*-hydroxysyccinimide ester, 91%; g) allyltrimethylsilane, $BF_3{\cdot}OEt_2$, CH_3NO_2, 89%; h) NaOMe, MeOH, 91%; i) TrtCl, Et_3N, DMF, 60°C, 79%; j) *p*-methoxybenzyl chloride, NaH, DMF, **18α**: 52%, **18β**: 28%.



Scheme 2. SPPS synthesis of glycopeptides **22** and **23**. *Reagents and conditions*: a) *i*. 20% piperidine, DMF; *ii*. Fmoc SPPS cycle for Lys(α-*C*-Man)-Lys(β-*C*-Gal)-Lys(α-*C*-Gal)-Lys(N_3)-Lys(Boc); *iii*. 20% piperidine, DMF; b) Ac_2O, DIPEA, DMF; c) TFA/TIS/H_2O (95/2.5/2.5 v/v/v), 3h; d) RP-HPLC; e) *i*. **2**, HCTU, DIPEA, DMSO, 50°C, 2h; *ii*. **2**, HCTU, DIPEA, DMSO, overnight; **22**) 3.4 mg, 5%; **23**) 1.8 mg, 2%.

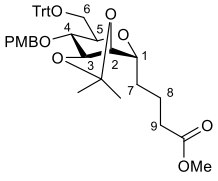
Conclusion

The synthesis of four *C*-glycosyl lysine building blocks and their application in SPPS is described. Key steps in the synthesis of the glycosyl amino acid are the installation of a *C*-allyl functionality on the carbohydrate and the ensuing Grubbs cross metathesis with methyl acrylate and subsequent reduction of the double bond. The building blocks were equipped with solely acid labile protecting groups to allow their concomitantly with release of the peptide from the resin, increasing assembly efficiency. Synthesis of a model penta- and hexapeptide showed that these building blocks are well suited to be used in an online solid phase peptide synthesis protocol. Despite its poor solubility, the GlcNAc building block could be coupled using DMSO as a solvent and coupling reactions at elevated temperature to provide the desired hexamer. The developed protecting group strategy allows one to combine the building blocks with many other functionalities in the target peptides, such as azide and alkyne click handles. The building blocks described in this Chapter will enable the rapid assembly of libraries of well-defined homo- and heteromultivalent glycopeptides and glycoclusters.

Experimental

All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3Å molecular sieves. All moisture and oxygen sensitive reactions were performed under an argon atmosphere. Column chromatography was performed on silica gel (Screening Devices BV, 40-63 µm, 60 Å). For TLC analysis, pre-coated silica gel aluminum sheets (Merck, silica gel 60, F254) were used with detection by UV-absorption (254/366 nm) where applicable. Compounds were visualized on TLC by UV absorption (245 nm), or by staining with one of the following TLC stain solutions: $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L), $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) and 10% H_2SO_4 in H_2O; bromocresol (0.4 g/L) in EtOH; $KMnO_4$ (7.5 g/L), K_2CO_3 (50 g/L) in H_2O. Staining was followed by charring at ~150°C. ^1H and ^{13}C spectra were recorded on a Bruker AV-400 (400/100 MHz) 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 $CDCl_3$ or via the solvent residual peak. Coupling constants (*J*) are given in Hz. LC-MS analysis were done on an Agilent Technologies 1260 Infinity system with a C18 Gemini 3 µm, C18, 110 Å, 50 x 4.6 mm column. Absorbance was measured at 214 nm and 256 nm and an Agilent Technologies 6120 Quadrupole mass spectrometer was used as detector. Automated solid phase peptide synthesis was performed on an Applied Biosystems 433A Peptide Synthesizer. The glycopeptides were purified with a Gilson GX-281 preparative HPLC with a Gemini-NX 5u, C18, 110 Å, 250 x 10.0 mm column with NH_4OAc. Optical rotations were measured on an Anton Paar Modular Circular Polarimeter MCP 100/150. High resolution mass spectra were recorded on 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.

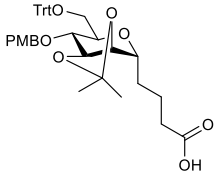
Methyl 4-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-*6*-*O*-trityl-*α*-D-mannopyranosyl)-butanoate (7)



Compound **6**[18] (5.7 g, 9.4 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere, before being dissolved in dry DCE (0.10 L). Methyl acrylate (2.4 mL, 26 mmol, 2.8 eq.), CuI (0.28 g, 1.5 mmol, 0.16 eq.) and Grubbs 2nd generation catalyst (0.32 g, 0.38 mmol, 0.04 eq.) were added and the flask was covered in aluminum foil. The suspension was heated to 50°C and stirred for 48 hours, after which it was concentrated *in vacuo* and co-evaporated with toluene (3x). Purification by column chromatography (10→70% Et_2O in pentane) afforded the intermediate (4.9 g, 7.4 mmol, 1.0 eq.), which was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry DCE (37 mL). Two empty balloons were placed on the flask, followed by the addition of ruthenium trichloride (0.29 g, 1.4 mmol, 0.19 eq.) and $NaBH_4$ (0.89 g, 24 mmol, 3.2 eq.) at 0°C. Methanol (6.0 mL, 0.15 mol, 20 eq.) was carefully added to the suspension over 20 minutes, after which the mixture was allowed to warm-up up to room temperature over 20 minutes. The mixture was subsequently heated to 45°C for 4 hours. The reaction mixture was cooled to room temperature, diluted with brine, filtered over

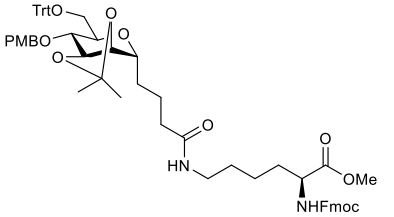
celite and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (20→60% Et_2O in pentane) gave compound **7** (4.6 g, 6.9 mmol, 73% over two steps). R_f: 0.22 (7/3 pentane/Et_2O); $[\alpha]_D^{25}$ +16.1° (*c* = 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.56 – 7.50 (m, 6H, Ar), 7.37 – 7.31 (m, 6H, Ar), 7.31 – 7.27 (m, 3H, Ar), 7.03 – 6.98 (m, 2H, Ar), 6.81 – 6.76 (m, 2H, Ar), 4.74 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.38 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.31 (t, 1H, *J* = 6.9 Hz, H-3), 4.08 (t, 1H, *J* = 6.4 Hz, H-2), 3.92 (q, 1H, *J* = 6.3 Hz, H-1), 3.84 – 3.69 (m, 5H, H-4, H-5, CH_3 PMB), 3.64 (s, 3H, OCH_3), 3.43 (dd, 1H, *J* = 9.9, 2.1 Hz, C*H*H-6), 3.23 (dd, 1H, *J* = 9.9, 5.0 Hz, C*H*H-6), 2.44 (t, 2H, *J* = 7.3 Hz, CH_2-9), 2.11 – 1.99 (m, 1H, C*H*H-8), 1.91 – 1.79 (m, 1H, C*H*H-8), 1.79 – 1.68 (m, 2H, CH_2-7), 1.55 (s, 3H, CH_3 isopropylidene), 1.42 (s, 3H, CH_3 isopropylidene); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.8 (C=O), 159.1, 144.0, 130.1 (C_q Ar), 129.7, 128.8, 127.8, 126.9, 113.6 (Ar), 109.2 (C_q isopropylidene), 86.4 (C_q Trt), 78.8 (C-3), 77.1 (C-2), 75.5 (C-4), 72.7 (C-5), 72.7 (CH_2 PMB), 72.5 (C-1), 63.4 (CH_2-6), 55.2 (CH_3 PMB), 51.5 (OCH_3), 33.8 (CH_2-9), 32.0 (CH_2-7), 27.7, 25.6 (CH_3 isopropylidene), 21.0 (CH_2-8); FT-IR (neat, cm^{-1}): 2987, 2934, 1736, 1612, 1514, 1491, 1449, 1380, 1302, 1246, 1217, 1162, 1071, 1034, 1002, 900 867, 822, 766, 748, 705, 633; HRMS: $[M+Na]^+$ calcd. for $C_{41}H_{46}O_8Na$: 689.30849, found 689.30821.

4-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-*6*-*O*-trityl-*α*-D-mannopyranosyl)-butanoic acid (8)



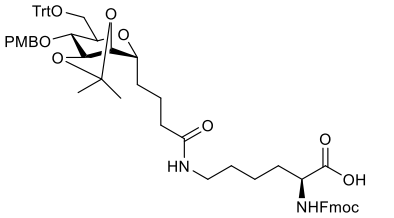
Methyl ester **7** (4.6 g, 6.9 mmol, 1.0 eq.) was dissolved in in a mixture of THF/H_2O/MeOH (7/1/2, v/v/v, 35 mL). LiOH (0.87 g, 21 mmol, 3.0 eq.) was added and the mixture was heated to 40°C for 8 hours, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was cooled to 0°C, acidified with 1 M HCl to pH = 6, diluted with H_2O and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The title compound was obtained (4.3 g, 6.6 mmol, 96%) and used without further purification. R_f: 0.85 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +16.0° (*c* = 0.43, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.51 – 7.43 (m, 6H, Ar), 7.34 – 7.20 (m, 9H), 7.01 – 6.92 (m, 2H, Ar), 6.79 – 6.71 (m, 2H, Ar), 4.69 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.34 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.26 (t, 1H, *J* = 6.9 Hz, H-3), 4.03 (t, 1H, *J* = 6.5 Hz, H-2), 3.91 – 3.81 (m, 1H, H-1), 3.79 (s, 3H, CH_3 PMB), 3.78 – 3.65 (m, 2H, H-4, H-5), 3.38 (dd, 1H, *J* = 9.9, 2.1 Hz, C*H*H-6), 3.18 (dd, 1H, *J* = 9.9, 4.9 Hz, C*H*H-6), 2.43 (t, 2H, *J* = 7.2 Hz, CH_2-9), 2.00 (m, 1H, C*H*H-8), 1.85 – 1.63 (m, 3H, C*H*H-8, CH_2-7), 1.50 (s, 3H, CH_3 isopropylidene), 1.38 (s, 3H, CH_3 isopropylidene); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 179.2 (C=O), 159.2, 144.1, 130.2 (C_q Ar), 129.8, 128.9, 127.9, 127.0, 113.7 (Ar), 109.4 (C_q isopropylidene), 86.5 (C_q Trt), 78.8 (C-3), 77.1 (C-2), 75.6 (C-4), 72.9 (C-5), 72.8 (CH_2 PMB), 72.6 (C-1), 63.4 (CH_2-6), 55.4 (CH_3 PMB), 33.8 (CH_2-9), 32.0 (CH_2-7), 27.8, 25.7 (CH_3 isopropylidene), 20.8 (CH_2-8); FT-IR (neat, cm^{-1}): 3058, 2987, 2934, 1707, 1612, 1586, 1513, 1490, 1449, 1381, 1302, 1245, 1216, 1160, 1068, 1034, 1002, 900, 866, 822, 777, 765, 737, 703, 644, 632; HRMS: $[M+Na]^+$ calcd. for $C_{40}H_{44}O_8Na$: 675.29284, found 675.29260.

N_α-Fmoc-N_ε-[butan-4-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-*6*-*O*-trityl-*α*-D-mannopyranosyl)-amide]-L-lysine-methyl ester (9)



Compound **8** (3.8 g, 5.8 mmol, 1.0 eq.) and Fmoc-L-lysine-OMe[21] (2.9 g, 7.0 mmol, 1.2 eq.) were dissolved in DMF (30 mL). HCTU (2.9 g, 7.0 mmol, 1.2 eq.) and DIPEA (3.0 mL, 17 mmol, 3.0 eq.) were added and the solution was stirred for 4 hours. The reaction mixture was diluted with EtOAc and was washed with 1 M HCl (1x), sat. aq. $NaHCO_3$ (1x), brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (2→30% acetone in DCM) yielded the title compound (5.6 g, 5.5 mmol, 95%). R_f: 0.38 (9/1 DCM/acetone); $[\alpha]_D^{25}$ +11.5° (*c* = 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.78 – 7.74 (m, 2H, Ar), 7.63 – 7.58 (m, 2H, Ar), 7.48 – 7.42 (m, 6H, Ar), 7.42 – 7.37 (m, 2H, Ar), 7.34 – 7.21 (m, 11H, Ar), 6.96 – 6.91 (m, 2H, Ar), 6.75 – 6.70 (m, 2H, Ar), 5.43 (d, 1H, *J* = 8.2 Hz, NH), 5.31 (t, 1H, *J* = 5.8 Hz, N*H*Fmoc), 4.68 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.47 – 4.35 (m, 2H, CH_2 Fmoc), 4.34 – 4.19 (m, 4H, H-3, CH L-Lys, C*H*H PMB, CH Fmoc), 3.99 (t, 1H, *J* = 6.7 Hz, H-2), 3.83 – 3.76 (m, 4H, H-1, CH_3 PMB), 3.74 (s, 3H, CH_3 PMB), 3.71 – 3.66 (m, 2H, H-4, H-5), 3.37 (dd, 1H, *J* = 9.9, 1.4 Hz, C*H*H-6), 3.18 – 3.11 (m, 1H, C*H*H-6), 3.07 – 2.93 (m, 2H, CH_2 ε-L-Lys), 2.22 – 2.14 (m, 2H, CH_2-9), 1.92 – 1.81 (m, 2H, CH_2-8), 1.79 – 1.53 (m, 4H, CH_2-7, 1x CH_2 β/γ/δ-L-Lys), 1.49 (s, 3H, CH_3 isopropylidene), 1.36 (s, 3H, CH_3 isopropylidene), 1.25 – 1.19 (m, 4H, 2x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 172.8, 159.2 (C=O), 156.1, 144.0, 143.9, 141.4, 130.1 (C_q Ar), 129.8, 128.9, 127.9, 127.8, 127.2, 125.2, 125.2, 120.1, 113.7 (Ar), 109.4 (C_q isopropylidene), 86.6 (C_q Trt), 78.9 (C-3), 77.2 (C-2), 75.5 (C-4), 73.1 (C-5), 72.7 (CH_2 PMB), 72.7 (C-1), 67.0 (CH_2 Fmoc), 63.4 (CH_2-6), 55.3 (CH_3 PMB), 53.8 (CH Fmoc), 52.5 (OCH_3), 47.3 (CH L-Lys), 38.9 (CH_2 ε-L-Lys), 36.1 (CH_2-9), 32.1 (CH_2-7), 31.9, 29.0 (CH_2 β/γ/δ-L-Lys), 27.7, 25.5 (CH_3 isopropylidene), 22.5 (CH_2 β/γ/δ-L-Lys), 21.9 (CH_2-8); FT-IR (neat, cm^{-1}): 3315, 2935, 1722, 1654, 1612, 1514, 1449, 1380, 1247, 1213, 1175, 1077, 1033, 849, 761, 740, 704, 633, 563; HRMS: $[M+Na]^+$ calcd. for $C_{62}H_{68}O_{11}N_2Na$: 1039.47153, found 1039.47134.

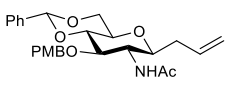
N_α-Fmoc-N_ε-[butan-4-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-*6*-*O*-trityl-*α*-D-mannopyranosyl)-amide]-L-lysine (1)



Compound **9** (3.05 g, 3.00 mmol, 1.0 eq.) was dissolved in THF (30 mL) and cooled to 0°C. A solution of LiOH in H_2O (0.30 M, 20 mL, 6.0 mmol, 2.0 eq.) was added and the mixture was stirred vigorously for 30 minutes, after which the mixture was diluted with EtOAc and acidified by the addition of 1 M HCl to pH = 3-4. The mixture was washed with brine (1x) and the aqueous layer was extracted with EtOAc (1x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. After purification by column chromatography (1→8% MeOH in DCM) the title compound (1.73 g, 1.72 mmol, 57%) was obtained as a white foam. R_f: 0.61 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +20.8° (*c* = 0.62, $CHCl_3$); ^{1}H NMR (MeOD, 400 MHz, HH-COSY,

HSQC): δ 7.77 (d, 2H, *J* = 7.5 Hz, Ar), 7.69 – 7.63 (m, 2H, Ar), 7.45 – 7.34 (m, 8H, Ar), 7.33 – 7.18 (m, 11H, Ar), 6.95 – 6.88 (m, 2H, Ar), 6.75 – 6.68 (m, 2H, Ar), 4.61 (d, 1H, *J* = 11.0 Hz, C*H*H PMB), 4.34 (d, 2H, *J* = 7.0 Hz, CH_2 Fmoc), 4.29 (d, 1H, *J* = 11.1 Hz, C*H*H PMB), 4.25 – 4.16 (m, 2H, H-3, CH Fmoc), 4.09 (dd, 1H, *J* = 9.4, 4.6 Hz, CH L-Lys), 4.01 (t, 1H, *J* = 6.4 Hz, H-2), 3.81 (dd, 1H, *J* = 7.7, 5.3 Hz, H-1), 3.75 (s, 3H, CH_3 PMB), 3.69 (dd, 1H, *J* = 9.5, 7.3 Hz, H-4), 3.62 – 3.55 (m, 1H, H-5), 3.35 – 3.30 (m, 1H, C*H*H-6), 3.11 (t, 2H, *J* = 6.7 Hz, CH_2 ε-L-Lys), 3.05 (dd, 1H, *J* = 9.9, 5.2 Hz, C*H*H-6), 2.26 – 2.16 (m, 2H, CH_2-9), 1.98 – 1.86 (m, 1H, C*H*H-8), 1.86 – 1.68 (m, 2H, C*H*H-8, C*H*H-7), 1.68 – 1.56 (m, 3H, C*H*H-7, 1x CH_2 β/γ/δ-L-Lys), 1.50 – 1.30 (m, 10H, 2x CH_2 β/γ/δ-L-Lys, 2x CH_3 isopropylidene); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 175.8, 160.7 (C=O), 145.3, 142.6, 131.3 (C_q Ar), 130.8, 130.0, 128.8, 128.2, 128.1, 126.3, 120.9, 114.5 (C_q Ar), 110.5 (C_q isopropylidene), 87.8 (C_q Trt), 80.0 (C-3), 78.4 (C-2), 76.6 (C-4), 74.0 (C-5), 73.8 (C-1), 73.6 (CH_2 PMB), 67.8 (CH_2 Fmoc), 64.6 (CH_2-6), 55.7 (CH_3 PMB), 55.4 (CH L-Lys) 48.4 (CH Fmoc), 40.1 (CH_2 ε-L-Lys), 36.8 (CH_2-9), 32.9 (CH_2-7), 30.3, 29.9 (CH_2 β/γ/δ-L-Lys), 28.0, 25.7 (CH_3 isopropylidene), 24.3 (CH_2 β/γ/δ-L-Lys), 23.3 (CH_2-8); FT-IR (neat, cm^{-1}): 3330, 2934, 1716, 1612, 1513, 1449, 1381, 1302, 1246, 1213, 1179, 1160, 1067, 1033, 1002, 900, 865, 822, 760, 735, 701, 646, 632, 621, 516; LC-MS: Rt = 13.48 min (Vydac 219TP 5 µm Diphenyl, 10 - 90% MeCN, 21 min run); ESI-MS: m/z 1025.4 $[M+Na]^+$; HRMS: $[M+H]^+$ calcd. for $C_{61}H_{67}O_2N_{11}$: 1003.47394, found 1003.47380.

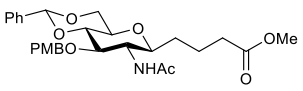
3-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-1-propene (11)



Alcohol **10**[28] (1.6 g, 4.9 mmol, 1.0 eq.) was co-evaporated with toluene (1x) under an argon atmosphere, followed by the addition of dry THF (0.12 L) and *p*-methoxybenzyl-2,2,2-trichloroacetimidate (3.1 mL, 15 mmol, 3.0 eq.). The suspension was cooled to 0°C and a solution of TfOH in THF (0.01 M, 50 mL, 0.5 mmol, 0.10 eq.) was added. After 15 minutes, the mixture was allowed to warm up to room temperature and stirred for 3 hours. The obtained solution was neutralized with Et_3N and diluted with EtOAc and washed with sat. aq. $NaHCO_3$ (1x) and brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was embedded on silica and purified by column chromatography (2→20% acetone in DCM). The title compound was obtained (1.7 g, 3.8 mmol, 78%) as a white solid. R_f: 0.45 (9/1 DCM/acetone); $[\alpha]_D^{25}$ -58.4° (*c* = 0.32, $CHCl_3$/MeOH 1/1); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.53 – 7.48 (m, 2H, Ar), 7.42 – 7.35 (m, 3H, Ar), 7.25 – 7.21 (m, 2H, Ar), 6.89 – 6.84 (m, 2H, Ar), 5.88 – 5.75 (m, 1H, CH_2-C*H*=CH_2), 5.58 (s, 1H, CH benzylidene), 5.15 – 5.00 (m, 3H, NH, CH_2-CH=C*H*$_2$), 4.82 (d, 1H, *J* = 11.8 Hz, C*H*H PMB), 4.61 (d, 1H, *J* = 11.7 Hz, C*H*H PMB), 4.32 (dd, 1H, *J* = 10.4, 5.0 Hz, C*H*H-6), 3.80 (s, 3H, CH_3 PMB), 3.77 – 3.64 (m, 4H, H-2, H-3, H-4, C*H*H-6), 3.51 – 3.44 (m, 1H, H-1), 3.44 – 3.37 (m, 1H, H-5), 2.39 – 2.31 (m, 1H, C*H*H-CH=CH_2), 2.28 – 2.18 (m, 1H, C*H*H-CH=CH_2), 1.90 (s, 3H, CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 170.3 (C=O), 159.5, 137.6 (C_q Ar), 134.4 (CH_2-*C*H=CH_2), 130.6 (C_q Ar), 130.2, 129.1, 128.4, 126.1 (Ar), 117.2 (CH_2-CH=*C*H_2), 113.9 (Ar), 101.2 (CH benzylidene), 83.0 (C-4), 79.2 (C-1), 77.8 (C-3), 73.5 (CH_2 PMB), 70.5 (C-5), 69.0 (CH_2-6), 55.4 (CH_3 PMB), 55.1 (C-2), 36.6 (*C*H_2-CH=CH_2), 23.7 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3277, 2871, 1651, 1615, 1555, 1514, 1454, 1371, 1302, 1249, 1172, 1132, 1102, 1034, 1015,

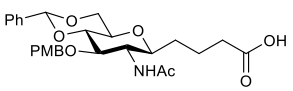
962, 919, 821, 749, 697, 592, 516; HRMS: $[M+H]^+$ calcd. for $C_{26}H_{32}O_6N$: 454.22241, found 454.22225.

Methyl 4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-butanoate (12)



Compound **11** (1.4 g, 3.1 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry DCE (31 mL). Methyl acrylate (0.78 mL, 8.6 mmol, 2.8 eq.), CuI (90 mg, 0.47 mmol, 0.15 eq.) and Grubbs 2nd generation catalyst (0.26 g, 0.31 mmol, 0.10 eq.) were added and the flask was covered in aluminum foil. The suspension was heated to 50°C overnight, after which it was concentrated *in vacuo* and co-evaporated with toluene (3x) under an argon atmosphere. The residue was dissolved in dry DCE (31 mL) and cooled to 0°C. Ruthenium trichloride (0.12 g, 0.58 mmol, 0.19 eq.) and $NaBH_4$ (0.37 g, 9.8 mmol, 3.2 eq.) were added, and an empty balloon was placed on the flask. Methanol (2.5 mL, 61 mmol, 20 eq.) was carefully added to the suspension over 20 minutes, after which the mixture was allowed to warm-up up to room temperature over 25 minutes. The mixture was subsequently heated to 45°C for 7 hours. The reaction mixture was cooled to room temperature, diluted with brine and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (8→15% acetone in DCM) gave compound **12** (1.1 g, 2.1 mmol, 68% over two steps). R_f: 0.41 (8/2 DCM/acetone); $[\alpha]_D^{25}$ -56.5° (*c* = 0.72, $CHCl_3$/MeOH 1/1); ^1H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.51 (dd, 2H, *J* = 7.7, 1.7 Hz, Ar), 7.42 – 7.35 (m, 3H, Ar), 7.23 (d, 2H, *J* = 8.6 Hz, Ar), 6.88 – 6.83 (m, 2H, Ar), 5.57 (s, 1H, CH benzylidene), 5.15 (d, 1H, *J* = 9.0 Hz, NH), 4.81 (d, 1H, *J* = 11.8 Hz, C*H*H PMB), 4.60 (d, 1H, *J* = 11.8 Hz, C*H*H PMB), 4.30 (dd, 1H, *J* = 10.5, 5.0 Hz, C*H*H-6), 3.79 (s, 3H, CH_3 PMB), 3.77 – 3.55 (m, 7H, H-2, H-3, H-4, C*H*H-6, OCH_3), 3.40 – 3.30 (m, 2H, H-1, H-5), 2.31 – 2.25 (m, 2H, CH_2-9), 1.89 (s, 3H, CH_3 Ac), 1.87 – 1.76 (m, 1H, C*H*H-8), 1.66 – 1.54 (m, 2H, C*H*H-7, C*H*H-8), 1.50 – 1.38 (m, 1H, C*H*H-7); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.1 (C=O), 170.3, 159.4, 137.6, 130.6 (C_q Ar), 130.1, 129.0, 128.4, 126.1, 113.9 (Ar), 101.2 (CH benzylidene), 83.0 (C-4), 79.6 (C-1), 77.9 (C-3), 73.4 (CH_2 PMB), 70.4 (C-5), 69.0 (CH_2-6), 55.4 (CH_3 PMB), 55.0 (C-2), 51.6 (OCH_3), 33.8 (CH_2-9), 31.4 (CH_2-7), 23.7 (CH_3 Ac), 21.1 (CH_2-8); FT-IR (neat, cm^{-1}): 3269, 2877, 1741, 1648, 1558, 1514, 1452, 1367, 1324, 1247, 1200, 1171, 1132, 1092, 1032, 957, 858, 822, 749, 695, 618; HRMS: $[M+H]^+$ calcd. for $C_{28}H_{36}O_8N_1$: 514.24354, found 514.24353.

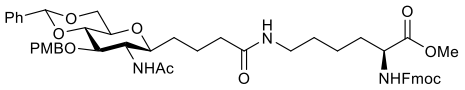
4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-butanoic acid (13)



To a white suspension of methyl ester **12** (1.0 g, 2.0 mmol, 1.0 eq.) in THF/H_2O/MeOH (7/1/2, v/v/v, 20 mL) was added LiOH (0.26 g, 6.2 mmol, 3.1 eq.). The mixture was heated to 40°C for 3 hours, after which TLC analysis showed complete conversion of the starting material. The reaction was diluted with EtOAc and acidified with 1 M HCl to pH = 6-7, followed by the extraction of the aqueous layer with EtOAc (1x) and DCM (1x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in*

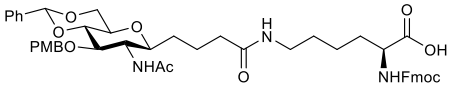
vacuo. The title compound was obtained (1.0 g, 2.0 mmol, 100%) and used without further purification. R_f: 0.45 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ -54.3° (*c* = 0.28, $CHCl_3$/MeOH 1/1); ^{1}H NMR (DMSO, 400 MHz, HH-COSY, HSQC): δ 11.99 (s, 1H, OH), 7.89 (d, 1H, *J* = 9.0 Hz, NH), 7.47 – 7.34 (m, 5H, Ar), 7.18 (d, 2H, *J* = 8.7 Hz, Ar), 6.85 (d, 2H, *J* = 8.7 Hz, Ar), 5.68 (s, 1H, CH benzylidene), 4.64 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.51 (d, 1H, *J* = 11.4 Hz, C*H*H PMB), 4.21 (dd, 1H, *J* = 10.1, 4.9 Hz, C*H*H-6), 3.77 – 3.53 (m, 7H, H-2, H-3, H-4, C*H*H-6, CH_3 PMB), 3.40 – 3.26 (m, 2H, H-1, H-5), 2.18 (t, 2H, *J* = 7.2 Hz, CH_2-9), 1.83 (s, 3H, CH_3 Ac), 1.73 – 1.62 (m, 1H, C*H*H-8), 1.58 – 1.41 (m, 2H, C*H*H-7, C*H*H-8), 1.34 – 1.22 (m, 1H, C*H*H-7); ^{13}C-APT NMR (DMSO, 101 MHz, HSQC): δ 174.4 (C=O), 169.2, 158.6, 137.8, 130.9 (C_q Ar), 129.0, 128.7, 128.1, 126.0, 113.4 (Ar), 100.0 (CH benzylidene), 81.4 (C-4), 79.7 (C-1), 79.0 (C-3), 72.9 (CH_2 PMB), 70.0 (C-5), 68.0 (CH_2-6), 55.0 (CH_3 PMB), 53.9 (C-2), 33.5 (CH_2-9), 30.8 (CH_2-7), 22.9 (CH_3 Ac), 20.8 (CH_2-8); FT-IR (neat, cm^{-1}): 2870, 2428, 1725, 1597, 1516, 1489, 1366, 1302, 1274, 1251, 1223, 1172, 1136, 1109, 1088, 1041, 1020, 966, 923, 822, 747, 694, 606, 539; HRMS: $[M+H]^+$ calcd. for $C_{27}H_{34}O_8N_1$: 500.22789, found 500.22784.

N_α-Fmoc-N_ε-[butan-4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-amide]-L-lysine-methyl ester (14)



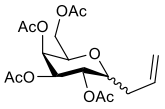
Acid **13** (0.97 g, 1.9 mmol, 1.0 eq.) and Fmoc-L-lysine-OMe[21] (0.98 g, 2.3 mmol, 1.2 eq.) were dissolved in DMF (20 mL), followed by the addition of HCTU (0.96 g, 2.3 mmol, 1.2 eq.) and DIPEA (1.0 mL, 5.7 mmol, 3.0 eq.). The reaction was stirred at room temperature for 4 hours, after which Et_2O was added. The precipitation was filtered and recrystallized with MeOH/DCM/Et_2O to obtain compound **14** (1.3 g, 1.5 mmol, 78%). R_f: 0.59 (9/1 DCM/MeOH). $[\alpha]_D^{25}$ -35.0° (*c* = 0.34, $CHCl_3$/MeOH 1/1); ^{1}H NMR (DMSO, 400 MHz, HH-COSY, HSQC): δ 7.92 – 7.86 (m, 3H, Ar, N*H*Ac), 7.79 (d, 1H, *J* = 7.8 Hz, N*H*Fmoc), 7.77 – 7.69 (m, 3H, Ar, NH), 7.45 – 7.36 (m, 7H, Ar), 7.33 (t, 2H, *J* = 7.4 Hz, Ar), 7.18 (d, 2H, *J* = 8.6 Hz, Ar), 6.84 (d, 2H, *J* = 8.6 Hz, Ar), 5.68 (s, 1H, CH benzylidene), 4.65 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.52 (d, 1H, *J* = 11.4 Hz, C*H*H PMB), 4.35 – 4.26 (m, 2H, CH_2 Fmoc), 4.26 – 4.16 (m, 2H, CH Fmoc, C*H*H-6), 4.04 – 3.96 (m, 1H, CH L-Lys), 3.75 – 3.69 (m, 4H, C*H*H-6, CH_3 PMB), 3.69 – 3.55 (m, 6H, H-2, H-3, H-4, OCH_3), 3.38 – 3.26 (m, 2H, H-1, H-5), 3.06 – 2.94 (m, 2H, CH_2 ε-L-Lys), 2.00 (t, 2H, *J* = 7.2 Hz, CH_2-9), 1.83 (s, 3H, CH_3 Ac), 1.74 – 1.18 (m, 10H, CH_2-7, CH_2-8, 3x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR (DMSO, 101 MHz, HSQC): δ 173.0, 171.8 (C=O), 169.2, 158.6, 156.1, 143.8, 143.8, 140.8, 137.8, 130.9 (C_q Ar), 129.0, 128.7, 128.1, 127.7, 127.1, 126.0, 125.3, 120.1, 113.4 (Ar), 100.0 (CH benzylidene), 81.5 (C-4), 79.7 (C-1), 79.1 (C-3), 72.9 (CH_2 PMB), 70.0 (C-5), 68.0 (CH_2-6), 65.6 (CH_2 Fmoc), 55.0 (CH_3 PMB), 54.0 (C-2), 53.8 (CH L-Lys), 51.9(OCH_3), 46.7 (CH Fmoc), 38.1 (CH_2 ε-L-Lys), 35.4 (CH_2-9), 31.1, 30.3, 28.7, 23.0 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys), 22.9 (CH_3 Ac), 21.8 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); FT-IR (neat, cm^{-1}): 3298, 2867, 1687, 1636, 1547, 1514, 1452, 1370, 1250, 1177, 1133, 1088, 1031, 819, 735, 695, 621, 539; HRMS: $[M+H]^+$ calcd. for $C_{49}H_{58}O_{11}N_3$: 864.40659, found 864.40649.

N_α-Fmoc-N_ε-[butan-4-(2-deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-amide]-L-lysine (2)



To a suspension of compound **14** (0.92 g, 1.1 mmol, 1.0 eq.) in THF (21 mL) was added a solution of LiOH (0.30 M, 7.1 mL, 2.1 mmol, 2.0 eq.) at room temperature. After stirring for 50 minutes, the obtained clear solution was neutralized with 1 M HCl (2.2 mL, 2.2 mmol, 2.0 eq.). $NaHCO_3$ (0.36 g, 4.3 mmol, 4.0 eq.) and Fmoc *N*-hydroxysyccinimide ester (0.72 g, 2.1 mmol, 2.0 eq.) were added and the mixture was stirred vigorously for 2 hours. Upon completion of the reaction determined by LC-MS, Et_2O was added at 0°C to precipitate the crude product. After filtration, the precipitate was purified by recrystallization (MeOH/DCM/Et_2O) to yield the title compound (0.88 g, 1.0 mmol, 91 %) as a white solid. R_f: 0.40 (85/15 DCM/MeOH); $[\alpha]_D^{25}$ +15.5° (*c* = 1.0, $CHCl_3$/MeOH 1/1); ^{1}H NMR (MeOD/$CDCl_2$: 1/1 v/v, 400 MHz, HH-COSY, HSQC): δ 7.80 – 7.74 (m, 2H, Ar), 7.68 – 7.60 (m, 2H, Ar), 7.51 – 7.44 (m, 2H, Ar), 7.43 – 7.32 (m, 5H, Ar), 7.35 – 7.26 (m, 2H, Ar), 7.23 – 7.15 (m, 2H, Ar), 6.86 – 6.78 (m, 2H, Ar), 5.58 (s, 1H, CH benzylidene), 4.75 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.59 – 4.54 (m, 1H, C*H*H PMB), 4.37 – 4.28 (m, 2H, CH_2 Fmoc), 4.31 – 4.18 (m, 2H, C*H*H-6, CH Fmoc), 4.02 (dd, 1H, *J* = 7.0, 5.0 Hz, CH L-Lys), 3.81 – 3.72 (m, 4H, H-2, CH_3 PMB), 3.74 – 3.59 (m, 3H, H-3, H-4, C*H*H-6), 3.44 – 3.32 (m, 2H, H-1, H-5), 3.20 – 3.08 (m, 2H, CH_2 ε-L-Lys), 2.13 (t, 2H, *J* = 7.6 Hz, CH_2-9), 1.91 (s, 3H, CH_3 Ac), 1.86 – 1.29 (m, 10H, CH_2-7, CH_2-8, 3x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 178.6, 175.2, 172.6 (C=O), 160.0, 157.3, 144.9, 144.7, 142.0, 138.5, 131.5 (C_q Ar), 130.2, 129.5, 128.8, 128.3, 127.7, 126.8, 125.8, 120.5, 114.2 (Ar), 101.9 (CH benzylidene), 83.3 (C-4), 80.4 (C-3), 79.8 (C-1), 74.6 (CH_2 PMB), 71.1 (C-5), 69.5 (CH_2-6), 67.3 (CH_2 Fmoc), 57.0 (CH L-Lys), 55.6 (CH_3 PMB), 55.4 (C-2), 48.0 (CH Fmoc), 39.8 (CH_2 ε-L-Lys), 36.6 (CH_2-9), 33.4, 32.0, 29.5, 25.7, 23.4 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys), 23.0 (CH_3 Ac), 22.6 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); FT-IR (neat, cm^{-1}): 3286, 1638, 1547, 1513, 1451, 1370, 1249, 1176, 1135, 1088, 1030, 820, 737, 696, 621, 541; LC-MS: Rt = 7.75 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 872.4 $[M+Na]^+$; HRMS: $[M+H]^+$ calcd. for $C_{48}H_{56}O_{11}N_3$: 850.39094, found 850.39103.

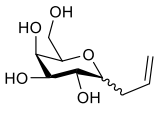
3-(2,3,4,6-tetra-*O*-acetyl-α/β-D-galactopyranosyl)-1-propene (15)



Acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (23.7 g, 60.8 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in CH_3NO_2 (0.24 L). Allyltrimethylsilane (20 mL, 0.13 mol, 2.1 eq.) was added, followed by the addition of $BF_3{\cdot}OEt_2$ (23 mL, 0.18 mol, 3.0 eq.) at 0°C. The yellow solution was allowed to stir at room temperature for 3 days. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ at 0°C, diluted with EtOAc and washed with brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10→50% Et_2O in pentane) afforded the title compound (20.1 g, 54.0 mmol, 89%) as a yellow oil with an α/β ratio of 2/1. R_f: 0.41 (1/1 pentane/Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.80 – 5.69 (m, 1H, CH_2-C*H*=CH_2), 5.43 – 5.38 (m, 1H, H-4), 5.26 (dd, 1H, *J* = 9.3, 5.0 Hz, H-2), 5.20 (dd, 1H, *J* = 9.4, 3.2 Hz, H-3), 5.12 – 5.06 (m, 2H, CH_2-CH=C*H*$_2$), 4.33 – 4.25 (m, 1H, H-1), 4.24 – 4.14 (m, 1H, C*H*H-6), 4.14 – 4.01 (m, 2H,

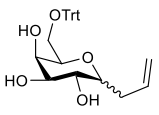
H-5, C*H*H-6), 2.54 – 2.38 (m, 1H, C*H*H-CH=CH_2), 2.35 – 2.21 (m, 1H, C*H*H-CH=CH_2), 2.11 (s, 3H, CH_3 Ac), 2.06 (s, 3H, CH_3 Ac), 2.05 – 2.00 (m, 6H, 2x CH_3 Ac); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 170.7, 170.2, 170.1, 170.0 (C=O), 133.4 (CH_2-*C*H=CH_2), 117.8 (CH_2-CH=*C*H_2), 71.6 (C-1), 68.4 (C-5), 68.0 (C-2), 67.8 (C-3), 67.7 (C-4), 61.6 (CH_2-6), 31.0 (*C*H_2-CH=CH_2), 20.9, 20.9, 20.8, 20.8, 20.8 (CH_3 Ac); FT-IR (neat, cm^{-1}): 2978, 1740, 1644, 1434, 1369, 1212, 1044, 909, 601; HRMS: $[M+Na]^+$ calcd for $C_{17}H_{24}O_9Na$: 395.1318, found 395.1316. *NMR analysis only given for the α-anomer.

3-(α/β-D-galactopyranosyl)-1-propene (16)



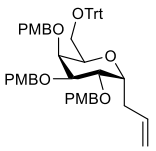
Compound **15** (20.0 g, 53.8 mmol, 1.0 eq.) was dissolved in MeOH (0.11 L). Sodium methoxide (5.4 M in MeOH, 4.0 mL, 22 mmol, 0.40 eq.) was added and the solution was stirred for 3 hours, after which it was acidified by the addition of amberlite H^+ resin. The mixture was filtered and concentrated *in vacuo*. The title compound (10.0 g, 49.2 mmol, 91%) was obtained as a yellow foam and used without further purification. R_f: 0.13 (9/1 DCM/MeOH); ^{1}H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 5.93 – 5.82 (m, 1H, CH_2-C*H*=CH_2), 5.17 – 5.06 (m, 2H, CH_2-CH=C*H*$_2$), 4.03 – 3.93 (m, 2H, H-1, H-2), 3.93 – 3.85 (m, 1H, H-3), 3.80 – 3.62 (m, 4H, H-4, H-5, CH_2-6), 2.52 – 2.32 (m, 2H, C*H*$_2$-CH=CH_2); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ 136.7 (CH_2-*C*H=CH_2), 116.9 (CH_2-CH=C*H*$_2$), 75.6 (C-1), 74.0, 71.9 (C-4, C-5), 70.1 (C-3), 70.0 (C-2), 61.9 (CH_2-6), 31.0 (*C*H_2-CH=CH_2); FT-IR (neat, cm^{-1}): 3352, 2919, 1642, 1416, 1073, 914, 515; HRMS: $[M+Na]^+$ calcd for $C_9H_{16}O_5Na$: 227.0895, found 227.0894. *NMR analysis only given for the α-anomer.

3-(6-*O*-trityl-α/β-D-galactopyranosyl)-1-propene (17)



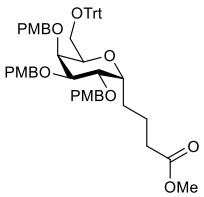
Trityl chloride (21 g, 75 mmol, 1.3 eq.) and Et_3N (17 mL, 0.12 mol, 2.5 eq.) were added to a solution of compound **16** (10.0 g, 48.9 mmol, 1.0 eq.) in DMF (0.16 L). The mixture was heated to 60°C overnight. The mixture was diluted with EtOAc and washed with brine (3x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (30→100% EtOAc in pentane) gave the title compound (17.3 g, 38.7 mmol, 79%). R_f: 0.36 (3/7 pentane/EtOAc); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.44 (m, 6H, Trt), 7.36 – 7.29 (m, 6H, Trt), 7.29 – 7.23 (m, 3H, Trt), 6.04 – 5.87 (m, 1H, CH_2-C*H*=CH_2), 5.24 – 5.03 (m, 2H, CH_2-CH=C*H*$_2$), 3.96 – 3.89 (m, 1H, H-1), 3.89 – 3.83 (m, 1H, H-5), 3.81 – 3.72 (m, 2H, H-2, H-4), 3.64 – 3.53 (m, 1H, H-3), 3.33 – 3.12 (m, 3H, C*H*H-6, 2x OH), 3.11 – 3.04 (m, 1H, C*H*H-6), 2.91 – 2.82 (m, 1H, OH), 2.52 – 2.42 (m, 1H, C*H*H-CH=CH_2), 2.39 – 2.29 (m, 1H, C*H*H-CH=CH_2); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ 145.3 (C_q Trt), 137.0 (CH_2-*C*H=CH_2), 129.6, 128.8, 128.0 (Ar), 116.9 (CH_2-CH=*C*H_2), 87.3 (C_q Trt), 75.4 (C-1), 71.4 (C-5), 70.8 (C-3), 70.2, 69.7 (C-2, C-4), 64.3 (CH_2-6),30.2 (*C*H_2-CH=CH_2); FT-IR (neat, cm^{-1}): 3391, 3059, 2929, 1642, 1597, 1490, 1448, 1265, 1222, 1153, 1069, 988, 901, 823, 762, 737, 704, 650, 632, 580, 536; HRMS: $[M+Na]^+$ calcd for $C_{28}H_{30}O_5Na$: 469.1991, found 469.1988. *NMR analysis only given for the α-anomer.

3-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-1-propene (18α)



Triol **17** (16.4 g, 36.8 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in DMF (0.37 L). Sodium hydride (60% dispersion in mineral oil, 5.3 g, 0.13 mol, 3.5 eq.) was added at 0°C over 30 minutes. After 1 hour, *p*-methoxybenzyl chloride (18 mL, 0.13 mol, 3.5 eq.) and tetrabutylammonium iodide (1.4 g, 3.8 mmol, 0.10 eq.) were added. Another portion of sodium hydride (60% dispersion in mineral oil, 2.3 g, 58 mmol, 1.6 eq.) was added after 1 hour and the miture was stirred at room temperature overnight. The reaction mixture was quenched with MeOH at 0°C, diluted with Et_2O and washed H_2O (3x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (10→30% Et_2O in pentane) gave compound **18α** (15.5 g, 19.2 mmol, 52%) and compound **18β** (8.23 g, 10.3 mmol, 28%). Analysis α-compound: R_f: 0.26 (7/3 pentane/Et_2O); $[\alpha]_D^{25}$ +35.9° (*c* = 0.44, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, HH-COSY, HSQC): δ 7.47 – 7.41 (m, 6H, Ar), 7.34 – 7.21 (m, 11H, Ar), 7.09 (dd, 4H, *J* = 19.9, 8.5 Hz, Ar), 6.90 (d, 2H, *J* = 8.6 Hz, Ar), 6.86 – 6.81 (m, 4H, Ar), 5.87 – 5.76 (m, 1H, CH_2-C*H*=CH_2), 5.17 – 5.03 (m, 2H, CH_2-CH=C*H*$_2$), 4.52 – 4.42 (m, 5H, 2x CH_2 PMB, 1x C*H*H PMB), 4.34 (d, 1H, *J* = 11.2 Hz, C*H*H PMB), 4.09 – 4.03 (m, 1H, H-5), 3.91 (dd, 1H, *J* = 4.3, 3.0 Hz, H-4), 3.82 – 3.74 (m, 11H, H-1, H-3, 3x CH_3 PMB), 3.62 – 3.55 (m, 2H, H-2, C*H*H-6), 3.17 (dd, 1H, *J* = 10.6, 3.2 Hz, C*H*H-6), 2.45 – 2.36 (m, 1H, C*H*H-CH=CH_2), 2.36 – 2.27 (m, 1H, C*H*H-CH=CH_2); ^{13}C-APT NMR (CD_3CN, 126 MHz, HSQC): δ 160.7, 160.6, 160.5, 145.7 (C_q Ar), 136.8 (CH_2-*C*H=CH_2), 132.3, 132.1, 130.8, 130.5, 130.2, 129.9, 128.9, 128.1 (Ar), 117.2 (CH_2-CH=*C*H_2), 115.0, 114.9 (Ar), 87.6 (C_q Trt), 77.2 (C-2), 76.8 (C-3), 75.3 (C-4), 74.4 (C-5), 73.4, 73.0 (CH_2 PMB), 71.2 (C-1), 62.5 (CH_2-6), 56.2 (CH_3 PMB), 33.8 (*C*H_2-CH=CH_2); FT-IR (neat, cm^{-1}): 2934, 2906, 2836, 1612, 1586, 1513, 1491, 1464, 1449, 1355, 1302, 1247, 1173, 1091, 1034, 995, 916, 821, 765, 748, 707, 649, 633, 568, 516; HRMS: $[M+Na]^+$ calcd for $C_{52}H_{54}O_8Na$: 829.3716, found 829.3735.

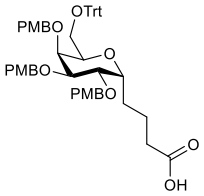
Methyl 4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-butanoate (19α)



Compound **18α** (15.2 g, 18.8 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere before being dissolved in dry DCE (0.19 L). Methyl acrylate (4.8 mL, 53 mmol, 2.8 eq.), CuI (0.54 g, 2.8 mmol, 0.15 eq.) and Grubbs 2nd generation catalyst (0.63 g, 0.74 mmol, 0.04 eq.) were added and the flask was covered in aluminum foil. The suspension was heated to 50°C for 48 hours, after which it was concentrated *in vacuo* and co-evaporated with toluene (2x) under an argon atmosphere. The obtained residue was dissolved in dry DCE (0.10 L) and cooled to 0°C. Two empty balloons were placed on the flask, followed by the addition of ruthenium trichloride (0.74 g, 3.6 mmol, 0.19 eq.) and $NaBH_4$ (2.3 g, 61 mmol, 3.2 eq.). Methanol (15 mL, 0.37 mol, 20 eq.) was carefully added to the suspension over 20 minutes, after which the mixture was allowed to warm-up up to room temperature over 30 minutes. The mixture was subsequently heated to 45°C for 6 hours. The reaction mixture was cooled to room temperature, diluted with brine and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and

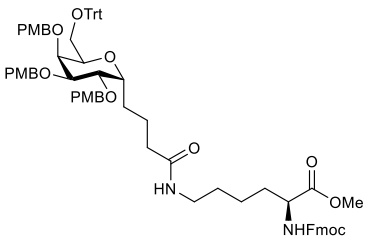
concentrated *in vacuo*. Purification by column chromatography (20→70% Et_2O in pentane) gave compound **19α** (11.8 g, 13.6 mmol, 73% over two steps). R_f: 0.43 (1/1 pentane/ Et_2O); $[\alpha]_D^{25}$ +23.0° (*c* = 0.43, $CHCl_3$); ^1H NMR (CD_3CN, 500 MHz, HH-COSY, HSQC): δ 7.44 (d, 6H, *J* = 8.1 Hz, Ar), 7.30 (t, 6H, *J* = 7.4 Hz, Ar), 7.28 – 7.20 (m, 5H, Ar), 7.13 (d, 2H, *J* = 8.4 Hz, Ar), 7.06 (d, 2H, *J* = 8.4 Hz, Ar), 6.89 (d, 2H, *J* = 8.6 Hz, Ar), 6.84 (dd, 4H, *J* = 8.6, 3.2 Hz, Ar), 4.54 – 4.41 (m, 5H, 2x CH_2 PMB, 1x C*H*H PMB), 4.34 (d, 1H, *J* = 11.1 Hz, C*H*H PMB), 3.97 – 3.92 (m, 1H, H-5), 3.86 (t, 1H, *J* = 3.4 Hz, H-4), 3.81 – 3.76 (m, 9H, 3x CH_3 PMB), 3.73 – 3.68 (m, 2H, H-1, H-3), 3.61 (s, 3H, OCH_3), 3.60 – 3.56 (m, 1H, H-2), 3.55 – 3.50 (m, 1H, C*H*H-6), 3.17 (dd, 1H, *J* = 10.5, 3.3 Hz, C*H*H-6), 2.33 (t, 2H, *J* = 7.2 Hz, CH_2-9), 1.76 – 1.58 (m, 2H, C*H*H-7, C*H*H-8), 1.58 – 1.45 (m, 2H, C*H*H-7, C*H*H-8); ^{13}C-APT NMR (CD_3CN, 126 MHz, HSQC): δ 174.8 (C=O), 160.7, 145.7 (C_q Ar), 132.4, 132.1, 130.8, 130.5, 130.3, 129.8, 128.9, 128.1, 115.0, 114.9 (Ar), 87.7 (C_q Trt), 77.5 (C-2), 77.4 (C-3), 75.6 (C-4), 73.9 (C-5), 73.4, 73.4, 73.3 (CH_2 PMB), 71.6 (C-1), 63.0 (CH_2-6), 56.2 (CH_3 PMB), 52.0 (OCH_3), 34.7 (CH_2-9), 28.0, 22.5 (CH_2-7, CH_2-8); FT-IR (neat, cm^{-1}): 2949, 1736, 1612, 1513, 1449, 1302, 1248, 1173, 1090, 1034, 821, 748, 707, 633; HRMS: $[M+Na]^+$ calcd. for $C_{54}H_{58}O_{10}Na$: 889.39222, found 889.39203.

4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-butanoic acid (20α)



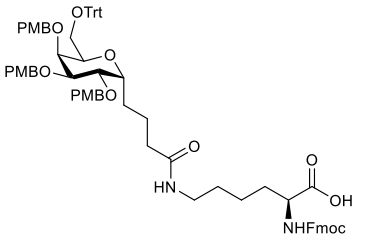
19α (11.7 g, 13.5 mmol, 1.0 eq.) was dissolved in in a mixture of THF/H_2O (4/1, v/v, 0.14 L), followed by the addition of LiOH (1.7 g, 41 mmol, 3.0 eq.). The mixture was heated to 40°C for 30 hours. The reaction mixture was cooled to 0°C, acidified with 3 M HCl to pH = 4/5, diluted with H_2O and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The title compound was obtained (10.5 g, 12.4 mmol, 92%) and used without further purification. R_f: 0.84 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +25.6° (*c* = 0.90, $CHCl_3$); ^1H NMR (CD_3CN, 500 MHz, HH-COSY, HSQC): δ 7.51 – 7.47 (m, 6H, Ar), 7.33 (t, 6H, *J* = 7.4 Hz, Ar), 7.30 – 7.24 (m, 5H, Ar), 7.18 (d, 2H, *J* = 8.5 Hz, Ar), 7.10 (d, 2H, *J* = 8.6 Hz, Ar), 6.92 (d, 2H, *J* = 8.6 Hz, Ar), 6.90 – 6.84 (m, 4H, Ar), 4.59 – 4.46 (m, 5H, 2x CH_2 PMB, 1x C*H*H PMB), 4.39 (d, 1H, *J* = 11.1 Hz, C*H*H PMB), 4.04 – 3.97 (m, 1H, H-5), 3.94 – 3.90 (m, 1H, H-4), 3.84 – 3.77 (m, 10H, H-1, 3x CH_3 PMB), 3.76 (dd, 1H, *J* = 6.9, 2.8 Hz, H-3), 3.69 – 3.64 (m, 1H, H-2), 3.64 – 3.58 (m, 1H, C*H*H-6), 3.24 (dd, 1H, *J* = 10.5, 3.4 Hz, C*H*H-6), 2.38 (t, 2H, *J* = 7.0 Hz, CH_2-9), 1.83 – 1.68 (m, 2H, C*H*H-7, C*H*H-8), 1.64 – 1.55 (m, 2H, C*H*H-7, C*H*H-8); ^{13}C-APT NMR (CD_3CN, 126 MHz, HSQC): δ 175.7 (C=O), 160.5, 160.4, 160.3, 145.5 (C_q Ar), 132.2, 132.0, 132.0, 130.6, 130.4, 130.1, 129.7, 128.8, 128.0, 114.9, 114.8, 114.8 (Ar), 87.6 (C_q Trt), 77.4 (C-2, C-3), 75.6 (C-4), 73.7 (C-5), 73.3 (CH_2 PMB), 71.7 (C-1), 63.0 (CH_2-6), 56.1, 56.1 (CH_3 PMB), 34.4 (CH_2-9), 27.8, 22.4 (CH_2-7, CH_2-8); FT-IR (neat, cm^{-1}): 2937, 1707, 1612, 1513, 1449, 1302, 1248, 1173, 1087, 1034, 821, 707, 633; HRMS: $[M+Na]^+$ calcd. for $C_{53}H_{56}O_{10}Na$: 875.37657, found 875.37656.

N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-amide]-L-lysine-methyl ester (21α)



Compound **01α** (4.3 g, 5.0 mmol, 1.0 eq.) and Fmoc-L-lysine-OMe[21] (2.5 g, 6.0 mmol, 1.2 eq.) were dissolved in DMF (25 mL). HCTU (2.5 g, 6.0 mmol, 1.2 eq.) and DIPEA (2.6 mL, 15 mmol, 3.0 eq.) were added and the solution was stirred for 2 hours. The reaction mixture was diluted with EtOAc and was washed with 1 M HCl (1x), sat. aq. $NaHCO_3$ (1x), brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (40→80% EtOAc in pentane) gave compound **21α** (5.5 g, 4.5 mmol, 90%) as an oil. R_f: 0.63 (3/7 pentane/EtOAc); $[\alpha]_D^{25}$ +23.3° (*c* = 0.46, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, HH-COSY, HSQC): δ 7.82 (d, 2H, *J* = 7.5 Hz, Ar), 7.65 (d, 2H, *J* = 7.1 Hz, Ar), 7.46 – 7.38 (m, 8H, Ar), 7.36 – 7.17 (m, 13H, Ar), 7.11 (d, 2H, *J* = 8.4 Hz, Ar), 7.05 (d, 2H, *J* = 8.5 Hz, Ar), 6.87 (d, 2H, *J* = 8.6 Hz, Ar), 6.82 (d, 4H, *J* = 8.5 Hz, Ar), 6.15 (br, 1H, NH), 5.91 (br, 1H, N*H*Fmoc), 4.51 – 4.39 (m, 5H, 2x CH_2 PMB, 1x C*H*H PMB), 4.39 – 4.29 (m, 3H, C*H*H PMB, CH_2 Fmoc), 4.23 (t, 1H, *J* = 6.8 Hz, CH Fmoc), 4.15 – (m, 1H, CH L-Lys), 3.96 – 3.90 (m, 1H, H-5), 3.86 – 3.82 (m, 1H, H-4), 3.81 – 3.75 (m, 9H, 3x CH_3 PMB), 3.72 – 3.63 (m, 5H, H-1, H-3, OCH_3), 3.59 – 3.48 (m, 2H, H-2, C*H*H-6), 3.17 – 3.08 (m, 3H, C*H*H-6, CH_2 ε-L-Lys), 2.13 (t, 2H, *J* = 6.9 Hz, CH_2-9), 1.82 – 1.27 (m, 10H, 2x CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR (CD_3CN, 126 MHz, HSQC): δ 145.7, 142.5 (C_q Ar), 130.8, 130.5, 130.3, 129.9, 129.0, 128.9, 128.3, 128.2, 126.4, 121.2, 118.2, 115.0, 114.9 (Ar), 87.7 (C_q Trt), 77.5 (C-2, C-3), 75.6 (C-4), 74.0 (C-5), 73.4, 73.2 (CH_2 PMB), 71.7 (C-1), 67.6 (CH_2 Fmoc), 63.0 (CH_2-6), 56.2 (CH_3 PMB), 55.4 (CH L-Lys), 52.8 (OCH_3), 48.5 (CH Fmoc), 39.6 (CH_2 ε-L-Lys), 37.1 (CH_2-9), 32.3, 30.2, 28.3, 23.8, 23.4 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); FT-IR (neat, cm^{-1}): 2950, 1723, 1653, 1612, 1514, 1450, 1248, 1174, 1088, 1034, 823, 743, 707; HRMS: $[M+H]^+$ calcd. for $C_{75}H_{81}O_{13}N_2$: 1217.57332, found 1217.57311.

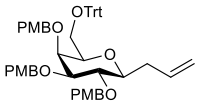
N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-amide]-L-lysine (4)



Compound **21α** (4.8 g, 4.0 mmol, 1.0 eq.) was dissolved in THF (55 mL) and cooled to 0°C. A solution of LiOH in H_2O (0.30 M, 27 mL, 8.1 mmol, 2.0 eq.) was added and the suspension was stirred vigorously for 1 hour, after which the obtained solution was acidified by the addition of 1 M HCl to pH = 5-6 and diluted with brine. The mixture was extracted with EtOAc (1x) and the organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. After purification by column chromatography (2→6% MeOH in DCM), the title compound (2.2 g, 1.8 mmol, 46%) was obtained as a white foam. R_f: 0.70 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +15.8° (*c* = 1.1, $CHCl_3$); ^{1}H NMR (CD_3CN, 500 MHz, HH-COSY, HSQC): δ 7.80 (d, 2H, *J* = 7.4 Hz, Ar), 7.64 (d, 2H, *J* = 7.1 Hz, Ar), 7.46 – 7.36 (m, 8H, Ar), 7.34 – 7.17 (m, 13H, Ar), 7.10 (d, 2H, *J* = 8.3 Hz, Ar), 7.04 (d, 2H, *J* = 8.4 Hz, Ar), 6.84 (dd, 6H, *J* = 23.1, 8.0 Hz, Ar), 6.23 (br, 1H, NH), 5.92

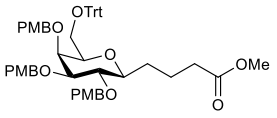
(br, 1H, N*H*Fmoc), 4.51 – 4.38 (m, 5H, 2x CH_2 PMB, 1x C*H*H PMB), 4.38 – 4.29 (m, 3H, C*H*H PMB, CH_2 Fmoc), 4.21 (t, 1H, *J* = 6.7 Hz, CH Fmoc), 4.11 (br, 1H, CH L-Lys), 3.97 – 3.90 (m, 1H, H-5), 3.88 – 3.82 (m, 1H, H-4), 3.82 – 3.73 (m, 9H, 3x CH_3 PMB), 3.73 – 3.65 (m, 2H, H-1, H-3), 3.60 – 3.48 (m, 2H, H-2, C*H*H-6), 3.23 – 3.07 (m, 3H, C*H*H-6, CH_2 ε-L-Lys), 2.17 – 2.10 (m, 2H, CH_2-9), 1.84 – 1.27 (m, 10H, 2x CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR (CD_3CN, 126 MHz, HSQC): δ 174.1, 160.7 (C=O), 160.5, 160.5, 145.6, 145.4, 145.4, 142.4, 132.4, 132.1 (C_q Ar), 130.8, 130.5, 130.3, 129.9, 129.0, 128.9, 128.3, 128.2, 126.4, 121.1, 115.0, 114.9 (Ar), 87.7 (C_q Trt), 77.5, 77.3 (C-2, C-3), 75.6 (C-4), 74.0 (C-5), 73.4, 73.3, 73.2 (CH_2 PMB), 71.7 (C-1), 67.6 (CH_2 Fmoc), 63.0 (CH_2-6), 56.2 (CH_3 PMB), 55.2 (CH L-Lys), 48.4 (CH Fmoc), 39.7 (CH_2 ε-L-Lys), 37.1 (CH_2-9), 32.3, 30.1, 28.2, 23.8, 23.4 (CH_2-7/8, 3x CH_2 β/γ/δ-L-Lys); FT-IR (neat, cm^{-1}): 2935, 1720, 1612, 1513, 1449, 1302, 1248, 1174, 1088, 1034, 822, 761, 743, 707, 633; LC-MS: Rt = 7.68 min (Vydac 219TP 5 µm Diphenyl, 50 - 90% MeCN, 21 min run); ESI-MS: m/z 1225.5 $[M+Na]^+$; HRMS: $[M+H]^+$ calcd. for $C_{74}H_{79}O_{13}N_2$: 1203.55767, found 1203.55754.

3-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-1-propene (18β)



See experimental of compound **18α**. Purification gave compound **18β** (8.23 g, 10.3 mmol, 28%). Analysis β-compound: R_f: 0.33 (7/3 pentane/Et_2O); $[\alpha]_D^{25}$ +9.3° (*c* = 0.52, $CHCl_3$); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.47 – 7.41 (m, 6H, Ar), 7.38 – 7.20 (m, 13H, Ar), 6.99 – 6.95 (m, 2H, Ar), 6.92 – 6.85 (m, 4H, Ar), 6.79 – 6.74 (m, 2H, Ar), 5.94 – 5.82 (m, 1H, CH_2-C*H*=CH_2), 5.13 – 4.97 (m, 2H, CH_2-CH=C*H*$_2$), 4.78 (d, 1H, *J* = 10.4 Hz, C*H*H PMB), 4.72 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.67 (d, 1H, *J* = 10.7 Hz, C*H*H PMB), 4.60 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.51 (d, 1H, *J* = 10.5 Hz, C*H*H PMB), 4.27 (d, 1H, *J* = 10.7 Hz, C*H*H PMB), 3.93 (dd, 1H, *J* = 2.9, 1.1 Hz, H-4), 3.82 – 3.71 (m, 9H, 3x CH_3 PMB), 3.63 – 3.54 (m, 2H, H-3, H-5), 3.43 (t, 1H, *J* = 9.3 Hz, H-2), 3.34 – 3.19 (m, 2H, H-1, C*H*H-6), 2.89 (dd, 1H, *J* = 9.3, 5.6 Hz, C*H*H-6), 2.60 – 2.49 (m, 1H, C*H*H-CH=CH_2), 2.23 – 2.11 (m, 2H, C*H*H-CH=CH_2); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ 160.3, 160.2, 160.1, 145.1 (C_q Ar), 136.5 (CH_2-*C*H=CH_2), 132.0, 131.9, 131.9 (C_q Ar), 130.7, 130.6, 130.5, 129.6, 128.8, 128.1 (Ar), 116.9 (CH_2-CH=*C*H$_2$), 114.7, 114.5, 114.4 (Ar), 87.5 (C_q Trt), 85.2 (C-3), 79.7 (C-1), 78.9 (C-2), 78.0 (C-5), 75.4 (C-4), 75.3, 74.9, 72.3 (CH_2 PMB), 64.7 (CH_2-6), 55.9, 55.9 (CH_3 PMB), 37.1 (*C*H_2-CH=CH_2); FT-IR (neat, cm^{-1}): 2907, 1613, 1583, 1513, 1491, 1449, 1362, 1302, 1248, 1173, 1076, 1034, 915, 821, 747, 707, 633, 518; HRMS: $[M+Na]^+$ calcd for $C_{52}H_{54}O_8Na$: 829.3716, found 829.3740.

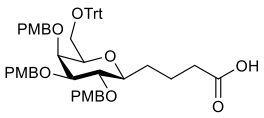
Methyl 4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-butanoate (19β)



Allyl **18β** (8.0 g, 9.9 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere before being dissolved in dry DCE (0.10 L). Methyl acrylate (2.6 mL, 29 mmol, 2.9 eq.), CuI (0.29 g, 1.5 mmol, 0.15 eq.) and Grubbs 2nd generation catalyst (0.34 g, 0.40 mmol, 0.04 eq.) were added and the flask was covered in aluminum foil. The suspension was heated to 50°C for 48 hours, after which it was concentrated *in vacuo* and co-evaporated with toluene (2x) under an argon atmosphere. The obtained residue was dissolved in dry DCE (50 mL) and cooled to 0°C.

Two empty balloons were placed on the flask, followed by the addition of ruthenium trichloride (0.39 g, 1.9 mmol, 0.19 eq.) and $NaBH_4$ (1.2 g, 32 mmol, 3.2 eq.). Methanol (8.0 mL, 0.18 mol, 20 eq.) was carefully added to the suspension over 30 minutes, after which the mixture was allowed to warm-up up to room temperature over 15 minutes. The mixture was subsequently heated to 45°C for 5 hours. The reaction mixture was cooled to room temperature, diluted with brine and extracted with DCM (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. NMR analysis showed still 20% alkene present, therefore the 2nd step was repeated using the same reaction conditions and heated for 7 hours. Purification by column chromatography (20→70% Et_2O in pentane) afforded the title compound (5.8 g, 6.7 mmol, 68% over two steps); R_f: 0.44 (1/1 pentane/ Et_2O); $[\alpha]_D^{25}$ +2.9° (*c* = 0.84, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.43 – 7.39 (m, 6H, Ar), 7.32 – 7.21 (m, 13H, Ar), 7.08 – 7.03 (m, 2H, Ar), 6.91 – 6.84 (m, 4H, Ar), 6.76 – 6.71 (m, 2H, Ar), 4.84 (d, 1H, *J* = 10.4 Hz, C*H*H PMB), 4.72 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.67 – 4.59 (m, 2H, CH_2 PMB), 4.54 (d, 1H, *J* = 10.4 Hz, C*H*H PMB), 4.45 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 3.88 (dd, 1H, *J* = 2.7, 1.0 Hz, H-4), 3.81 (d, 6H, *J* = 3.7 Hz, 2x CH_3 PMB), 3.78 (s, 3H, CH_3 PMB), 3.61 (s, 3H, OCH_3), 3.58 – 3.49 (m, 2H, H-2, H-3), 3.49 – 3.42 (m, 1H, C*H*H-6), 3.32 (t, 1H, *J* = 6.2 Hz, H-5), 3.16 – 3.04 (m, 2H, H-1, C*H*H-6), 2.30 (t, 2H, *J* = 7.3 Hz, CH_2-9), 1.92 – 1.79 (m, 2H, C*H*H-7, C*H*H-8), 1.75 – 1.63 (m, 1H, C*H*H-8), 1.52 – 1.40 (m, 1H, C*H*H-7); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.1 (C=O), 159.3, 159.2, 159.0 (C_q Ar), 144.0, 130.9, 130.7, 130.7, 129.9, 129.7, 129.2, 128.7, 127.9, 127.0, 113.8, 113.5 (Ar), 86.8 (C_q Trt), 84.7 (C-3), 79.3 (C-1), 78.8 (C-2), 77.5 (C-5), 75.1 (CH_2 PMB), 73.8 (C-4), 73.8, 72.0 (CH_2 PMB), 63.3 (CH_2-6), 55.3, 55.3, 55.3 (CH_3 PMB), 51.4 (OCH_3), 34.0 (CH_2-9), 31.1 (CH_2-7), 21.3 (CH_2-8); FT-IR (neat, cm^{-1}): 2949, 1736, 1612, 1586, 1513, 1491, 1449, 1362, 1302, 1248, 1173, 1076, 1033, 822, 748, 707, 633; HRMS: $[M+Na]^+$ calcd. for $C_{54}H_{58}O_{10}Na$: 889.39222, found 889.39207.

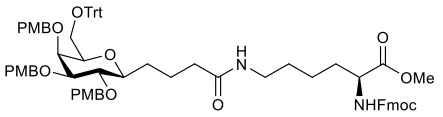
4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-butanoic acid (20β)



Compound **19β** (5.8 g, 6.7 mmol, 1.0 eq.) was dissolved in in a mixture of THF/H_2O (4/1, v/v, 65 mL), followed by the addition of LiOH (0.85 g, 20 mmol, 3.0 eq.). The mixture was heated to 40°C for 30 hours. The reaction mixture was cooled to 0°C, acidified with 3 M HCl to pH = 4/5, diluted with H_2O and extracted with DCM (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The title compound was obtained (5.5 g, 6.4 mmol, 96%) and used without further purification. R_f: 0.89 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +3.6° (*c* = 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.44 – 7.39 (m, 6H, Ar), 7.34 – 7.20 (m, 13H, Ar), 7.09 – 7.04 (m, 2H, Ar), 6.93 – 6.82 (m, 4H, Ar), 6.77 – 6.71 (m, 2H, Ar), 4.84 (d, 1H, *J* = 10.4 Hz, C*H*H PMB), 4.73 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.68 – 4.60 (m, 2H, CH_2 PMB), 4.54 (d, 1H, *J* = 10.5 Hz, C*H*H PMB), 4.45 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 3.89 (dd, 1H, *J* = 2.7, 1.0 Hz, H-4), 3.82 (s, 3H, CH_3 PMB), 3.80 – 3.76 (m, 6H, 2x CH_3 PMB), 3.60 – 3.41 (m, 3H, H-2, H-2, H-3, C*H*H-6), 3.31 (t, 1H, *J* = 6.2 Hz, H-5), 3.17 – 3.06 (m, 2H, H-1, C*H*H-6), 2.34 (t, 2H, *J* = 7.2 Hz, CH_2-9), 1.93 – 1.81 (m, 2H, C*H*H-8, C*H*H-7), 1.74 – 1.62 (m, 1H, C*H*H-8), 1.52 – 1.42 (m, 1H, C*H*H-7); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 179.2 (C=O), 159.4, 159.3, 159.1, 144.1, 131.0, 130.8, 130.7 (C_q Ar), 130.0, 129.8, 129.3, 128.8,

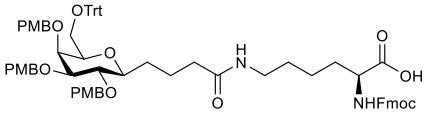
128.0, 127.1, 113.9, 113.6 (Ar), 86.9 (C_q Trt), 84.7 (C-3), 79.4 (C-1), 78.7 (C-2), 77.6 (C-5), 75.2, 73.9 (CH_2 PMB), 73.9 (C-4), 72.1 (CH_2 PMB), 63.3 (CH_2-6), 55.4, 55.4, 55.4 (CH_3 PMB), 33.9 (CH_2-9), 31.0 (CH_2-7), 21.1 (CH_2-8); FT-IR (neat, cm^{-1}): 2935, 1707, 1612, 1586, 1513, 1449, 1302, 1247, 1173, 1075, 1033, 821, 748, 706, 633; HRMS: $[M+Na]^+$ calcd. for $C_{53}H_{56}O_{10}Na$: 875.37657, found 875.37650.

N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-amide]-L-lysine-methyl ester (21β)



Compound **20β** (3.4 g, 4.0 mmol, 1.0 eq.) and Fmoc-L-lysine-OMe[21] (2.0 g, 4.8 mmol, 1.2 eq.) were dissolved in DMF (20 mL). HCTU (2.0 g, 4.8 mmol, 1.2 eq.) and DIPEA (2.1 mL, 12 mmol, 3.0 eq.) were added and the solution was stirred for 2 hours. The reaction mixture was diluted with EtOAc and was washed with 1 M HCl (1x), sat. aq. $NaHCO_3$ (1x), brine (1x). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (30→80% EtOAc in pentane) gave compound **21β** (4.5 g, 3.7 mmol, 93%) as an oil. R_f: 0.53 (4/6 pentane/EtOAc); $[\alpha]_D^{25}$ +8.7° (*c* = 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.77 – 7.72 (m, 2H, Ar), 7.61 (dd, 2H, *J* = 7.6, 3.1 Hz, Ar), 7.42 – 7.36 (m, 8H, Ar), 7.34 – 7.17 (m, 15H, Ar), 7.05 – 7.00 (m, 2H, Ar), 6.90 – 6.81 (m, 4H, Ar), 6.74 – 6.69 (m, 2H, Ar), 5.49 (t, 1H, *J* = 5.8 Hz, NH), 5.40 (d, 1H, *J* = 8.2 Hz, N*H*Fmoc), 4.82 (d, 1H, *J* = 10.4 Hz, C*H*H PMB), 4.72 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.67 – 4.58 (m, 2H, CH_2 PMB), 4.52 (d, 1H, *J* = 10.5 Hz, C*H*H PMB), 4.45 – 4.33 (m, 3H, C*H*H PMB, CH_2 Fmoc), 4.32 – 4.25 (m, 1H, CH L-Lys), 4.22 (t, 1H, *J* = 7.0 Hz, CH Fmoc), 3.86 – 3.82 (m, 1H, H-4), 3.82 – 3.75 (m, 9H, 3x CH_3 PMB), 3.73 (s, 3H, OCH_3), 3.59 – 3.40 (m, 3H, H-2, H-3, C*H*H-6), 3.35 (t, 1H, *J* = 6.0 Hz, H-5), 3.19 – 2.93 (m, 4H, H-1, C*H*H-6, CH_2 ε-L-Lys), 2.26 – 2.08 (m, 2H, CH_2-9), 1.91 – 1.69 (m, 4H, CH_2-7, CH_2-8), 1.61 – 1.40 (m, 2H, 1x CH_2 β/γ/δ-L-Lys), 1.30 – 1.15 (m, 4H, 2x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 173.3, 159.4 (C=O), 159.1, 156.2, 144.0, 143.9, 141.4, 130.9, 130.7, 130.7 (C_q Ar), 129.9, 129.8, 129.3, 128.8, 128.0, 127.8, 127.2, 127.2, 125.2, 120.1, 113.9, 113.9, 113.6 (Ar), 86.9 (C_q Trt), 84.7 (C-3), 79.7 (C-1), 78.8 (C-2), 77.7 (C-5), 75.2 (CH_2 PMB), 73.9 (C-4), 73.9, 72.1 (CH_2 PMB), 67.1 (CH_2 Fmoc), 63.7 (CH_2-6), 55.4, 55.4 , 55.4 (CH_3 PMB), 53.8 (CH L-Lys), 52.5 (OCH_3), 47.3 (CH Fmoc), 38.9 (CH_2 ε-L-Lys), 36.6 (CH_2-9), 32.1 (CH_2 β/γ/δ-L-Lys), 30.8 (CH_2-7), 29.1, 22.6 (CH_2 β/γ/δ-L-Lys), 22.5 (CH_2-8); FT-IR (neat, cm^{-1}): 3330, 2936, 1722, 1652, 1612, 1586, 1513, 1449, 1302, 1247, 1174, 1076, 1033, 900, 822, 761, 740, 706, 651, 633, 558; HRMS: $[M+H]^+$ calcd. for $C_{75}H_{81}O_{13}N_2$: 1217.57332, found 1217.57314.

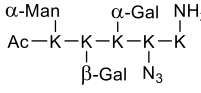
N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-amide]-L-lysine (3)



Compound **21β** (4.0 g, 3.3 mmol, 1.0 eq.) was dissolved in THF (37 mL) and cooled to 0°C. A solution of LiOH in H_2O (0.30 M, 22 mL, 6.6 mmol, 2.0 eq.) was added and the suspension was stirred vigorously for 75 minutes, after which the obtained solution was acidified by the addition of 1 M HCl to pH = 5-6 and diluted with brine. The mixture was extracted

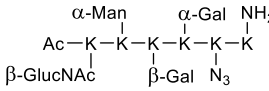
with EtOAc (2x) and the organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. After purification by column chromatography (2→8% MeOH in DCM), the title compound (1.9 g, 1.58 mmol, 48%) was obtained as a white foam. R_f: 0.64 (9/1 DCM/MeOH); $[\alpha]_D^{25}$ +35.6° (*c* = 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.74 (d, 2H, *J* = 7.6 Hz, Ar), 7.60 (t, 2H, *J* = 7.2 Hz, Ar), 7.44 – 7.33 (m, 8H, Ar), 7.33 – 7.18 (m, 15H, Ar), 7.05 – 6.99 (m, 2H, Ar), 6.93 – 6.87 (m, 2H, Ar), 6.87 – 6.81 (m, 2H, Ar), 6.76 – 6.69 (m, 2H, Ar), 5.78 – 5.67 (m, 2H, NH, N*H*Fmoc), 4.84 (d, 1H, *J* = 10.5 Hz, C*H*H PMB), 4.74 (d, 1H, *J* = 11.2 Hz, C*H*H PMB), 4.65 (s, 2H, CH_2 PMB), 4.55 (d, 1H, *J* = 10.5 Hz, C*H*H PMB), 4.44 (d, 1H, *J* = 11.3 Hz, C*H*H PMB), 4.36 (d, 2H, *J* = 7.2 Hz, CH_2 Fmoc), 4.33 – 4.25 (m, 1H, CH L-Lys), 4.20 (t, 1H, *J* = 7.1 Hz, CH Fmoc), 3.84 (d, 1H, *J* = 2.8 Hz, H-4), 3.83 – 3.73 (m, 9H, 3x CH_3 PMB), 3.63 (t, 1H, *J* = 9.3 Hz, H-2), 3.50 (dd, 1H, *J* = 9.3, 2.8 Hz, H-3), 3.43 (dd, 1H, *J* = 9.6, 6.4 Hz, C*H*H-6), 3.32 (t, 1H, *J* = 6.2 Hz, H-5), 3.19 – 3.10 (m, 2H, H-1, C*H*H ε-L-Lys), 3.10 – 2.95 (m, 2H, C*H*H-6, C*H*H ε-L-Lys), 2.30 – 2.11 (m, 2H, CH_2-9), 1.92 – 1.37 (m, 6H, CH_2-7, CH_2-8, 1x CH_2 β/γ/δ-L-Lys), 1.36 – 1.09 (m, 4H, 2x CH_2 β/γ/δ-L-Lys); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ 174.0, 159.3 (C=O), 159.3, 159.2, 156.1, 144.1, 143.9, 141.4, 130.6, 130.4 (C_q Ar), 130.0, 129.3, 128.7, 128.0, 127.8, 127.2, 127.2, 125.3, 120.0, 113.9, 113.9, 113.6 (Ar), 87.0 (C_q Trt), 84.5 (C-3), 80.2 (C-1), 78.5 (C-2), 77.7 (C-5), 75.2 (CH_2 PMB), 74.0 (C-4), 73.9, 72.2 (CH_2 PMB), 67.0 (CH_2 Fmoc), 63.6 (CH_2-6), 55.4, 55.3 (CH_3 PMB), 53.6 (CH L-Lys), 47.2 (CH Fmoc), 38.9 (CH_2 ε-L-Lys), 36.4 (CH_2-9), 31.7 (CH_2 β/γ/δ-L-Lys), 30.3 (CH_2-7), 28.8, 22.9 (CH_2 β/γ/δ-L-Lys), 21.8 (CH_2-8); FT-IR (neat, cm^{-1}): 2935, 1717, 1612, 1586, 1512, 1449, 1302, 1246, 1173, 1153, 1074, 1032, 900, 821, 760, 735, 704, 651, 633, 621, 541, 516; LC-MS: Rt = 7.96 min (Vydac 219TP 5 µm Diphenyl, 50 - 90% MeCN, 21 min run); ESI-MS: m/z 1225.6 $[M+Na]^+$; HRMS: $[M+H]^+$ calcd. for $C_{74}H_{79}O_{13}N_2$: 1203.55767, found 1203.55765.

Acetyl-Lys(*N*ε-[butan-4-(α-D-mannosyl)-amide])-Lys(*N*ε-[butan-4-(β-D-galactosyl)-amide])-Lys(*N*ε-[butan-4-(α-D-galactosyl)-amide])-Lys(N_3)-Lys-NH_2 (22)



Tentagel S Ram resin on 100 µmol scale was treated with 20% piperidine in DMF for 10 min and subsequently elongated with Fmoc-Lys(Boc)-OH, Fmoc-Lys(N_3)-OH, **4**, **3** and **1** using 2.0 equivalents of each amino acid and two hours coupling time at 50°C. After deprotection of the Fmoc, 50 µmol of the resin was capped by treatment with a mixture of 20% Ac_2O in 0.1 M DIPEA in DMF. The resin was washed with DCM and treated with the standard cleavage cocktail (TFA/TIS/H_2O, 95/2.5/2.5 v/v/v, 2.0 mL) for three hours. The suspension was filtered and the product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, conjugate **22** (3.4 mg, 2.4 µmol, 5%) was obtained as a white solid. LC-MS: Rt = 6.88 min (C18 Gemini, 5 - 20% MeCN, 15 min run); ESI-MS: m/z 1422.8 $[M+H]^+$; HRMS: $[M+H]^{2+}$ calcd. for $C_{62}H_{113}N_{13}O_{24}$: 711.90052, found 711.89978.

Acetyl-Lys(*N*ε-[butan-4-(2-deoxy-2-*N*-acetyl-β-D-glucosyl)-amide])-Lys(*N*ε-[butan-4-(α-D-mannosyl)-amide])-Lys(*N*ε-[butan-4-(β-D-galactosyl)-amide])-Lys(*N*ε-[butan-4-(α-D-galactosyl)-amide])-Lys(N_3)-Lys-NH_2 (23)



The previously obtained 50 µmol pentamer on resin (see **22**) was treated with a mixture of **2** (2.0 eq.), HCTU (2.0 eq.), DIPEA (4.0 eq.) in DMSO (2.0 mL) at 50 °C for two hours. LC-

MS analysis showed the presence of pentamer. Therefore the resin was treated with the same mixture at room temperature overnight. After deprotection of the Fmoc, the resin was capped by treatment with a mixture of 20% Ac_2O in 0.1 M DIPEA in DMF. The resin was washed with DCM and treated with the standard cleavage cocktail (TFA/TIS/H_2O, 95/2.5/2.5 v/v/v, 2.0 mL) for three hours. The suspension was filtered and the product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, conjugate **23** (1.8 mg, 1.0 µmol, 2%) was obtained as a white solid. LC-MS: Rt = 7.33 min (C18 Gemini, 5 - 20% MeCN, 15 min run); ESI-MS: m/z 1824.0 $[M+H]^+$; HRMS: $[M+H]^{2+}$ calcd. for $C_{80}H_{144}N_{16}O_{31}$: 912.50862, found 912.50813.

Footnotes and References

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(26) Treatment of this mixture with *N*-bromosuccinimide in THF did not result in selective cyclization as was shown in Chapter 4 and 5 for respectively mannose and rhamnose.
(27) The low yields (48% and 46%) can be explained by partly removal of the Fmoc during the reaction and the trityl during the work-up.
(28) See compound **9a** of Chapter 3.