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Glycopeptides

Synthesis of *C*-Glycosyl Amino Acid Building Blocks Suitable for the Solid-Phase Synthesis of Multivalent Glycopeptide Mimics

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Abstract: Five *C*-glycosyl functionalized lysine building blocks, featuring *C*-glycosidic derivatives of α-rhamnose, α-mannose, α-galactose, β-galactose, and β-*N*-acetyl glucosamine have been designed and synthesized. These derivatives, equipped with acid-labile protecting groups, are eminently suitable for solid-phase synthesis of multivalent glycopeptides. The lysine building blocks were prepared from *C*-allyl glycosides that underwent a Grubbs cross-metathesis with an acrylate, followed by a reduction of the C=C double bond in the resulting α,β-unsaturated esters, and liberation of the carboxylate to allow condensation with a lysine side chain. The thus obtained *C*-glycosides, five in total, were applied in the solid-phase peptide synthesis (SPPS) of three glycopeptides, showing the potential of the described building blocks in the assembly of well-defined mimics of homo- and heteromultivalent glycopeptides and glycoclusters.

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 function as pattern recognition receptors. In innate immunity, they promote the secretion of cytokines and in adaptive immunity, they contribute to endocytosis.[1,2] Examples of these receptors are DC-SIGN and the mannose receptor, both *C*-type lectins that are present on dendritic cells. Since the binding interactions between lectins and their carbohydrate-binding partners are often relatively weak, strong interactions depend on the multivalent binding. Therefore, many multivalent carbohydrate structures such as polymers, glycoconjugates, and dendrimers have been designed, synthesized, and evaluated for the development of 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 as well as in cancer immunotherapeutic strategies.[3–6] Such multivalent conjugates can not only be tailored to effectively mimic complex glycan structures,[7–9] but also their physical properties can be changed and tuned.[4,10,11] Another example is the exploitation of the abundance of the circulating anti-L-rhamnose antibodies in human blood,[12,13] that can be recruited using multivalent rhamnose conjugates.[14–18]

We reasoned that the development of automated solid phase techniques to generate clusters of (different) carbohydrates would be attractive to rapidly assemble multivalent glycoconjugates.[6] Ponader et al. developed a solid-phase synthesis method to obtain homo- and hetero-multivalent glycooligomers using alkyne-functionalized building blocks functionalized by a Cu-catalyzed azide-alkyne [2+3] cycloaddition with mannose, galactose or glucose synthons equipped with an azide.[19] This approach, however, cannot be used to install different glycan entities and requires "post-assembly" synthetic steps making the approach overall more elaborate. In addition, *O*-glycosides[20] are generated that can be degraded enzymatically. To prevent hydrolysis of the glycosidic linkage, *C*-glycosides[21,22] have been developed and incorporated into *C*-glycosyl amino acid building blocks[6,23–26] allowing the online solid-phase peptide synthesis (SPPS) of glycopeptides.

Here, we report on the development of a methodology for the generation of a library of *C*-glycoside functionalized lysine SPPS building blocks, including α-rhamnose **1**, α-mannose **2**, α-galactose **3**, β-galactose **4**, and β-*N*-acetylglucosamine **5** functionalized lysine synthons, as depicted in Figure 1A. These building blocks have been designed to be suitable for contemporary Fmoc-based SPPS chemistry and can be used for the synthesis of homo- and heteromultivalent glycomimetics. The synthesis of building blocks **1–5** hinges on the introduction of

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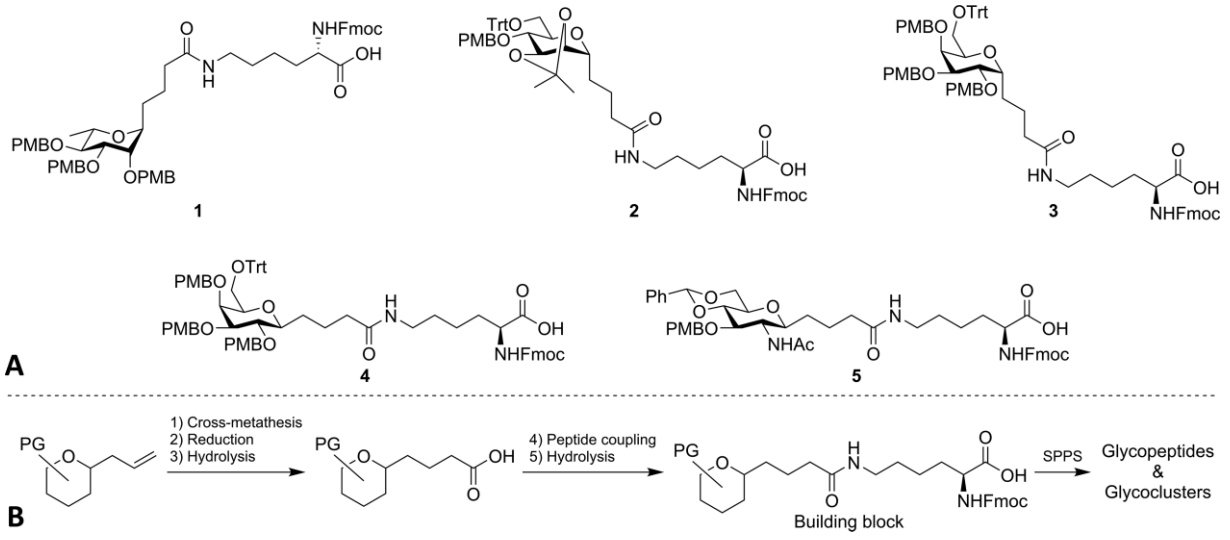


Figure 1. A) Structures of the *C*-glycoside SPPS building blocks **1–5**; **B)** synthesis of the *C*-glycosidic SPPS building blocks; PG: protecting group.

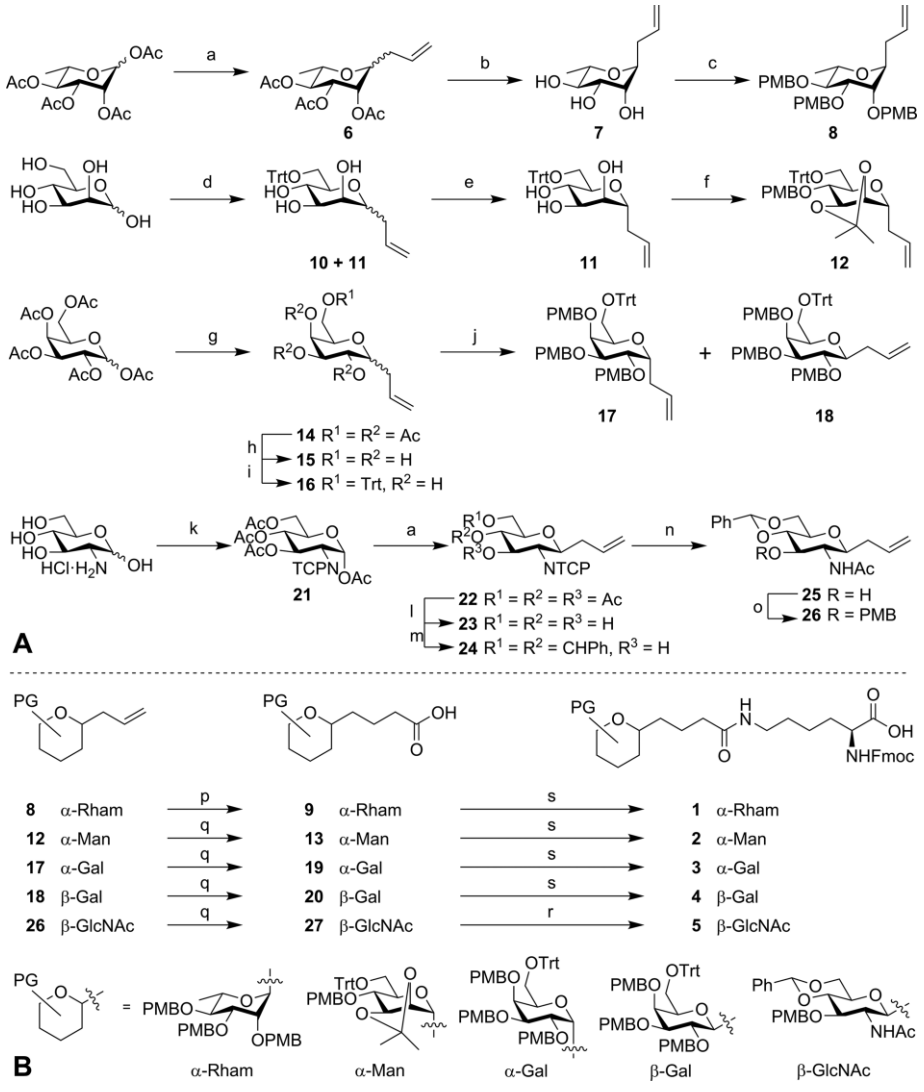
an anomeric *C*-allyl group,[27] cross-metathesis to install a carboxylic acid functionality and condensation with a suitably protected lysine (See Figure 1B). The monosaccharides are protected with acid-labile trityl, *p*-methoxybenzyl, isopropylidene, and/or benzylidene groups to allow a one-step protocol in the final stage of the SPPS that simultaneously removes all protecting groups and releases the glycopeptides or glycoclusters from the resin.

Results and Discussion

The synthesis of the target *C*-glycosyl lysine building blocks is depicted in Scheme 1. The preparation of *C*-rhamnose **6** started with the treatment of peracetylated rhamnopyranose with allyltrimethylsilane under the agency of $BF_2OTf{\cdot}OEt_2$ as Lewis acid[28] generated in situ from $BF_3{\cdot}OEt_2$ and TMSOTf, which afforded the allyl rhamnoside **6** as an inseparable 5:1 α/β-mixture. This mixture was resolved in the following manner. Following sodium methoxide-mediated deacetylation, treatment of the mixture of rhamnosyl-*C*-glycosides with *N*-bromosuccinimide led to the corresponding mixture of bromonium ions, as shown in Figure 2. The nucleophilic attack of the *C*-2-OH in the β-rhamnoside on the formed bromonium ion occurred rapidly. The reaction of the *C*-2-OH in the α-rhamnose, in contrast, is hampered, because of the requirement to adopt an energetically unfavorable $^4C_1$ conformation and the formation of the *trans*-fused 5,6-bicyclic ring system (see Figure 2). The cyclized β-product and unreacted α-rhamnose could be readily separated via column chromatography, giving pure α-compound **7** in 86 % over three steps.[29] Next, triol **7** was alkylated with *p*-methoxybenzyl chloride in the presence of sodium hydride to provide the fully protected *C*-rhamnoside **8**.

Installation of the required carboxylic acid functionality was achieved by a cross-metathesis with benzyl acrylate under the influence of Grubbs 2nd generation catalyst to give the α,β-unsaturated ester in excellent yield (Scheme 1B). The double bond was chemoselectively reduced with $NaBH_4$ and ruthenium trichloride.[30,31] Saponification of the ester then set the stage for the condensation with Fmoc-L-lysine-OMe. Under the influence of HCTU and DIPEA, *C*-rhamnose **9** and the lysine building block were condensed in 80 % yield. Subsequent hydrolysis of the methyl ester with LiOH at 0 °C gave Fmoc-protected *C*-rhamnose-functionalized lysine building block **1** in 25 % yield over 9 steps.

The first step in the optimized synthesis route[6] towards mannose building block **2** comprised the *C*-allylation at the anomeric position of mannose. Girard et al. achieved a stereoselective allylation using a Sakurai-type reaction on per-benzylated methyl α-D-mannopyranoside.[32] However, debenzylation and purification proved to be problematic when performed on a larger scale. Therefore, an alternative procedure was followed in which per-acetyl-D-mannose was treated with a mixture of allyltrimethylsilane, $BF_3{\cdot}OEt_2$, and TMSOTf in MeCN to give the desired allyl mannoside as a 4.2:1 α/β mixture (Scheme 1A). Known methods[33,34] to separate the α/β mixture of *C*-allyl mannose could not be reproduced on a large scale, and therefore a procedure, analogous to the one described above for the rhamnose synthon, was used. The primary alcohol in the crude α/β-*C*-allyl mannose was protected with a trityl, to produce a mixture of **10** and **11** (55 % yield over four steps). Next, the two anomers were treated with *N*-bromosuccinimide in THF. This led to the selective formation of the cyclic β-anomer that could be separated from α-*C*-allyl mannose **11**, which was isolated in 91 % yield. Based on our previous synthesis of a mannose-6-*C*-phosphonate building block[35] the *C*-2-OH and *C*-3-OH were masked with an isopropylidene ketal, and, next, by the installation of a *p*-methoxybenzyl at the *C*-4-OH to give fully protected **12**. This compound was transformed into the required *C*-mannosyl lysine building block following the sequence of reactions performed for the rhamnose building block. Thus, a cross-metathesis of **12** with methyl acrylate was followed by a reduction of the obtained α,β-unsaturated ester with $NaBH_4$, and ruthenium trichloride to give the desired intermediate in 73 % yield over the two steps. Saponification of the methyl ester us-



Scheme 1. A) Synthesis of *C*-allyl building blocks. *Reagents and conditions*: a) allyltrimethylsilane, $BF_3{\cdot}OEt_2$, TMSOTf, MeCN, **6**: 86 %, **22**: 58 %; b) *i*. NaOMe, MeOH; *ii*. *N*-bromosuccinimide, THF, 3 h, then $Na_2S_2O_3$, 79 % over two steps; c) *p*-methoxybenzyl chloride, NaH, TBAI, DMF, 80 %; d) *i*. Ac_2O, pyridine, *ii*. allyltrimethylsilane, $BF_3{\cdot}OEt_2$, TMSOTf, MeCN; *iii*. NaOMe, MeOH; *iv*. TrtCl, Et_3N, DMF, 60 °C, 55 % over four steps; e) *N*-bromosuccinimide, THF, 3 h, 91 %; f) *i*. *p*-toluenesulfonic acid, 2,2-dimethoxypropane, 93 %; *ii*. *p*-methoxybenzyl chloride, NaH, DMF, 97 %; 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, **17**: 52 %, **18**: 28 %; k) *i*. tetrachlorophthalic anhydride, NaOMe, Et_3N, MeOH, 50 °C; *ii*. Ac_2O, pyridine, 51 % over two steps; l) AcCl, MeOH, 94 %; m) benzaldehyde dimethylacetal, *p*-toluenesulfonic acid, DMF/MeCN, 60 °C, 87 %; n) *i*. ethylene diamine, EtOH, 90 °C; *ii*. Ac_2O, $NaHCO_3$, THF/H_2O, 83 % over two steps; o) *p*-methoxybenzyl-2,2,2-trichloroacetimidate, TfOH, THF, 78 %; **B)** synthesis of *C*-glycoside-functionalized lysines **1–5**. *Reagents and conditions*: p) *i*. benzyl acrylate, Grubbs 2nd gen. catalyst, DCM, 50 °C; *ii*. $NaBH_4$, $RuCl_3$, MeOH, DCE, 40 °C; *iii*. LiOH, THF/MeOH/H_2O, 40 °C, **9**: 89 over three steps; q) *i*. methyl acrylate, CuI, Grubbs 2nd gen. catalyst, DCE, 50 °C; *ii*. $NaBH_4$, $RuCl_3$, MeOH, DCE, 45 °C; *iii*. LiOH, THF/H_2O/MeOH or THF/H_2O, 40 °C, **13**: 70 %, **19**: 67 %, **20**: 65 %, **27**: 68 % over three steps; r) *i*. Fmoc-L-Lys-OMe, HCTU, DIPEA, DMF; *ii*. LiOH, THF/H_2O, 0 °C, 71 %, **1**: 57 %, **2**: 54 %, **3**: 41 %, **4**: 45 % over two steps; s) *i*. Fmoc-L-Lys-OMe, HCTU, DIPEA, DMF; *ii*. LiOH, THF/H_2O, then 1 M HCl, then $NaHCO_3$, Fmoc *N*-hydroxysuccinimide ester, **5**: 91 % over two steps.

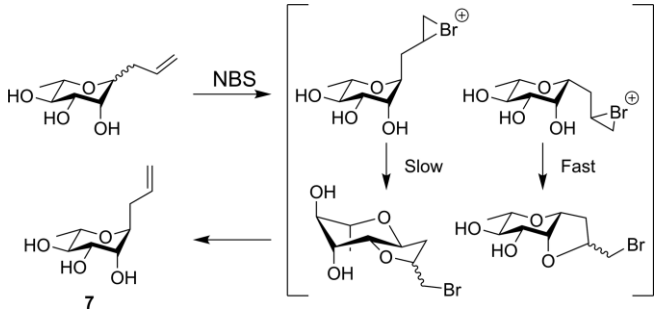


Figure 2. Cyclization of *C*-rhamnose to obtain pure **7**.

ing LiOH yielded acid **13**, which was condensed with Fmoc-L-lysine-OMe under the influence of HCTU and DIPEA. The obtained methyl ester was then treated with LiOH at 0 °C to obtain to SPPS building block **2** in 17 % yield over 12 steps.

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. Performing this reaction in MeCN gave **14** as a 6:1 α/β mixture (98 % yield), while in nitromethane a 2:1 α/β mixture (89 % yield) was obtained. Of note, these reactions show, in line with literature precedents,[36,37] that neighbouring group participation of the *C*-2-*O*-acetate is not a decisive factor in determining the stereochemi-

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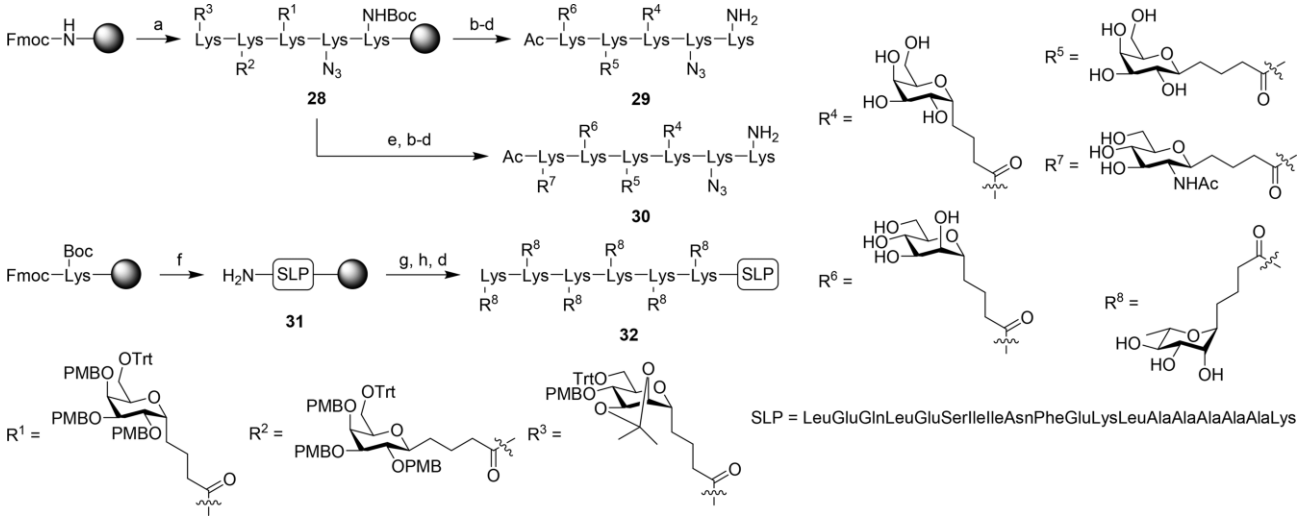
cal outcome of these reactions. The preference for the formation of the α-product can be accounted for by the reactivity of the galactopyranosyl oxocarbenium ion.[38] Deacetylation of **15** with sodium methoxide and subsequent tritylation of the primary alcohol with TrtCl and Et_3N, produced compound **16** as an inseparable α/β mixture. In this case, treatment with NBS did not result in a selective cyclization as both the α- and the β-compound underwent the cyclization at the same rate. Fortunately, after alkylation with *p*-methoxybenzyl chloride, the anomers could be separated by silica gel column chromatography yielding α-anomer **17** and β-anomer **18** in diastereomerically pure forms. Both anomers were subjected to the previously described cross-metathesis-reduction-saponification reaction sequence to furnish acids **19** and **20** in good overall yield. The acids were condensed with Fmoc-L-lysine-OMe in the presence of HCTU and DIPEA, followed again by carefully hydrolysis with LiOH at 0 °C, providing galactose SPPS building blocks **3** and **4** in respectively 9 % and 6 % yield over 9 steps.

The last *C*-glycosyl lysine building block to be synthesized comprised glucosamine synthon **5**. En route to this building block, a TCP protecting group was installed on glucosamine, which was followed by acetylation to give donor **21**. 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.[39] To shorten the synthesis of **22**, donor **21** was used directly for the *C*-glycosylation. After substantial optimization of the reaction conditions, it was found that sonication of **21** with allyltrimethylsilane (5.0 equiv.), and $BF_3{\cdot}OEt_2$ (5.0 equiv.) and TMSOTf (1.0 equiv.) delivered the *C*-glycoside **22** in 58 % yield on a 40 mmol scale. Deacetylation with in situ generated HCl (0.8 equiv.) gave triol **23** in 94 %. The use of more HCl or the use of sodium methoxide resulted in lower yields as a result of the ring-opening of the TCP protecting group. Subsequent installation of the benzylidene protecting group gave alcohol **24** in 87 %. Removal of the TCP protecting group with ethylene diamine followed by selective *N*-acetylation gave **25** in 83 % yield. Alkylation of the *C*-3-OH was accomplished by treatment of **25** with *p*-methoxybenzyl-2,2,2-trichloroacetimidate and a catalytic amount of TfOH giving **26** in 78 % yield. Subsequently, cross-metathesis with methyl acrylate, reduction of the resulting double bond, and hydrolysis of the obtained methyl ester led to acid **27** in a 68 % yield over three steps. After coupling with Fmoc-L-lysine-OMe, the obtained methyl ester was isolated by crystallization. Selective hydrolysis of the methyl ester in the fully protected amino acid *C*-glycoside proved challenging due to poor solubility. 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 $NaHCO_3$ and Fmoc-*N*-hydroxysuccinimide ester to reinstall the Fmoc-protecting group. Subsequent precipitation with Et_2O and recrystallization from MeOH/DCM/Et_2O gave SPPS building block **5** in 91 % yield, completing the set of target compounds in 8 % yield over 13 steps.

With the target *C*-glycosyl building blocks in hand, we set out to probe their efficacy in SPPS. To this end, the four *C*-glycoside SPPS building blocks (α-Man **2**, α-Gal **3**, β-Gal **4**, and β-GlcNAc **5**) were used in the synthesis of glycopeptides **29** and **30** (Scheme 2), which also featured a 6-azido lysine, to illustrate the compatibility with these two functionalities. One of the major advantages of the use of the building blocks designed here for the assembly of glycopeptides is the fact that one does not have to rely on post-assembly azide-alkyne click-chemistry, allowing the use of these functionalities for other conjugation purposes. Thus, using standard Fmoc-SPPS protocols, immobilized peptide **28** was generated carrying an α-Gal, β-Gal, and α-Man appendage. Cleavage of the glycopeptide from the resin was achieved by treatment with a cocktail of TFA/TIS/H_2O (95:2.5:2.5 v/v/v) for three hours. Triisopropylsilane (TIS) and



Scheme 2. SPPS synthesis of glycopeptides **29**, **30**, and **32**. *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), 3 h; d) RP-HPLC; e) *i*. **5**, HCTU, DIPEA, DMSO, 50 °C, 2 h; *ii*. **5**, HCTU, DIPEA, DMSO, overnight; *iii*. 20 % piperidine, DMF f) *i*. Fmoc SPPS cycle for LEQLESIINFEKLAAAAA; *ii*. 20 % piperidine, NMP; g) *i*. **1**, PyBOP, NMM, NMP; *ii*. 20 % piperidine, NMP; *iii*. repeat of conditions *i*. and *ii*. five more times; h) TFA/TIS/H_2O (93:2:5 v/v/v). Yield glycopeptides: **29**) 3.4 mg, 5 %; **30**) 1.8 mg, 2 %; **32**) 2.6 mg, 6 %.

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H_2O effectively scavenged the cations released upon acidic deprotection of the glycosyl lysine moieties. After precipitation of the peptide from Et_2O, it was purified by RP-HPLC to give **29** (3.4 mg) in 5 % yield. Elongation of immobilized peptide **28** with building block **5** proved challenging due to the poor solubility of the building block. The use of a condensation cocktail of **5**, HCTU, and DIPEA in DMSO proved effective and after a double treatment of **28** and ensuing cleavage and RP-HPLC purification target peptide **30** was obtained in 2 % yield. To illustrate the use of the *C*-rhamnosyl lysine building block in a relevant larger oligopeptide, we functionalized the ovalbumin derived peptide LEQLESIINFEKLAAAAAK, harboring the MHC–I epitope SIINFEKL, which can be used as a model antigen with six *C*-rhamnoses. After an automated synthesis of immobilized peptide **31**, it was elongated at the *N*-terminus with six rhamnose-functionalized lysines to obtain the protected resin-bound conjugate. After TFA/TIS/H_2O-mediated cleavage and RP-HPLC purification, conjugate **32** (2.6 mg) was obtained in a 6 % yield, showing the applicability of the *C*-rhamnosyl building block in the synthesis of the more complex peptides and the compatibility of the protecting group strategy with common Fmoc-SPPS for the generation of *C*-glycosylated peptides.

Conclusion

With the increasing interest in glycosylated polymers, dendrimers, and peptides, more effective tools are required for their assembly. In line with this, we have here developed glycosylated lysine building blocks that can be used in (automated) solid-phase peptide syntheses. Five *C*-glycosyl lysine SPPS building blocks, featuring α-rhamnose, α-mannose, α-galactose, β-galactose, and β-*N*-acetyl glucosamine moiety have been synthesized and their application in SPPS is described. The building blocks were equipped with solely acid-labile protecting groups to allow deprotection of the moieties, concomitantly with the release of the peptides from the resin, increasing assembly efficiency. Key steps in the synthesis of the glycosyl amino acids are the installation of a *C*-allyl functionality on the carbohydrates, the ensuing Grubbs cross-metathesis with an acrylate synthon, and the chemoselective reduction of the C=C double bond in the α,β-unsaturated ester. The *C*-allylation reactions proceeded to provide mixtures of α- and β-anomers. The undesired β-anomers of the *C*-allyl rhamnoside and *C*-allyl mannoside could be separated from the target α-anomers using a selective cyclization reaction of the β-anomer using *N*-bromosuccinimide. The application of the *C*-glycosyl lysines was investigated by the synthesis of a model peptide in which the building blocks were combined with an azido lysine. Although the solubility of the GlcNAc-amino acid initially posed a challenge, conditions have been found to effectively use the building blocks in an SPPS assembly. The *C*-rhamnose building block was used in the assembly of a larger antigenic peptide, containing the MHC–I epitope, SIINFEKL, to deliver a conjugate that can be used as a model antigen, functionalized with an antibody targeting rhamnose cluster. 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 described building blocks will enable the rapid assembly of libraries of well-defined mimics for homo- and hetero-oligovalent glycopeptides and glycoclusters and the chemistry described can be applied to generate other *C*-glycosyl amino acids, featuring different monosaccharides.

Experimental Section

General 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 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 ca. 150 °C. ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 (400/100/162 MHz) spectrometer or a Bruker AV-500 Ultrashield (500/126/202 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 analyses were done on an Agilent Technologies 1260 Infinity system with a C18 Gemini 3 μm, C18, 110 Å, 50 × 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. Peptides and conjugates were purified with a Gilson GX-281 preparative HPLC with a Gemini-NX 5u, C18, 110 Å, 250 × 10.0 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.

General Procedure Glycopeptides Synthesis: The automated solid-phase peptide synthesis was performed 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: 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 × 4.6 mm column. 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 washed with 0.6 mL of the cleavage cocktail, and the combined TFA solutions were added dropwise to cold Et_2O and stored at −20 °C

overnight. The obtained suspension of the product in Et_2O was centrifuged, Et_2O was removed and the precipitant was dissolved in $CH_3CN/H_2O/t$BuOH (1:1:1 v/v/v). Purification was performed on a Gilson GX-281 preparative RP-HPLC with a Gemini-NX 5u, C18, 110 Å, 250 × 10.0 mm column.

General Procedure Rhamnose-conjugate Synthesis: The synthesis of the peptide components of the constructs was performed as has been described before.[40] In short, the peptides were synthesized using solid-phase peptide synthesis on a Tentagel S Ac resin (Rapp, Tübingen) using a Syro II peptide synthesizer (MultiSyntech, Witten, Germany). Normal couplings (1.5 h – 2 h) were performed using Fmoc amino acids carrying acid labile side chain protection groups (where required). Activation of Fmoc amino acids was performed with PyBop and NMM, azidopropionic acid was coupled using the succinimidyl ester. Fmoc deprotection was performed with 20 vol.-% piperidine in NMP. Washings were performed with NMP. Cleavage from the resin and side chain deprotection was performed with TFA containing 5 vol.-% water and 2 vol.-% triethylsilane. Purification was performed with rpHPLC. Analysis of the purified peptide was performed with UPLC-MS (Acquity, Waters) and showed the expected molecular masses.

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

3-(α-L-Rhamnosyl)-1-propene (7): Compound **6** (36.3 g, 115 mmol, 1.0 equiv., α/β ratio: 5:1) was co-evaporated with toluene (3 ×) under argon atmosphere and dissolved in MeOH (0.58 L). Sodium methoxide (5.4 M in MeOH, 2.2 mL, 12 mmol, 0.1 equiv.) was added and the solution was stirred for 2 hours, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was acidified by the addition of amberlite H^+ resin, filtered and concentrated in vacuo. The obtained residue was co-evaporated with toluene (1 ×) under argon atmosphere and dissolved in THF (1.2 L). *N*-bromosuccinimide (10 g, 55 mmol, 0.48 equiv.) was added and the reaction was stirred for 3 hours, after which the reaction was quenched with an aqueous solution of $Na_2S_2O_3$ (4.4 M, 40 mL). The mixture was further diluted with toluene and concentrated in vacuo. The crude product was embedded on silica and purified by column chromatography (2→8 % MeOH in DCM) yielding compound **7** (14.2 g, 75.4 mmol, 79 %) as a white solid. R_f: 0.24 (9:1 DCM/MeOH); $[\alpha]_D^{20}$ +18.0° (*c* = 1.0, MeOH); ^{1}H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 5.89–5.75 (m, 1H, CH_2-C*H*=CH_2), 5.17–5.03 (m, 2H, CH_2-CH=C*H*$_2$), 3.91–3.82 (m, 1H, H-1), 3.81–3.75 (m, 1H, H-2), 3.65 (dd, 1H, *J* = 8.9, 3.4 Hz, H-3), 3.54–3.45 (m, 1H, H-5), 3.40 (t, 1H, *J* = 8.9 Hz, H-4), 2.54–2.42 (m, 1H, C*H*H-CH=CH_2), 2.38–2.26 (m, 1H, C*H*H-CH=CH_2), 1.24 (d, 3H, *J* = 6.1 Hz, CH_3-6); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ = 135.9 (CH_2-*C*H=CH_2), 117.4 (CH_2-CH=*C*H_2), 78.5 (C-1), 74.3 (C-4), 72.4 (C-3), 72.3 (C-2), 71.0 (C-5), 34.7 (*C*H_2-CH=CH_2), 18.3 (CH_3-6); FT-IR (neat, cm^{-1}): 3371, 2977, 2934, 2361, 1644, 1418, 1253, 1140, 1057, 981, 916, 825, 779, 668, 550; HRMS: $[M + Na]^+$ calcd. for $C_9H_{16}O_4Na$: 211.0946, found 211.0944.

3-(2,3,4-Tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-1-propene (8): Compound **7** (1.92 g, 10.2 mmol, 1.0 equiv.) was co-evaporated with toluene (1 ×) under argon atmosphere and dissolved in DMF (0.10 L). Sodium hydride (60 % dispersion in mineral oil, 1.47 g, 36.5 mmol, 3.6 equiv.) was added at 0 °C. After 20 minutes, *p*-methoxybenzyl chloride (5.0 mL, 37 mmol, 3.6 equiv.) and TBAI (0.38 g, 1.0 mmol, 0.1 equiv.) were added. The reaction was warmed-up to room temperature. After 6 hours, another portion of sodium hydride (60 % dispersion in mineral oil, 0.40 g, 10 mmol, 1.0 equiv.) was added and the reaction was stirred overnight. The reaction was quenched with MeOH at 0 °C, diluted with H_2O and extracted with DCM. The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (10→20 % Et_2O in pentane) gave compound **8** (4.4 g, 8.0 mmol, 79 %) as a white solid. R_f: 0.84 (8:2 pentane/EtOAc); $[\alpha]_D^{20}$ +20.0° (*c* = 2.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.32 (d, 2H, Ar), 7.27 (d, 4H, Ar), 6.93–6.84 (m, 6H, Ar), 5.79–5.66 (m, 1H, CH_2-C*H*=CH_2), 5.08–4.98 (m, 2H, CH_2-CH=C*H*$_2$), 4.78 (d, 1H, *J* = 10.8 Hz, C*H*H PMB), 4.65–4.49 (m, 5H, 2 × CH_2 PMB, C*H*H PMB), 4.05–3.97 (m, 1H, H-1), 3.79 (s, 9H, 3 × CH_3 PMB), 3.73 (dd, 1H, *J* = 7.9, 3.1 Hz, H-3), 3.70–3.65 (m, 1H, H-4), 3.62 (t, 1H, *J* = 3.3 Hz, H-5), 3.60–3.54 (m, 1H, H-2), 2.42–2.32 (m, 1H, C*H*H-CH=CH_2), 2.30–2.20 (m, 1H, C*H*H-CH=CH_2), 1.34 (d, 3H, *J* = 6.3 Hz, H-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ = 159.1 (C_q Ar), 134.2 (CH_2-*C*H=CH_2), 130.5, 130.3, 130.2 (C_q Ar), 129.5, 129.5, 129.3, 128.3 (Ar), 116.9 (CH_2-CH=*C*H_2), 113.6, 113.6, 113.5 (Ar), 79.5 (C-2), 77.3 (C-3), 74.5 (C-5), 74.0 (CH_2 PMB), 72.7 (C-1), 71.4, 71.1 (CH_2 PMB), 69.5 (C-4), 64.4 (CH_2 PMB), 55.0 (CH_3 PMB), 34.2 (*C*H_2-CH=CH_2), 17.9 (C-6); FT-IR (neat, cm^{-1}): 2934, 2836, 2360, 1641, 1612, 1586, 1512, 1464, 1421, 1358, 1302, 1245, 1173, 1079, 1033, 917, 820, 783, 755, 710, 668, 637, 587, 517; HRMS: $[M + Na]^+$ calcd. For $C_{33}H_{40}O_7Na$: 571.2672, found 571.2670.

3-(2,3,4-Tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-butanoic Acid (9): Compound **8** (25.1 g, 45.8 mmol, 1.0 equiv.) and benzyl acrylate (19.3 mL, 128 mmol, 2.8 equiv.) were co-evaporated with toluene (1 ×) under argon atmosphere. The mixture was dissolved in DCM (0.23 L) and the flask was shielded from light with aluminum foil. Grubbs 2nd gen. catalyst (0.78 g, 0.92 mmol, 0.02 equiv.) was added and the reaction was continued to reflux overnight at 50 °C. Upon completion determined by TLC analysis, the reaction mixture was filtered through Celite® and concentrated in vacuo. Purification by column chromatography (10→25 % EtOAc in pentane) gave the alkene intermediate (28.9 g, 42.2 mmol, 92 %) as a white solid. The obtained alkene (15.4 g, 22.6 mmol, 1.0 equiv.) was co-evaporated with toluene (1 ×) under argon atmosphere and dissolved in DCE (90 mL). $RuCl_3$ (0.89 g, 4.2 mmol, 0.19 equiv.) was added and the argon balloon was replaced with an empty balloon. The reaction was cooled to 0 °C, $NaBH_4$ (2.7 g, 72.3 mmol, 3.2 equiv.) was added, after which MeOH (9.15 mL) was carefully added. The mixture was heated to 40 °C for 4.5 hours, subsequently quenched with MeOH at 0 °C. The reaction mixture was diluted with DCM and washed with brine (1 ×). The organic layer was dried with $MgSO_4$, filtered

and concentrated in vacuo. Purification by column chromatography (1→4 % acetone in DCM) yielded the intermediate (13.3 g, 22.6 mmol, 86 %) as a transparent oil. 22.7 g of the intermediate (33.2 mmol, 1.0 equiv.) was dissolved in a mixture of THF/MeOH/H_2O (7:2:1 v/v/v, 0.11 L). The reaction was cooled to 0 °C and LiOH•H_2O (3.5 g, 83 mmol, 2.5 equiv.) was added. The reaction was heated to 40 °C for 4 hours, after which TLC analysis showed full conversion of the starting material. The reaction mixture was acidified with 1 M HCl to pH = 4–5 and extracted with DCM (2 ×). The combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (1→20 % acetone in DCM + 0.1 % AcOH) addorded the title compound (18.3 g, 31 mmol, 96 %) as a yellow oil. R_f: 0.32 (4:1 DCM/acetone); $[\alpha]_D^{20}$ +37.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.30–7.18 (m, 6H, Ar), 6.90–6.81 (m, 6H, Ar), 4.72 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.58–4.48 (m, 5H, 2 × CH_2 PMB, C*H*H PMB), 3.92–3.85 (m, 1H, H-1), 3.80 (s, 9H, 3 × CH_3 PMB), 3.67 (dd, 1H, *J* = 7.7, 3.1 Hz, H-3), 3.63–3.56 (m, 1H, H-5), 3.54–3.48 (m, 2H, H-2, H-4), 2.32 (t, 2H, *J* = 7.1 Hz, CH_2-9), 1.77–1.63 (m, 1H, C*H*H-8), 1.63–1.51 (m, 2H, C*H*H-8, C*H*H-7), 1.47–1.36 (m, 1H, C*H*H-7), 1.30 (d, 3H, *J* = 6.3 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ = 178.9 (C=O), 159.3, 130.7, 130.6, 130.4 (C_q PMB), 129.8, 129.7, 129.5, 113.9, 113.9 (Ar), 79.7 (C-2), 77.9 (C-3), 75.6 (C-4), 74.2 (CH_2 PMB), 73.0 (C-1), 71.8, 71.4 (CH_2 PMB), 69.6 (C-5), 55.4 (CH_3 PMB), 33.5 (CH_2-9), 28.7 (CH_2-7), 21.2 (CH_2-8), 18.1 (CH_3-6); FT-IR (neat, cm^{-1}): 2934, 2836, 1721, 1707, 1611, 1586, 1512, 1463, 1359, 1302, 1245, 1173, 1108, 1077, 1032, 819, 756, 710, 637, 584, 516; HRMS: [M + Na]$^+$ calcd. for $C_{34}H_{42}O_9Na$: 617.2727, found 617.2736.

N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-α-L-rhamnosyl)-amide]-L-lysine (1): Compound **9** (3.0 g, 5.0 mmol, 1.0 equiv.) and Fmoc-L-lysine-OMe (2.4 g, 6.3 mmol, 1.3 equiv.) were co-evaporated with toluene (2 ×) under argon atmosphere. The mixture was dissolved in DMF (25 mL). HCTU (2.49 g, 6.0 mmol, 1.2 equiv.) and DIPEA (2.6 mL, 15 mmol, 3.0 equiv.) were subsequently added at 0 °C. The reaction was warmed-up to room temperature and stirred for 5 hours. The reaction was quenched with H_2O and diluted with EtOAc. The organic layer was subsequently washed with 1 M HCl (2 ×), sat. aq. $NaHCO_3$ (1 ×) and brine (1 ×). The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (10→70 % acetone in DCM) gave the intermediate (3.8 g, 4.0 mmol, 80 %) as a white solid, of which 2.8 g (2.9 mmol, 1.0 equiv.) was dissolved in THF (40 mL) and cooled to 0 °C. An aqueous solution of LiOH (0.30 M, 19 mL, 5.7 mmol, 2.0 equiv.) was slowly added. After 40 minutes, the solution was diluted with EtOAc and acidified with 1 M HCl. The aqueous layer was extracted with EtOAc (2 ×) and the combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (15→25 % acetone in DCM + 0.1 % AcOH) afforded the title compound (1.94 g, 2.06 mmol, 71 %) as a white solid. R_f: 0.26 (4:1 DCM/acetone + 0.1 % AcOH); $[\alpha]_D^{20}$ +39.0° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.76 (d, 2H, *J* = 7.6 Hz, Ar), 7.62 (t, 2H, *J* = 7.3 Hz, Ar), 7.43–7.36 (m, 2H, Ar), 7.35–7.18 (m, 9H, Ar, Ar), 6.91–6.82 (m, 6H, Ar), 5.89 (t, 1H, *J* = 5.8 Hz, NH), 5.78 (d, 1H, *J* = 7.9 Hz, N*H*Fmoc), 4.71 (d, 1H, *J* = 10.9 Hz, C*H*H PMB), 4.61–4.46 (m, 5H, 2 × CH_2 PMB, C*H*H PMB), 4.44–4.34 (m, 3H, CH_2 Fmoc, CH L-Lys), 4.22 (t, 1H, *J* = 7.0 Hz CH Fmoc), 3.96–3.88 (m, 1H, H-1), 3.81 (s, 9H, 3 × CH_3 PMB), 3.73–3.62 (m, 2H, H-3, H-5), 3.59–3.50 (m, 2H, H-2, H-4), 3.31–3.17 (m, 2H, CH_2 ε-L-Lys), 2.16 (t, 2H, CH_2-9), 1.95–1.84 (m, 1H, C*H*H-8), 1.83–1.75 (m, 1H, C*H*H-8), 1.74–1.64 (m, 1H, C*H*H-7), 1.64–1.35 (m, 7H, C*H*H-7, 3 × CH_2 β/γ/δ-L-Lys), 1.32 (d, 3H, *J* = 6.4 Hz, CH_3-6); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ = 174.2, 173.7 (C=O), 159.4, 159.3, 156.2, 144.0, 143.9, 141.4, 130.5, 130.4, 129.8 (C_q Ar), 129.6, 127.8, 127.2, 125.3, 120.1, 113.9 (Ar), 79.3 (C-2), 77.3 (C-3), 75.6 (C-4), 74.0 (CH_2 PMB), 72.7 (C-1), 71.8, 71.5 (CH_2 PMB), 70.0 (C-5), 67.1 (CH_2 Fmoc), 55.4 (CH_3 PMB), 53.6 (CH L-Lys), 47.2 (CH Fmoc), 39.1 (CH_2 ε-L-Lys), 36.1 (CH_2-9), 31.8 (CH_2-7), 29.0, 28.7, 22.3, 22.0 (CH_2-8, CH_2 β/γ/δ-L-Lys), 17.9 (CH_3-6); FT-IR (neat, cm^{-1}): 2930, 1719, 1612, 1512, 1451, 1302, 1247, 1174, 1080, 1034, 821, 741; LC-MS: Rt = 9.03 min (Gemini C_{18}, 10–90 % MeCN, 12.5 min run); ESI-MS (*m*/*z*): [M + Na]$^+$ calcd. for $C_{61}H_{66}N_2O_{11}Na$: 967.4, found 967.4; HRMS: [M + Na]$^+$ calcd. for $C_{55}H_{64}N_2O_{12}Na$: 967.4357, found 967.4385.

3-(6-*O*-Trityl-α/β-D-mannopyranosyl)-1-propene (10 + 11): D-Mannose (52.3 g, 302 mmol, 1.0 equiv.) was dissolved in pyridine (0.43 L) and the reaction mixture was cooled to 0 °C. Acetic anhydride (0.20 L, 2.1 mol, 7.0 equiv.) and DMAP (3.69 g, 30.2 mmol, 0.1 equiv.) were added. After stirring for 25 minutes, the solution was warmed-up to room temperature and stirring was continued overnight. The mixture was subsequently cooled to 0 °C and quenched with MeOH. The solution was diluted with EtOAc and washed with 1 M HCl (5 ×). The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The residue was co-evaporated with toluene (2 ×), which gave acetyl 2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranoside as a clear oil which solidified on bench in quantitative yield (124 g). The intermediate was co-evaporated with toluene (2 ×) and dissolved in MeCN (1.20 L) under an argon atmosphere. After cooling the mixture to 0 °C, allyltrimethylsilane (95 mL, 0.62 mol, 2.0 equiv.), BF_3•OEt_2 (0.19 L, 1.5 mol, 4.9 equiv.) and TMSOTf (11 mL, 62 mmol, 0.2 equiv.) were added, respectively. After stirring for 30 minutes, the reaction mixture was warmed-up to room temperature and stirring continued for 3 days. The reaction mixture was cooled to 0 °C, diluted with EtOAc and quenched with Et_3N to pH 8. The organic layer was washed with sat. aq. $NaHCO_3$ (1 ×), dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (10→60 % Et_2O in pentane) gave a mixture (86.3 g) of 3-(α/β-D-mannopyranosyl)-1-propene and unreacted acetyl 2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranoside. After dissolving the mixture in MeOH (0.60 L), sodium methoxide (5.4 M in MeOH, 22 mL, 0.12 mol, 0.4 equiv.) was added and the solution was stirred for 1.5 hours. TLC analysis showed complete conversion into a lower running spot (R_f = 0.19 (MeOH/DCM: 1:9 v/v) and the reaction was quenched using amberlite H^+ resin to pH 2–3. The reaction mixture was filtered and concentrated in vacuo, which gave a mixture of the fully deacetylated intermediates (47.2 g, max. 231 mmol) as an oil. After co-evaporating with dioxane (1 ×) under an argon atmosphere, the residue was dissolved in DMF (0.77 L). Trityl chloride (100 g, 348 mmol, 1.5 equiv.) and Et_3N (80 mL, 0.57 mol, 2.5 equiv.) were added and the suspension was heated to 60 °C. After stirring for 2.5 h, TLC analysis showed complete conversion of the starting material. The reaction mixture was cooled to room temperature, diluted with H_2O and extracted with EtOAc (2 ×). The combined organic layers were dried with $MgSO_4$, filtered and concentrated in vacuo. After purification by column chromatography (20→40 % EtOAc in pentane) compounds **10** and **11** (74.5 g, 167 mmol, 55 % over four steps) were obtained as a foam with an α/β ratio of 4.2:1. R_f: 0.46 (1:4 pentane/EtOAc); See compound **11** for analysis.

3-(6-*O*-Trityl-α-D-mannopyranosyl)-1-propene (11): A solution of compound **10** and **11** (31.4 g, 70.3 mmol, 1.0 equiv., α/β: 4.2:1) and *N*-bromosuccinimide (6.3 g, 35 mmol, 0.5 equiv.) in THF (0.70 L) was stirred for 2 h. The reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ (0.50 L). After stirring for an additional 10 minutes, the mixture was further diluted with sat. aq. $Na_2S_2O_3$ (0.25 L) and extracted with DCM (1 ×). The organic layer was washed with sat. aq. $NaHCO_3$ (1 ×), dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (10→60 % acetone

in DCM + 0.1 % Et_3N) yielded the title compound (23.1 g, 51.7 mmol, 91 %) as a white foam. R_f: 0.42 (7:3 DCM/acetone); $[\alpha]_D^{25}$ −18.2° (*c* = 0.72, $CHCl_3$); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.54–7.44 (m, 6H, Ar), 7.36–7.29 (m, 6H, Ar), 7.29–7.23 (m, 3H, Ar), 6.04–5.90 (m, 1H, CH_2-C*H*=CH_2), 5.26–5.11 (m, 2H, CH_2-CH=CH_2), 3.88 (ddd, 1H, *J* = 9.5, 5.4, 2.5 Hz, H-1), 3.71–3.64 (m, 2H, H-2, H-5), 3.60 (ddd, 1H, *J* = 9.3, 6.0, 3.5 Hz, H-4), 3.45–3.37 (m, 1H, H-3), 3.25–3.12 (m, 3H, CH_2-6, OH), 3.05 (t, 2H, *J* = 4.6 Hz, 2 × OH), 2.60–2.51 (m, 1H, C*H*H-CH=CH_2), 2.35–2.27 (m, 1H, C*H*H-CH=CH_2); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ = 145.3 (C_q Trt), 136.3 (CH_2-*C*H=CH_2), 129.6, 128.8, 128.0 (Ar), 117.3 (CH_2-CH=*C*H_2), 87.2 (C_q Trt), 77.4 (C-1), 74.4 (C-5), 72.5 (C-4), 71.6 (C-2), 69.7 (C-3), 65.1 (CH_2-6), 34.4 (*C*H_2-CH=CH_2); FT-IR (neat, cm^{-1}): 3402, 3060, 2928, 1708, 1643, 1597, 1490, 1449, 1221, 1073, 1033, 989, 901, 827, 765, 748, 701, 633, 529; HRMS: $[M + Na]^+$ calcd. for $C_{28}H_{30}O_5Na$: 469.1991, found 496.1991.

3-(2,3-*O*-Isopropylidene-4-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-mannopyranosyl)-1-propene (12): Compound **11** (43.9 g, 98.3 mmol, 1.0 equiv.) was dissolved in 2,2-dimethoxypropane (0.50 L) and cooled to 0 °C. *p*-Toluenesulfonic acid (2.88 g, 15.1 mmol, 0.15 equiv.) was added and the reaction mixture was stirred for 10 minutes, after which TLC analysis showed complete conversion of the starting material. The reaction was quenched by the addition of Et_3N (7 mL), diluted with DCM and washed with a mixture of sat. aq. $NAHCO_3$/brine (1:1, v/v, 1 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (10→50 % Et_2O in pentane + 0.1 % Et_3N) gave the intermediate (44.6 g, 89.1 mmol, 91 %) as a clear oil. After co-evaporating with toluene (2 ×) under an argon atmosphere, the intermediate (49.9 g, 102.5 mmol, 1.0 equiv.) was dissolved in DMF (0.50 L) and cooled to 0 °C. Sodium hydride (60 % dispersion in mineral oil, 4.95 g, 123 mmol, 1.2 equiv.) and *p*-methoxybenzyl chloride (17.0 mL, 125 mmol, 1.2 equiv.) were added and the suspension was warmed-up up to room temperature after 20 minutes. After stirring at room temperature for an additional hour, TLC analysis showed complete conversion of the starting material. The reaction was quenched by the addition of MeOH at 0 °C, diluted with Et_2O and washed with H_2O (2 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (5→20 % Et_2O in pentane + 0.1 % Et_3N) yielded the title compound (60.3 g, 99.4 mmol, 97 %) as a clear oil. R_f: 0.63 (pentane/Et_2O); $[\alpha]_D^{25}$ +12.7° (*c* = 0.67, $CHCl_3$); ^{1}H NMR (CD_3CN, 400 MHz, HH-COSY, HSQC): δ 7.49–7.44 (m, 6H, Ar), 7.35–7.24 (m, 9H, Ar), 6.96–6.92 (m, 2H, Ar), 6.80–6.75 (m, 2H, Ar), 6.06–5.93 (m, 1H, CH_2-C*H*=CH_2), 5.24–5.10 (m, 2H, CH_2-CH=CH_2), 4.62 (d, 1H, *J* = 11.0 Hz, C*H*H PMB), 4.31–4.21 (m, 2H, H-3, C*H*H PMB), 4.08 (dd, 1H, *J* = 6.4, 5.4 Hz, H-2), 3.99–3.92 (m, 1H, H-1), 3.75 (s, 3H, CH_3 PMB), 3.70–3.60 (m, 2H, H-4, H-5), 3.31 (dd, 1H, *J* = 9.9, 2.1 Hz, C*H*H-6), 3.08 (dd, 1H, *J* = 9.8, 5.0 Hz, C*H*H-6), 2.42 (t, 2H, *J* = 6.9 Hz, CH_2-CH=CH_2), 1.46 (s, 3H, CH_3 isopropylidene), 1.34 (s, 3H, CH_3 isopropylidene); ^{13}C-APT NMR (CD_3CN, 101 MHz, HSQC): δ = 160.1 (C_q PMB), 145.1 (C_q Trt), 135.9 (CH_2-*C*H=CH_2), 131.3 (C_q PMB), 130.5, 129.6, 128.8, 128.1 (Ar), 117.6 (CH_2-CH=*C*H_2), 114.4 (Ar), 109.9 (C_q isopropylidene), 87.2 (C_q Trt), 79.2 (C-3), 77.2 (C-2), 76.5 (C-4), 73.7 (C-1), 73.2 (CH_2 PMB), 73.0 (C-5), 64.4 (CH_2-6), 55.8 (CH_3 PMB), 37.4 (*C*H_2-CH=CH_2), 28.1, 26.0 (CH_3 isopropylidene); FT-IR (neat, cm^{-1}): 2987, 2934, 1613, 1514, 1491, 1449, 1381, 1302, 1247, 1212, 1172, 1069, 1034, 1002, 915, 868, 822, 765, 747, 704, 633, 518; HRMS: $[M + Na]^+$ calcd. for $C_{39}H_{42}O_6Na$: 629.2879, found 629.2881.

4-(2,3-*O*-Isopropylidene-4-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-mannopyranosyl)-butanoic Acid (13): Compound **12** (5.7 g, 9.4 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) under an argon atmosphere, before being dissolved in dry DCE (0.10 L). Methyl acrylate (2.4 mL, 26 mmol, 2.8 equiv.), CuI (0.28 g, 1.5 mmol, 0.16 equiv.) and Grubbs 2nd generation catalyst (0.32 g, 0.38 mmol, 0.04 equiv.) 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 (3 ×). Purification by column chromatography (10→70 % Et_2O in pentane) afforded the intermediate (4.9 g, 7.4 mmol, 1.0 equiv.), which was co-evaporated with toluene (2 ×) under an argon atmosphere and dissolved in dry DCE (37 mL). Two empty balloons were placed on the flask, followed by the addition of $RuCl_3$ (0.29 g, 1.4 mmol, 0.19 equiv.) and $NaBH_4$ (0.89 g, 24 mmol, 3.2 equiv.) at 0 °C. Methanol (6.0 mL, 0.15 mol, 20 equiv.) was carefully added to the suspension over 20 minutes, after which the mixture was warmed-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 through celite and extracted with DCM (2 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (20→60 % Et_2O in pentane) gave the intermediate (4.6 g, 6.9 mmol, 73 % over two steps), which was dissolved in a mixture of THF/H_2O/MeOH (7:1:2, v/v/v, 35 mL). LiOH (0.87 g, 21 mmol, 3.0 equiv.) 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 (2 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. The title compound was obtained (4.3 g, 6.6 mmol, 96 %). 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 (2): Compound **13** (3.8 g, 5.8 mmol, 1.0 equiv.) and Fmoc-L-lysine-OMe (2.9 g, 7.0 mmol, 1.2 equiv.) were dissolved in DMF (30 mL). HCTU (2.9 g, 7.0 mmol, 1.2 equiv.) and DIPEA (3.0 mL, 17 mmol, 3.0 equiv.) were added and the solution was stirred for 4 hours. The reaction mixture was diluted with EtOAc and washed with 1 M HCl (1 ×), sat. aq. $NaHCO_3$ (1 ×), brine (1 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (2→30 % acetone in DCM) yielded the intermediate (5.6 g, 5.5 mmol, 95 %) of which 3.05 g (3.00 mmol, 1.0 equiv.) was dissolved in THF (30 mL) and cooled to 0 °C. An aqueous solution of LiOH (0.30 M, 20 mL, 6.0 mmol, 2.0 equiv.) 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 (1 ×) and the aqueous layer was extracted with EtOAc (1 ×). The combined or-

ganic layers were dried with 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, 1 × CH_2 β/γ/δ-L-Lys), 1.50–1.30 (m, 10H, 2 × CH_2 β/γ/δ-L-Lys, 2 × 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*): $[M + Na]^+$ calcd. for $C_{61}H_{66}N_2O_{11}Na$: 1025.5, found 1025.4; HRMS: $[M + H]^+$ calcd. for $C_{61}H_{67}O_2N_{11}$: 1003.47394, found 1003.47380.

3-(2,3,4,6-Tetra-*O*-acetyl-α/β-D-galactopyranosyl)-1-propene (14): Acetyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (23.7 g, 60.8 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) under an argon atmosphere and dissolved in CH_3NO_2 (0.24 L). Allyltrimethylsilane (20 mL, 0.13 mol, 2.1 equiv.) was added, followed by the addition of BF_3•OEt_2 (23 mL, 0.18 mol, 3.0 equiv.) 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 (1 ×). The organic layer was dried with 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, 2 × 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 (CH_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 (15): Compound **14** (20.0 g, 53.8 mmol, 1.0 equiv.) was dissolved in MeOH (0.11 L). Sodium methoxide (5.4 M in MeOH, 4.0 mL, 22 mmol, 0.40 equiv.) 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 (CH_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 (16): Trityl chloride (21 g, 75 mmol, 1.3 equiv.) and Et_3N (17 mL, 0.12 mol, 2.5 equiv.) were added to a solution of compound **15** (10.0 g, 48.9 mmol, 1.0 equiv.) in DMF (0.16 L). The mixture was heated to 60 °C overnight. The mixture was diluted with EtOAc and washed with brine (3 ×). The organic layer was dried with 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, 2 × 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 (CH_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 (17): Triol **16** (16.4 g, 36.8 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) 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 equiv.) was added at 0 °C over 30 minutes. After 1 hour, *p*-methoxybenzyl chloride (18 mL, 0.13 mol, 3.5 equiv.) and tetrabutylammonium iodide (1.4 g, 3.8 mmol, 0.10 equiv.) were added. Another portion of sodium hydride (60 % dispersion in mineral oil, 2.3 g, 58 mmol, 1.6 equiv.) was added after 1 hour and the mixture was stirred at room temperature overnight. The reaction was quenched with MeOH at 0 °C, diluted with Et_2O and washed H_2O (3 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (10→30 % Et_2O in pentane) gave compound **17** (15.5 g, 19.2 mmol, 52 %) and compound **18** (8.23 g, 10.3 mmol, 28 %). 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, 2 × CH_2 PMB, 1 × 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, 3 × 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-bbdec NMR (CD_3CN, 126 MHz, HSQC): δ = 160.7, 160.6, 160.5, 145.7 (C_q Ar), 136.8 (CH_2-CH=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 (CH_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.

3-(2,3,4-Tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-1-propene (18): See experimental of compound **17**. Purification gave compound **18** (8.23 g, 10.3 mmol, 28 %). 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, 3 × 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=CH_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 (CH_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.

4-(2,3,4-Tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-butanoic Acid (19): Compound **17** (15.2 g, 18.8 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) under an argon atmosphere before being dissolved in dry DCE (0.19 L). Methyl acrylate (4.8 mL, 53 mmol, 2.8 equiv.), CuI (0.54 g, 2.8 mmol, 0.15 equiv.) and Grubbs 2nd generation catalyst (0.63 g, 0.74 mmol, 0.04 equiv.) 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 (2 ×) 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 $RuCl_3$ (0.74 g, 3.6 mmol, 0.19 equiv.) and $NaBH_4$ (2.3 g, 61 mmol, 3.2 equiv.). Methanol (15 mL, 0.37 mol, 20 equiv.) was carefully added to the suspension over 20 minutes, after which the mixture was warmed-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 (3 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (20→70 % Et_2O in pentane) gave the intermediate (11.8 g, 13.6 mmol, 73 % over two steps) of which 11.7 g (13.5 mmol, 1.0 equiv.) was dissolved 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 equiv.). 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 (2 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. The title compound was obtained (10.5 g, 12.4 mmol, 92 %). R_f: 0.84 (9:1 DCM/MeOH); $[\alpha]_D^{25}$ +25.6° (c = 0.90, $CHCl_3$); ^{1}H 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, 2 × CH_2 PMB, 1 × 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, 3 × 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-bbdec 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.

4-(2,3,4-Tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-butanoic Acid (20): Allyl **18** (8.0 g, 9.9 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) under an argon atmosphere before being dissolved in dry DCE (0.10 L). Methyl acrylate (2.6 mL, 29 mmol, 2.9 equiv.), CuI (0.29 g, 1.5 mmol, 0.15 equiv.) and Grubbs 2nd generation catalyst (0.34 g, 0.40 mmol, 0.04 equiv.) 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 (2 ×) 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 $RuCl_3$ (0.39 g, 1.9 mmol, 0.19 equiv.) and $NaBH_4$ (1.2 g, 32 mmol, 3.2 equiv.). Methanol (8.0 mL, 0.18 mol, 20 equiv.) was carefully added to the suspension over 30 minutes, after which the mixture was warmed-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 (3 ×). The combined organic layers were dried with 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 intermediate (5.8 g, 6.7 mmol, 68 % over two steps), which was was dissolved 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 equiv.). 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 (2 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. The title compound was obtained (5.5 g, 6.4 mmol, 96 %). 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, 2 × 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*$_\alpha$-Fmoc-*N*$_\varepsilon$-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-galactopyranosyl)-amide]-L-lysine (3):** Compound **19** (4.3 g, 5.0 mmol, 1.0 equiv.) and Fmoc-L-lysine-OMe (2.5 g, 6.0 mmol, 1.2 equiv.) were dissolved in DMF (25 mL). HCTU (2.5 g, 6.0 mmol,

1.2 equiv.) and DIPEA (2.6 mL, 15 mmol, 3.0 equiv.) were added and the solution was stirred for 2 hours. The reaction mixture was diluted with EtOAc and washed with 1 M HCl (1 ×), sat. aq. $NaHCO_3$ (1 ×), brine (1 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (40→80 % EtOAc in pentane) gave the intermediate (5.5 g, 4.5 mmol, 90 %) as an oil, of which 4.8 g (4.0 mmol, 1.0 equiv.) was dissolved in THF (55 mL) and cooled to 0 °C. An aqueous solution of LiOH (0.30 M, 27 mL, 8.1 mmol, 2.0 equiv.) 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 (1 ×) and the organic layer was dried with 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, 2 × CH_2 PMB, 1 × 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, 3 × 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, 2 × CH_2-7/8, 3 × CH_2 β/γ/δ-L-Lys); ^{13}C-bbdec 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, 3 × 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): $[M + Na]^+$ calcd. for $C_{74}H_{78}N_2O_{13}Na$: 1225.5, found 1225.5; HRMS: $[M + H]^+$ calcd. for $C_{74}H_{79}O_{13}N_2$: 1203.55767, found 1203.55754.

N_α-Fmoc-N_ε-[butan-4-(2,3,4-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactopyranosyl)-amide]-L-lysine (4): Compound **20** (3.4 g, 4.0 mmol, 1.0 equiv.) and Fmoc-L-lysine-OMe (2.0 g, 4.8 mmol, 1.2 equiv.) were dissolved in DMF (20 mL). HCTU (2.0 g, 4.8 mmol, 1.2 equiv.) and DIPEA (2.1 mL, 12 mmol, 3.0 equiv.) were added and the solution was stirred for 2 hours. The reaction mixture was diluted with EtOAc and washed with 1 M HCl (1 ×), sat. aq. $NaHCO_3$ (1 ×), brine (1 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (30→80 % EtOAc in pentane) gave the intermediate (4.5 g, 3.7 mmol, 93 %) as an oil, of which 4.0 g (3.3 mmol, 1.0 equiv.) was dissolved in THF (37 mL) and cooled to 0 °C. An aqueous solution of LiOH (0.30 M, 22 mL, 6.6 mmol, 2.0 equiv.) 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 (2 ×) and the organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. After purification by column chromatography (2→8 % MeOH in DCM), the title compound (1.9 g, 1.6 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, 3 × 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, 1 × CH_2 β/γ/δ-L-Lys), 1.36–1.09 (m, 4H, 2 × 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): $[M + Na]^+$ calcd. for $C_{74}H_{78}N_2O_{13}Na$: 1225.5, found 1225.6; HRMS: $[M + H]^+$ calcd. for $C_{74}H_{79}O_{13}N_2$: 1203.55767, found 1203.55765.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-α-D-glucopyranoside (21): Glucosamine hydrochloride (21.6 g, 100 mmol, 1.0 equiv.) was added to a solution of sodium methoxide (1.0 M in MeOH, 0.10 L, 1.0 equiv.) 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 equiv.). After 20 minutes, additional tetrachlorophthalic anhydride (14.3 g, 50.0 mmol, 0.5 equiv.) and Et_3N (10 mL, 0.10 mol, 1.0 equiv.) 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 Ac_2O (0.15 L, 1.6 mol, 16.0 equiv.). 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 (3 ×). The combined organic layers were subsequently washed with a 1 M HCl (2 ×), sat. aq. $NaHCO_3$ (2 ×) and brine (1 ×). The organic layer was dried with $MgSO_4$, filtered, concentrated in vacuo and co-evaporated with toluene (1 ×). 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); $[\alpha]_D^{25}$ = +96.6° (c = 1.0, DCM); ^{1}H 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, C*H*H-6), 4.13 (dd, 1H, J = 12.2, 1.8 Hz, C*H*H-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: $[M + Na]^+$ calcd. for $C_{22}H_{19}Cl_4NO_{11}Na$ 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 equiv.) was co-evaporated with toluene (3 ×) 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 equiv.) was added, followed by slow addition of TMSOTf (7.2 mL, 40 mmol, 1.0 equiv.) and BF_3•OEt_2 (25 mL, 0.20 mol, 5.0 equiv.). 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. $NaHCO_3$ (1 ×) and brine (1 ×). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (10→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); $[\alpha]_D^{25}$ = +74.4° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.78–5.65 (m, 2H, H-3, CH_2-C*H*=CH_2), 5.12 (t, 1H, *J* = 10.2 Hz, H-4), 5.00–4.87 (m, 2H, CH_2-CH=C*H*$_2$), 4.47–4.34 (m, 1H, H-1), 4.27 (dd, 1H, *J* = 12.3, 4.9 Hz, C*H*H-6), 4.21 (t, 1H, *J* = 10.2 Hz, H-2), 4.10 (dd, 1H, *J* = 12.3, 2.3 Hz, C*H*H-6), 3.79–3.73 (m, 1H, H-5), 2.26 (t, 2H, *J* = 6.8 Hz, C*H*$_2$-CH=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 (CH_2-*C*H=CH_2), 130.2, 130.0, 127.1, 126.8 (C-Cl), 118.1 (CH_2-CH=*C*H$_2$), 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 (*C*H$_2$-CH=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: $[M + H]^+$ calcd. for $C_{23}H_{22}Cl_4NO_9$ 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 equiv.) was added to a solution of compound **22** (17.1 g, 28.7 mmol, 1.0 equiv.) in a mixture of DCM/MeOH (1:4 v/v, 0.29 L) at 0 °C. After stirring for 1 hour, the reaction mixture was warmed-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 (2 ×) and purified by column chromatography (1→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° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 5.76–5.63 (m, 1H, CH_2-C*H*=CH_2), 4.88 (t, 2H, CH_2-CH=C*H*$_2$), 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, C*H*$_2$-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, C*H*$_2$-CH=CH_2); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ = 163.9, 163.8 (C=O), 140.4, 140.4 (C_q Ar), 133.4 (CH_2-*C*H=CH_2), 130.1, 129.7, 127.3, 127.3 (C-Cl), 117.5 (CH_2-CH=*C*H$_2$), 79.2 (C-5), 74.2 (C-1), 71.8 (C-3), 71.4 (C-4), 62.0 (CH_2-6), 57.5 (C-2), 37.2 (*C*H$_2$-CH=CH_2); 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 $C_{17}H_{15}Cl_4NO_6Na$ 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 equiv.) was co-evaporated with toluene (3 ×) under an argon atmosphere. The residue was dissolved in a mixture of DMF/acetonitrile (9:1 v/v, 113 mL). Benzylaldehyde dimethyl acetal (6.9 mL, 45 mmol, 2.0 equiv.) and *p*-toluenesulfonic acid (0.43 g, 2.3 mmol, 0.1 equiv.) were added and the mixture was heated to 60 °C. After stirring overnight, the mixture was cooled to 0 °C and quenched with Et_3N to pH = 7. The solution was diluted with EtOAc and the organic layer washed with H_2O (3 ×), dried with $MgSO_4$, filtered and concentrated in vacuo. Purification by column chromatography (10→40 % Et_2O in pentane) gave compound **24** (11.0 g, 19.7 mmol, 87 %) as a white solid. R_f: 0.8 (1:1 pentane/Et_2O); $[\alpha]_D^{20}$ = +17.5° (*c* = 1.0, DCM); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.39–7.27 (m, 5H, Ar), 5.78–5.64 (m, 1H, CH_2-C*H*=CH_2), 5.49 (s, 1H, CH benzylidene), 4.94 (t, 2H, *J* = 9.4 Hz, CH_2-CH=C*H*$_2$), 4.60 (t, 1H, *J* = 10.2, 9.0 Hz, H-3), 4.33 (dd, 1H, *J* = 10.2, 4.7 Hz, C*H*H-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, C*H*H-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, C*H*$_2$-CH=CH_2); ^{13}C-APT NMR ($CDCl_3$, 101 MHz, HSQC): δ = 163.7, 163.2 (C=O), 140.4, 140.3 (C-Cl), 136.9 (C_q Ar), 133.0 (CH_2-*C*H=CH_2), 130.1, 129.8 (C-Cl), 129.3, 128.3 (Ar), 127.1, 127.1 (C-Cl), 126.0 (Ar), 117.7 (CH_2-CH=*C*H$_2$), 101.7 (CH benzylidene), 82.5 (C-4), 75.0 (C-1), 70.1 (C-5), 68.8 (CH_2-6), 68.6 (C-3), 57.1 (C-2), 37.0 (*C*H$_2$-CH=CH_2); 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 $C_{24}H_{20}Cl_4NO_6$ 558.0039, found 558.0047.

3-*C*-(2-Deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-β-D-glucopyranosyl)-1-propene (25): To a solution of compound **24** (3.8 g, 6.8 mmol, 1.0 equiv.) in EtOH (70 mL) was added ethylenediamine (23 mL, 0.34 mol, 50 equiv.) 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 (3 ×) and embedded 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 equiv.) was dissolved in a mixture of THF/H_2O (1:1 v/v, 50 mL). Sodium bicarbonate (5.6 g, 66 mmol, 10 equiv.) and Ac_2O (3.1 mL, 33 mmol, 5.0 equiv.) 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. $NaHCO_3$ (1 ×) and brine (1 ×). The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo. The remaining crude product was crystallized using DCM/MeOH/pentane giving compound **25**. The remaining residue was embedded 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° (*c* = 0.3, MeOH); ^{1}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, CH_2-C*H*=CH_2), 5.59 (s, 1H, CH benzylidene), 5.03 (t, 2H, *J* = 17.5, 8.7 Hz, CH_2-CH=C*H*$_2$), 4.25 (dd, 1H, *J* = 10.3, 5.0 Hz, C*H*H-6), 3.75 (q, 2H, *J* = 11.5, 10.0 Hz, H-2, C*H*H-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, C*H*H-CH=CH_2), 2.26–2.13 (m, 1H, C*H*H-CH=CH_2), 1.99 (s, 3H, C*H*$_3$ Ac); ^{13}C-APT NMR (MeOD, 101 MHz, HSQC): δ = 173.7 (C=O), 139.2 (C_q Ar), 135.7 (CH_2-C*H*=CH_2), 129.9, 129.0, 127.5 (Ar), 117.2 (CH_2-CH=*C*H$_2$), 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 (*C*H$_2$-CH=CH_2), 22.9 (CH_3 Ac); FT-IR (neat, cm^{-1}): 3380, 2361, 1630, 1377, 1125, 1033, 999, 698; HRMS: $[M + H]^+$ calcd. for $C_{18}H_{24}NO_5$ 334.1655, found 334.1654.

3-(2-Deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-1-propene (26): Alcohol **25** (1.6 g, 4.9 mmol, 1.0 equiv.) was co-evaporated with toluene (1 ×) 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 equiv.). The suspension was cooled to 0 °C and a solution of TfOH in THF (0.01 M, 50 mL, 0.50 mmol, 0.10 equiv.) was added. After 15 minutes, the mixture was warmed 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. $NaHCO_3$ (1 ×) and brine (1 ×). The organic layer was dried with 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=CH_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-CH=CH_2), 130.6 (C_q Ar), 130.2, 129.1, 128.4, 126.1 (Ar), 117.2 (CH_2-CH=CH_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 (CH_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.

4-(2-Deoxy-2-*N*-acetyl-4,6-*O*-di-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-butanoic Acid (27): Compound **26** (1.4 g, 3.1 mmol, 1.0 equiv.) was co-evaporated with toluene (2 ×) under an argon atmosphere and dissolved in dry DCE (31 mL). Methyl acrylate (0.78 mL, 8.6 mmol, 2.8 equiv.), CuI (90 mg, 0.47 mmol, 0.15 equiv.) and Grubbs 2nd generation catalyst (0.26 g, 0.31 mmol, 0.10 equiv.) 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 (3 ×) under an argon atmosphere. The residue was dissolved in dry DCE (31 mL) and cooled to 0 °C. $RuCl_3$ (0.12 g, 0.58 mmol, 0.19 equiv.) and $NaBH_4$ (0.37 g, 9.8 mmol, 3.2 equiv.) were added, and an empty balloon was placed on the flask. Methanol (2.5 mL, 61 mmol, 20 equiv.) was carefully added to the suspension over 20 minutes, after which the mixture was warmed-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 (3 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. Purification by column chromatography (8→15 % acetone in DCM) gave the intermediate (1.1 g, 2.1 mmol, 68 % over two steps), of which 1.0 g (1.0 g, 2.0 mmol, 1.0 equiv.) was suspended in THF/H_2O/MeOH (7:1:2, v/v/v, 20 mL). LiOH (0.26 g, 6.2 mmol, 3.1 equiv.) was added and 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 (1 ×) and DCM (1 ×). The combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo. The title compound was obtained (1.0 g, 2.0 mmol, quant.). 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 (5): Acid **27** (0.97 g, 1.9 mmol, 1.0 equiv.) and Fmoc-L-lysine-OMe (0.98 g, 2.3 mmol, 1.2 equiv.) were dissolved in DMF (20 mL), followed by the addition of HCTU (0.96 g, 2.3 mmol, 1.2 equiv.) and DIPEA (1.0 mL, 5.7 mmol, 3.0 equiv.). The reaction was stirred at room temperature for 4 hours, after which Et_2O was added. The precipitate was filtered and recrystallized with MeOH/DCM/Et_2O to obtain the intermediate (1.3 g, 1.5 mmol, 78 %), of which 0.92 g (1.1 mmol, 1.0 equiv.) was suspended in THF (21 mL). An aqueous solution of LiOH (0.30 M, 7.1 mL, 2.1 mmol, 2.0 equiv.) was added at room temperature and stirring was continued for 50 minutes, before the obtained clear solution was neutralized with 1 M HCl (2.2 mL, 2.2 mmol, 2.0 equiv.). $NaHCO_3$ (0.36 g, 4.3 mmol, 4.0 equiv.) and Fmoc *N*-hydroxysuccinimide ester (0.72 g, 2.1 mmol, 2.0 equiv.) 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, 3 × 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, 3 × CH_2 β/γ/δ-L-Lys), 23.0 (CH_3 Ac), 22.6 (CH_2-7/8, 3 × 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*): [M + Na]$^+$ calcd. for $C_{48}H_{55}N_3O_{11}Na$: 872.4, found 872.4 [M + Na]$^+$; HRMS: [M + H]$^+$ calcd. for $C_{48}H_{56}O_{11}N_3$: 850.39094, found 850.39103.

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 (29): 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, **3**, **4** and **2** using 2.0 equivalents of each amino acid and 2 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 3 hours. The suspension was filtered and the product was precipitated with Et_2O. After purification by RP-HPLC and lyophilization, conjugate **29** (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*): [M + H]$^+$ calcd. for $C_{62}H_{112}N_{13}O_{24}$: 1422.8, found 1422.8; HRMS: [M+2H]$^{2+}$ 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 (30): The previously obtained 50 μmol pentamer on resin (see **29**) was treated with a mixture of **5**

(2.0 equiv.), HCTU (2.0 equiv.), DIPEA (4.0 equiv.) in DMSO (2.0 mL) at 50 °C for 2 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 3urs. The suspension was filtered and the product was precipitated with Et_2O. After purification by RP-HPLC and lyophilisation, conjugate **30** (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*): $[M + H]^+$ calcd. for $C_{80}H_{143}N_{16}O_{31}$: 1824.0, found 1824.0; HRMS: $[M+2H]^{2+}$ calcd. for $C_{80}H_{144}N_{16}O_{31}$: 912.50862, found 912.50813.

Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Lys(N_ε-[butan-4-(α-L-rhamnosyl)-amide])-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Ala-Lys-OH (32): Tentagel S Ac resin loaded with LEQLESIINFEKLAAAAAK on 10 μmol scale was elongated six times with **1** (3.0 equiv., 2 hours coupling time). After cleaving from the resin, the peptide was purified by RP-HPLC. After lyophilisation, conjugate 32 (2.6 mg, 0.63 μmol, 6 %) was obtained as a white solid. UPLC-MS: Rt = 3.34 min (ACQUITY UPLC BEH C18, 5–75 % MeCN, 10 min run); MALDI-TOF MS (*m/z*): $[M + Na]^+$ calcd. for $C_{189}H_{324}N_{35}O_{65}$: 4124.9, found 4128.7; HRMS: $[M+3H]^{3+}$ calcd. for $C_{189}H_{325}O_{65}N_{35}$: 1375.44212, found 1375.44806.

Keywords: Carbohydrates · Glycoconjugates · Glycopeptides · Metathesis · Synthetic methods

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