



Universiteit
Leiden
The Netherlands

Synthetic carbohydrate ligands for immune receptors

Reintjens, N.R.M.

Citation

Reintjens, N. R. M. (2020, February 27). *Synthetic carbohydrate ligands for immune receptors*. Retrieved from <https://hdl.handle.net/1887/85676>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/85676>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/85676> holds various files of this Leiden University dissertation.

Author: Reintjens, N.R.M.

Title: Synthetic carbohydrate ligands for immune receptors

Issue Date: 2020-02-27

Chapter 4

*Synthesis of multivalent MPR ligand–antigen conjugates**

Introduction

Carbohydrates play an important role in many biological processes, such as cell-cell communication, pathogen recognition and protein folding. Mannose-6-phosphate (M6P), a D-mannopyranose bearing a phosphate group at C-6 position, serves as a signaling moiety on the termini of glycan branches mounted on newly synthesized proteins in the trans-Golgi network (TGN) and it is essential for the transportation of these enzymes to the late endosomes and lysosomes. The mannose-6-phosphate receptor (MPR), a P-type lectin, plays an important role in this transportation through binding to the M6P.^{1,2} There are two members of this lectin family: the cation-independent mannose-6-phosphate receptor (CI-MPR) and the cation-dependent mannose-6-phosphate receptor (CD-MPR). The first one is a ~300 kDa transmembrane glycoprotein that consists of 15 extracellular repeating domains (Figure 1A), has a low affinity for M6P and is able to bind to both M6P and other ligands, such as insulin growth factor II (IGFII).^{3–5} On the other hand, the CI-MPR has high affinity for ligands containing multiple M6Ps due to the two M6P binding domains (3 and 9), which can

*The data presented in this Chapter were gathered in collaboration with Christopher Vis, Toroa McGlinn, Nico J. Meeuwenoord, Tim P. Hogervorst, Herman S. Overkleeft, Dmitri V. Filippov, Gijsbert A. van der Marel and Jeroen D. C. Codée.

simultaneously bind two M6Ps. Binding to the two M6P domains can also lead to dimerization between two receptors. The group of Berkowitz studied the ligand-receptor interactions with mono- and bivalent probes and two dimeric models for multivalent binding of the receptor were suggested: the “ladder” and the “hook” model (Figure 1A and 1B, respectively).^{6,7} The CD-MPR is a ~46 kDa transmembrane protein that consists of only one M6P binding site. It does not bind to IGFII and can be found as a dimer (Figure 1C). A small fraction of MPRs can be found on the cell surface, where only CI-MPR binds and internalizes M6P-bound enzymes (Figure 1D).⁸ The optimal binding of the CD-MPR and domain 9 of the CI-MPR is reported to be at a pH of 6.0-6.5 and 6.4-6.5, while domain 3 of the CI-MPR binds at a higher pH of 6.9-7.0. These observations explain why only the CI-MPR binds M6P-bound enzymes at the cell surface (pH = ~7.4).^{3,9,10}

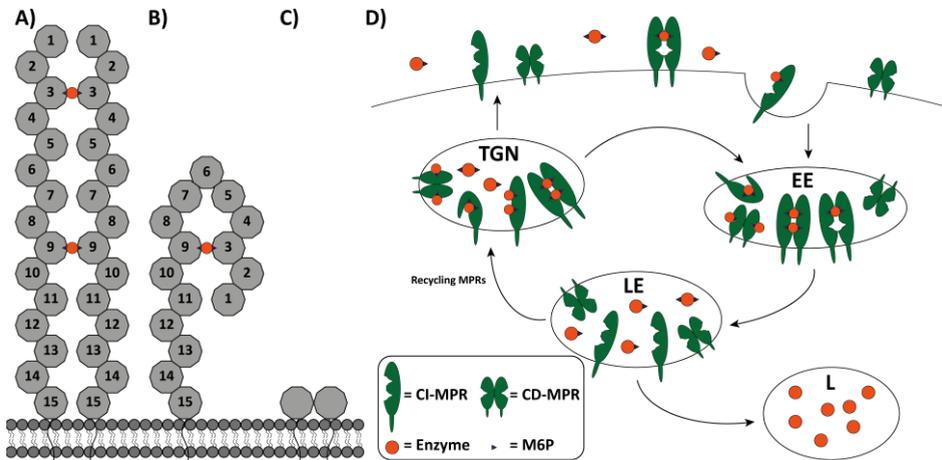


Figure 1. Proposed binding models and schematic representations of: A) Bivalent M6P ligand bound to two monomers of CI-MPR; B) Bivalent M6P ligand bound to the same receptor; C) CD-MPR; D) MPR-pathway: lysosomal enzymes in the TGN are transported to the early endosome. From there, the enzymes are transported to the late endosome, where the enzyme is dissociated from the MPR. The enzymes are packaged into the lysosome and the MPR is recycled back to the TGN, from where a small portion is moved to the cell surface. LE = late endosome; EE = early endosome, L = lysosome.

The MPR can be used as an efficient tool for targeted delivery to the endosomes as the conjugation of M6P analogues to acid α -glucosidase improved the treatment of the lysosomal myopathy Pompe disease¹¹. The MPR has also been exploited in the delivery of doxorubicin via mannose-6-phosphate-modified human serum albumin as carrier or *N*-hexanoyl-*D*-erythro-sphingosine with M6P-functionalized liposomes.^{12,13} Hogendoorn *et al.* have reported the synthesis of a M6P cluster attached to a covalent cathepsin inhibitor and a fluorescent BODIPY dye, which was used to label proteases

along the endocytic pathway.¹⁴ It was shown that the MPR can also be exploited as an effective pathway for immunogenicity.¹⁵ This led to the idea that conjugate vaccines in which a M6P moiety is covalently bound to an epitope will have improved uptake ensuring a more efficient delivery to the endosomes. One of the potential drawbacks of the use of M6P is dephosphorylation by endogenous phosphatases. To prevent this, several M6P analogues have previously been evaluated, such as a malonyl ether and a malonate or a C-phosphonate ester.^{6,16} The C-phosphonate proved to be a stable and effective replacement for the phosphate monoester.

This chapter describes the incorporation of a cluster of mannose-6-phosphonates (M6Po) in two types of peptide antigen-conjugates (**1-8**, Figure 2), wherein two different M6Po's (**9** and **10**, respectively) are conjugated to either the *N*- or the *C*-terminus of a synthetic long peptide (SLP). In the first type of conjugates (**1-4**), DEVA₅K was used as SLP. This model epitope contains the MHC-I antigen SIINFEKL, incorporated in a longer peptide motif DEVSGLEQLESIINFEKLAAAAAK. The α -propargyl mannose-6-phosphonate (*O*-M6Po) building block (**9**) was developed since an α -configured linker is tolerated by the MPR.^{7,14} The alkyne function in building block **9** allows the copper mediated 1,3-dipolar cycloaddition to azide-functions in the SLP.¹⁷ As it was shown that bi- or multivalent M6P-ligands are more effective in binding the CI-MPR than ligands with only one M6P, six M6Po's were incorporated in each conjugate.^{3,18,19} The objective to enhance the immune response after transferring the conjugate to the endosomes by the MPR, led to the design of bis-conjugates (**2** and **4**) in which a Toll-like receptor ligand 7 (TLR7L) is added at either the *N*- or the *C*-terminus of the SLP. In the second type of conjugates (**5-8**) the ovalbumin derived (HAAHA) peptide ISQAVHAAHAEINEAGRK, which contains an MHC-II epitope, was used as SLP. *C*-M6Po building block **10** was developed as a building block that can be used in the online solid phase peptide synthesis (SPPS) of conjugates **5-8**. This building block is a *C*-analogue of M6Po, in which the anomeric oxygen is replaced with a CH₂, preventing hydrolysis by the acidic conditions used in SPPS. An additional advantage of the SPPS compatible building blocks is the possibility to prepare conjugates of peptides that are not suitable for copper mediated 1,3-dipolar cycloaddition. For example, the HAAHA peptide, used for the second type of conjugates, contains two histidines, which can coordinate to copper and thereby inhibit the reduction of Cu(II) to Cu(I).²⁰ The SPPS building block **10** is equipped with acid labile protecting groups, which will be removed at the end of the SPPS concomitantly with the other acid labile peptide protecting groups and release of the peptide from the resin.

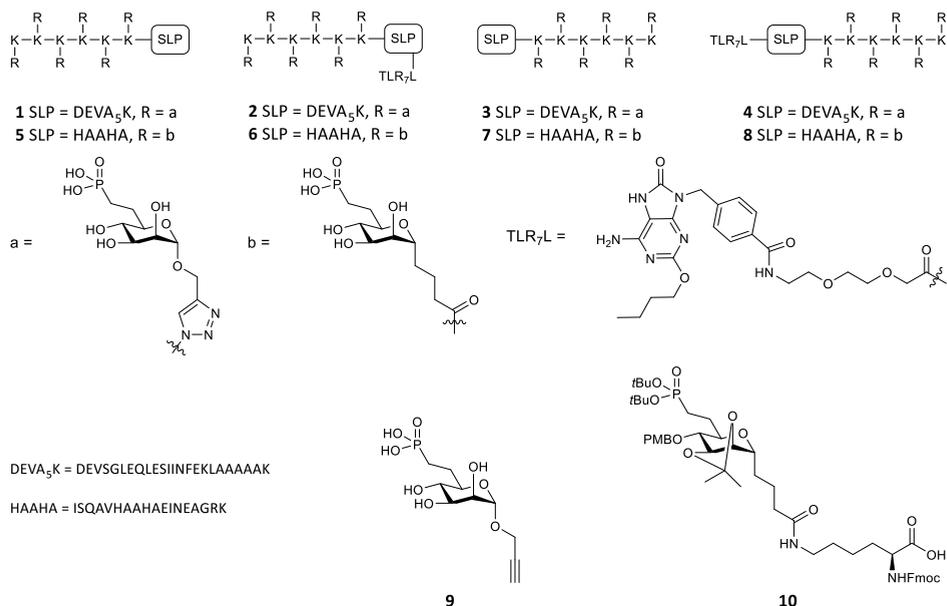


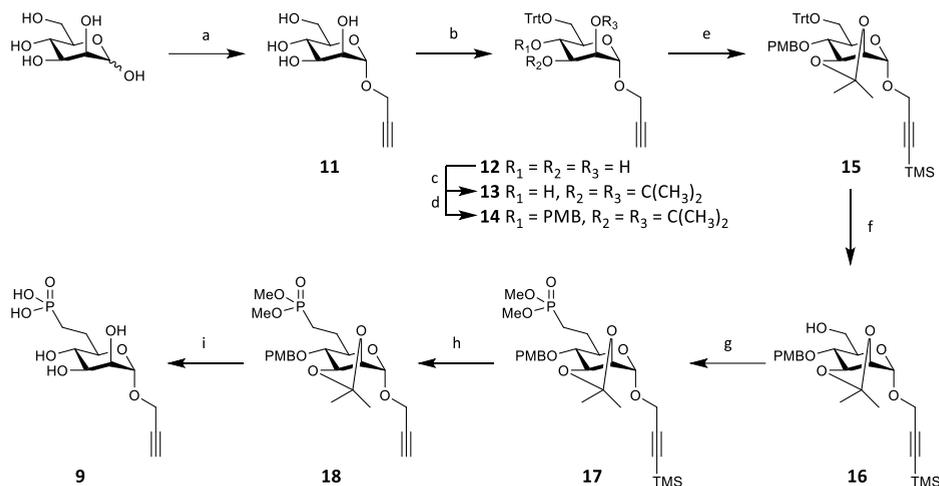
Figure 2. Structures of the 1st type *O*-M6Po conjugates **1-4**, the 2nd type *C*-M6Po conjugates **5-8** and building blocks **9** and **10**.

Results and Discussion

O-M6Po-SIINFEKL conjugates

The first type of *O*-M6Po conjugates comprises the six-fold addition of α -propargyl mannose-6-phosphonate (*O*-M6Po) building block (**9**) to azide containing peptides. Synthesis of the required building block **9** started with the acetylation of *D*-mannose, followed by $\text{BF}_3 \cdot \text{OEt}_2$ catalyzed glycosylation of propargyl alcohol and deacetylation to give crystalline compound **11** (Scheme 1). Subsequently, the following protective group manipulations were performed: tritylation of the primary 6-OH in **11**, isopropylidation of **12**, and *p*-methoxybenzylation of **13** to give fully protected mannose **14**. The presence of the isopropylidene group proved to be an essential conformational lock, preventing intramolecular cyclization during the installation of the phosphonate.²¹⁻²³ Prior to the installation of the phosphonate, the terminal alkyne in **14** was protected with a TMS group using TMSCl and *n*-BuLi at -78°C . Removal of the trityl in thus obtained **15** with a catalytic amount of *p*-toluenesulfonic acid in DCM/MeOH was accompanied by partial removal of the isopropylidene ketal. Reinstallation of the isopropylidene and subsequent deprotection of the mixed acetal formed on the primary alcohol gave **16** in 98% over three steps. Alcohol **16** was converted into a triflate using Tf_2O and pyridine at -40°C . A fast work-up was necessary due to the instability of the formed triflate.²⁴

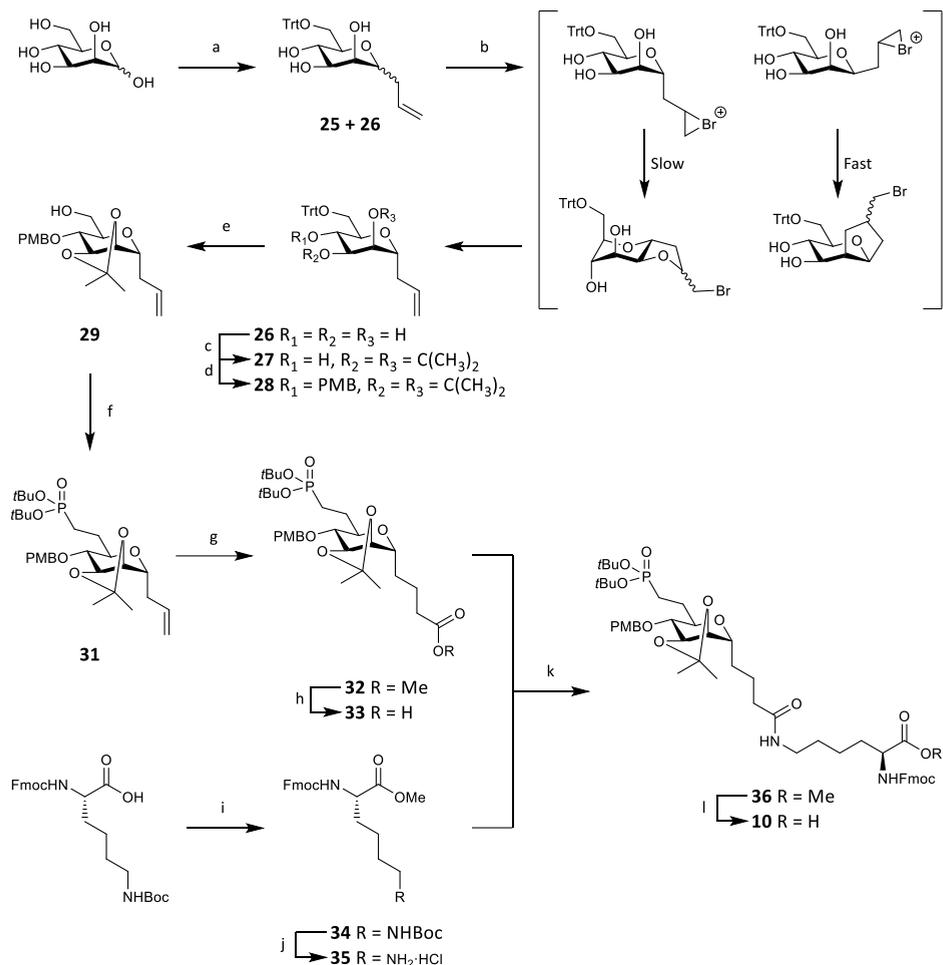
The obtained crude triflate was added to a solution of deprotonated dimethyl methylphosphonate in THF at -70°C , giving compound **17** in 72% over two steps. Removal of the TMS protecting group gave **18**, which was transformed into key building block **9** by a two-step deprotection sequence. In the first step, the phosphonate was deprotected using TMSBr. Next, the *p*-methoxybenzyl and isopropylidene were removed by treatment of the intermediate with AcOH/H₂O at 90°C . Compound **9** was obtained in 27% over 15 steps starting from D-mannose.



Scheme 1. Synthesis of alkyne building block **9**. *Reagents and conditions:* a) *i.* Ac₂O, pyridine; *ii.* propargyl alcohol, BF₃·OEt₂, 50°C ; *iii.* NaOMe, MeOH, 70% over three steps; b) TrtCl, Et₃N, DMF, 60°C , 83%; c) *p*-toluenesulfonic acid, 2,2-dimethoxypropane, 87%; d) *p*-methoxybenzyl chloride, NaH, DMF, 95%; e) TMSCl, *n*-BuLi, THF, -78°C , 97%; f) *i.* *p*-toluenesulfonic acid, DCM/MeOH; *ii.* *p*-toluenesulfonic acid, 2,2-dimethoxypropane; *iii.* 1 M HCl, EtOAc, 0°C , 98% over three steps; g) *i.* Tf₂O, pyridine, DCM, -40°C ; *ii.* *n*-BuLi, dimethyl methylphosphonate, THF, -70°C to -50°C , 72% over two steps; h) TBAF, THF, quant.; i) *i.* TMSBr, pyridine, MeCN; *ii.* AcOH/H₂O, 90°C , 81% over two steps.

Next the assembly of the (O-M6Po)₆-SIINFEKL conjugates was undertaken. Immobilized peptides **19** and **22** were prepared through standard SPPS HCTU/Fmoc chemistry using Tentagel S Ram as solid support (Scheme 2). TFA/TIS/H₂O (95/2.5/2.5 v/v/v) treatment removed all protecting groups and cleaved the peptides from the resin to give peptides **20** and **23** in 1% and 6%, respectively, after purification. Alternatively, the MMT protecting group at the C-terminal lysine of **19** was selectively deprotected with a cocktail of TFA/TIS/DCM (2/2/96 v/v/v) and the released amine was subsequently coupled with the spacer {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid and the Boc-protected TLR7-ligand building block.²⁵ After deprotection, release from the resin and RP-HPLC purification peptides **21** and **24** were both obtained in 2% yield. Coupling of O-

benzylated methyl α -D-mannopyranoside.²⁷ However, debenylation and purification turned out to be problematic when this was performed on large scale. Therefore an alternative procedure was followed in which D-mannose was acetylated and treated with a mixture of allyltrimethylsilane, $\text{BF}_3 \cdot \text{OEt}_2$, and TMSOTf in MeCN to give the desired allyl mannoside as a 4.2/1 α/β mixture. Known methods^{28,29} to separate the α/β mixture of C-allyl mannose could not be reproduced on large scale, and therefore the following procedure was developed. The primary alcohol in the crude α/β -C-allyl mannose was protected with a trityl, to produce a mixture of **25** and **26** (55% yield over 4 steps). Next, the two anomers were treated with *N*-bromosuccinimide in THF. The formed bromonium ion allows a fast, intramolecular cyclization of the β -mannose while the α -mannose cyclizes slowly due to the unfavorable ring flip, necessary for nucleophilic attack of the 2-O at the bromonium ion. After column purification pure α -C-allyl mannose **26** was recovered in 91%. Protection of the C-2-OH and C-3-OH with an isopropylidene ketal was followed by installation of a *p*-methoxybenzyl at the C-4-OH to give compound **28**. Removal of the trityl group was performed with the same conditions used in the synthesis of **16**, giving **29** in 72% over three steps. Conversion of **29** to the primary triflate, followed by nucleophilic attack of the anion of di-*tert*-butyl methylphosphonate **30**³⁰ gave phosphonate **31** in 72% over two steps on 3 mmol scale. The yield dropped to 49% when increasing the scale to 30 mmol due to the instability of the primary triflate. Cross metathesis with methyl acrylate, followed by the reduction of the double bond with NaBH_4 and ruthenium trichloride gave compound **32**.^{31,32} Hydrolysis of the obtained methyl ester using LiOH gave acid intermediate **33**. Although carboxylic acid **33** is suitable for SPPS the spacer length was adjusted to that of the first type of conjugates. For this purpose, Fmoc-L-Lys-OMe was condensed with **33** under the influence of HCTU and DIPEA to give **34** in 86%. Hydrolysis of **34** with LiOH at 0°C, left the Fmoc group unaffected and gave SPPS building block **10** in 80%.



Scheme 3. Synthesis of building block compound **10**. *Reagents and conditions:* a) *i.* Ac_2O , pyridine, *ii.* allyltrimethylsilane, $BF_3 \cdot OEt_2$, TMSOTf, MeCN; *iii.* NaOMe, MeOH; *iv.* TrtCl, Et_3N , DMF, $60^\circ C$, 55% over four steps; b) *N*-bromosuccinimide, THF, 3 h, 91%; c) *p*-toluenesulfonic acid, 2,2-dimethoxypropane, 93%; d) *p*-methoxybenzyl chloride, NaH, DMF, 97%; e) *i.* *p*-toluenesulfonic acid, DCM/MeOH; *ii.* *p*-toluenesulfonic acid, 2,2-dimethoxypropane; *iii.* 1 M HCl, EtOAc, $0^\circ C$, 75% over three steps; f) *i.* Tf_2O , pyridine, DCM, $-40^\circ C$; *ii.* *n*-BuLi, di-*tert*-butyl methylphosphonate (**30**), THF, $-70^\circ C$ to $-50^\circ C$, 72% over two steps; g) *i.* methyl acrylate, CuI, Grubbs 2nd gen. catalyst, DCE, $60^\circ C$; *ii.* $NaBH_4$, $RuCl_3$, MeOH, DCE, $45^\circ C$, 72% over two steps; h) LiOH, THF/ H_2O , quant; i) MeI, K_2CO_3 , DMF, 93%; j) 4 M HCl in dioxane, dioxane, 98%; k) HCTU, DIPEA, DMF, 86%; l) LiOH, THF/ H_2O , $0^\circ C$, 80%.

With the desired building block **10** in hand, conjugates **5-8** were prepared using semi-automated SPPS (Scheme 4). Tentagel S Ram resin was elongated with ISQAVHAAHAEINEAGRK using automated SPPS, wherein the lysine(MMT) at the C-terminus will be used for elongation at a later stage of the synthesis. Six subsequent

Conclusion

This chapter describes the synthesis of two type of peptide conjugates (**1-4** and **5-8**, respectively) having a hexavalent M6Po cluster incorporated at either the *N*- or the *C*-terminus. These ligands for the mannose-6-phosphate receptors were designed and synthesized to improve the uptake of these conjugates resulting in an enhanced immunogenicity. To prevent dephosphorylation by endogenous phosphatases, *C*-phosphonates are applied in the conjugates. The assembly of the first type uses copper mediated 1,3-dipolar cycloaddition (click chemistry) to append six *O*-M6Po residues to the separately prepared peptides in one event. For this purpose *O*-M6Po building block **9** was developed. The second type comprises an online synthesis of the conjugates requiring a mannose building block with an anomeric center, resisting the acid conditions of SPPS. A new method was found to synthesize an α -*C*-allyl-mannoside that was further elaborated to building block **10**. The designed *C*-M6P building block **10** proved to be well suited for SPPS allowing for the streamlined assembly of the conjugates. To further improve the immune response bis-conjugates were assembled provided with a TLR7-ligand. Presently, the immunological evaluation is ongoing.

Experimental

All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3Å molecular sieves. All moisture and oxygen sensitive reactions were performed under an argon atmosphere. Column chromatography was performed on silica gel (Screening Devices BV, 40-63 μm , 60 Å). For TLC analysis, pre-coated silica gel aluminum sheets (Merck, silica gel 60, F254) were used with detection by UV-absorption (254/366 nm) where applicable. Compounds were visualized on TLC by UV absorption (245 nm), or by staining with one of the following TLC stain solutions: $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$ (25 g/L), $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot 2\text{H}_2\text{O}$ (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 $\sim 150^\circ\text{C}$. ^1H , ^{13}C and ^{31}P 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 analysis were done on an Agilent Technologies 1260 Infinity system with a C18 Gemini 3 μm , C18, 110 Å, 50 x 4.6 mm column. Absorbance was measured at 214 nm and 256 nm and an Agilent Technologies 6120 Quadrupole mass spectrometer was used as detector. Peptides and conjugates were purified with a Gilson GX-281 preparative HPLC with a Gemini-NX 5 μ , C18, 110 Å, 250 x 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.

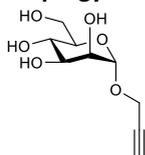
Automated solid phase synthesis general experimental information

The automated solid-phase peptide synthesis was performed on a 250 μmol scale on a Protein Technologies Tribute-UV IR Peptide Synthesizer applying Fmoc based protocol starting from Tentagel S RAM resin (loading 0.22 mmol/g). The synthesis was continued with Fmoc-amino acids specific for each peptide. The consecutive steps performed in each cycle for HCTU chemistry on 250 μmol scale: 1) Deprotection of the Fmoc-group with 20% piperidine in DMF for 10 min; 2) DMF wash; 3) Coupling of the appropriate amino acid using a four-fold excess. Generally, the Fmoc amino acid (1.0 mmol) was dissolved in 0.2 M HCTU in DMF (5 mL), the resulting solution was transferred to the reaction vessel followed by 0.5 mL of 0.5 M DIPEA in DMF to initiate the coupling. The reaction vessel was then shaken for 30 min at 50°C; 4) DMF wash; 5) capping with 10% Ac_2O in 0.1 M DIPEA in DMF; 6) DMF wash; 7) DCM wash. Aliquots of resin of the obtained sequences were checked on an analytical Agilent Technologies 1260 Infinity system with a Gemini 3 μm , C18, 110 Å, 50 x 4.6 mm column. The Fmoc amino acids applied in the synthesis were: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(N_3)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(MMT)-OH, Fmoc-Phe-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Val-OH.

General procedure for cleavage from the resin, deprotection and purification

30 μmol resin was washed with DMF, DCM and dried after the last synthesis step followed by a treatment for 180 minutes with 0.6 mL cleavage cocktail of 95% TFA, 2.5% TIS and 2.5% H_2O . The suspension was filtered, the resin was washed with 0.6 mL of the cleavage cocktail, and the combined TFA solutions were added dropwise to cold Et_2O and stored at -20°C overnight. The obtained suspension of the product in Et_2O was centrifuged, Et_2O was removed and the precipitant was dissolved in $\text{CH}_3\text{CN}/\text{H}_2\text{O}/t\text{BuOH}$ (1/1/1 v/v/v) or $\text{DMSO}/\text{CH}_3\text{CN}/\text{H}_2\text{O}/t\text{BuOH}$ (3/1/1/1 v/v/v/v). Purification was performed on a Gilson GX-281 preparative RP-HPLC with a Gemini-NX 5 μ , C18, 110 Å, 250 x 10.0 mm column.

Propargyl α -D-mannopyranoside (11)

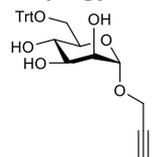


To a solution of D-mannose (28.1 g, 156 mmol, 1.0 eq.) in pyridine (0.22 L) was added acetic anhydride (0.10 L, 1.1 mol, 6.8 eq.) and DMAP (0.37 g, 3.0 mmol, 0.02 eq.) at 0°C. After 30 minutes the solution was allowed to warm-up to room temperature and stirred for an additional 5.5 hours. The reaction mixture was cooled to 0°C and quenched by slowly

adding MeOH. The mixture was diluted with EtOAc, washed with 1 M HCl (3x), dried over MgSO_4 , filtered and concentrated *in vacuo*. Co-evaporation with toluene (2x) under an argon atmosphere yielded acetyl 2,3,4,6-tetra-O-acetyl- α/β -D-mannoside as an oil, which was dissolved in DCM (0.45 L). Propargyl alcohol (14.0 mL, 240 mmol, 1.5

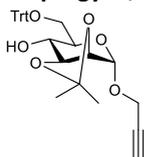
eq.) and $\text{BF}_3 \cdot \text{OEt}_2$ (30.0 mL, 239 mmol, 1.5 eq.) were added and the solution was stirred overnight at 50°C . TLC analysis showed complete conversion and the reaction mixture was cooled to 0°C , followed by quenching with Et_3N to pH 8. The dark solution was washed with sat. aq. NaHCO_3 (1x), dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Purification by flash column chromatography (50 \rightarrow 100% Et_2O in pentane) yielded propargyl 2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (55.3 g, 143 mmol), which was dissolved in MeOH (0.30 L). Sodium methoxide (5.4 M in MeOH, 11 mL, 60 mmol, 0.40 eq.) was added and the solution was stirred for 30 minutes, after which it was acidified by the addition of amberlite H^+ resin. The mixture was filtered and concentrated *in vacuo*. Crystallization by EtOH/pentane afforded the title compound (22.9 g, 105 mmol, 67% over three steps) as a white solid. R_f : 0.39 (1/4 MeOH/DCM); $[\alpha]_D^{25} +145^\circ$ ($c = 0.53$, MeOH); ^1H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 4.96 (d, 1H, $J = 1.6$ Hz, H-1), 4.27 (d, 2H, $J = 2.4$ Hz, CH_2 propargyl), 3.84 (dd, 1H, $J = 11.8$, 2.3 Hz, CHH-6), 3.80 (dd, 1H, $J = 3.2$, 1.7 Hz, H-2), 3.74 – 3.65 (m, 2H, H-3, CHH-6), 3.62 (t, 1H, $J = 9.4$ Hz, H-4), 3.51 (ddd, 1H, $J = 8.7$, 5.8, 2.2 Hz, H-5), 2.86 (t, 1H, $J = 2.4$ Hz, CH propargyl); ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 99.7 (C-1), 80.0 (C_q propargyl), 76.0 (CH propargyl), 75.0 (C-5), 72.4 (C-3), 72.0 (C-2), 68.4 (C-4), 62.8 (C-6), 54.8 (CH_2 propargyl); FT-IR (neat, cm^{-1}): 3370, 2931, 2584, 1982, 1639, 1365, 1263, 1132, 1058, 1007, 965, 912, 880, 812, 685, 515; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_9\text{H}_{14}\text{O}_6\text{Na}$: 241.0688, found 241.0684.

Propargyl 6-*O*-trityl- α -D-mannopyranoside (12)

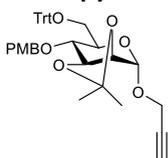


Trityl chloride (57.2 g, 205 mmol, 1.5 eq.) and Et_3N (46 mL, 0.33 mol, 2.5 eq.) were added to a solution of compound **11** (29.0 g, 133 mmol, 1.0 eq.) in DMF (0.44 L). The mixture was heated to 60°C for 4 hours, followed by addition of trityl chloride (38.1 g, 137 mmol, 1.0 eq.) and Et_3N (28 mL, 0.20 mol, 1.5 eq.). After stirring for one hour, TLC analysis

showed complete conversion of the starting material and the reaction mixture was cooled to room temperature. The mixture was diluted with EtOAc, washed with H_2O (3x), dried over Na_2SO_4 , filtered and concentrated *in vacuo*. After purification by column chromatography (30 \rightarrow 100% EtOAc in pentane), the title compound (50.4 g, 109 mmol, 82%) was obtained as a white foam. R_f : 0.65 (1/4 pentane/EtOAc); $[\alpha]_D^{25} +45.3^\circ$ ($c = 0.91$, CHCl_3); ^1H NMR (CD_3CN , 400 MHz, HH-COSY, HSQC): δ 7.53 – 7.45 (m, 6H, Ar), 7.38 – 7.30 (m, 6H, Ar), 7.30 – 7.22 (m, 3H, Ar), 4.96 (d, 1H, $J = 1.2$ Hz, H-1), 4.44 – 4.30 (m, 2H, CH_2 propargyl), 3.81 – 3.75 (m, 1H, H-2), 3.67 (ddd, 1H, $J = 9.1$, 7.1, 1.7 Hz, H-5), 3.55 (ddd, 1H, $J = 9.5$, 6.3, 3.5 Hz, H-3), 3.52 – 3.42 (m, 1H, H-4), 3.36 – 3.28 (m, 2H, CHH-6, OH), 3.26 (d, 1H, $J = 6.4$ Hz, OH), 3.18 (dd, 1H, $J = 9.9$, 7.0 Hz, CHH-6), 3.11 (d, 1H, $J = 5.0$ Hz, OH), 2.78 (t, 1H, $J = 2.4$ Hz, CH propargyl); ^{13}C -APT NMR (CD_3CN , 101 MHz, HSQC): δ 145.2 (C_q Trt), 129.6, 128.8, 128.0 (Ar), 99.1 (C-1), 87.2 (C_q Trt), 80.0 (C_q propargyl), 76.1 (CH propargyl), 73.3 (C-5), 72.5 (C-3), 71.2 (C-2), 68.6 (C-4), 64.7 (CH_2 -6), 54.4 (CH_2 propargyl); FT-IR (neat, cm^{-1}): 3412, 3290, 3059, 3033, 2928, 2119, 1597, 1490, 1449, 1377, 1320, 1221, 1184, 1134, 1074, 1049, 1005, 986, 900, 844, 810, 765, 748, 702, 650, 633, 582, 531; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{28}\text{H}_{28}\text{O}_6\text{Na}$: 483.1784, found 483.1780.

Propargyl 2,3-di-O-isopropylidene-6-O-trityl- α -D-mannopyranoside (13)

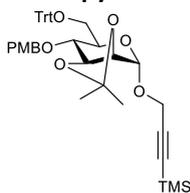
To a solution of compound **12** (50.4 g, 109 mmol, 1.0 eq.) in 2,2-dimethoxypropane (0.55 L) was added *p*-toluenesulfonic acid (3.22 g, 16.9 mmol, 0.15 eq.) at 0°C. After stirring for 1.5 hours, TLC analysis showed complete conversion of the starting material. The mixture was quenched by the addition of Et₃N (8 mL), diluted with brine and extracted with DCM (2x). The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→50% Et₂O in pentane) and crystallization in DCM/pentane yielded compound **13** (47.7 g, 95.4 mmol, 87%) as a white solid. *R*_f: 0.20 (7/3 pentane/Et₂O); [α]_D²⁵ +23.0° (*c* = 0.67, CHCl₃); ¹H NMR (CD₃CN, 400 MHz, HH-COSY, HSQC): δ 7.52 – 7.45 (m, 6H, Ar), 7.38 – 7.30 (m, 6H, Ar), 7.30 – 7.24 (m, 3H, Ar), 5.21 (s, 1H, H-1), 4.50 – 4.35 (m, 2H, CH₂ propargyl), 4.14 (d, 1H, *J* = 5.6 Hz, H-2), 3.98 – 3.92 (m, 1H, H-3), 3.69 – 3.61 (m, 1H, H-5), 3.49 – 3.42 (m, 1H, H-4), 3.34 (dd, 1H, *J* = 10.1, 1.7 Hz, *CHH*-6), 3.24 – 3.14 (m, 2H, *CHH*-6, OH), 2.79 (t, 1H, *J* = 2.4 Hz, CH propargyl), 1.45 (s, 3H, CH₃ isopropylidene), 1.32 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 145.1 (C_q Trt), 129.5, 128.8, 128.1 (Ar), 110.0 (C_q isopropylidene), 96.2 (C-1), 87.3 (C_q Trt), 79.7 (C_q propargyl), 79.6 (C-3), 76.4 (C-2, CH propargyl), 70.9 (C-5), 69.9 (C-4), 64.3 (C-6), 54.5 (CH₂ propargyl), 28.2, 26.5 (CH₃ isopropylidene); FT-IR (neat, cm⁻¹): 3279, 2935, 1490, 1448, 1374, 1225, 1168, 1136, 1103, 1075, 1047, 1029, 992, 918, 898, 851, 822, 786, 767, 743, 705, 696, 650, 634, 583, 543, 532, 471; HRMS: [M+Na]⁺ calcd. for C₃₁H₃₂O₆Na: 523.2097, found 523.2095.

Propargyl 2,3-di-O-isopropylidene-4-O-*p*-methoxybenzyl-6-O-trityl- α -D-mannopyranoside (14)

After co-evaporation with toluene (1x) under an argon atmosphere, compound **13** (47.7 g, 95.4 mmol, 1.0 eq.) was dissolved in DMF (0.48 L) and cooled to 0°C. Sodium hydride (60% dispersion in mineral oil, 4.59 g, 115 mmol, 1.2 eq.) and *p*-methoxybenzyl chloride (15.6 mL, 115 mmol, 1.2 eq.) were added to the mixture. After 3 hours stirring at 0°C, the suspension was allowed to warm-up to room temperature and stirred for an additional 2 hours. The reaction mixture was quenched by the addition of MeOH at 0°C, diluted with H₂O and extracted with EtOAc (2x). The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. After purification by column chromatography (5→40% Et₂O in pentane) and crystallization in DCM/pentane the title compound (56.4 g, 90.9 mmol, 95%) was obtained as a white solid. *R*_f: 0.50 (7/3 pentane/Et₂O); [α]_D²⁵ +29.8° (*c* = 0.94, CHCl₃); ¹H NMR (CD₃CN, 400 MHz, HH-COSY, HSQC): δ 7.49 – 7.43 (m, 6H, Ar), 7.36 – 7.25 (m, 9H, Ar), 6.94 – 6.89 (m, 2H, Ar), 6.79 – 6.74 (m, 2H, Ar), 5.24 (s, 1H, H-1), 4.59 (d, 1H, *J* = 11.1 Hz, *CHH* PMB), 4.47 – 4.34 (m, 2H, CH₂ propargyl), 4.29 (d, 1H, *J* = 11.1 Hz, *CHH* PMB), 4.21 – 4.15 (m, 2H, H-2, H-3), 3.76 (s, 3H, CH₃ PMB), 3.69 (ddd, 1H, *J* = 10.2, 6.2, 1.6 Hz, H-5), 3.46 (dd, 1H, *J* = 10.3, 6.4 Hz, H-4), 3.38 (dd, 1H, *J* = 10.1, 1.7 Hz, *CHH*-6), 3.09 (dd, 1H, *J* = 10.1, 6.3 Hz, *CHH*-6), 2.78 (t, 1H, *J* = 2.4 Hz, CH propargyl), 1.49 (s, 3H, CH₃ isopropylidene), 1.35 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 160.1 (C_q PMB), 145.0 (C_q Trt), 131.2 (C_q PMB), 130.5, 129.6, 128.8, 128.1, 114.4 (Ar), 110.1 (C_q isopropylidene), 96.2 (C-1), 87.2 (C_q Trt), 79.7 (C_q propargyl), 79.5 (C-3), 76.4 (C-2), 76.4

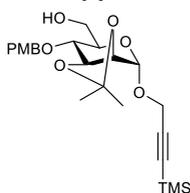
(CH propargyl), 76.3 (C-4), 73.1 (CH₂ PMB), 69.7 (C-5), 64.1 (CH₂-6), 55.8 (CH₃ PMB), 54.6 (CH₂ propargyl), 28.2, 26.5 (CH₃ isopropylidene); FT-IR (neat, cm⁻¹): 3286, 1612, 1586, 1513, 1490, 1449, 1372, 1302, 1245, 1220, 1171, 1145, 1076, 1059, 1029, 998, 915, 899, 863, 821, 777, 765, 737, 699, 644, 632, 587, 550, 518, 468; HRMS: [M+Na]⁺ calcd. for C₃₉H₄₀O₇Na: 643.2672, found 643.2677.

Trimethylsilylpropargyl 2,3-di-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-6-*O*-trityl- α -*D*-mannopyranoside (**15**)



Compound **14** (38.1 g, 61.3 mmol, 1.0 eq.) was co-evaporated twice with toluene under an argon atmosphere and dissolved in THF (0.61 L). The solution was cooled to -78°C, followed by the addition of *n*-butyllithium (1.6 M in hexane, 46 mL, 74 mmol, 1.2 eq.). After 15 minutes, TMSCl (12 mL, 95 mmol, 1.5 eq.) was added dropwise to the pink mixture. The resulting yellow mixture was allowed to warm-up to -50°C over two hours and the reaction was quenched by the addition of sat. aq. NH₄Cl. The mixture was diluted with EtOAc and washed with sat. aq. NH₄Cl (1x) and sat. aq. NaHCO₃ (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (2→15% Et₂O in pentane) gave compound **15** in quantitative yield (44.1 g). R_f: 0.38 (9/1 pentane/Et₂O); [α]_D²⁵ +33.8° (*c* = 0.45, CHCl₃); ¹H NMR (CD₃CN, 400 MHz, HH-COSY, HSQC): δ 7.49 – 7.43 (m, 6H, Ar), 7.36 – 7.24 (m, 9H, Ar), 6.94 – 6.89 (m, 2H, Ar), 6.79 – 6.73 (m, 2H, Ar), 5.24 (s, 1H, H-1), 4.59 (d, 1H, *J* = 11.1 Hz, *CHH* PMB), 4.47 – 4.34 (m, 2H, CH₂ propargyl), 4.30 (d, 1H, *J* = 11.1 Hz, *CHH* PMB), 4.21 – 4.15 (m, 2H, H-2, H-3), 3.75 (s, 3H, CH₃ PMB), 3.71 – 3.65 (m, 1H, H-5), 3.48 (dd, 1H, *J* = 10.4, 6.2 Hz, H-4), 3.39 (dd, 1H, *J* = 10.0, 1.7 Hz, *CHH*-6), 3.10 (dd, 1H, *J* = 10.0, 6.2 Hz, *CHH*-6), 1.50 (s, 3H, CH₃ isopropylidene), 1.35 (s, 3H, CH₃ isopropylidene), 0.15 (s, 9H, 3x CH₃ TMS); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 160.1 (C_q PMB), 145.0 (C_q Trt), 131.2 (C_q PMB), 130.4, 129.6, 128.8, 128.1, 114.4 (Ar), 110.1 (C_q isopropylidene), 101.7 (C \equiv C), 96.3 (C-1), 92.4 (C \equiv C), 87.3 (C_q Trt), 79.5 (C-3), 76.5 (C-2), 76.3 (C-4), 73.1 (CH₂ PMB), 69.7 (C-5), 64.1 (CH₂-6), 55.8 (CH₃ PMB), 55.3 (CH₂ propargyl), 28.3, 26.6 (CH₃ isopropylidene), -0.1 (CH₃ TMS); FT-IR (neat, cm⁻¹): 3059, 3033, 2988, 2934, 2179, 1613, 1587, 1514, 1491, 1449, 1381, 1372, 1302, 1248, 1221, 1171, 1146, 1082, 1031, 999, 966, 946, 899, 846, 763, 747, 708, 633, 588, 551, 522, 475; HRMS: [M+Na]⁺ calcd. for C₄₂H₄₈O₇SiNa: 715.3067, found 715.3068.

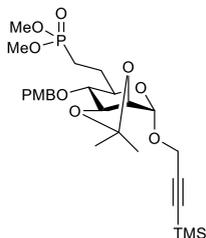
Trimethylsilylpropargyl 2,3-di-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl- α -*D*-mannopyranoside (**16**)



A mixture of compound **15** (42.8 g, 61.3 mmol, 1.0 eq.), *p*-toluenesulfonic acid (4.76 g, 24.5 mmol, 0.4 eq.) in DCM/MeOH (2/1 v/v, 0.42 L) was stirred at room temperature for 1.5 hours, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was quenched by the addition of sat. aq. NaHCO₃ (0.50 L) and extracted with EtOAc (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The obtained residue was dissolved in a mixture of 2,2-dimethoxypropane/DCM (4/1, 0.35 L). *p*-Toluenesulfonic acid (1.17 g, 6.02 mmol, 0.1 eq.) was added and the mixture was

stirred at room temperature for 25 minutes. The reaction mixture was quenched with sat. aq. NaHCO₃ (0.50 L) and extracted with EtOAc (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The obtained intermediates were dissolved in EtOAc (0.28 L) and cooled to 0°C. 1 M HCl (30 mL) was added and the mixture was allowed to warm-up to room temperature. After stirring for 1 hour, TLC analysis showed complete conversion and the reaction was quenched with sat. aq. NaHCO₃ (0.50 L) at 0°C, followed by extraction with EtOAc (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (20→60% Et₂O in pentane) yielded the title compound (26.0 g, 57.8 mmol, 94%) as an oil. R_f: 0.47 (4/1 pentane/EtOAc); [α]_D²⁵ +79.6° (c = 0.57, CHCl₃); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.31 – 7.23 (m, 2H, Ar), 6.90 – 6.81 (m, 2H, Ar), 5.23 (s, 1H, H-1), 4.82 (d, 1H, J = 11.1 Hz, CHH PMB), 4.56 (d, 1H, J = 11.1 Hz, CHH PMB), 4.36 – 4.29 (m, 1H, H-3), 4.23 (d, 2H, J = 2.3 Hz, CH₂ propargyl), 4.18 (d, 1H, J = 6.2 Hz, H-2), 3.86 – 3.81 (m, 1H, CHH-6), 3.80 (s, 3H, CH₃ PMB), 3.76 – 3.68 (m, 1H, CHH-6), 3.64 – 3.57 (m, 1H, H-5), 3.56 – 3.49 (m, 1H, H-4), 1.94 (br, 1H, OH), 1.52 (s, 3H, CH₃ isopropylidene), 1.38 (s, 3H, CH₃ isopropylidene), 0.17 (s, 9H, 3x CH₃ TMS); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 159.4, 130.3 (C_q PMB), 129.8, 113.9 (Ar), 109.6 (C_q isopropylidene), 100.1 (C≡C), 95.7 (C-1), 92.3 (C≡C), 78.8 (C-3), 75.8 (C-2), 75.7 (C-4), 72.7 (CH₂ PMB), 69.0 (C-5), 62.6 (CH₂-6), 55.4 (CH₃ PMB), 55.1 (CH₂ propargyl), 28.1, 26.5 (CH₃ isopropylidene), -0.1 (CH₃ TMS); FT-IR (neat, cm⁻¹): 3493, 2936, 2178, 1613, 1587, 1514, 1457, 1372, 1302, 1246, 1220, 1171, 1142, 1075, 1033, 994, 965, 948, 914, 842, 788, 760, 737, 701, 650, 637, 580, 515; HRMS: [M+Na]⁺ calcd. for C₂₃H₃₄O₇SiNa: 473.1971, found 473.1968.

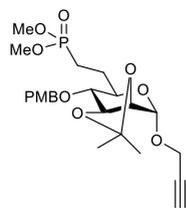
Trimethylsilylpropargyl 6-deoxy-2,3-di-O-isopropylidene-4-O-p-methoxybenzyl-6-dimethoxyphosphonomethyl- α -D-mannopyranoside (17)



Alcohol **16** (9.05 g, 20.1 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry DCM (0.10 L). The solution was cooled to -40°C, followed by the addition of pyridine (4.0 mL, 50.0 mmol, 2.5 eq.) and trifluoromethanesulfonic anhydride (5.1 mL, 30.3 mmol, 1.5 eq.). After stirring for 80 minutes at -40°C, the mixture was diluted with cold DCM and washed with cold sat. aq. CuSO₄ (1x), cold sat. aq. NaHCO₃ (1x) and cold brine (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo* at 30°C. The obtained triflate was co-evaporated with toluene (2x) under an argon atmosphere and used without further purification. Dimethyl methylphosphonate (10 g) was co-evaporated with toluene (2x) under an argon atmosphere. 3.0 equivalents of dimethyl methylphosphonate (7.65 g, 60.8 mmol) were dissolved in dry THF (40 mL) and cooled to -70°C, followed by the addition of *n*-butyllithium (1.6 M in hexane, 38 mL, 60.8 mmol, 3.0 eq.). After 1.5 hours, a solution of the obtained triflate in dry THF (2x 17 mL) was added via a canula over 30 minutes. The reaction mixture was allowed to warm-up to -50°C over 2 hours. The reaction was subsequently quenched by the addition of a solution of AcOH in THF (60 mL, 2 M, 6.0 eq.) and diluted with EtOAc. The organic layer was washed with sat. aq. NaHCO₃ (1x), dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by

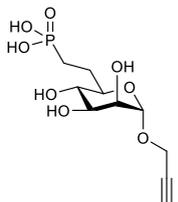
column chromatography (5→50% acetone in DCM + 0.1% Et₃N) afforded the title compound (7.31 g, 13.1 mmol, 65%) as an oil. *R*_f: 0.55 (4/1 DCM/acetone); [α]_D²⁵ +70.6° (*c* = 0.49, CHCl₃); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.04 (d, 2H, *J* = 8.6 Hz, Ar), 6.63 (d, 2H, *J* = 8.6 Hz, Ar), 4.94 (s, 1H, H-1), 4.59 (d, 1H, *J* = 11.3 Hz, CHH PMB), 4.31 (d, 1H, *J* = 11.3 Hz, CHH PMB), 4.03 (t, 1H, *J* = 6.3 Hz, H-3), 3.98 (d, 2H, *J* = 10.6 Hz, CH₂ propargyl), 3.95 – 3.90 (m, 1H, H-2), 3.54 (s, 3H, CH₃ PMB), 3.50 (s, 3H, OCH₃), 3.47 (s, 3H, OCH₃), 3.34 – 3.24 (m, 1H, H-5), 3.00 (dd, 1H, *J* = 9.9, 7.0 Hz, H-4), 1.97 – 1.83 (m, 1H, CHH-6), 1.73 – 1.58 (m, 1H, CHH-7), 1.58 – 1.38 (m, 2H, CHH-6, CHH-7), 1.28 (s, 3H, CH₃ isopropylidene), 1.14 (s, 3H, CH₃ isopropylidene), -0.04 (s, 9H, 3x CH₃ TMS); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 158.8, 129.7 (C_q Ar), 129.2, 113.2 (Ar), 108.7 (C_q isopropylidene), 99.8 (C≡C), 94.8 (C-1), 91.4 (C≡C), 78.1 (C-3), 77.6 (C-4), 75.2 (C-2), 71.7 (CH₂ PMB), 67.6, 67.4 (C-5), 54.6 (CH₃ PMB), 54.3 (CH₂ propargyl), 51.8, 51.7, 51.7, 51.7 (OCH₃), 27.6, 25.8 (CH₃ isopropylidene), 24.0 (CH₂-6), 20.5, 19.1 (CH₂-7), -0.7 (CH₃ TMS); ³¹P-APT NMR (CDCl₃, 162 MHz): δ 35.0; FT-IR (neat, cm⁻¹): 2176, 1612, 1586, 1515, 1458, 1372, 1302, 1245, 1220, 1170, 1140, 1062, 1029, 916, 842, 808, 760, 736, 701, 636, 584, 523, 486; HRMS: [M+Na]⁺ calcd. for C₂₆H₄₁O₉PSiNa: 579.2155, found 579.2158.

Propargyl 6-deoxy-2,3-di-O-isopropylidene-4-O-p-methoxybenzyl-6-dimethoxyphosphonomethyl- α -D-mannopyranoside (18)

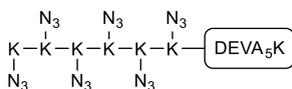


TBAF (1 M in THF, 24.5 mL, 2.0 eq.) was added to a solution of compound **17** (6.86 g, 12.1 mmol, 1.0 eq.) in THF (60 mL) at 0°C. After stirring for 15 minutes, TLC analysis showed complete conversion of the starting material. The mixture was diluted with EtOAc and washed with sat. aq. NaHCO₃ (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (5→15% acetone in DCM + 0.1% Et₃N)

gave compound **18** in quantitative yield (5.94 g). *R*_f: 0.44 (4/1 DCM/acetone); [α]_D²⁵ +67.9° (*c* = 1.3, CHCl₃); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.25 (m, 2H, Ar), 6.84 (d, 2H, *J* = 8.0 Hz, Ar), 5.16 (s, 1H, H-1), 4.79 (d, 1H, *J* = 11.1 Hz, CHH PMB), 4.50 (d, 1H, *J* = 11.1 Hz, CHH PMB), 4.27 – 4.21 (m, 1H, H-3), 4.18 (dd, 2H, *J* = 8.1, 2.4 Hz, CH₂ propargyl), 4.12 (d, 1H, *J* = 5.6 Hz, H-2), 3.77 (s, 3H, CH₃ PMB), 3.71 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 3.54 – 3.46 (m, 1H, H-5), 3.21 (dd, 1H, *J* = 9.8, 7.0 Hz, H-4), 2.44 (t, 1H, *J* = 2.4 Hz, CH propargyl), 2.17 – 2.05 (m, 1H, CHH-6), 1.97 – 1.79 (m, 1H, CHH-7), 1.79 – 1.60 (m, 2H, CHH-6, CHH-7), 1.48 (s, 3H, CH₃ isopropylidene), 1.34 (s, 3H, CH₃ isopropylidene); ¹³C NMR (101 MHz, CDCl₃) δ 159.3, 130.2 (C_q Ar), 129.8, 113.8 (Ar), 109.4 (C_q isopropylidene), 95.4 (C-1), 78.6 (C-3), 78.5 (C_q propargyl), 78.4 (C-4), 75.7 (C-2), 75.3 (CH propargyl), 72.5 (CH₂ PMB), 68.3, 68.1 (C-5), 55.3 (CH₃ PMB), 54.2 (CH₂ propargyl), 52.4, 52.4 (OCH₃), 28.1, 26.3 (CH₃ isopropylidene), 24.6 (CH₂-6), 21.1, 19.7 (CH₂-7); ³¹P-APT NMR (CDCl₃, 162 MHz): δ 35.2; FT-IR (neat, cm⁻¹): 3280, 2936, 1612, 1514, 1458, 1373, 1302, 1244, 1221, 1171, 1140, 1064, 1031, 916, 853, 810, 637, 591, 521; HRMS: [M+Na]⁺ calcd. for C₂₃H₃₃O₉PNa: 507.1760, found 507.1760.

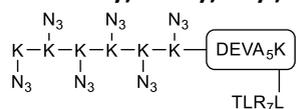
Propargyl 6-deoxy-6-phosphonomethyl- α -D-mannopyranoside (9)


Compound **18** (1.0 g, 2.1 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry MeCN (25 mL). Pyridine (1.8 mL, 22 mmol, 11 eq.) was added and the solution was cooled to 0°C. TMSBr (5.6 mL, 42 mmol, 20 eq.) was added and a glass stopper was put on the flask. After stirring at 0°C for 2 hours, the mixture was quenched with pyridine and diluted with H₂O. The obtained mixture was concentrated *in vacuo* and co-evaporated with dioxane (2x). The residue was dissolved in EtOAc and washed with brine (2x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The obtained intermediate (*R*_f = 0.14 (MeOH/DCM: 2/3 v/v) was dissolved in a mixture of AcOH/Milli-Q H₂O (1/1, v/v, 25 mL) and heated to 90°C for 8 hours, after which it was cooled to room temperature. The reaction mixture was diluted with Milli-Q H₂O and concentrated *in vacuo*. After co-evaporation with Milli-Q H₂O (5x), the residue was dissolved in Milli-Q H₂O, washed with DCM (4x) and concentrated *in vacuo*. Lyophilization yielded the title compound (0.50 g, 1.7 mmol, 81%) as a white solid. *R*_f: 0.40 (3/2 DCM/MeOH); ¹H NMR (D₂O, 500 MHz, HH-COSY, HSQC): δ 4.97 (d, 1H, *J* = 1.7 Hz, H-1), 4.36 – 4.23 (m, 2H, CH₂ propargyl), 3.92 (dd, 1H, *J* = 3.4, 1.8 Hz, H-2), 3.73 (dd, 1H, *J* = 9.3, 3.5 Hz, H-3), 3.60 – 3.47 (m, 2H, H-4, H-5), 2.90 (t, 1H, *J* = 2.4 Hz, CH propargyl), 2.13 – 2.00 (m, 1H, CHH-6), 1.90 – 1.75 (m, 1H, CHH-7), 1.75 – 1.52 (m, 2H, CHH-6, CHH-7); ¹³C-APT NMR (D₂O, 126 MHz, HSQC): δ 98.7 (C-1), 78.9 (C_q propargyl), 76.3 (CH propargyl), 73.0, 72.9 (C-5), 70.4 (C-3), 70.0 (C-4), 70.0 (C-2), 54.6 (CH₂ propargyl), 25.2 (CH₂-6), 24.4, 23.3 (CH₂-7); ³¹P-APT NMR (D₂O, 162 MHz, HMBC): δ 26.1; HRMS: [M+Na]⁺ calcd. for C₁₀H₁₇O₈PNa: 319.0559, found 319.0566.

Ac-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-NH₂ (20)


100 μmol of crude H-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Lys(MMT)-Tentagel S Ram was elongated with Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃) with standard HCTU/Fmoc chemistry concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was treated with a mixture of Ac₂O/DMF/DIPEA (2x 2.0 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (4.0 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (4.0 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **20** (4.6 mg, 1.3 μmol, 1%) was obtained as a white solid. LC-MS: *R*_t = 6.91 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: *m/z* 1757.0 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₅₀H₂₄₉N₅₃O₄₅: 1756.44071, found 1756.44051.

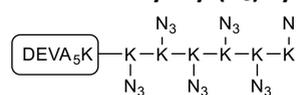
Ac-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-NH₂ (21)



50 μmol of crude H-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-

Lys(MMT)-Tentagel S Ram was elongated with Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃) with standard HCTU/Fmoc chemistry concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was treated with a mixture of Ac₂O/DMF/DIPEA (2x 1.0 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 15 mL) over 5 minutes. The resin was washed subsequently with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL), DCM (3x) and DMF (3x). A solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (41 mg, 106 μmol , 2.1 eq.) and HCTU (42 mg, 101 μmol , 2.0 eq.) in DMF (1.0 mL) and DIPEA (35 μL , 201 μmol , 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (1.0 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (47 mg, 102 μmol , 2.0 eq.) and HCTU (42 mg, 100 μmol , 2.0 eq.) and DIPEA (35 μL , 200 μmol , 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (2.0 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (2.0 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **21** (4.0 mg, 1.1 μmol , 2%) was obtained as a white solid. LC-MS: Rt = 7.13 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1999.8 [M+H]²⁺; HRMS: [M+H]³⁺ calcd. for C₁₇₃H₂₇₈N₅₉O₅₁: 1332.69857, found 1332.69879.

Ac-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-NH₂ (23)

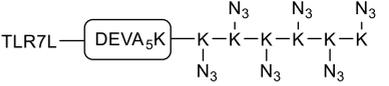


100 μmol of crude H-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-

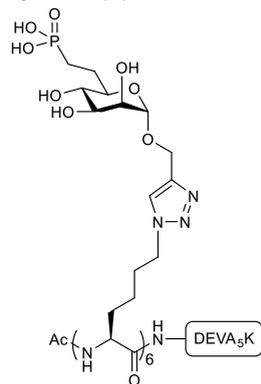
Lys(MMT)-Tentagel S Ram was elongated with Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃) with standard HCTU/Fmoc chemistry concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was treated with a mixture of Ac₂O/DMF/DIPEA (2x 2.0 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (4.0 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (4.0 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **23** (17.4 mg, 5.5 μmol , 6%) was obtained as a white solid. LC-MS: Rt = 7.11 min (C18 Gemini, 10 - 90% MeCN, 11

min run); ESI-MS: m/z 1757.4 $[M+H]^{2+}$; HRMS: $[M+H]^{2+}$ calcd. for $C_{150}H_{249}N_{53}O_{45}$: 1756.44071, found 1756.44041.

(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzamide)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-NH₂ (24)

TLR7L— 50 μ mol of crude H-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT)-Tentagel S Ram was elongated with Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃) with standard HCTU/Fmoc chemistry concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). The resin was washed with DMF (5x), followed by the addition of a solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (41 mg, 106 μ mol, 2.1 eq.) and HCTU (42 mg, 101 μ mol, 2.0 eq.) in DMF (1.0 mL) and DIPEA (35 μ L, 201 μ mol, 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (1.0 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((tert-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (47 mg, 102 μ mol, 2.0 eq.) and HCTU (42 mg, 100 μ mol, 2.0 eq.) and DIPEA (35 μ L, 200 μ mol, 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (2.0 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (2.0 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **24** (8.2 mg, 2.1 μ mol, 2%) was obtained as a white solid. LC-MS: Rt = 7.11 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1978.7 $[M+H]^{2+}$; HRMS: $[M+H]^{3+}$ calcd. for $C_{171}H_{276}N_{59}O_{50}$: 1318.69505, found 1318.69517.

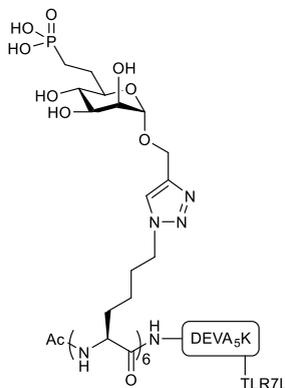
Ac-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-NH₂ (1)



$[M+H]^{3+}$ calcd. for $C_{210}H_{352}N_{53}O_{93}P_6$: 1763.42844, found 1763.42972.

Azide **20** (4.24 mg, 1.21 μ mol, 1.0 eq.) was dissolved in DMSO (641 μ L), followed by the addition of a solution of compound **9** in DMSO (91.1 mM, 120 μ L, 10.9 μ mol, 9.0 eq.). 20 mM Tris/150 mM NaCl buffer (787 μ L) and click mix (24 μ L, 26 mg/mL CuSO₄ in H₂O, 120 mg/mL NaAsc in H₂O, 52 mg/mL TBTA in DMSO) were added. The reaction vessel was shaken for 3 hours, after which LC-MS analysis showed complete conversion. The reaction was quenched by the addition of EDTA (6.6 μ L, 0.5 M in H₂O) and mixed for 15 minutes. After purification by RP-HPLC and lyophilisation, compound **1** (0.3 mg, 64 nmol, 5%) was obtained as a white solid. LC-MS: Rt = 4.56 min (C18 Gemini, 10 - 90% MeCN, 11 min run); HRMS:

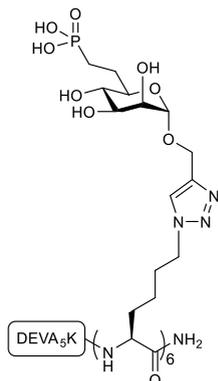
Ac-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-NH₂ (2)



Azide **21** (3.7 mg, 0.93 μmol , 1.0 eq.) was dissolved in DMSO (491 μL), followed by the addition of a solution of compound **9** in DMSO (91.1 mM, 92 μL , 8.33 μmol , 9.0 eq.). 20 mM Tris/150 mM NaCl buffer (604 μL) and click mix (18 μL , 26 mg/mL CuSO_4 in H_2O , 120 mg/mL NaAsc in H_2O , 52 mg/mL TBTA in DMSO) were added. The reaction vessel was shaken for 3 hours, after which LC-MS analysis showed complete conversion. The reaction was quenched by the addition of EDTA (5.0 μL , 0.5 M in H_2O) and mixed for 15 minutes. After purification by RP-HPLC and lyophilisation, compound **2** (1.0 mg, 165 nmol, 18%) was obtained as a white solid. LC-MS: Rt = 4.89 min (C18 Gemini, 10 - 90% MeCN, 11 min run); HRMS: $[\text{M}+\text{H}]^{3+}$

calcd. for $\text{C}_{233}\text{H}_{380}\text{N}_{59}\text{O}_{99}\text{P}_6$: 1924.83078, found 1924.83260.

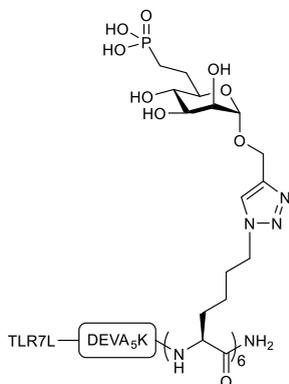
Ac-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-NH₂ (3)



Azide **23** (3.2 mg, 0.92 μmol , 1.0 eq.) was dissolved in DMSO (480 μL), followed by the addition of a solution of compound **9** in DMSO (91.1 mM, 100 μL , 9.11 μmol , 9.0 eq.). 20 mM Tris/150 mM NaCl buffer (600 μL) and click mix (18 μL , 26 mg/mL CuSO_4 in H_2O , 120 mg/mL NaAsc in H_2O , 52 mg/mL TBTA in DMSO) were added. The reaction vessel was shaken for 2.5 hours, after which LC-MS analysis showed complete conversion. The reaction was quenched by the addition of EDTA (10 μL , 0.5 M in H_2O) and mixed for 15 minutes. After purification by RP-HPLC and lyophilisation, compound **3** (0.9 mg, 0.17 μmol , 18%) was obtained as a white solid. LC-MS: Rt = 4.45 min (C18 Gemini, 10 - 90% MeCN, 11 min run); HRMS: $[\text{M}+\text{H}]^{3+}$ calcd. for

$\text{C}_{210}\text{H}_{352}\text{N}_{53}\text{O}_{93}\text{P}_6$: 1763.42844, found 1763.43069.

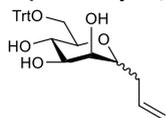
(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-Lys(M6Po)-NH₂ (4)



Azide **24** (7.4 mg, 1.86 μmol , 1.0 eq.) was dissolved in DMSO (989 μL), followed by the addition of a solution of compound **9** in DMSO (91.1 mM, 184 μL , 16.8 μmol , 9.0 eq.). 20 mM Tris/150 mM NaCl buffer (1.21 mL) and click mix (36 μL , 26 mg/mL CuSO₄ in H₂O, 120 mg/mL NaAsc in H₂O, 52 mg/mL TBTA in DMSO) were added. The reaction vessel was shaken for 3 hours, after which LC-MS analysis showed complete conversion. The reaction was quenched by the addition of EDTA (10 μL , 0.5 M in H₂O) and mixed for 15 minutes. After purification by RP-HPLC and lyophilisation, compound **4** (3.3 mg, 0.58 μmol , 31%) was obtained as a white solid. LC-MS: Rt = 4.71 min (C18 Gemini, 10 - 90% MeCN, 11 min run); HRMS: [M+H]⁴⁺

calcd. for C₂₃₁H₃₇₉N₅₉O₉₈P₆: 1433.37226, found 1433.37178.

3-(6-O-trityl- α/β -D-mannopyranosyl)-1-propene (25 + 26)

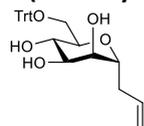


D-Mannose (52.3 g, 302 mmol, 1.0 eq.) was dissolved in pyridine (0.43 L) and the reaction mixture was cooled to 0°C. Acetic anhydride (0.20 L, 2.1 mol, 7.0 eq.) and DMAP (3.69 g, 30.2 mmol, 0.1 eq.) were added.

After stirring for 25 minutes, the solution was allowed to warm-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 (5x). The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was co-evaporated with toluene (2x), 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 (2x) 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 eq.), BF₃·OEt₂ (0.19 L, 1.5 mol, 4.9 eq.) and TMSOTf (11 mL, 62 mmol, 0.2 eq.) were added, respectively. After stirring for 30 minutes, the reaction mixture was allowed to warm-up to room temperature and stirring continued for 3 days. The reaction mixture was cooled to 0°C, diluted with EtOAc and quenched with Et₃N to pH 8. The organic layer was washed with sat. aq. NaHCO₃ (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→60% Et₂O 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 eq.) 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-

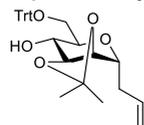
evaporating with dioxane (1x) under an argon atmosphere, the residue was dissolved in DMF (0.77 L). Trityl chloride (100 g, 348 mmol, 1.5 eq.) and Et₃N (80 mL, 0.57 mol, 2.5 eq.) were added and the suspension was heated to 60°C. After stirring for 2.5 h, TLC analysis showed complete conversion of the starting material. The reaction mixture was cooled to room temperature, diluted with H₂O and extracted with EtOAc (2x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. After purification by column chromatography (20→40% EtOAc in pentane) compounds **25** and **26** (74.5 g, 167 mmol, 55% over 4 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 **26** for analysis.

3-(6-*O*-trityl-α-*D*-mannopyranosyl)-1-propene (**26**)



A solution of compound **25** and **26** (31.4 g, 70.3 mmol, 1.0 eq., α/β: 4.2/1) and *N*-bromosuccinimide (6.3, 35 mmol, 0.5 eq.) in THF (0.70 L) was stirred for 2 h, after which LC-MS analysis showed complete conversion of the β-mannose. The mixture was quenched by the addition of sat. aq. Na₂S₂O₃ (0.50 L). After stirring for an additional 10 minutes, the mixture was further diluted with sat. aq. Na₂S₂O₃ (0.25 L) and extracted with DCM (1x). The organic layer was washed with sat. aq. NaHCO₃ (1x), dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→60% acetone in DCM + 0.1% Et₃N) yielded the title compound (23.1 g, 51.7 mmol, 91%) as a white foam. R_f: 0.42 (7/3 DCM/acetone); [α]_D²⁵ -18.2° (*c* = 0.72, CHCl₃); ¹H NMR (CD₃CN, 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, CH₂-CH=CH₂), 5.26 – 5.11 (m, 2H, CH₂-CH=CH₂), 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, CH₂-6, OH), 3.05 (t, 2H, *J* = 4.6 Hz, 2x OH), 2.60 – 2.51 (m, 1H, CHH-CH=CH₂), 2.35 – 2.27 (m, 1H, CHH-CH=CH₂); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 145.3 (C_q Trt), 136.3 (CH₂-CH=CH₂), 129.6, 128.8, 128.0 (Ar), 117.3 (CH₂-CH=CH₂), 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 (CH₂-6), 34.4 (CH₂-CH=CH₂); FT-IR (neat, cm⁻¹): 3402, 3060, 2928, 1708, 1643, 1597, 1490, 1449, 1221, 1073, 1033, 989, 901, 827, 765, 748, 701, 633, 529; LC-MS: Rt = 7.15 min (Gemini C₁₈, 10 – 90% MeCN, 11 min run); HRMS: [M+Na]⁺ calcd. for C₂₈H₃₀O₅Na: 469.1991, found 496.1991.

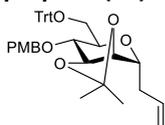
3-(2,3-*O*-isopropylidene-6-*O*-trityl-α-*D*-mannopyranosyl)-1-propene (**27**)



Compound **26** (43.9 g, 98.3 mmol, 1.0 eq.) 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 eq.) 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 Et₃N (7 mL), diluted with DCM and washed with a mixture of sat. aq. NaHCO₃/brine (1/1, v/v, 1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→50% Et₂O in pentane + 0.1% Et₃N) gave compound **27** (44.6 g, 89.1 mmol, 91%) as a clear oil. R_f: 0.24 (7/3 pentane/Et₂O); [α]_D²⁵ -15.7° (*c* = 0.19, CHCl₃); ¹H NMR (CD₃CN, 400 MHz, HH-COSY, HSQC): δ 7.50 – 7.44 (m, 6H, Ar), 7.36 – 7.30 (m, 6H, Ar), 7.29 – 7.23 (m, 3H, Ar), 6.02 – 5.91 (m, 1H, CH₂-CH=CH₂),

5.22 – 5.07 (m, 2H, CH₂-CH=CH₂), 4.07 – 3.98 (m, 2H, H-2, H-3), 3.92 – 3.86 (m, 1H, H-1), 3.73 – 3.66 (m, 1H, H-4), 3.59 – 3.53 (m, 1H, H-5), 3.25 – 3.20 (m, 2H, CHH-6, OH), 3.16 (dd, 1H, *J* = 10.0, 5.7 Hz, CHH-6), 2.40 (t, 2H, *J* = 6.9 Hz, CH₂-CH=CH₂), 1.39 (s, 3H, CH₃ isopropylidene), 1.30 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 145.2 (C_q Trt), 135.9 (CH₂-CH=CH₂), 129.6, 128.8, 128.1 (Ar), 117.5 (CH₂-CH=CH₂), 109.8 (C_q isopropylidene), 87.2 (C_q Trt), 79.3 (C-3), 77.1 (C-2), 74.4 (C-5), 73.6 (C-1), 69.6 (C-4), 64.5 (CH₂-6), 37.7 (CH₂-CH=CH₂), 28.1, 25.9 (CH₃ isopropylidene); FT-IR (neat, cm⁻¹): 2936, 1612, 1514, 1458, 1373, 1302, 1244, 1221, 1171, 1140, 1064, 1031, 916, 853, 810, 637, 591, 521; HRMS: [M+Na]⁺ calcd. for C₃₁H₃₄O₅Na: 509.2304, found 509.2305.

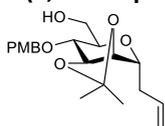
3-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-6-*O*-trityl- α -D-mannopyranosyl)-1-propene (28)



After co-evaporating with toluene (2x) under an argon atmosphere, compound **27** (49.9 g, 102.5 mmol, 1.0 eq.) 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 eq.) and *p*-methoxybenzyl chloride (17.0 mL,

125 mmol, 1.2 eq.) were added and the suspension was allowed to warm-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 mixture was quenched by the addition of MeOH at 0°C, diluted with Et₂O and washed with H₂O (2x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (5→20% Et₂O in pentane + 0.1% Et₃N) yielded the title compound (60.3 g, 99.4 mmol, 97%) as a clear oil. R_f: 0.63 (pentane/Et₂O); [α]_D²⁵ +12.7° (*c* = 0.67, CHCl₃); ¹H NMR (CD₃CN, 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, CH₂-CH=CH₂), 5.24 – 5.10 (m, 2H, CH₂-CH=CH₂), 4.62 (d, 1H, *J* = 11.0 Hz, CHH PMB), 4.31 – 4.21 (m, 2H, H-3, CHH 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, CH₃ PMB), 3.70 – 3.60 (m, 2H, H-4, H-5), 3.31 (dd, 1H, *J* = 9.9, 2.1 Hz, CHH-6), 3.08 (dd, 1H, *J* = 9.8, 5.0 Hz, CHH-6), 2.42 (t, 2H, *J* = 6.9 Hz, CH₂-CH=CH₂), 1.46 (s, 3H, CH₃ isopropylidene), 1.34 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CD₃CN, 101 MHz, HSQC): δ 160.1 (C_q PMB), 145.1 (C_q Trt), 135.9 (CH₂-CH=CH₂), 131.3 (C_q PMB), 130.5, 129.6, 128.8, 128.1 (Ar), 117.6 (CH₂-CH=CH₂), 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 (CH₂ PMB), 73.0 (C-5), 64.4 (CH₂-6), 55.8 (CH₃ PMB), 37.4 (CH₂-CH=CH₂), 28.1, 26.0 (CH₃ 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 C₃₉H₄₂O₆Na: 629.2879, found 629.2881.

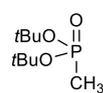
3-(2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl- α -D-mannopyranosyl)-1-propene (29)

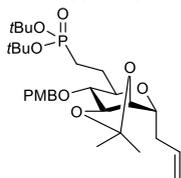


A solution of compound **28** (60.3 g, 99.4 mmol, 1.0 eq.) and *p*-toluenesulfonic acid (7.70 g, 39.7 mmol, 0.4 eq.) in DCM/MeOH (2/1 v/v, 0.66 L) was stirred for one hour, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was quenched by the addition of sat. aq. NaHCO₃ (0.50 L) and extracted with EtOAc (3x).

The combined organic layers were dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The obtained residue was dissolved in a mixture of 2,2-dimethoxypropane/DCM (4/1, 0.50 L). *p*-Toluenesulfonic acid (1.93 g, 9.94 mmol, 0.1 eq.) was added and the mixture was stirred for 5 minutes, after which it was quenched with sat. aq. NaHCO_3 (0.50 L) and extracted with EtOAc (1x). The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The obtained intermediates were dissolved in EtOAc (0.45 L) and cooled to 0°C . 1 M HCl (45 mL) was added and after 30 minutes the mixture was allowed to warm-up to room temperature. After stirring for an additional 30 minutes, TLC analysis showed complete conversion and the reaction was quenched with sat. aq. NaHCO_3 (0.50 L) at 0°C . The organic layer was separated and the aqueous layer was extracted with EtOAc (1x). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated *in vacuo*. After purification by column chromatography (20→70% Et₂O in pentane) the title compound (35.2 g, 96.6 mmol, 97%) was obtained as a clear oil. R_f : 0.53 (2/3 pentane/Et₂O); $[\alpha]_D^{25} +29.8^\circ$ ($c = 1.0$, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 7.30 – 7.24 (m, 2H, Ar), 6.90 – 6.84 (m, 2H, Ar), 5.88 – 5.76 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.17 – 5.09 (m, 2H, $\text{CH}_2\text{-CH=CH}_2$), 4.81 (d, 1H, $J = 11.1$ Hz, *CHH* PMB), 4.55 (d, 1H, $J = 11.1$ Hz, *CHH* PMB), 4.30 (t, 1H, $J = 6.7$ Hz, H-3), 4.07 (dd, 1H, $J = 6.4$, 4.5 Hz, H-2), 3.96 – 3.90 (m, 1H, H-1), 3.80 (s, 3H, CH_3 PMB), 3.73 (dd, 1H, $J = 11.5$, 3.0 Hz, *CHH*-6), 3.67 – 3.54 (m, 2H, H-4, *CHH*-6), 3.53 – 3.45 (m, 1H, H-5), 2.43 – 2.31 (m, 2H, $\text{CH}_2\text{-CH=CH}_2$), 1.97 (br, 1H, OH), 1.52 (s, 3H, CH_3 isopropylidene), 1.38 (s, 3H, CH_3 isopropylidene); $^{13}\text{C-APT NMR}$ (CD_3CN , 101 MHz, HSQC): δ 159.5 (C_q PMB), 133.9 ($\text{CH}_2\text{-CH=CH}_2$), 130.2 (C_q PMB), 129.9 (Ar), 118.0 ($\text{CH}_2\text{-CH=CH}_2$), 113.9 (Ar), 109.4 (C_q isopropylidene), 78.7 (C-3), 76.3 (C-2), 75.9 (C-4), 72.7 (C-1), 72.7 (CH_2 PMB), 71.9 (C-5), 62.9 (CH_2 -6), 55.4 (CH_3 PMB), 36.4 ($\text{CH}_2\text{-CH=CH}_2$), 27.9, 25.9 (CH_3 isopropylidene); FT-IR (neat, cm^{-1}): 3480, 2985, 2935, 1642, 1612, 1587, 1514, 1457, 1381, 1302, 1245, 1218, 1168, 1139, 1062, 1034, 992, 918, 863, 821, 638, 582, 515; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{20}\text{H}_{28}\text{O}_6\text{Na}$: 387.1784, found 387.1786.

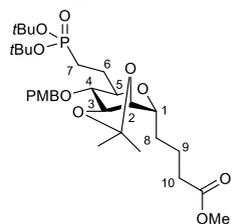
Di-*tert*-butyl methylphosphonate (30)

 To a solution of di-*tert*-butyl phosphite (26 g, 0.13 mol, 1.0 eq.) in THF (0.25 L) was added slowly a solution of *n*-butyllithium (1.6 M in hexane, 99 ml, 0.16 mol, 1.2 eq.) at -78°C under an argon atmosphere. After stirring for 1 h, a solution of iodomethane in THF (2 M, 85 ml, 0.17 mol, 1.3 eq.) was added and the reaction was allowed to warm up to room temperature overnight. Concentration *in vacuo* and purification by column chromatography (0→50% EtOAc in pentane) yielded the title compound as a slightly yellow liquid (21.7 g, 104 mmol, 80%). $^1\text{H NMR}$ (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 1.50 (s, 18H, 6x CH_3 *t*Bu), 1.42 (d, 3H, $J = 17.4$ Hz, CH_3); $^{13}\text{C NMR}$ (CDCl_3 , 101 MHz, HSQC): δ 81.4, 81.3 (C_q *t*Bu), 30.4 (CH_3 *t*Bu), 17.1, 15.7 (CH_3); $^{31}\text{P NMR}$ (CDCl_3 , 162 MHz): δ 21.9; FT-IR (neat, cm^{-1}): 2980, 1370, 1310, 1257, 1173, 1040, 983, 771; HRMS: $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_9\text{H}_{21}\text{O}_3\text{PNa}$: 231.11205, found 231.11206.

3-(6-deoxy-2,3-O-isopropylidene-4-O-p-methoxybenzyl-6-di-tert-butoxyphosphonomethyl- α -D-mannopyranosyl)-1-propene (31)

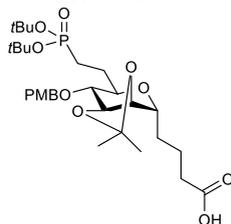
Alcohol **29** (1.11 g, 3.04 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere and dissolved in dry DCM (15 mL). The solution was cooled to -40°C , followed by the addition of pyridine (0.6 mL, 7.4 mmol, 2.4 eq.) and trifluoromethanesulfonic anhydride (0.8 mL, 4.8 mmol, 1.6 eq.). After stirring for one hour at -40°C , TLC analysis showed complete conversion of the starting

material. The reaction mixture was diluted with cold DCM and washed with cold sat. aq. CuSO_4 (1x), cold sat. aq. NaHCO_3 (1x) and cold brine (1x). The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo* at 30°C . The obtained triflate was co-evaporated with toluene (2x) under an argon atmosphere and used without further purification. Di-*tert*-butyl methylphosphonate **30** (4 g) was co-evaporated with toluene (2x) under an argon atmosphere. Di-*tert*-butyl methylphosphonate **30** (1.85 g, 8.88 mmol, 2.9 eq.) was dissolved in dry THF (6.0 mL) and cooled to -78°C , followed by the addition of *n*-butyllithium (1.6 M in hexane, 5.7 mL, 9.1 mmol, 3.0 eq.). After two hours, a solution of the crude triflate in dry THF (2x 2.5 mL) was added via a canula over 10 minutes. The reaction mixture was allowed to warm-up to -50°C over 2.5 hours. The reaction was subsequently quenched by the addition of a solution of AcOH in THF (36 M, 14 mL) and diluted with EtOAc. The organic layer was washed with sat. aq. NaHCO_3 (1x), dried over Na_2SO_4 , filtered and concentrated *in vacuo*. Purification by column chromatography (5 \rightarrow 25% acetone in DCM + 0.1% Et_3N) and size exclusions (DCM/MeOH: 1/1) gave the title compound (1.22 g, 2.20 mmol, 72%). R_f : 0.52 (9/1 DCM/acetone); $[\alpha]_D^{25} +0.87^{\circ}$ ($c = 0.11$, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz, HH-COSY, HSQC): δ 7.27 (d, 2H, $J = 4.2$ Hz, Ar), 6.88 – 6.82 (m, 2H, Ar), 5.88 – 5.73 (m, 1H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 5.14 – 5.02 (m, 2H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 4.80 (d, 1H, $J = 11.0$ Hz, CHH PMB), 4.55 (d, 1H, $J = 11.1$ Hz, CHH PMB), 4.23 (t, 1H, $J = 6.4$ Hz, H-3), 4.03 (dd, 1H, $J = 6.2$, 4.9 Hz, H-2), 3.85 – 3.76 (m, 4H, H-1, CH_3 PMB), 3.45 – 3.31 (m, 2H, H-4, H-5), 2.43 – 2.24 (m, 2H, $\text{CH}_2\text{-CH}=\text{CH}_2$), 2.07 – 1.79 (m, 2H, $\text{CH}_2\text{-6/7}$), 1.76 – 1.54 (m, 2H, $\text{CH}_2\text{-6/7}$), 1.47 (s, 21H, CH_3 isopropylidene, 6x CH_3 *t*Bu), 1.37 (s, 3H, CH_3 isopropylidene); ^{13}C -APT NMR (CDCl_3 , 101 MHz, HSQC): δ 159.4 (C_q PMB), 134.2 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 130.3 (C_q PMB), 129.9 (Ar), 117.7 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 113.9 (Ar), 109.3 (C_q isopropylidene), 81.4, 81.3 (C_q *t*Bu), 79.1 (C-4), 78.5 (C-3), 76.4 (C-2), 72.7 (CH_2 PMB), 72.3, 72.2 (C-5), 71.9 (C-1), 55.4 (CH_3 PMB), 36.7 ($\text{CH}_2\text{-CH}=\text{CH}_2$), 30.6, 30.5 (CH_3 *t*Bu), 28.0 (CH_3 isopropylidene), 27.4, 26.1, 26.1 ($\text{CH}_2\text{-6/7}$), 26.0 (CH_3 isopropylidene), 25.9 ($\text{CH}_2\text{-6/7}$); ^{31}P -APT NMR (CDCl_3 , 162 MHz): δ 24.1; FT-IR (neat, cm^{-1}): 2979, 2934, 1643, 1613, 1586, 1514, 1458, 1393, 1369, 1302, 1244, 1219, 1171, 1077, 1036, 972, 916, 867, 822, 737, 696, 639, 519, 486; HRMS: $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{29}\text{H}_{48}\text{O}_8\text{P}$: 555.30813, found 555.30800.

Methyl 4-(6-deoxy-2,3-O-isopropylidene-4-O-p-methoxybenzyl-6-di-tert-butoxyphosphonomethyl- α -D-mannopyranosyl)-butanoate (32)


Compound **31** (8.26 g, 14.9 mmol, 1.0 eq.) was co-evaporated with toluene (2x) under an argon atmosphere, before being dissolved in dry DCE (0.15 L). Methyl acrylate (3.8 mL, 42 mmol, 2.8 eq.), CuI (0.43 g, 2.3 mmol, 0.15 eq.) and Grubbs 2nd generation catalyst (0.76 g, 0.89 mmol, 0.06 eq.) were added and the flask was covered in aluminum foil. The suspension was heated to 60°C and stirred for 48 hours, after which it was concentrated *in vacuo* and co-evaporated with toluene (5x) under an argon atmosphere. The residue was dissolved in dry DCE (75 mL) and cooled to 0°C. Two empty balloons were placed on the flask, followed by the addition of ruthenium trichloride (0.59 g, 2.8 mmol, 0.19 eq.) and NaBH₄ (1.80 g, 47.6 mmol, 3.2 eq.). Methanol (12.0 mL, 0.3 mol, 20 eq.) was carefully added to the suspension over 30 minutes, after which the mixture was allowed to warm-up up to room temperature over 15 minutes. The mixture was subsequently heated to 45°C for 5.5 hours. The reaction mixture was cooled to room temperature, diluted with brine and extracted with DCM (3x). The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→30% acetone in pentane + 0.1% Et₃N) afforded compound **32** (6.59 g, 10.7 mmol, 72% over two steps). R_f: 0.73 (7/3 DCM/acetone); [α]_D²⁵ +15.5° (*c* = 0.53, CHCl₃); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.23 (d, 2H, *J* = 8.7 Hz, Ar), 6.83 – 6.77 (m, 2H, Ar), 4.75 (d, 1H, *J* = 11.1 Hz, CHH PMB), 4.50 (d, 1H, *J* = 11.1 Hz, CHH PMB), 4.17 (t, 1H, *J* = 6.4 Hz, H-3), 3.93 (dd, 1H, *J* = 6.2, 4.9 Hz, H-2), 3.73 (s, 3H, CH₃ PMB), 3.71 – 3.65 (m, 1H, H-1), 3.60 (s, 3H, OCH₃), 3.36 – 3.27 (m, 2H, H-4, H-5), 2.32 – 2.23 (m, 2H, CH₂-10), 2.02 – 1.89 (m, 1H, CHH-6/7/8/9), 1.89 – 1.69 (m, 2H, CH₂-6/7/8/9), 1.69 – 1.49 (m, 5H, CH₂-6/7/8/9), 1.47 (s, 3H, CH₃ isopropylidene), 1.45 – 1.40 (m, 18H, 6x CH₃ *t*Bu), 1.31 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 173.6 (C=O), 159.2, 130.2 (C_q PMB), 129.7, 113.7 (Ar), 109.1 (C_q isopropylidene), 81.4, 81.3, 81.3, 81.3 (C_q *t*Bu), 78.8 (C-4), 78.3 (C-3), 76.9 (C-2), 72.5 (CH₂ PMB), 71.9, 71.8 (C-5), 71.7 (C-1), 55.2 (CH₃ PMB), 51.5 (OCH₃), 33.5 (CH₂-10), 31.2 (CH₂-9), 30.4, 30.4, 30.3, 30.3 (CH₃ *t*Bu), 27.8 (CH₃ isopropylidene), 27.1, 26.0, 26.0 (CH₂-6 or CH₂-7), 25.8 (CH₃ isopropylidene), 25.6 (CH₂-6 or CH₂-7), 21.0 (CH₂-8); ³¹P-APT NMR (CDCl₃, 162 MHz): δ 24.5; FT-IR (neat, cm⁻¹): 2979, 2935, 1737, 1612, 1514, 1457, 1393, 1369, 1302, 1244, 1219, 1170, 1082, 1037, 1006, 974, 918, 866, 823, 791, 739, 695, 520, 487; HRMS: [M+Na]⁺ calcd. for C₃₁H₅₁O₁₀PNa: 637.3118, found 637.3124.

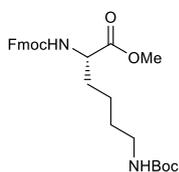
4-(6-deoxy-2,3-O-isopropylidene-4-O-p-methoxybenzyl-6-di-tert-butoxyphosphonomethyl- α -D-mannopyranosyl)-butanoic acid (**33**)



Methyl ester **32** (6.59 g, 10.7 mmol, 1.0 eq.) was dissolved in a mixture of THF/H₂O (4/1, v/v, 0.10 L). LiOH (1.35 g, 32 mmol, 3.0 eq.) was added and the mixture was stirred for 7 hours, after which TLC analysis showed complete conversion of the starting material. The reaction mixture was cooled to 0°C, acidified with 3 M HCl to pH = 4–5 and extracted with DCM (2x).

The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. The title compound was obtained in quantitative yield (6.65 g) and used without further purification. R_f: 0.28 (7/3 DCM/acetone); [α]_D²⁵ +20.0° (c = 0.18, CHCl₃); ¹H NMR (CDCl₃, 400 MHz, HH-COSY, HSQC): δ 7.27 (d, 2H, J = 8.7 Hz, Ar), 6.85 (d, 2H, J = 8.6 Hz, Ar), 4.80 (d, 1H, J = 11.1 Hz, CHH PMB), 4.54 (d, 1H, J = 11.1 Hz, CHH PMB), 4.22 (t, 1H, J = 6.6 Hz, H-3), 3.97 (t, 1H, J = 6.1 Hz, H-2), 3.79 (s, 3H, CH₃ PMB), 3.73 – 3.65 (m, 1H, H-1), 3.43 – 3.34 (m, 2H, H-4, H-5), 2.35 (t, 2H, J = 7.2 Hz, CH₂-10), 2.03 – 1.57 (m, 8H, CH₂-6/7/8/9), 1.56 – 1.42 (m, 21H, CH₃ isopropylidene, 6x CH₃ tBu), 1.36 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (CDCl₃, 101 MHz, HSQC): δ 176.1 (C=O), 159.4, 130.4 (C_q PMB), 129.9, 113.9 (Ar), 109.4 (C_q isopropylidene), 82.4, 82.3, 82.3, 82.2 (C_q tBu), 79.1 (C-4), 78.7 (C-3), 77.3 (C-2), 72.6 (CH₂ PMB), 72.4, 72.2 (C-5), 71.7 (C-1), 55.4 (CH₃ PMB), 34.1 (CH₂-10), 31.9 (CH₂-8), 30.5, 30.5, 30.5, 30.5 (CH₃ tBu), 27.9 (CH₃ isopropylidene), 27.0, 26.1, 26.1 (CH₂-6/7), 25.7 (CH₃ isopropylidene), 25.5 (CH₂-6/7), 21.2 (CH₂-9); ³¹P-APT NMR (CDCl₃, 162 MHz): δ 24.8; FT-IR (neat, cm⁻¹): 2980, 2935, 1724, 1613, 1586, 1514, 1458, 1394, 1370, 1302, 1245, 1217, 1158, 1081, 1037, 980, 918, 867, 822, 793, 735, 701, 661, 519, 486; HRMS: [M+Na]⁺ calcd. for C₃₀H₄₉O₁₀PNa: 623.2961, found 623.2971.

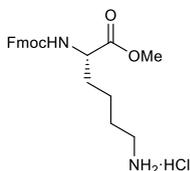
Fmoc-L-Lys(Boc)-OMe (**34**)



K₂CO₃ (16.8 g, 0.122 mol, 3.0 eq.) was added to a solution of Fmoc-L-Lys(Boc)-OH (18.8 g, 40 mmol, 1.0 eq.) in DMF (0.20 L). The mixture was cooled to 0°C and MeI (7.5 mL, 0.12 mol, 3.0 eq.) was slowly added. The reaction mixture was allowed to warm-up to room temperature and stirred for 2 hours. The reaction mixture was quenched with H₂O and the obtained solution was extracted with Et₂O (5x). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→100% Et₂O in pentane) gave the title compound (17.9 g, 37.1 mmol, 93%). R_f: 0.60 (2/8 pentane/Et₂O); [α]_D²⁰ -6.0° (c = 1.0, DCM); ¹H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.80 (d, 2H, J = 7.5 Hz, Ar), 7.71 – 7.64 (m, 2H, Ar), 7.42 – 7.36 (m, 2H, Ar), 7.35 – 7.27 (m, 2H, Ar), 4.41 – 4.31 (m, 2H, CH₂ Fmoc), 4.22 (t, 1H, J = 7.0 Hz, CH Fmoc), 4.15 (dd, 1H, J = 9.3, 4.8 Hz, CH-L-Lys), 3.71 (s, 3H, OCH₃), 3.03 (t, 2H, J = 6.8 Hz, CH₂ ϵ -L-Lys), 1.87 – 1.76 (m, 1H, CHH β -L-Lys), 1.73 – 1.62 (m, 1H, CHH β -L-Lys), 1.51 – 1.35 (m, 13H, CH₂ γ -L-Lys, CH₂ δ -L-Lys, 3x CH₃ Boc); ¹³C-APT NMR (MeOD, 101 MHz, HSQC): δ 174.7, 158.7 (C=O), 145.3, 145.2, 142.6 (C_q Ar), 128.8, 128.2, 126.2, 120.9 (Ar), 67.9 (CH₂ Fmoc), 55.4 (CH-Lys), 52.7 (OCH₃), 48.4 (CH₂ Fmoc), 41.0 (CH₂ ϵ -L-Lys), 32.2 (CH₂ β -L-Lys), 30.5 (CH₂ γ -L-Lys), 28.8 (CH₃ Boc), 24.2 (CH₂ δ -L-Lys); FT-IR (neat, cm⁻¹): 2973, 1687, 1421, 1365,

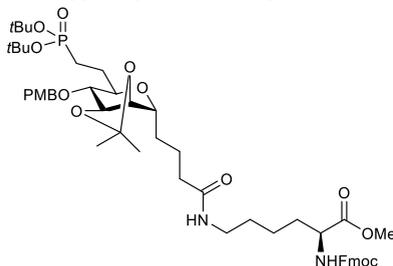
1167, 1046, 759, 739; HRMS: $[M+H]^+$ calcd. for $C_{27}H_{35}N_2O_6$: 483.24896, found 483.24895.

Fmoc-L-Lys-OMe-HCl (35)



Compound **34** (17.8 g, 37 mmol, 1.0 eq.) was suspended in dioxane (10 mL) and cooled to 0°C, followed by the addition of 4 M HCl in dioxane (90 mL). The reaction mixture was stirred for 3.5 hours and the mixture was concentrated *in vacuo*. Crystallization with dioxane/EtOAc/pentane gave the title compound (15.2 g, 36.3 mmol, 98%) as a white solid. R_f : 0.14 (9/1 DCM/MeOH); $[\alpha]_D^{20} +3.8^\circ$ ($c = 2.0$, MeOH); 1H NMR (MeOD, 400 MHz, HH-COSY, HSQC): δ 7.79 (d, 2H, $J = 7.5$ Hz, Ar), 7.71 – 7.62 (m, 2H, Ar), 7.43 – 7.35 (m, 2H, Ar), 7.35 – 7.27 (m, 2H, Ar), 4.40 (dd, 1H, $J = 10.6, 6.9$ Hz, *CHH* Fmoc), 4.33 (dd, 1H, $J = 10.5, 6.9$ Hz, *CHH* Fmoc), 4.23 – 4.15 (m, 2H, CH Fmoc, CH-L-Lys), 3.71 (s, 3H, OCH₃), 2.95 – 2.87 (m, 2H, CH₂ ϵ -L-Lys), 1.92 – 1.81 (m, 1H, *CHH* β -L-Lys), 1.77 – 1.61 (m, 3H, *CHH* β -Lys, CH₂ γ -L-Lys), 1.52 – 1.39 (m, 2H, CH₂ δ -L-Lys). ^{13}C -APT NMR (MeOD, 101 MHz, HSQC): δ 174.3, 158.7 (C=O), 145.3, 145.1, 142.6 (C_q Ar), 128.8, 128.2, 128.1, 126.2, 126.2, 120.9 (Ar), 67.9 (CH₂ Fmoc), 55.1 (CH-Lys), 52.8 (OCH₃ Lysine), 48.4 (CH₂ Fmoc), 40.5 (CH₂ ϵ -Lys), 32.0 (CH₂ β -Lys), 28.0 (CH₂ γ -Lys), 23.8 (CH₂ δ -Lys); FT-IR (neat, cm⁻¹): 3302, 2862, 1725, 1689, 1582, 1544, 1478, 1466, 1447, 1396, 1355, 1306, 1289, 1274, 1239, 1209, 1171, 1149, 1135, 1109, 1083, 1047, 1023, 1007, 959, 928, 894, 785, 757, 739, 657, 620, 594, 533, 499, 462; HRMS: $[M+H]^+$ calcd. for $C_{22}H_{27}N_2O_4$: 383.19653, found 383.19633.

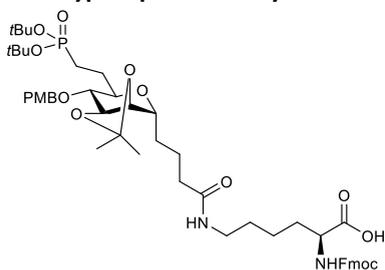
N_α -Fmoc- N_ϵ -[butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide]-L-lysine-methyl ester (36)



Compound **33** (1.78 g, 2.97 mmol, 1.0 eq.) and lysine **35** (1.39 g, 3.32 mmol, 1.12 eq.) were dissolved in DMF (15 mL). HCTU (1.47 g, 3.55 mmol, 1.2 eq.) and DIPEA (1.6 mL, 9.2 mmol, 3.0 eq.) were added and the solution was stirred for 2 hours. The reaction mixture was diluted with EtOAc and was washed with 1 M HCl (1x), sat. aq. NaHCO₃ (1x), brine (1x). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. Purification by column chromatography (10→100% acetone in DCM) gave compound **36** (2.25 g, 2.33 mmol, 78%) as an oil. R_f : 0.31 (9/1 DCM/MeOH); $[\alpha]_D^{25} +14.7$ ($c = 0.44$, CHCl₃); 1H NMR (CDCl₃, 500 MHz, HH-COSY, HSQC): δ 7.76 (d, 2H, $J = 7.5$ Hz, Ar), 7.61 (t, 2H, $J = 8.1$ Hz, Ar), 7.40 (t, 2H, $J = 7.4$ Hz, Ar), 7.31 (t, 2H, $J = 7.5$ Hz, Ar), 7.29 – 7.23 (m, 2H, Ar), 6.85 (d, 2H, $J = 8.5$ Hz, Ar), 6.68 (br, 1H, NH), 5.60 (d, 1H, $J = 8.1$ Hz, *NHFmoc*), 4.78 (d, 1H, $J = 11.2$ Hz, *CHH* PMB), 4.52 (d, 1H, $J = 11.3$ Hz, *CHH* PMB), 4.45 – 4.38 (m, 1H, *CHH* Fmoc), 4.38 – 4.30 (m, 2H, CH L-Lys, *CHH* Fmoc), 4.26 – 4.18 (m, 2H, H-3, CH Fmoc), 3.95 (t, 1H, $J = 6.2$ Hz, H-2), 3.79 (s, 3H, CH₃ PMB), 3.74 (s, 3H, OCH₃), 3.63 – 3.55 (m, 1H, H-1), 3.42 – 3.32 (m, 2H, H-4, H-5), 3.32 – 3.20 (m, 1H, *CHH* ϵ -L-Lys), 3.20 – 3.12 (m, 1H, *CHH* ϵ -L-Lys), 2.25 – 2.13 (m, 2H, CH₂-10), 1.99 – 1.52 (m, 10H, 5x CH₂-6/7/8/9, $\beta/\gamma/\delta$ -L-Lys), 1.51 – 1.43 (m, 23H, 1x CH₂-6/7/8/9, $\beta/\gamma/\delta$ -L-Lys,

CH₃ isopropylidene, 6x CH₃ tBu), 1.40 – 1.32 (m, 5H, 1x CH₂-6/7/8/9, β/γ/δ-L-Lys, CH₃ isopropylidene); ¹³C-APT NMR (CDCl₃, 126 MHz, HSQC): δ 173.1 (C=O), 159.4 (C_q PMB), 156.2 (C=O), 144.1, 143.9, 141.4 (C_q Fmoc), 130.4 (C_q PMB), 129.9, 127.8, 127.2, 125.3, 125.2, 120.1, 113.9 (Ar), 109.4 (C_q isopropylidene), 81.8, 81.8, 81.7, 81.6 (C_q tBu), 79.1 (C-4), 78.6 (C-3), 78.6 (C-2), 72.8, 72.6 (C-5), 72.6 (CH₂ PMB), 71.6 (C-1), 67.1 (CH₂ Fmoc), 55.4 (CH₃ PMB), 53.9 (CH L-Lys), 52.5 (OCH₃), 47.3 (CH Fmoc), 39.0 (CH₂ ε-L-Lys), 36.2 (CH₂-10), 32.1 (CH₂ β-L-Lys), 31.9 (CH₂ δ-L-Lys), 30.6, 30.5, 30.5 (CH₃ tBu), 29.2, 27.8 (CH₃ isopropylidene), 27.2, 26.2, 26.2, 26.0 (CH₂-6/7), 25.6 (CH₃ isopropylidene), 22.6 (CH₂ γ-L-Lys), 22.2 (CH₂-9); ³¹P-APT NMR (CDCl₃, 162 MHz): δ 24.3; FT-IR (neat, cm⁻¹): 3281, 2980, 2935, 1721, 1650, 1613, 1514, 1451, 1370, 1246, 1172, 1082, 1037, 981, 916, 867, 823, 760, 732, 646, 621, 538; HRMS: [M+Na]⁺ calcd. for C₅₂H₇₃N₂O₁₃PNa: 987.4748, found 987.4761.

N_α-Fmoc-N_ε-[butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-p-methoxybenzyl-6-di-tert-butoxyphosphonomethyl-α-D-mannopyranosyl)-amide]-L-lysine (10)

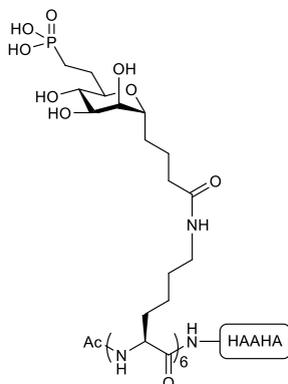


Compound **36** (2.20 g, 2.28 mmol, 1.0 eq.) was dissolved in THF (23 mL) and cooled to 0°C. A solution of LiOH in H₂O (0.30 M, 15 mL, 4.5 mmol, 2.0 eq.) was added and the mixture was stirred vigorously for 40 minutes, after which the mixture was acidified by the addition of 1 M HCl to pH = 3–4 and diluted with brine. The mixture was extracted with EtOAc (2x) and the combined organic layers were dried over Na₂SO₄, filtered

and concentrated *in vacuo*. After purification by column chromatography (2→10% MeOH in DCM + 0.1% AcOH), the title compound (1.68 g, 1.77 mmol, 78%) was obtained as a white foam. R_f: 0.36 (9/1 DCM/MeOH + 0.1% AcOH); [α]_D²⁵ +31.9° (c = 0.32, DCM); ¹H NMR (MeOD, 500 MHz, HH-COSY, HSQC): δ 7.78 (d, 2H, J = 7.6 Hz, Ar), 7.67 (t, 2H, J = 8.4 Hz, Ar), 7.38 (td, 2H, J = 7.4, 1.1 Hz, Ar), 7.30 (td, 2H, J = 7.5, 1.2 Hz, Ar), 7.28 – 7.23 (m, 2H, Ar), 6.89 – 6.84 (m, 2H, Ar), 4.75 (d, 1H, J = 11.3 Hz, CH₂ PMB), 4.53 (d, 1H, J = 11.3 Hz, CH₂ PMB), 4.35 (dd, 2H, J = 7.0, 2.4 Hz, CH₂ Fmoc), 4.23 – 4.18 (m, 2H, H-3, CH Fmoc), 4.13 (dd, 1H, J = 9.2, 4.6 Hz, CH L-Lys), 4.00 – 3.96 (m, 1H, H-2), 3.77 (s, 3H, CH₃ PMB), 3.71 – 3.65 (m, 1H, H-1), 3.40 – 3.33 (m, 2H, H-4, H-5), 3.19 – 3.13 (m, 2H, CH₂ ε-L-Lys), 2.22 – 2.15 (m, 2H, CH₂-10), 1.99 – 1.38 (m, 35H, 7x CH₂-6/7/8/9, β/γ/δ-L-Lys, CH₃ isopropylidene, 6x CH₃ tBu), 1.33 (s, 3H, CH₃ isopropylidene); ¹³C-APT NMR (MeOD, 126 MHz, HSQC): δ 175.6, 160.8 (C=O), 158.6 (C_q PMB), 145.4, 145.2, 142.6 (C_q Fmoc), 131.6 (C_q PMB), 130.9, 128.8, 128.2, 128.2, 126.3, 126.2, 121.0, 114.7 (Ar), 110.3 (C_q isopropylidene), 83.5, 83.5, 83.5, 83.5 (C_q tBu), 79.7 (C-4), 79.7 (C-3), 78.2 (C-2), 73.2 (CH₂ PMB), 73.2 (C-1), 72.8, 72.7 (C-5), 67.9 (CH₂ Fmoc), 55.7 (CH₃ PMB), 55.5 (CH Fmoc), 48.4 (CH L-Lys), 40.1 (ε-L-Lys), 36.6 (CH₂-10), 32.5 (CH₂ β-L-Lys), 32.3 (CH₂ δ-L-Lys), 30.8, 30.8, 30.7, 30.7, 30.0 (CH₃ tBu), 28.2 (CH₃ isopropylidene), 27.6, 27.1, 27.1, 26.4 (CH₂-6/7), 25.9 (CH₃ isopropylidene), 24.3 (CH₂ γ-L-Lys), 23.2 (CH₂-9); ³¹P-APT NMR (MeOD, 202 MHz, HMBC): δ 25.7; FT-IR (neat, cm⁻¹): 3301, 2980, 2935, 1716, 1650, 1613, 1514, 1451, 1394, 1370, 1246, 1161, 1081, 1038, 985, 912, 866, 823, 760, 730, 647, 621, 540;

LC-MS: Rt = 8.72 min (C18 Gemini, 10 - 50% MeCN, 11 min run); HRMS: $[M+Na]^+$ calcd. for $C_{51}H_{71}N_2O_{13}PNa$: 973.4591, found 973.4603.

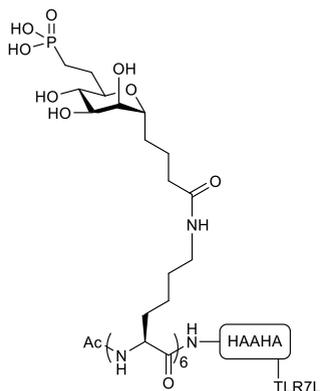
Ac-[Lys(butan-4-(6-deoxy-6-phosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys-NH₂ (5)



100 μ mol of crude H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-Tentagel S Ram was washed with DMF (5x) and elongated by the addition of a solution of acid **10** (0.19 g, 0.20 mmol, 2.0 eq.) and HCTU (84 mg, 0.20 μ mol, 2.0 eq.) in DMF (2.0 mL) and DIPEA (70 μ L, 0.40 mmol, 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (2.0 mL, 2x 20 min) and washed with DMF (5x). This was repeated another 5 times, after which the resin was treated with a mixture of Ac_2O /DMF/DIPEA (2x 2.0 mL, 20 min), and washed with

DMF (3x) and DCM (3x). 30 μ mol of crude Ac-[Lys(butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-Tentagel S Ram was taken and the peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **5** (13.3 mg, 2.9 μ mol, 10%) was obtained as a white solid. LC-MS: Rt = 6.19 min (C18 Gemini, 0 - 50% MeCN, 11 min run); ESI-MS: m/z 1525.6 $[M+H]^3+$; HRMS: $[M+H]^4+$ calcd. for $C_{184}H_{325}N_{41}O_{80}P_6$: 1143.77568, found 1143.77633.

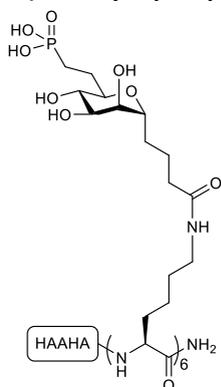
Ac-[Lys(butan-4-(6-deoxy-6-phosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-NH₂ (6)



40 μ mol of crude Ac-[Lys(butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-Tentagel S Ram was treated with a continuous flow of a mixture of AcOH/TFE/DCM (1/2/7 v/v/v, 30 mL) over 15 minutes. The resin was washed subsequently with DCM (5x), AcOH/TFE/DCM (1/2/7 v/v/v, 4 mL), DCM (5x), 1 M DIPEA in NMP (2x 3 mL), DCM (3x) and DMF (3x). A solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (34 mg, 88 μ mol, 2.2

eq.) and HCTU (34 mg, 82 μmol , 2.1 eq.) in DMF (0.8 mL) and DIPEA (28 μL , 0.16 mmol, 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.8 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (38 mg, 83 μmol , 2.1 eq.) and HCTU (34 mg, 82 μmol , 2.1 eq.) and DIPEA (28 μL , 0.16 mmol, 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (3x), DCM (3x) and the peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.6 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.6 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **6** (3.1 mg, 0.61 μmol , 2%) was obtained as a white solid. LC-MS: Rt = 5.73 min (C18 Gemini, 10 - 50% MeCN, 11 min run); ESI-MS: m/z 1686.9 [M+H]³⁺; HRMS: [M+H]⁴⁺ calcd. for C₂₀₇H₃₅₃N₄₇O₈₆P₆: 1264.82744, found 1264.82866.

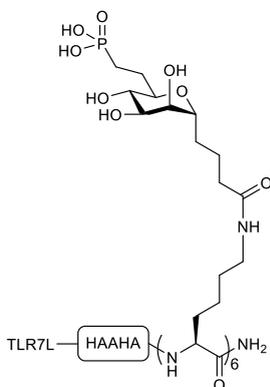
Ac-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys-Lys(butan-4-(6-deoxy-6-phosphonomethyl- α -D-mannopyranosyl)-amide)]₆-NH₂ (7**)**



100 μmol of H-Tentagel S Ram was washed with DMF (5x) and elongated by the addition of a solution of acid **10** (0.19 g, 0.20 mmol, 2.0 eq.) and HCTU (84 mg, 0.20 mmol, 2.0 eq.) in DMF (2.0 mL) and DIPEA (70 μL , 0.40 mmol, 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (2.0 mL, 2x 20 min) and washed with DMF (5x). This was repeated another 5 times, after which the resin was elongated using the synthesizer with Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT) with standard HCTU/Fmoc chemistry concluding in final Fmoc removal with a solution of 20% piperidine in DMF (3x 3 min). 30 μmol of crude

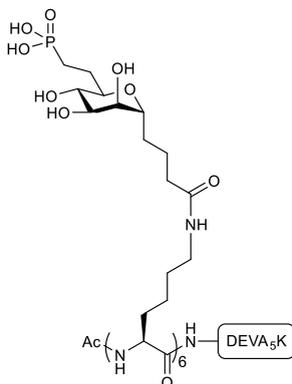
H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-[Lys(butan-4-(6-deoxy-2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Tentagel S Ram was treated with a mixture of Ac₂O/DMF/DIPEA (2x 1.2 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **7** (11.0 mg, 2.4 μmol , 8%) was obtained as a white solid. LC-MS: Rt = 6.00 min (C18 Gemini, 0 - 50% MeCN, 11 min run); ESI-MS: m/z 1525.6 [M+H]³⁺; HRMS: [M+H]⁴⁺ calcd. for C₁₈₄H₃₂₅N₄₁O₈₀P₆: 1143.77568, found 1143.77518.

4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys-[Lys(butan-4-(6-deoxy-6-phosphonomethyl- α -D-mannopyranosyl)-amide)]₆-NH₂ (8**)**



30 μ mol of crude H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-[Lys(butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Tentagel S Ram was treated with a solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (27 mg, 70 μ mol, 2.3 eq.) and HCTU (25 mg, 60 μ mol, 2.0 eq.) in DMF (0.6 mL) and DIPEA (21 μ L, 0.12 mmol, 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.6 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (29 mg, 63 μ mol, 2.1 eq.) and HCTU (25 mg, 60 μ mol, 2.0 eq.) and DIPEA (21 μ L, 0.12 mmol, 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (3x), DCM (3x) and the peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **8** (17.0 mg, 3.4 μ mol, 11%) was obtained as a white solid. LC-MS: Rt = 5.91 min (C18 Gemini, 10 - 50% MeCN, 11 min run); ESI-MS: m/z 1673.1 [M+H]²⁺; HRMS: [M+H]⁴⁺ calcd. for C₂₀₅H₃₅₁N₄₇O₈₅P₆: 1254.32479, found 1254.32528.

Ac-[Lys(butan-4-(6-deoxy-6-phosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH₂ (43**)**



100 μ mol of crude H-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT) was washed with DMF (5x) and elongated by the addition of a solution of acid **10** (0.19 g, 0.20 mmol, 2.0 eq.) and HCTU (84 mg, 0.20 μ mol, 2.0 eq.) in DMF (2.0 mL) and DIPEA (70 μ L, 0.40 mmol, 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (2.0 mL, 2x 20 min) and washed with DMF (5x). This was repeated another 5 times. 40 μ mol of crude H-[Lys(butan-4-(6-deoxy-2,3-O-isopropylidene-4-O-*p*-methoxybenzyl-6-di-*tert*-butoxyphosphonomethyl- α -D-mannopyranosyl)-amide)]₆-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT)-Tentagel S Ram

was treated with a mixture of Ac₂O/DMF/DIPEA (2x 0.8 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.6 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.6 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **43** (8.2 mg, 1.6 μmol, 4%) was obtained as a white solid. LC-MS: Rt = 4.74 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1740.1 [M+H]²⁺; HRMS: [M+H]³⁺ calcd. for C₂₁₆H₃₇₆N₄₁O₉₃P₆: 1739.47874, found 1739.47817.

Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH₂ (39)

DEVA₅K

See compound **45**, chapter 2. LC-MS: Rt = 4.88 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1273.7 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₁₂H₁₈₇N₂₉O₃₈: 1273.17904, found 1273.17779.

Ac-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-NH₂ (40)

DEVA₅K

30 μmol of crude H-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-TLR7L Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT)-Tentagel S Ram was treated with a mixture of Ac₂O/DMF/DIPEA (2x 0.6 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 11 mL) over 15 minutes. The resin was washed subsequently with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL), DCM (3x) and DMF (3x). A solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (24 mg, 62 μmol, 2.1 eq.) and HCTU (25 mg, 60 μmol, 2.0 eq.) in DMF (0.6 mL) and DIPEA (21 μL, 121 μmol, 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.6 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((tert-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (28 mg, 61 μmol, 2.0 eq.) and HCTU (26 mg, 62 μmol, 2.1 eq.) and DIPEA (21 μL, 121 μmol, 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **40** (9.4 mg, 3.1 μmol, 10%) was obtained as a white solid. LC-MS: Rt = 5.41 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1536.9 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₃₇H₂₁₇N₃₅O₄₅: 1536.28784, found 1536.28769.

4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Lys-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-NH₂ (41)

TLR7L—DEVA₅K 30 μmol of crude H-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Lys(N₃)-Asp(OtBu)-Glu(OtBu)-Val-Ser(tBu)-Gly-Leu-Glu(OtBu)-Gln(Trt)-Leu-Glu(OtBu)-Ser(tBu)-Ile-Ile-Asn(Trt)-Phe-Glu(OtBu)-Lys(Boc)-Leu-Ala-Ala-Ala-Ala-Ala-Lys(MMT)-Tentagel S Ram was washed with DMF (5x), followed by the addition of a solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (25 mg, 65 μmol, 2.2 eq.) and HCTU (25 mg, 60 μmol, 2.0 eq.) in DMF (0.6 mL) and DIPEA (21 μL, 121 μmol, 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.6 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (28 mg, 61 μmol, 2.0 eq.) and HCTU (25 mg, 60 μmol, 2.0 eq.) and DIPEA (21 μL, 121 μmol, 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **41** (4.1 mg, 1.4 μmol, 5%) was obtained as a white solid. LC-MS: Rt = 5.34 min (C18 Gemini, 10 - 90% MeCN, 11 min run); ESI-MS: m/z 1516.0 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₃₅H₂₁₅N₃₅O₄₄: 1515.78424, found 1515.78242.

Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys-NH₂ (42)

HAAHA Tentagel S Ram resin loaded with H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-NH₂ on 30 μmol scale was washed with DCM (5x). The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2/2/1 v/v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **42** (16.8 mg, 8.8 μmol, 30%) was obtained as a white solid. LC-MS: Rt = 3.70 min (C18 Gemini, 10 - 50% MeCN, 11 min run); ESI-MS: m/z 1901.0 [M+H]⁺; HRMS: [M+H]²⁺ calcd. for C₈₀H₁₃₅N₂₉O₂₅: 951.00865, found 951.00848.

Ac-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys(4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-NH₂ (43)

HAAHA 30 μmol of crude H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-
TLR7L Lys(MMT)-Tentagel S Ram was treated with a mixture of Ac₂O/DMF/DIPEA (2x 0.6 mL, 20 min), and washed with DMF (3x) and DCM (3x). The peptide was treated with a continuous flow of a mixture of TFA/TIS/DCM (96/2/2 v/v/v, 11 mL) over 15 minutes. The resin was washed subsequently with DCM (5x), TFA/TIS/DCM (96/2/2 v/v/v, 2 mL), DCM (5x), 1 M DIPEA in NMP (2 mL), DCM (3x) and DMF (3x). A solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (24 mg, 62 μmol,

2.1 eq.) and HCTU (25 mg, 60 μmol , 2.0 eq.) in DMF (0.6 mL) and DIPEA (21 μL , 121 μmol , 4.0 eq.) were added and the suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.6 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (28 mg, 61 μmol , 2.0 eq.) and HCTU (26 mg, 62 μmol , 2.1 eq.) and DIPEA (21 μL , 121 μmol , 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **43** (7.6 mg, 3.1 μmol , 10%) was obtained as a white solid. LC-MS: Rt = 6.47 min (C18 Gemini, 10 - 50% MeCN, 11 min run); ESI-MS: m/z 1214.3 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₀₅H₁₆₅N₃₅O₃₂: 1214.11745, found 1214.11682.

4-((6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)-N-(2-(2-(2-amino-2-oxoethoxy)ethoxy)ethyl)benzamide)-Ile-Ser-Gln-Ala-Val-His-Ala-Ala-His-Ala-Glu-Ile-Asn-Glu-Ala-Gly-Arg-Lys-NH₂ (44**)**

TLR7L—HAAHA 30 μmol of crude H-Ile-Ser(OtBu)-Gln(Trt)-Ala-Val-His(Trt)-Ala-Ala-His(Trt)-Ala-Glu(OtBu)-Ile-Asn(Trt)-Glu(OtBu)-Ala-Gly-Arg(Pbf)-Lys(MMT)-Tentagel S Ram was washed with DMF (5x), followed by the addition of a solution of {2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid (24 mg, 62 μmol , 2.1 eq.) and HCTU (25 mg, 60 μmol , 2.0 eq.) in DMF (0.6 mL) and DIPEA (21 μL , 121 μmol , 4.0 eq.). The suspension was shaken overnight. The resin was washed with DMF (5x), treated with 20% piperidine in DMF (0.6 mL, 2x 20 min) and washed with DMF (5x). A solution of 4-((2-butoxy-6-((*tert*-butoxycarbonyl)amino)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzoid acid (28 mg, 61 μmol , 2.0 eq.) and HCTU (26 mg, 60 μmol , 2.0 eq.) and DIPEA (21 μL , 121 μmol , 4.0 eq.) were added and the suspension was shaken overnight. The peptide was cleaved from the resin after treatment with TFA/TIS/H₂O (95/2.5/2.5 v/v/v) (1.2 mL) for 3 hours the suspension was filtered and the residue was washed with the cleavage cocktail (1.2 mL). The product was precipitated with Et₂O. After purification by RP-HPLC and lyophilisation, compound **44** (10.3 mg, 4.3 μmol , 14%) was obtained as a white solid. LC-MS: Rt = 6.17 min (C18 Gemini, 10 - 50% MeCN, 11 min run); ESI-MS: m/z 1193.7 [M+H]²⁺; HRMS: [M+H]²⁺ calcd. for C₁₀₃H₁₆₃N₆₅O₃₁: 1193.11216, found 1193.11176.

Footnotes and References

- (1) Dahms, N. *Biochim. Biophys. Acta - Gen. Subj.* **2002**, *1572* (2–3), 317–340.
- (2) Ghosh, P.; Dahms, N. M.; Kornfeld, S. *Nat. Rev. Mol. Cell Biol.* **2003**, *4* (3), 202–213.
- (3) Tong, P. Y.; Gregory, W.; Kornfeld, S. *J. Biol. Chem.* **1989**, *264* (14), 7962–7969.
- (4) Kang, J. X.; Bell, J.; Beard, R. L.; Chandraratna, R. A. *Cell Growth Differ.* **1999**, *10* (8), 591–600.
- (5) Godár, S.; Hořejší, V.; Weidle, U. H.; Binder, B. R.; Hansmann, C.; Stockinger, H. *Eur. J. Immunol.* **1999**, *29* (3), 1004–1013.
- (6) Berkowitz, D. B.; Maiti, G.; Charette, B. D.; Dreis, C. D.; MacDonald, R. G. *Org. Lett.* **2004**, *6* (26), 4921–4924.
- (7) Fei, X.; Connolly, C. M.; MacDonald, R. G.; Berkowitz, D. B. *Bioorg. Med. Chem. Lett.* **2008**, *18* (10), 3085–3089.

- (8) Prydz, K.; Brändli, A. W.; Bomsel, M.; Simons, K. *J. Biol. Chem.* **1990**, *265* (21), 12629–12635.
- (9) Marron-Terada, P. G.; Hancock, M. K.; Haskins, D. J.; Dahms, N. M. *Biochemistry* **2000**, *39* (9), 2243–2253.
- (10) Watanabe, H.; Grubb, J. H.; Sly, W. S. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87* (20), 8036–8040.
- (11) El Cheikh, K.; Basile, I.; Da Silva, A.; Bernon, C.; Cérutti, P.; Salgues, F.; Perez, M.; Maynadier, M.; Gary-Bobo, M.; Caillaud, C.; *et al.* *Angew. Chemie Int. Ed.* **2016**, *55* (47), 14774–14777.
- (12) Greupink, R.; Bakker, H. I.; Bouma, W.; Reker-Smit, C.; Meijer, D. K. F.; Beljaars, L.; Poelstra, K. **2006**.
- (13) Minnelli, C.; Cianfruglia, L.; Laudadio, E.; Galeazzi, R.; Pisani, M.; Crucianelli, E.; Bizzaro, D.; Armeni, T.; Mobbili, G. *J. Drug Target.* **2018**, *26* (3), 242–251.
- (14) Hoogendoorn, S.; van Puijvelde, G. H. M.; Kuiper, J.; van der Marel, G. A.; Overkleeft, H. S. *Angew. Chemie Int. Ed.* **2014**, *53* (41), 10975–10978.
- (15) Parra-López, C. A.; Lindner, R.; Vidavsky, I.; Gross, M.; Unanue, E. R. *J. Immunol.* **1997**, *158* (6), 2670–2679.
- (16) Barragan-Montero, V.; Awwad, A.; Combemale, S.; de Santa Barbara, P.; Jover, B.; Molès, J.-P.; Montero, J.-L. *ChemMedChem* **2011**, *6* (10), 1771–1774.
- (17) This conjugation strategy was chosen, since the synthesis of a phosphonate in the presence of an azide was unsuccessful.
- (18) Distler, J. J.; Guo, J. F.; Jourdain, G. W.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1991**, *266* (32), 21687–21692.
- (19) York, S. J.; Arneson, L. S.; Gregory, W. T.; Dahms, N. M.; Kornfeld, S. *J. Biol. Chem.* **1999**, *274* (2), 1164–1171.
- (20) Zhou, J.; Xu, K.; Zhou, P.; Zheng, O.; Lin, Z.; Guo, L.; Qiu, B.; Chen, G. *Biosens. Bioelectron.* **2014**, *51*, 386–390.
- (21) Mikkelsen, L. M.; Krintel, S. L.; Jiménez-Barbero, J.; Skrydstrup, T. *J. Org. Chem.* **2002**, *67* (18), 6297–6308.
- (22) Liu, L.; Wang, C.-Q.; Liu, D.; He, W.-G.; Xu, J.-Y.; Lin, A.-J.; Yao, H.-Q.; Tanabe, G.; Muraoka, O.; Xie, W.-J.; *et al.* *Org. Lett.* **2014**, *16* (19), 5004–5007.
- (23) Unpublished results.
- (24) Decomposition was observed when concentrating in vacuo at 40°C instead of 30°C.
- (25) Gentil, G. P. P.; Hogervorst, T. P.; Tondini, E.; van de Graaff, M. J.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A.; Ossendorp, F.; Filippov, D. V. *Bioorg. Med. Chem. Lett.* **2019**.
- (26) Liu, Q.; Kistemaker, H. A. V.; Bhogaraju, S.; Dikic, I.; Overkleeft, H. S.; van der Marel, G. A.; Ovaa, H.; van der Heden van Noort, G. J.; Filippov, D. V. *Angew. Chemie Int. Ed.* **2018**, *57* (6), 1659–1662.
- (27) Girard, C.; Miramon, M.-L.; de Solminihac, T.; Herscovici, J. *Carbohydr. Res.* **2002**, *337* (19), 1769–1774.
- (28) Nicolaou, K. C.; Hwang, C. K.; Duggan, M. E. *J. Am. Chem. Soc.* **1989**, *111* (17), 6682–6690.
- (29) Kramer, J. R.; Deming, T. J. *J. Am. Chem. Soc.* **2010**, *132* (42), 15068–15071.
- (30) It was found to be important to co-evaporate the phosphonate reagents with toluene before treatment with *n*-BuLi.
- (31) Sharma, P. K.; Kumar, S.; Kumar, P.; Nielsen, P. *Tetrahedron Lett.* **2007**, *48* (49), 8704–8708.
- (32) Voigtritter, K.; Ghorai, S.; Lipshutz, B. H. *J. Org. Chem.* **2011**, *76* (11), 4697–4702.