Chapter 4

Synthesis and immunological evaluation of a small library of mannosylated peptides[1]

Introduction

Antigen presenting cells (APCs), such as dendritic cells (DCs) play a pivotal role in the mammalian innate and adaptive immune system and thereby in the defence against external challenges, such as bacteria, viruses and toxins, but also internal challenges, such as malignantly transformed self-cells.[2] APCs recognize pathogen related molecular structures with the aid of various classes of receptors such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). This recognition event can result in the upregulation of pro-inflammatory signalling molecules (both surface-bound and soluble), that alert the immune system to the presence of danger.[3] Concomitantly, exogenous material is taken up by APCs through endocytosis, phagocytosis or marcopynocitic internalization. After uptake, the cargo is proteolytically degraded in the endo-lysosomal system by a family of proteases[4] and further processed in the lysosomes.[5] The degradation of proteins ultimately results in presentation of a peptide antigen on major

histocompatibility complex II (MHC class II) at the outer membrane of APCs.[6] The combination of the expression of pro-inflammatory receptors and MHC-II loaded with exogenous peptides will lead to the activation of CD4+ T-helper cells.

Malignantly transformed self-cells and cells harbouring pathogens in their cytosol are cleared using a different pathway: the MHC-I-pathway of antigen presentation. On the MHC-I molecule 8-9-mer peptides derived from cytosolic and ER-associated proteins are continually expressed from various stages of the protein life cycle.[7] In this cytosolic pathway to antigen presentation, proteins from within the cell are processed by proteasomes and downstream aminopeptidases and presented on MHC class I.[8,9] In a process referred to as antigen cross-presentation[10] DCs take up exogenous antigen and let this antigenic material 'escape' from the endosomally-restricted MHC-II pathway into their own MHC-I-loading pathway.[11] Cross-presentation is the subject of intense study due to its relevance to disease and its mechanistic complexity. Besides the above mentioned PRRs that detect pathogen associated molecular patterns (PAMPs), APCs express various C-type lectin receptors (CLRs) which bind specific carbohydrates of pathogens but also carbohydrates structures of self-glycoproteins.[12] It has been hypothesized that these lectins play a crucial role in antigen cross-presentation.[13] Binding of a glycoconjugate by CLRs on APCs is followed by internalization thereby facilitating the processing and finally antigen presentation. There are multiple carbohydrate receptors on the surface of APCs and their expression varies between cell types. They have been shown to have overlapping binding specificity, which has complicated their structure. Despite this absence of knowledge about the exact structure of the carbohydrate-ligands for specific CLRs, considerable attention is directed to explore CLRs with the aid of natural and artificial glycoconjugates. Examples of CLRs that recognise mannose-containing structures are Dectin-2, Mincle, DC-SIGN, SIGNR1, Langerin, and the Mannose Receptor (MR). As discussed in Chapters 2 and 3 (oligo)mannose conjugates are regularly exploited in targeting strategies to enable improved uptake of specific molecules, such as (fluorescently) labeled

inhibitors.[14,15] In addition (oligo)mannose conjugates are used to target antigens to APCs to improve the uptake of antigens.[16]

Recently it has been shown that the MR can strongly enhance antigen cross-presentation by routing glycoprotein ligands to a very mild endosome (low proteolysis, near neutral pH), from which cross-presentation is favoured.[17] In line with these results, it was recently reported that a bismannosylated synthetic long peptide showed enhanced cross-presentation in an MR-dependent manner[18] and the chemical introduction of additional glycans onto the glycoprotein ovalbumin was shown to further enhance cross-presentation.[19] However, it is still unclear how different glycans exactly enhance cross-presentation, which in part is due to the extensive heterogeneity of glycoproteins that have been studied.[20,21] The position, branching, spacing and multivalency of (oligo)mannoses in a glycoconjugate are decisive for uptake by a mannose recognising CLR, the subsequent routing and finally for antigen presentation.

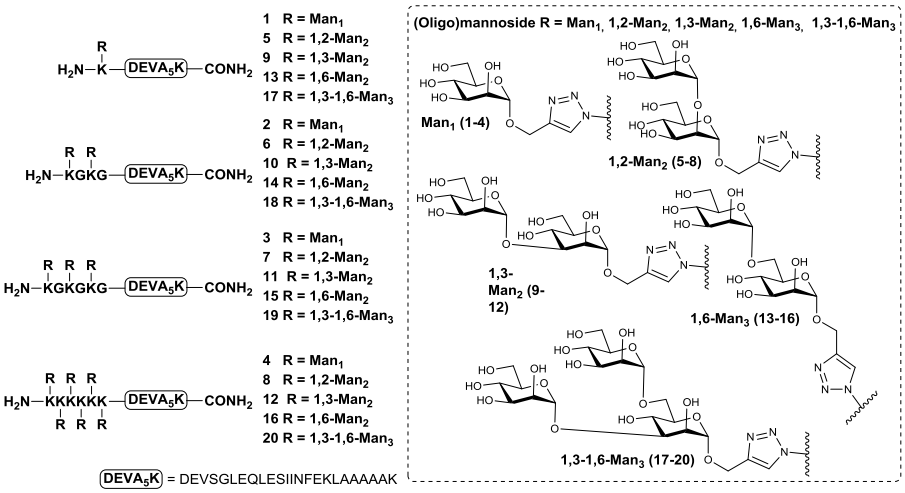


Figure 1: Target compounds 1-20 discussed in this chapter.

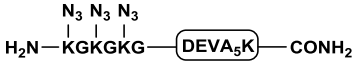
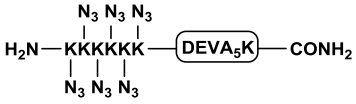
To gain more insight which carbohydrate structures promote cross-presentation, this chapter presents the synthesis and initial immunological evaluation of a library of mannosylated peptides, based on the MHC-class-I

epitope DEVSGLEQLESIINFEKLAAAAAK ($DEVA_5K$).[22] A set of 5 different (oligo)mannosides were attached to the side chain of azido lysine residues. The resulting library of twenty mannosylated peptides (**1-20**, Figure 1) has been evaluated on their ability to promote antigen presentation.

Result and discussion

The assembly of the mannosylated peptides **1-20** entails the following stages; i) the solution phase synthesis of five propargylated mono-, di- and tri-mannosides (Scheme 1), ii) the automated solid phase synthesis of four oligopeptides containing one, two, three or six azide functions **26-29** (Table 1), iii) the assembly of the library of twenty mannosylated peptides by the solution phase conjugation of the propargylated mannosides and azide containing peptides as depicted in Figure 2.

Table 1: Peptide sequence of azidolysine-$DEVA_5K$ peptides **26-29**.

Compound		Peptide Sequence
26	Az_1DEVA_5K	
27	Az_2DEVA_5K	
28	Az_3DEVA_5K	
29	Az_6DEVA_5K	

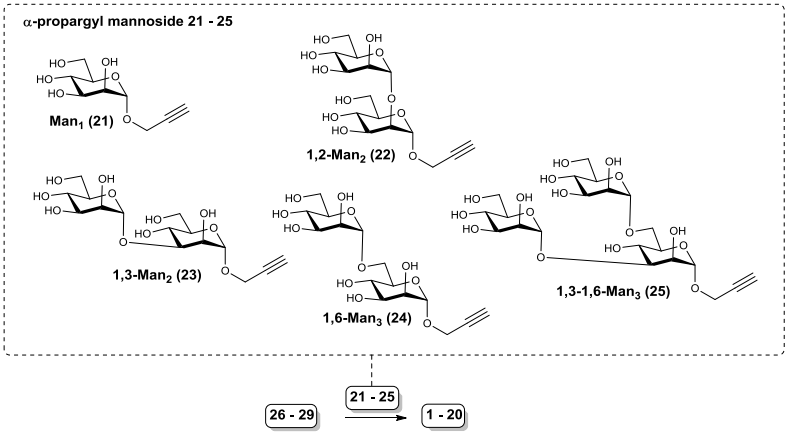
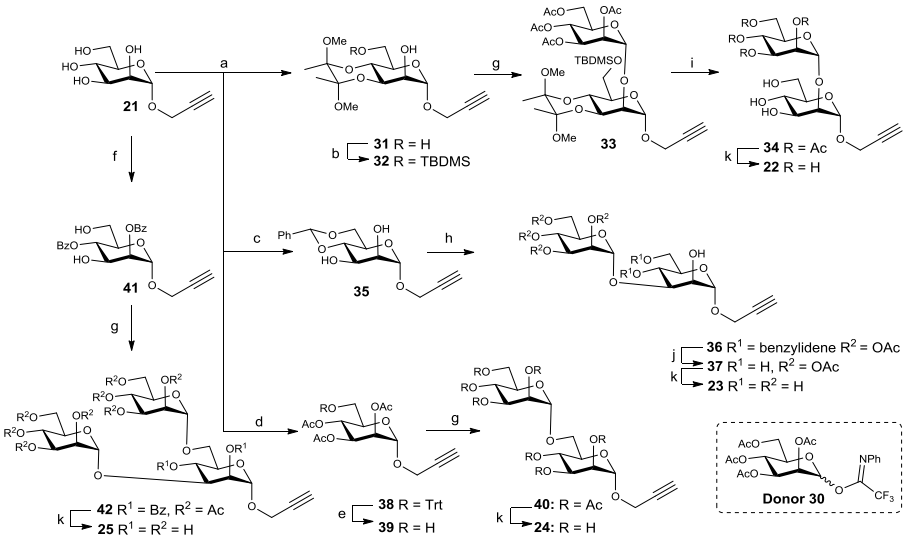


Figure 2: Peptide conjugate assembly.

Propargyl oligomannoside synthesis

The propargyl mannopyranosides **21-25** were prepared using propargyl α-D-mannopyranoside **21**[23] as starting compound (Scheme 1). In order to keep the anomeric alkyne moiety in the mannosides intact reductive transformations were avoided and acid/base labile protective groups were applied. En route to α-linked 1,2-di-mannoside (1,2-Man_2) **(22)** the equatorial hydroxyl functions in propargyl α–D-mannopyranose **21** were selectively masked with a 1,2-butane diacetal moiety to give **31** in 66% yield. Subsequent silylation of the primary alcohol in **31** using TBDMS chloride and imidazole gave acceptor **32** in quantitative yield. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **30** and acceptor **32** under influence of TfOH provided fully protected α-(1-2) linked dimer **33** in 71% yield. Both the butane diacetal and the silyl ether in dimer **33** were removed by treatment with 90% TFA. Cleavage of the glycosidic bonds could not be detected under these acidic conditions. Subsequently dimer **34** was globally deacetylated under standard Zémplen conditions providing 1,2-Man_2 **22** in 58% yield over two steps.

Scheme 1: Synthesis of α-propargyl (oligo)mannosides **21**-**25**.



Reagents and conditions: (a) 2,3-butanedione, $HC(OMe)_3$, MeOH, CSA, reflux, 66%; (b) TBDMSCl, imidazole, DMF, quantitative; (c) $PhCH(OMe)_2$, CSA, DMF, 200 mBar, 60 °C 55%; (d) *i*. TrtCl, DABCO, DCM; *ii*. Ac_2O, DMAP, 97%; (e) $BF_3{\cdot}Et_2O$, MeOH/toluene (1:1) 70%; (f) *i*. $PhC(OMe)_3$, CSA, MeCN; *ii*. H_2O, 52%; (g) Donor **30**, TfOH, DCM, activated molecular sieves, -25 to 0 °C, (**33**: 82%, **40**: 79%, **42**: 82%); (h) Donor **30**, TMSOTf, DCM, activated molecular sieves, -20 to 0 °C, 80%; (i) TFA/H_2O (9:1), 71%; (j) 70% AcOH (aq.), 55 °C, 84%; (k) NaOMe, MeOH, (**22**: 82%, **23**: 84%, **24**: 66%, **25**: 75%).

To obtain α-linked 1,3-di-mannoside (1,3-Man_2) **23**, imidate donor **30** and propargyl 4,6-*O*-benzylidene mannopyranose **35** were coupled in a regio and stereoselective fashion to give target dimer **36** in 80% yield together with the corresponding mannose trimer as minor by-product. Acidolysis of the benzylidene acetal and NaOMe mediated removal of the acetyl groups provided 1,3-Man_2 **23**.

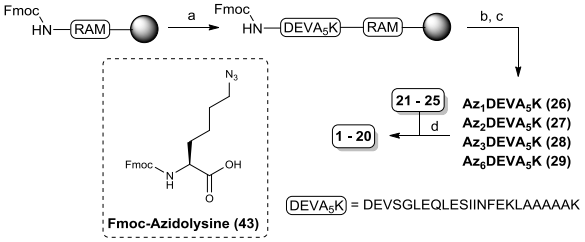
Acceptor **39**, needed to obtain α-linked 1,6-di-mannoside (1,6-Man_2) **24**, was synthesized by a two-step one-pot tritylation/acetylation procedure[24] followed by detritylation. Ensuing coupling with mannopyranosyl trichloroacetimidate **30** led to the isolation of fully acylated 1,6-Man_2 dimer **40** in 79%. Deprotection using NaOMe in MeOH gave 1,6-Man_2 **24** in 66% yield.

Propargyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (1,3-1,6-Man_3) **25** was obtained by double mannosylation of diol acceptor **41**. Treatment of propargyl α–D-mannopyranose **21** with trimethyl orthobenzoate in presence of a catalytic amount of CSA followed by *in situ* hydrolysis of the formed orthoesters delivered after purification 2,4-di-*O*-benzoylated mannose **41** in 52% yield and 2,6-di-*O*-benzoylated mannose in 35% yield. The 2,4-di-*O*-benzoylated acceptor **41** was coupled with imidate donor **30** giving the peracylated mannose trimer **42** in 82% yield. Subsequently the trimer was saponificated under standard Zémplen conditions providing 1,3-1,6-Man_3 **25** in 75%. With all the propargyl mannosides in hand attention was directed to the solid phase synthesis of the azide containing oligopeptides (**26**-**29**, Figure 2).

Peptide synthesis

The synthesis of the oligopeptides was carried out with an automated peptide synthesizer using standard Fmoc-chemisty (Scheme 2). Tentagel Rink amide was used as solid support together with commercially available Fmoc-protected amino acids and HCTU as condensing agent. Fmoc-protected azido lysine **43**[25] was prepared using a reported procedure. The four target oligopeptides **26**-**29**, contain one, two, three or six azide functions at the N-terminal end of the common DEVSGLEQLESIINFEKLAAAAAK ($DEVA_5K$) sequence. After construction of the immobilized protected $DEVA_5K$ elongation with azido lysine was undertaken. Incorporation of one azido lysine gave oligopeptide **26** and incorporation of six azido lysines resulted in oligopeptide **29**. The remaining immobilized oligopeptide **27** and **28** having two or three azide lysine residues were obtained by alternating coupling of azido lysine and glycine.

Scheme 2: Synthesis of mannosyl-DEVA$_5$K peptide conjugates **1-20**



Reagens and conditions: (a) SPPS using repetitive cycle of: *i*. 20% piperidine/DMF; *ii*. Fmoc-AA-OH, HCTU, DiPEA, NMP; *iii*. Ac_2O, DiPEA, HOBt, NMP; (b) SPPS using repetitive cycle of: *i*. 20% piperidine/DMF; *ii*. Fmoc-azidolysine **43** or glycine, HCTU, DiPEA, NMP; *iii*. Ac_2O, DiPEA, HOBt, NMP; (c) *i*. 95%TFA, 2.5% H_2O, 2.5% TIS. *ii*. RP-HPLC purification; (d) 0.05 M propargyl mannose sugar (aq.), 0.05 M sodium ascorbate (aq.), 0.05 M $CuSO_4$ (aq.), rt.

All protected immobilized peptides were cleaved from the solid support by treatment with 95% TFA and purified by RP-HPLC to yield Az_1DEVA_5K **26**, Az_2DEVA_5K **27**, Az_3DEVA_5K **28** and Az_6DEVA_5K **29**. With the peptide backbones in hand the peptides were subjected to conjugation with the set of propargyl mannosides.

Propargyl mannosides Man_1 (**21**), 1,2-Man_2 (**22**), 1,3-Man_2 (**23**), 1,6-Man_2 (**24**) and 1,3-1,6-Man_3 (**25)** were conjugated to oligopeptides Az_1DEVA_5K (**26),** Az_2DEVA_5K (**27**), Az_3DEVA_5K (**28**) and Az_6DEVA_5K (**29**) by Cu-catalyzed azide-alkyne cycloaddition. In this reaction 1 equivalent of $CuSO_4$ and (n + 2) equivalents of both the propargyl mannoside and sodium ascorbate with respect to the number of azides in the oligopeptide were used and each reaction was monitored by LCMS. After completion of the reaction, the mixture was subjected to gel filtration (HW-40) to remove compounds of low molecular weight. The retention time of the target mannosylated peptides prove to be shorter than expected and Cu-cluster formation of the products was assumed. Subsequent HPLC purification gave the mannosylated peptides conjugated with Man_1 **1-4**, 1,2-Man_2 **5-8** or 1,3-1,6-Man_3 **17-20** in relatively low yields (Table 1). In an alternative procedure HW-40 gel filtration of the crude cyclo-addition products was replaced by treatment with Cu-ion exchange resin CupriSorb^{TM} prior to HPLC purification.[26] This procedure led

to the isolation of a set of mannosylated peptides in an increased yield (Table 1).

Table 2: Results assembly of mannosyl-DEVA$_5$K conjugates **1-20**.

Peptide	Man$_1$[a]	1,2-Man$_2$[a]	1,3-Man$_2$[b]	1,6-Man$_2$[b]	1,3-1,6-Man$_3$[a]
26	**(1)** 13%	**(5)** 9%	**(9)** 25%	**(13)** 48%	**(17)** 12%
27	**(2)** 5%	**(6)** 8%	**(10)** 17%	**(14)** 22%	**(18)** 6%
28	**(3)** 7%	**(7)** 8%	**(11)** 20%	**(15)** 14%	**(19)** 5%
29	**(4)** 7%	**(8)** 14%	**(12)** 43%	**(16)** 35%	**(20)** 12%

[a)] Size-exclusion followed by HPLC purification. [b)] Treated with CupriSorb™ followed by HPLC purification.

Preliminary biological results

Immunological evaluation

In order to study whether the different glycan glycosylation pattern lead to different cross-presentation, the conjugates (**1-20**) were analysed for their ability to activate CD8+ T-cells, which reflects the degree of antigen presentation. Mouse bone marrow dendritic cells (BM-DCs) were incubated for 3h at 37 ^{0}C with an aliquot of each conjugate.[27] After this incubation the DCs were washed and the B3Z T-cell hybridoma were added.[28] These T-cells express the B3 T-cell receptor, which specifically recognises the peptide SIINFEKL presented in the H2-K^b subtype of MHC-I. Upon recognition of this peptide MHC-I complex, the B3Z is activated resulting in IL-2 production, which can be quantified. To allow for spectrophotometric activation of the T-cells, expression of a β-galactosidase reporter protein has been coupled to the IL-2 promotor. The expressed β-Gal can be quantified using chlorophenol β-D-galactopyranoside (CPRG) as β-galactosidase

substrate, that delivers a red colour upon hydrolysis by the enzyme.[29] The T-cell responses to conjugate **1**-**20** are plotted in Figure 4.

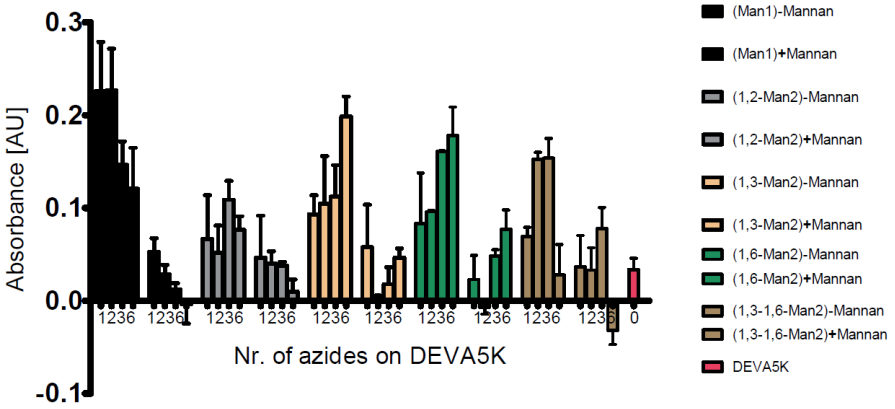


Figure 3: T-cell response of mannosyl-DEVA$_5$K conjugates **1**-**20**.

As shown in Figure 4 monomannoside conjugate $(Man_1)_1$ **1** and $(Man_1)_2$ **2** provided with one or two monomannosides showed the highest T cell response compared to the other constructs. Increasing the number of monomannose copies (Man_1) to three $(Man_1)_3$ **3** or six $(Man_1)_6$ **4** led to a decrease of T cell response. Surprisingly, the highest mannosylated conjugate $(1,3\text{-}1,6\text{-}Man_3)_6$ **20**, having six copies of the trimannoside gave the lowest T cell response of all. Conjugates provided with dimannosides (1,2-Man_2, 1,3-Man_2, 1,6-Man_2) **5**-**16** and in particular (1,2-Man_2) **5**-**8** showed a lower T cell response in comparison with the monomannoside conjugates (Man_1). Whereas increasing the number of monomannosides $(Man_1)_x$ to three (x=3) or six (x=6) copies led to a lower T cell response, an opposite trend was found for dimannosides $(1,3\text{-}Man_2)_x$ **9**-**12** and $(1,6\text{-}Man_2)_x$ **13**-**16**. By increasing the number of dimannosides (x= 2, 3, 6) an increase in T cell response was observed. Varying the number of dimannosides in the conjugate $(1,2\text{-}Man_2)_x$ did not show a significant difference in T cell response. The T cell response of the conjugates having a trimannosides $(1,3\text{-}1,6\text{-}Man_3)_x$ showed the highest T cell response with two (x=2, **18**) or three (x=3, **19**) copies. Surprisingly, T cell activation of the conjugate $(1,3\text{-}1,6\text{-}man_3)_x$ was completely diminished by the presence of

six trimannosides (x = 6, **20**). To assess the involvement of the mannose receptor in the uptake of the conjugates analogous experiments were performed in the presence of mannan, to block mannose receptor mediated uptake. Incubation in the presence of mannan reduced the T cell response to a level close to parent peptide $DEVA_5K$. This strongly indicates a mannose receptor mediated uptake of the mannosylated peptide conjugates. As control, DCs were incubated with all the unconjugated azido peptides (**26-29**) and with the short peptide $DEVA_5K$. All peptides lacking mannose sugars showed similar T cell response as the $DEVA_5K$ peptide (data not shown). Furthermore the presence of mannan did not affect the response of the unconjugated peptides indicating a mannose receptor independent mediated uptake of the control peptides.

Conclusion

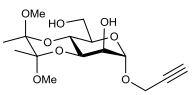
In this chapter the synthesis and preliminary immunological evaluation of a set of twenty mannosylated peptide conjugates is described. A mono-mannoside, tri di-mannosides and one tri-mannoside (**21-25**) equipped with an alkyne were synthesized uneventfully on mmole scale. The propargylated (oligo)mannosides were conjugated to MHC-class-I epitope DEVSGLEQLESIINFEKLAAAAAK provided with one, two, three or six azido lysines via Cu(I) catalysed click chemistry. The isolation procedure and hence the yield of the conjugates could be considerably improved by the application of CupriSorb^{TM} prior before HPLC purification. All mannosylated conjugates showed an increase in T cell response in comparison with a parent peptide. Reduction of T cell response of the conjugates in the presence of mannan to the level of the parent peptide is an indication of mannose receptor mediated uptake. The monomannoside conjugate $(Man_1)_1$ **1** and $(Man_1)_2$ **2** provided with one or two mono-mannosides showed the highest T cell response compared to the other constructs. The relation of the structure of the mannosylated conjugates and the degree of T cell response is rather

unforeseen. The effectiveness of both the mannose receptor mediated uptake and the subsequent processing of the conjugates are the main issues that contribute to the degree of T cell response. Both a low mannose mediated uptake of a specific conjugate and/or a reduced proteolytic susceptibility and processability may explain a decreased T cell response.

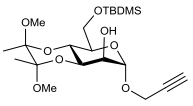
Experimental

General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under an argon atmosphere. Propargyl alcohol was distilled over K_2CO_3 prior to use. All other solvents and chemicals (Acros, Fluca, Merck) were of analytical grade and used as received. Column chromatography was performed on Screening Device silica gel 60 (0.040-0.063 mm). Size exclusion was performed on Sephadex LH20 (eluent DCM/MeOH, 1:1). TLC analysis was conducted on HPTLC aluminium sheet (Merck, TLC silica gel 60, F_{254}). Copper was removed using CupriSorb™ (Seachem) chelating resin. Compounds were visualized by UV absorption (λ = 254 nm), staining with *p*-anisaldehyde (3.7 mL in 135 mL EtOH, 1.5 mL AcOH and 5 mL H_2SO_4), 20% H_2SO_4 in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) in 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H, ^{13}C and 2D-HMBC (^1J-coupling) NMR were recorded on a Bruker DPX 300 (300 and 75 MHz respectively), Bruker AV 400 (400 and 100 MHz respectively), Bruker DMX 400 (400 and 100 MHz respectively), or Bruker DMX 600 (600 and 125 MHz respectively). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. *J* couplings are given in Hz. Optical rotations were measured on a Propol automatic polarimeter. IR spectra (thin film) were conducted on a Perkin Elmer FTIR Spectrum Two UATR (Single reflection diamond). LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI+) coupled to a Thermo Finnigan Surveyor HPLC system equipped with a

standard C_{18} (Gemini, 4.6 mm x 50 mm, 5 µm particle size, Phenomenex) analytical column and buffers A: H_2O, B: MeCN, C: 0.1% TFA (aq.). High-resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer.



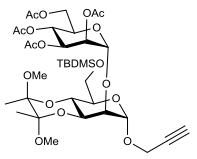
Propargyl 3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-α-D-mannopyranoside (31): To a solution of propargyl mannose **21** (2.2 g, 10 mmol) in MeOH (100 mL) was added butane-2,3-dione (1.1 mL, 12 mmol), trimethyl orthoformate (6 mL, 55 mmol), CSA (0.232 g, 1 mmol) and the reaction mixture was refluxed overnight. The reaction mixture was cooled to rt., neutralized with Et_3N, concentrated *in vacuo* and directly purified by column chromatography without further aqueous workup. Purification by column chromatography yielded butane-2,3-acetal protected mannose **31** as a colourless amorphous solid (2.2 g, 6.6 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 (s, 1H), 4.24 (d, *J* = 2.4 Hz, 2H), 4.13 (t, *J* = 9.8 Hz, 1H), 4.03 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.96 (s, 1H), 3.87 – 3.74 (m, 3H), 3.28 (s, 3H), 3.27 (s, 3H), 2.46 (t, *J* = 2.4 Hz, 1H), 1.33 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.5, 100.0, 98.7, 78.8, 75.1, 71.3, 69.6, 68.1, 62.9, 61.2, 54.5, 48.3, 48.0, 17.9. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_8$ 333.15439, found 333.15444.



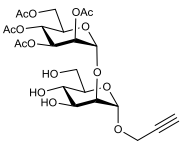
Propargyl 3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-6-*O*-*tert*butyldimethylsilyl-α-D-mannopyranoside (32): To a solution of butane-2,3-acetal protected mannose **31** (332 mg, 1,0 mmol) in DMF (10 mL) was added TBDMSCl (0.19 g, 1.25 mmol) and the mixture was cooled to 0 °C. To the mixture was added imidazole (85 mg, 1.25 mmol) and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with MeOH. The mixture was concentrated *in vacuo* and dissolved in Et_2O. The organic layer was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose acceptor **32** as a colourless oil (473 mg, 1 mmol,

quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 1H), 4.20 (d, *J* = 2.4 Hz, 2H), 4.03 – 3.94 (m, 2H), 3.91 (s, 1H), 3.82 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.77 (dd, *J* = 11.3, 5.0 Hz, 1H), 3.68 (ddt, *J* = 7.3, 4.8, 2.2 Hz, 1H), 3.25 (s, 3H), 3.22 (s, 3H), 2.48 (s, 1H), 2.40 (t, *J* = 2.4 Hz, 1H), 1.30 (s, 3H), 1.26 (s, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.4, 99.9, 98.3, 79.0, 74.7, 72.1, 69.6, 68.4, 62.8, 61.6, 53.8, 48.2, 48.0, 25.9, 18.4, 17.9, 17.8, -5.0, -5.3. HRMS: $[M+H]^+$ calculated for $C_{21}H_{39}O_8Si$ 447.24087, found 447.24090.

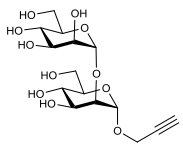
Propargyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-6-*O*-*tert*butyldimethylsilyl-α-D-mannopyranoside (33): 2,3-Acetal protected mannose acceptor **32** (223 mg, 0.5 mmol) and trichloro imidate donor **30** (389 mg, 0.75 mmol) were dissolved in DCM (10 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (4.4 µl, 50 µmol) was added. The reaction mixture was allowed to gradually warm up and the reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography yielded dimer **33** as an colourless oil (320 mg, 0.41 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.35 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.24 (t, *J* = 9.9 Hz, 1H), 5.20 (d, *J* = 1.9 Hz, 1H), 4.97 (s, 1H), 4.23 (dd, *J* = 12.1, 5.0 Hz, 1H), 4.20 (d, *J* = 1.5 Hz, 2H), 4.13 – 4.07 (m, 1H), 4.09 – 4.01 (m, 1H), 4.01 – 3.97 (m, 2H), 3.91 (s, 1H), 3.84 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.77 (dd, *J* = 11.3, 5.8 Hz, 1H), 3.71 – 3.61 (m, 1H), 3.22 (s, 3H), 3.20 (s, 3H), 2.41 (t, *J* = 2.4 Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.22 (s, 3H), 1.18 (s, 3H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 169.9, 169.6, 169.6, 100.1, 100.1, 99.6, 99.0, 97.3, 78.7, 75.8, 74.9, 72.8, 69.5, 69.1, 68.9, 68.5, 66.4, 63.1, 62.5, 61.8, 53.7, 48.2, 47.9, 25.9, 20.9, 20.9, 20.8, 20.8, 18.3, 17.7, 17.5,



-5.1, -5.3. HMBC: $^{1}J_{\alpha\text{-}1,2\text{-}man\ C\text{-}H}$ = 176 Hz. HRMS: $[M+H]^+$ calculated for $C_{35}H_{57}O_{17}Si$ 777.33595, found 777.33598.

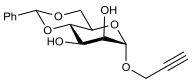


Propargyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (34): Dimer **33** (311 mg, 0.4 mmol) was dissolved in TFA/H_2O (9:1, 12.3 mL) and stirred at rt. After 30 min. the reaction mixture was diluted with toluene and concentrated *in vacuo*. The crude was co-evaporated with toluene (3x) and directly purified by column chromatography without further aqueous workup. Purification by column chromatography yielded dimer **34** as a colourless amorphous solid (159 mg, 0.29 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.31 (dd, *J* = 10.0, 3.3 Hz, 1H), 5.24 (d, *J* = 1.6 Hz, 1H), 5.20 (t, *J* = 10.0 Hz, 1H), 5.05 (d, *J* = 1.8 Hz, 1H), 4.29 (dd, *J* = 6.7, 1.6 Hz, 1H), 4.25 (s, 1H), 4.24 – 4.21 (m, 2H), 4.16 – 4.12 (m, 1H), 4.10 (d, *J* = 6.9 Hz, 1H), 3.99 – 3.87 (m, 3H), 3.83 (dd, *J* = 12.3, 3.0 Hz, 1H), 3.57 (dt, *J* = 9.7, 3.3 Hz, 1H), 3.37 (s, 3H), 2.49 (t, *J* = 2.4 Hz, 1H), 2.14 (s, 6H), 2.08 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 170.7, 170.1, 170.0, 99.6, 96.9, 80.3, 78.6, 75.3, 73.1, 70.7, 69.4, 69.2, 68.9, 67.4, 66.2, 63.1, 61.7, 54.5, 21.0, 20.9, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{23}H_{33}O_{15}$ 549.18140, found 549.18138.

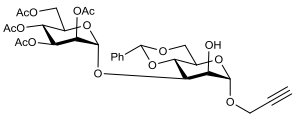


Propargyl 2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (22): To a solution of mannose dimer **34** (137 mg, 0.25 mmol) in MeOH (2.5 mL) was added NaOMe (1.3 mg, 0.025 mmol) and the mixture was stirred at room temperature. After 4h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The formed precipitate was filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,2-Man_2 dimer **22** as a white powder (78 mg, 0.21 mmol, 82%). ^{1}H NMR (400 MHz, MeOD) δ 5.20 (d, *J* = 1.7 Hz, 1H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.26 (dd, *J* = 4.3, 2.4 Hz, 2H), 3.99

(dd, *J* = 3.1, 1.7 Hz, 1H), 3.88 – 3.76 (m, 4H), 3.73 (t, *J* = 4.2 Hz, 1H), 3.73 – 3.57 (m, 5H), 3.50 (ddd, *J* = 8.9, 5.5, 1.9 Hz, 1H), 2.86 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.2, 98.6, 80.3, 80.1, 76.0, 75.0, 74.8, 72.4, 72.0, 71.8, 68.7, 68.4, 62.8, 62.7, 55.1. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13911.

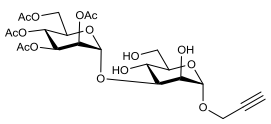


Propargyl 4,6-*O*-benzylidene-α-D-mannopyranoside (35): To a solution of propargyl mannose **21** (436 mg, 2 mmol) in MeCN (10 mL) was added benzaldehyde dimethyl acetal (0.36 mL, 2.4 mmol), trichlorotriazine (0.1 g, 0.6 mmol) and sonicated at 60 °C. After complete conversion of the starting material the reaction mixture was concentrated *in vacuo*. Purification by column chromatography yielded compound **35** as a white amorphous solid (330 mg, 1.1 mmol, 55%). ^{1}H NMR (400 MHz, MeOD) δ 7.50 (dd, *J* = 6.7, 3.1 Hz, 2H), 7.34 (dd, *J* = 5.1, 2.0 Hz, 3H), 5.60 (s, 1H), 4.99 (d, *J* = 1.3 Hz, 1H), 4.29 (dd, *J* = 2.4, 0.9 Hz, 2H), 4.21 (ddd, *J* = 9.3, 4.1, 1.5 Hz, 1H), 4.00 – 3.89 (m, 2H), 3.92 – 3.85 (m, 1H), 3.84 – 3.77 (m, 1H), 3.75 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.91 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 139.2, 129.9, 129.0, 127.5, 103.3, 101.1, 80.0, 76.2, 72.4, 69.6, 69.5, 65.6, 55.2. HRMS: $[M+H]^+$ calculated for $C_{16}H_{19}O_6$ 307.11761, found 307.11763.



Propargyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-4,6-*O*-benzylidene-α-D-mannopyranoside (36): Benzylidene acceptor **35** (153 mg, 0.5 mmol) and trichloro imidate donor **30** (271 mg, 0.55 mmol) were dissolved in DCM (5 mL) and stirred over activated molecular sieves for 0.5 h at rt. The mixture was cooled to -20 °C and TMSOTf (4.5 µl, 25 µmol) was added. The reaction was stirred for 2 h at -20 °C and quenched with Et_3N (0.1 mL) at that temperature. The mixture was filtered and concentrated *in vacuo*. Purification by size-exclusion yielded dimer **36** as a colourless amorphous foam (253 mg, 0.4 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.40 (m, 2H), 7.33 (dd, *J* = 5.0, 1.9 Hz, 3H), 5.59 (s, 1H), 5.39 (dd, *J* =

3.5, 1.8 Hz, 1H), 5.34 (dd, *J* = 9.8, 3.5 Hz, 1H), 5.27 (d, *J* = 1.8 Hz, 1H), 5.22 (t, *J* = 9.9 Hz, 1H), 5.09 (d, *J* = 1.5 Hz, 1H), 4.28 (d, *J* = 5.3 Hz, 1H), 4.26 (d, *J* = 2.5 Hz, 2H), 4.25 – 4.19 (m, 1H), 4.20 – 4.13 (m, 2H), 4.13 – 4.04 (m, 2H), 3.86 (d, *J* = 9.6 Hz, 1H), 3.85 (d, *J* = 9.8 Hz, 1H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.60 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.2, 169.8, 169.7, 137.2, 128.9, 128.2, 126.1, 101.5, 99.0, 98.5, 78.5, 78.3, 75.3, 73.7, 70.9, 69.2, 69.1, 69.0, 68.6, 66.4, 64.1, 62.8, 54.6, 20.8, 20.8. HMBC: $^{1}J_{\alpha\text{-}1,3\text{-}man\ C\text{-}H}$ = 176 Hz. HRMS: $[M+H]^+$ calculated for $C_{30}H_{37}O_{15}$ 637.21270, found 637.21264.

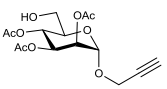


Propargyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (37): Dimer **36** (0.172 g, 0.27 mmol) was dissolved in 70% AcOH (aq.) (2.7 mL) and stirred for 4h at 55 °C. The mixture was cooled to rt, diluted with H_2O and transferred to a separation funnel. To the solution was added solid $NaHCO_3$ till neutral pH after which the aqueous layer was extracted with DCM (5x). The combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded dimer **37** as a colourless oil (0.124 g, 0.23 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 – 5.36 (m, 2H), 5.30 – 5.20 (m, 2H), 5.04 (s, 1H), 4.31 – 4.18 (m, 4H), 4.13 (d, *J* = 10.3 Hz, 1H), 4.06 (s, 1H), 3.99 (d, *J* = 13.5 Hz, 1H), 3.94 (d, *J* = 11.5 Hz, 1H), 3.84 (d, *J* = 11.8 Hz, 1H), 3.62 (d, *J* = 9.5 Hz, 1H), 3.11 (s, 4H), 2.41 (t, *J* = 2.1 Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.9, 170.7, 169.9, 99.5, 98.6, 79.5, 78.8, 75.0, 73.0, 70.7, 69.7, 69.5, 69.1, 66.3, 65.6, 62.9, 61.4, 54.6, 21.1, 21.0, 20.9. HRMS: $[M+H]^+$ calculated for $C_{23}H_{33}O_{15}$ 549.18140, found 549.18142.

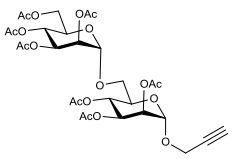
Propargyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (23): To a solution of mannose dimer **37** (91.1 mg, 0.17 mmol) in MeOH (1.7 mL) was added NaOMe (0.9 mg, 17 µmol) and the mixture was stirred at room temperature. After 6h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added drop wise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding dimer **23** as a white powder (61 mg, 0.16 mmol, 95%). ^{1}H NMR (400 MHz, MeOD) δ 5.07 (d, *J* = 1.7 Hz, 1H), 4.95 (d, *J* = 1.8 Hz, 1H), 4.28 (d, *J* = 2.4 Hz, 2H), 4.04 (dd, *J* = 2.9, 1.9 Hz, 1H), 3.97 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.85 (t, *J* = 2.4 Hz, 1H), 3.82 (d, *J* = 2.3 Hz, 1H), 3.81 – 3.75 (m, 4H), 3.73 (dd, *J* = 5.8, 2.4 Hz, 1H), 3.69 (dd, *J* = 5.8, 1.8 Hz, 1H), 3.62 (t, *J* = 9.5 Hz, 1H), 3.55 (ddd, *J* = 6.7, 3.8, 3.0 Hz, 1H), 2.87 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 103.9, 99.9, 80.5, 79.9, 76.1, 75.3, 74.9, 72.4, 72.1, 71.3, 68.7, 67.4, 62.8, 62.7, 54.8. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13915.

Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranoside (38): To a solution of propargyl mannose **21** (1.6 g, 7.5 mmol) in pyridine (30 mL) was added trityl chloride (2.6 g, 9.4 mmol) and the mixture was stirred till complete conversion of the starting material. The reaction mixture was cooled to 0 °C and Ac_2O (3.2 mL, 33.8 mmol) was added dropwise. The reaction mixture was allowed to warm to rt and the reaction was stirred overnight at rt. The mixture was cooled to 0 °C and the reaction was quenched with MeOH. Solvents were removed *in vacuo* and the crude was dissolved in EtOAc. The organic layer was washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (1x), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding propargyl mannose **38** in qunantitative yield. The crude trityl mannose **38** was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 7.2

Hz, 6H), 7.29 (t, *J* = 7.4 Hz, 6H), 7.22 (t, *J* = 7.2 Hz, 3H), 5.31 (d, *J* = 2.4 Hz, 1H), 5.30 – 5.26 (m, 2H), 5.08 (d, *J* = 1.6 Hz, 1H), 4.33 (d, *J* = 1.0 Hz, 2H), 3.90 (tt, *J* = 5.6, 2.9 Hz, 1H), 3.23 (dd, *J* = 10.3, 2.6 Hz, 1H), 3.18 (dd, *J* = 10.3, 5.1 Hz, 1H), 2.47 (t, *J* = 2.3 Hz, 1H), 2.17 (s, 3H), 1.96 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 170.0, 169.4, 143.8, 128.8, 127.9, 127.1, 95.8, 86.7, 78.3, 75.5, 70.7, 69.7, 69.3, 66.6, 62.4, 54.5, 21.0, 20.8, 20.6. HRMS: $[M+H]^+$ calculated for $C_{34}H_{35}O_9$ 587.22756, found 587.22762.



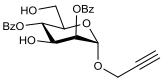
Propargyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (39): To a solution of trityl mannose **38** (4.4 g, 7.5 mmol) in a MeOH/toluene mixture (1:1, 75 mL) was added dropwise $BF_3{\cdot}Et_2O$ (1.0 mL, 8.25 mmol) and stirred till complete conversion of the starting material. The reaction mixture was diluted with EtOAc and washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded propargyl mannose acceptor **39** as a white amorphous solid (1.81 g, 5.25 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (dd, *J* = 10.2, 3.4 Hz, 1H), 5.29 (dd, *J* = 3.6, 1.8 Hz, 1H), 5.26 (t, *J* = 10.2 Hz, 1H), 5.05 (d, *J* = 1.8 Hz, 1H), 4.28 (d, *J* = 2.4 Hz, 2H), 3.82 (ddd, *J* = 10.1, 4.1, 2.3 Hz, 1H), 3.72 (ddd, *J* = 12.8, 8.5, 2.3 Hz, 1H), 3.63 (ddd, *J* = 12.8, 5.6, 4.1 Hz, 1H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.40 (dd, *J* = 8.5, 5.7 Hz, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.66 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.1, 170.0, 96.5, 78.2, 75.7, 71.3, 69.5, 68.8, 66.5, 61.3, 55.1, 21.0, 20.9, 20.8. HRMS: $[M+H]^+$ calculated for $C_{15}H_{21}O_9$ 345.11801, found 345.11804.



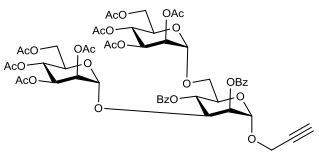
Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (40): Mannose acceptor **39** (0.479 g, 0.156 mmol) and trichloro imidate donor **30** (0.373 g, 0.72 mmol) were dissolved in DCM (9.6 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (13 µl, 0.144 µmol) was added. The reaction mixture was allowed to gradually warm up and the

reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion yielded dimer **40** as an colourless oil (0.257 g, 0.38 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 – 5.31 (m, 3H), 5.30 – 5.26 (m, 3H), 5.02 (d, *J* = 1.7 Hz, 1H), 4.86 (d, *J* = 1.7 Hz, 1H), 4.30 (d, *J* = 2.4 Hz, 2H), 4.27 (dd, *J* = 12.2, 5.2 Hz, 1H), 4.13 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.11 – 4.04 (m, 1H), 3.99 (ddd, *J* = 10.1, 5.7, 2.4 Hz, 1H), 3.79 (dd, *J* = 11.0, 5.7 Hz, 1H), 3.59 (dd, *J* = 11.0, 2.5 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.2, 170.1, 170.0, 169.9, 169.9, 169.9, 97.6, 96.1, 78.1, 75.7, 69.9, 69.4, 69.4, 69.1, 69.1, 68.7, 66.7, 66.5, 66.0, 62.5, 55.0, 21.0, 20.9, 20.9, 20.8, 20.8, 20.8. HMBC: $^1J_{\alpha\text{-}1,6\text{-man C-H}}$ = 173 Hz. HRMS: $[M+H]^+$ calculated for $C_{29}H_{40}O_{18}$ 675.21309, found 675.21300.

Propargyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (24): To a solution of mannose dimer **40** (0.257 g, 0.38 mmol) in MeOH (3.8 mL) was added NaOMe (5 mg, 0.095 mmol) and the mixture was stirred at room temperature. After 1h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,6-Man_2 dimer **24** as a white powder (94 mg, 0.25 mmol, 66%). ^{1}H NMR (400 MHz, MeOD) δ 4.93 (d, *J* = 1.6 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.25 (t, *J* = 2.2 Hz, 2H), 3.92 (dd, *J* = 11.0, 5.2 Hz, 1H), 3.87 (d, *J* = 2.8 Hz, 1H), 3.83 (d, *J* = 11.9 Hz, 2H), 3.78 – 3.69 (m, 3H), 3.67 (d, *J* = 3.4 Hz, 2H), 3.67 – 3.61 (m, 3H), 2.88 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 101.4, 100.0, 80.0, 76.1, 74.3, 73.5, 72.6, 72.0, 71.9, 68.5, 68.3, 67.2, 62.8, 54.9. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13913.

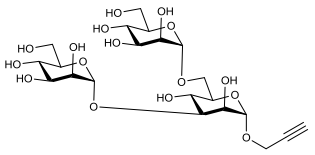


Propargyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (41): To a suspension of propargyl mannose **21** (0.218 g, 1.15 mmol) in MeCN (11.5 mL) was added $PhC(OMe)_3$ (0.6 mL, 3.5 mmol) and CSA (26 mg, 115 µmol) and the mixture was stirred rt. After 2h H_2O (0.5 mL) was added and the mixture was stirred overnight at rt. The reaction was mixture was neutralized wit Et_3N, concentrated *in vacuo* and co-evaporated wit toluene (3x). Purification by column chromatography yielded benzoylated mannose **41** as a white amorphous solid (254 mg, 0.6 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.03 (m, 4H), 7.65 – 7.53 (m, 2H), 7.51 – 7.41 (m, 3H), 5.52 (t, *J* = 9.9 Hz, 1H), 5.44 (dd, *J* = 3.5, 1.7 Hz, 1H), 5.24 (d, *J* = 1.7 Hz, 1H), 4.44 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.32 (d, *J* = 2.5 Hz, 2H), 3.99 (ddd, *J* = 10.1, 4.2, 2.3 Hz, 1H), 3.81 (dd, *J* = 12.6, 2.4 Hz, 1H), 3.73 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.40 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 166.1, 133.8, 133.7, 130.1, 129.3, 129.1, 128.7, 128.7, 96.7, 78.4, 75.6, 72.8, 71.2, 70.3, 68.7, 61.5, 55.4. HRMS: $[M+H]^+$ calculated for $C_{23}H_{24}O_8$ 427.13874, found 427.13869.



Propargyl 2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (42): Benzoylated mannose acceptor **41** (0.107 g, 0.25 mmol) and trichloro imidate **30** (390 mg, 0.75 mmol) were dissolved in DCM (5 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (4.4 µl, 50 µmol) was added. The reaction mixture was allowed to gradually warm up and the reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion yielded trimer **42** as an colourless oil (223 mg, 0.2 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.5, 1.5 Hz, 2H), 8.03 (dd, *J* = 8.5, 1.3 Hz, 2H), 7.67 – 7.50 (m, 4H), 7.45 (t, *J* = 7.8 Hz, 2H), 5.65 (t, *J* =

10.0 Hz, 1H), 5.55 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.34 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.26 (d, *J* = 10.1 Hz, 2H), 5.23 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.12 – 5.07 (m, 2H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.88 (dd, *J* = 2.8, 1.8 Hz, 1H), 4.81 (d, *J* = 1.7 Hz, 1H), 4.49 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.37 (d, *J* = 2.4 Hz, 2H), 4.24 – 4.04 (m, 5H), 4.03 (t, *J* = 2.2 Hz, 1H), 4.00 (t, *J* = 2.3 Hz, 1H), 3.91 (dd, *J* = 10.8, 6.7 Hz, 1H), 3.62 (dd, *J* = 10.8, 2.2 Hz, 1H), 2.56 (t, *J* = 2.4 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.94 (d, *J* = 0.9 Hz, 6H), 1.85 (s, 3H), 1.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.6, 170.0, 169.8, 169.7, 169.2, 169.1, 166.0, 165.3, 133.7, 133.7, 130.1, 130.0, 129.1, 128.9, 128.8, 128.6, 99.5, 97.3, 96.2, 78.1, 75.8, 75.0, 71.6, 70.1, 69.4, 69.3, 69.1, 68.8, 68.7, 68.3, 66.8, 66.1, 65.9, 62.4, 62.4, 55.1, 20.9, 20.8, 20.8, 20.7, 20.7, 20.5, 20.5. HRMS: $[M+H]^+$ calculated for $C_{51}H_{59}O_{26}$ 1087.32891, found 1087.32883.



Propargyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (25): To a solution of mannose trimer **42** (223 mg, 0.2 mmol) in MeOH (4 mL) was added NaOMe (5.4 mg, 0.1 mmol) and the mixture was stirred at room temperature. After 1h the reaction was quenched with Amberlite IR-120 H^+ untill pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,3-1,6-Man_3 trimer **25** as a white powder (82 mg, 0.15 mmol, 75%). ^{1}H NMR (400 MHz, MeOD) δ 5.07 (d, *J* = 1.7 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.7 Hz, 1H), 4.26 (t, *J* = 2.4 Hz, 2H), 4.04 (dd, *J* = 2.9, 1.7 Hz, 1H), 3.99 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.94 (dd, *J* = 11.1, 5.3 Hz, 1H), 3.88 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.86 – 3.81 (m, 10H), 3.80 (t, *J* = 3.6 Hz, 1H), 3.78 – 3.71 (m, 2H), 3.71 – 3.68 (m, 1H), 3.66 – 3.61 (m, 2H), 2.89 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.0, 101.4, 100.1, 80.6, 79.9, 76.2, 74.9, 74.3, 73.8, 72.6, 72.4, 72.0, 72.0, 71.2, 68.7,

68.5, 67.2, 67.0, 62.8, 55.0. HRMS: $[M+H]^+$ calculated for $C_{21}H_{35}O_{16}$ 543.19196, found 543.19200.

General procedure peptide synthesis

The solid-phase peptide synthesis was performed on 100 µmol scale according to established methods on an ABI 433A (Applied Biosystems) automated instrument applying an Fmoc based protocol[30] starting from Tentagel-S-RAM resin (loading 0.23 mmol/g). The consecutive steps performed in each cycle for HCTU chemistry on µmol scale: 1) Deprotection of the Fmoc-group with 20% piperidine in NMP for 15 min; 2) NMP wash; 3) Coupling of the appropriate amino acid using a five-fold excess. Generally, the Fmoc amino acid (0.25 mmol) was dissolved in 0.25 M HCTU in NMP (1 mL), the resulting solution was transferred to the reaction vessel followed by 0.5 mL of 1.0 M DiPEA in NMP to initiate the coupling. The reaction vessel was shaken for 30 min; 4) NMP wash; 5) capping with 0.5 M acetic anhydride in NMP in presence of 0.5 mmol DiPEA; 6) NMP wash; 7) DCM wash.

H-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (26): Az_1DEVA_5K **26** (49.0 mg, 18.1 µmol, 18% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{118}H_{197}N_{33}O_{39}$ 1350.72, found 1350.73.

H-Lys(N_3)-Gly-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (27): Az_2DEVA_5K **27** (49.0 mg, 16.8 µmol, 17% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{126}H_{210}N_{38}O_{41}$ 1456.27, found 1456.27.

H-Lys(N_3)-Gly-Lys(N_3)-Gly-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (28): Az_3DEVA_5K **28** (31.1 mg, 10.0 µmol, 10% based on theoretical

loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{134}H_{223}N_{43}O_{43}$ 1561.82, found 1561.87.

H-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (29): Az_6DEVA_5K **29** (18.0 mg, 5.2 µmol, 5% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{148}H_{247}N_{53}O_{44}$ 1735.93, found 1735.93.

General procedure (oligo)mannoside conjugation

The peptide was dissolved in a 0.05M propargyl mannose sugar solution (n_{azide} + 2 eq.) (aq.). To the solution was added a 0.05M sodium ascorbate solution (n_{azide} + 2 eq.) (aq.) and a 0.05M $CuSO_4$ solution (2 eq.) (aq.) and the reaction was monitored by HPLC. After full conversion to the product, two workup procedures were performed prior before purification by RP-HPLC. Gel filtration: The reaction mixture was diluted with MiliQ and purified by gel filtration (HW-40). The combined frations were concentrated, centrifuged and the supernatant was purified by RP-HPLC. Resin purification: The reaction mixture was diluted with MiliQ water and CupriSorb™ (50 mg) was added to the reaction mixture for 2 in which the solution turned from light blue to colourless. The resin was filtered and rinsed with MiliQ water. The filtrate was centrifuged and the supernatant was directly purified by RP-HPLC.

H-Lys(Man_1)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (1): $(Man_1)_1DEVA_5K$ **1** was conjugated following the general procedure using: Az_1DEVA_5K (6.80 mg, 2.52 µmol), 0.05M propargyl mannose **21** sol. (151.2 µl, 7.56 µmol), 0.05M sodium ascorbate sol. (151.2 µl, 7.56 µmol) and 0.05M $CuSO_4$ (50.4 µl, 2.52 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded $(Man_1)_1DEVA_5K$ **1** as a white powder (0.97 mg, 0.33 µmol, 13%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.067 min, ESI/MS (*m/z*): $[M+2H]^{2+}$

calculated for $C_{127}H_{211}N_{33}O_{45}$ 1459.76, found 1459.73; $[M+3H]^{3+}$ calculated for $C_{127}H_{212}N_{33}O_{45}$ 973.50, found 973.80.

H-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (2): (Man$_1$)$_2$DEVA$_5$K **2** was conjugated following the general procedure using: Az$_2$DEVA$_5$K **27** (8.35 mg, 2.87 µmol), 0.05M propargyl mannose **21** sol. (229.6 µl, 11.48 µmol), 0.05M sodium ascorbate sol. (229.6 µl, 11.48 µmol) and 0.05M $CuSO_4$ (5.74 µl, 114.8 µmol). Gel filtration followed by RP-HPLC purification by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (Man$_1$)$_2$DEVA$_5$K **2** as a white powder (0.50 mg, 0.15 µmol, 5%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.033 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{144}H_{238}N_{38}O_{53}$ 1673.35, found 1674.27; $[M+3H]^{3+}$ calculated for $C_{144}H_{239}N_{38}O_{53}$ 1116.57, found 1116.67.

H-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (3): (Man$_1$)$_3$DEVA$_5$K **3** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (4.85 mg, 1.55 µmol), 0.05M propargyl mannose **21** sol. (155.0 µl, 7.75 µmol), 0.05M sodium ascorbate sol. (155.0 µl, 7.75 µmol) and 0.05M $CuSO_4$ (4.65 µl, 93.0 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (Man$_1$)$_3$DEVA$_5$K **3** as a white powder (0.42 mg, 0.11 µmol, 7%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.027 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{161}H_{265}N_{43}O_{61}$ 1888.94, found 1889.13; $[M+3H]^{3+}$ calculated for $C_{161}H_{266}N_{43}O_{61}$ 1259.63, found 1259.53.

H-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (4): (Man$_1$)$_6$DEVA$_5$K **4** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.40 mg, 0.69 µmol),

0.05M propargyl mannose **21** sol. (110.4 µl, 5.52 µmol), 0.05M sodium ascorbate sol. (110.4 µl, 5.52 µmol) and 0.05M $CuSO_4$ (4.14 µl, 82.8 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(Man_1)_6DEVA_5K$ **4** as a white powder (0.22 mg, 0.05 µmol, 7%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.900 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{202}H_{333}N_{53}O_{80}$ 1594.11, found 1594.20.

H-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (5): $(1,2\text{-}Man_2)_1DEVA_5K$ **5** was conjugated following the general procedure using: Az_1DEVA_5K **26** (9.06 mg, 3.36 µmol), 0.05M 1,2-Man_2 **22** sol. (201.6 µl, 10.08 µmol), 0.05M sodium ascorbate sol. (201.6 µl, 10.08 µmol) and 0.05M $CuSO_4$ (3.36 µl, 67.2 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(1,2\text{-}Man_2)_1DEVA_5K$ **5** as a white powder (0.98 mg, 0.32 µmol, 9%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.060 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, found 1540.80; $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.40.

H-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (6): $(1,2\text{-}Man_2)_2DEVA_5K$ **6** was conjugated following the general procedure using: Az_2DEVA_5K **27** (8.41 mg, 2.89 µmol), 0.05M 1,2-Man_2 **22** solution (231.2 µl, 11.56 µmol), 0.05M sodium ascorbate sol. (231.2 µl, 11.56 µmol) and 0.05M $CuSO_4$ (5.78 µl, 115.6 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(1,2\text{-}Man_2)_2DEVA_5K$ **6** as a white powder (0.83 mg, 0.23 µmol, 8%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.990 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$ 1836.40, found 1836.27; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.53.

H-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (7): (1,2-Man$_2$)$_3$DEVA$_5$K **7** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (5.37 mg, 1.72 µmol), 0.05M 1,2-Man$_2$ **22** sol. (172.0 µl, 8.60 µmol), 0.05M sodium ascorbate sol. (172.0 µl, 8.60 µmol) and 0.05M $CuSO_4$ (5.16 µl, 103.2 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,2-Man$_2$)$_3$DEVA$_5$K **7** as a white powder (0.55 mg, 0.13 µmol, 8%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.937 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1422.07.

H-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (8): (1,2-Man$_2$)$_6$DEVA$_5$K **8** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (3.20 mg, 0.92 µmol), 0.05M 1,2-Man$_2$ **22** sol. (147.2 µl, 7.36 µmol), 0.05M sodium ascorbate sol. (147.2 µl, 7.36 µmol) and 0.05M $CuSO_4$ (5.52 µl, 110.4 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,2-Man$_2$)$_6$DEVA$_5$K **8** as a white powder (0.72 mg, 0.13 µmol, 14%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.990 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.33.

H-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (9): (1,3-Man$_2$)$_1$DEVA$_5$K **9** was conjugated following the general procedure using: Az$_1$DEVA$_5$K **26** (7.32 mg, 2.71 µmol), 0.05M 1,3-Man$_2$ **23** sol. (162.6 µl, 8.13 µmol), 0.05M sodium ascorbate sol. (162.6 µl, 8.13 µmol) and 0.05M $CuSO_4$ (2.71 µl, 54.2 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-

$Man_2)_1DEVA_5K$ **9** as a white powder (2.11 mg, 0.68 µmol, 25%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 5.017 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, 1540.73; $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.53.

H-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (10) (1,3-$Man_2)_2DEVA_5K$ **10** was conjugated following the general procedure using: Az_2DEVA_5K **27** (7.41 mg, 2.55 µmol), 0.05M 1,3-Man_2 **23** sol. (204.0 µl, 10.2 µmol), 0.05M sodium ascorbate sol. (204.0 µl, 10.2 µmol) and 0.05M $CuSO_4$ (102.0 µl, 5.1 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_2DEVA_5K$ **10** as a white powder (1.61 mg, 0.44 µmol, 17%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.957 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$ 1836.40, found 1836.33; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.67.

H-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (11): (1,3-$Man_2)_3DEVA_5K$ **11** was conjugated following the general procedure using: Az_3DEVA_5K **28** (5.09 mg, 1.63 µmol), 0.05M 1,3-Man_2 **23** sol. (163.0 µl, 8.15 µmol), 0.05M sodium ascorbate sol. (163.0 µl, 8.15 µmol) and 0.05M $CuSO_4$ (97.8 µl, 4.89 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_3DEVA_5K$ **11** as a white powder (0.1.42 mg, 0.33 µmol, 20%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.903 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1422.67.

H-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-

Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (12): (1,3-$Man_2)_6$DEVA$_5$K **12** was conjugated following the general procedure using: Az_6DEVA$_5$K **29** (2.7 mg, 0.78 µmol), 0.05M 1,3-Man_2 **23** sol. (124.8 µl, 6.24 µmol), 0.05M sodium ascorbate sol. (124.8 µl, 6.24 µmol) and 0.05M $CuSO_4$ (93.6 µl, 4.68 µmol). Resin purification followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_6$DEVA$_5$K **12** as a white powder (1.93 mg, 0.34 µmol, 43%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.813 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.07.

H-Lys(1,6-Man_2)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (13): (1,6-$Man_2)_1$DEVA$_5$K **13** was conjugated following the general procedure using: Az_1DEVA$_5$K **26** (7.40 mg, 2.74 µmol), 0.05M 1,6-Man_2 **24** sol. (164.4 µl,8.22 µmol), 0.05M sodium ascorbate sol. (164.4 µl, 8.22 µmol) and 0.05M $CuSO_4$ (54.8 µl, 2.74 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-$Man_2)_1$DEVA$_5$K **13** as a white powder (3.99 mg, 1.3 µmol, 48%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 5.013 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, found 1540.73, $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.87.

H-Lys(1,6-Man_2)-Gly-Lys(1,6-Man_2)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (14): (1,6-$Man_2)_2$DEVA$_5$K **14** was conjugated following the general procedure using: Az_2DEVA$_5$K **27** (8.11 mg, 2.79 µmol), 0.05M 1,6-Man_2 **24** sol. (223.2 µl, 11.16 µmol), 0.05M sodium ascorbate sol. (223.2 µl, 11.16 µmol) and 0.05M $CuSO_4$ (111.6 µl, 5.58 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-$Man_2)_2$DEVA$_5$K **14** as a white powder (2.30 mg, 0.63 µmol, 22%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.970 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$

1836.40, found 1836.27; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.47.

H-Lys(1,6-Man$_2$)-Gly-Lys(1,6-Man$_2$)-Gly-Lys(1,6-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (15): (1,6-Man$_2$)$_3$DEVA$_5$K **15** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (5.02 mg, 1.61 µmol), 0.05M 1,6-Man$_2$ **24** sol. (161.0 µl, 8.0.5 µmol), 0.05M sodium ascorbate sol. (161.0 µl, 8.05 µmol) and 0.05M $CuSO_4$ (69.6 µl, 3.48 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-Man$_2$)$_3$DEVA$_5$K **15** as a white powder (0.93 mg, 0.22 µmol, 14%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.893 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1421.93.

H-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (16): (1,6-Man$_2$)$_6$DEVA$_5$K **16** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.6 mg, 0.75 µmol), 0.05M 1,6-Man$_2$ **24** sol. (120.0 µl, 6.0 µmol), 0.05M sodium ascorbate sol. (120.0 µl, 6.0 µmol) and 0.05M $CuSO_4$ (90.0 µl, 4.5 µmol). Resin purification followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,6-Man$_2$)$_6$DEVA$_5$K **16** as a white powder (1.33 mg, 0.23 µmol, 35%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.810 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.27.

H-Lys(1,3-1,6-Man$_3$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (17): (1,3-1,6-Man$_3$)$_1$DEVA$_5$K **17** was conjugated following the general procedure using: Az$_1$DEVA$_5$K **26** (7.45 mg, 2.76 µmol), 0.05M 1,3-1,6-Man$_3$ **25** sol. (165.6 µl, 8.28 µmol), 0.05M sodium ascorbate sol. (165.5 µl, 8.28 µmol) and 0.05M

$CuSO_4$ (55.2 µl, 2.76 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_1$$DEVA_5K$ **17** as a white powder (1.11 mg, 0.34 µmol, 12%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.993 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{139}H_{231}N_{33}O_{55}$ 1621.31, found 1621.53; $[M+3H]^{3+}$ calculated for $C_{139}H_{231}N_{33}O_{55}$ 1081.54, found 1081.20.

H-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (18): (1,3-1,6-Man_3)$_2$$DEVA_5K$ **18** was conjugated following the general procedure using: Az_2DEVA_5K **27** (8.34 mg, 2.87 µmol), 0.05M 1,3-1,6-Man_3 **25** sol. (229.6 µl, 11.48 µmol), 0.05M sodium ascorbate sol. (229.6 µl, 11.48 µmol) and 0.05M $CuSO_4$ (114.8 µl, 5.74 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_2$$DEVA_5K$ **18** as a white powder (0.73 mg, 0.18 µmol, 6%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.903 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{168}H_{278}N_{38}O_{73}$ 1998.45, found 1998.87; $[M+3H]^{3+}$ calculated for $C_{168}H_{279}N_{38}O_{73}$ 1332.64, found 1332.93.

H-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (19): (1,3-1,6-Man_3)$_3$$DEVA_5K$ **19** was conjugated following the general procedure using: Az_3DEVA_5K **28** (4.90 mg, 1.57 µmol), 0.05M 1,3-1,6-Man_3 **25** sol. (157.0 µl, 7.85 µmol), 0.05M sodium ascorbate sol. (157.0 µl, 7.85 µmol) and 0.05M $CuSO_4$ (94.2 µl, 4.71 µmol). Gel filtration followed by RP-HPLC (linear gradient 25 → 50% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_3$$DEVA_5K$ **19** as a white powder (0.36 mg, 0.08 µmol, 5%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.837 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{197}H_{326}N_{43}O_{91}$ 1584.07, found 1584.60.

H-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (20): (1,3-1,6-Man$_3$)$_6$DEVA$_5$K **20** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.50 mg, 0.72 µmol), 0.05M 1,6-Man$_2$ **25** sol. (115.2 µl, 5.76 µmol), 0.05M sodium ascorbate sol. (115.2 µl, 5.76 µmol) and 0.05M $CuSO_4$ (86.4 µl, 4.32 µmol). Gel filtration followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man$_3$)$_6$DEVA$_5$K **20** as a white powder (0.58 mg, 0.09 µmol, 12%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.717 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{274}H_{452}N_{53}O_{140}$ 2243.0, found 2242.9; $[M+4H]^{4+}$ calculated for $C_{274}H_{453}N_{53}O_{140}$ 1682.5, found 1683.1; $[M+5H]^{5+}$ calculated for $C_{274}H_{453}N_{53}O_{140}$ 1346.2, found 1346.5.

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