Chapter 2

Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates[1]

Introduction

The targeted delivery of chemotherapeutics through the intermediacy of cell surface receptors represents an attractive means to selectively deliver cargo to target cells or subcellular compartments[2]. Conceptually distinct approaches have been developed over the years to selectively deliver therapeutics and diagnostics to specific cell types through receptor-mediated uptake. Various ligands have been used as a homing device, including antibodies[3,4] and small synthetic molecules such as folic acid,[5] peptides[6] and carbohydrates.[7,8,9] Lectins are carbohydrate-binding receptors involved in a wide variety of cellular recognition and communication processes.[10] They are abundantly expressed on dendritic cells and macrophages, the guardians of our innate immune system, to survey their surroundings and detect pathogens and danger signals.[11,12] Many of the lectins found on these cells are members of the C-type lectin family and these include the mannose receptor (MR),[12] Dectin-1 and 2 as well as DC-SIGN.[13] These carbohydrate-

binding receptors have been exploited in various antigen-targeting strategies to enable both the efficient uptake of antigens (as described in chapter 4) and simultaneous stimulation of the immune cells. Proteases play a key role in the generation of peptide antigens and as such in antigen presentation. To probe the activity of cathepsins in living DCs Hoogendoorn *et al.* reported the adaptation of the broad-spectrum cathepsin inhibitor, DCG-04 to obtain targeted activity-based cathepsin probes.[14] DCG-04 was originally reported by Bogyo and co-workers, in a seminal paper,[15] which, together with the first paper by Cravatt and co-workers on serine hydrolase probes,[16] shaped the field of activity-based protein profiling. Taking the natural product and broad-spectrum cysteine protease inhibitor, E-64, as a basis, Bogyo and co-workers appended both a biotin and – in a later contribution – a set of different fluorophores and showed that all these structures retain potency and (broad-spectrum) specificity against numerous mammalian cathepsin cysteine proteases.[17] From these studies, which yielded activity-based probes currently widely used by the chemical biology community, it became apparent that cathepsin cysteine proteases tolerate a wide variety of functional groups appended to the dipeptide epoxysuccinate core.

Functionalization of the irreversible cathepsin inhibitor, DCG-04 with a BODIPY dye and a mannose cluster gave activity-based probe (ABP) **1** (UHG392[18] as depicted in Figure 1), which was used probe the activity of cathepsins in living DCs. ABP **1** contains an artificial mannose cluster built up from mono-mannosides covalently attached through a triazole linker to a hexalysine scaffold. The nature of the mannose ligand may influence recognition by the cell surface lectins and consequently uptake and routing of the conjugates. For example, it is known that the prevalent carbohydrate binding lectins on DCs, DC-SIGN and the MR, bind oligomannosides better than monomannosides.[19,20] Glycan microarray studies have revealed that DC-SIGN strongly binds high mannose type structures[21] and available crystal structures of DC-SIGN bound to natural ligands show that a terminal branched trimannose structure, featuring α-(1,3) and α-(1,6) mannose branches on a core mannose residue, fits well in the carbohydrate binding

site of this signaling receptor.[22] The MR also binds oligomannosides and a preference for the same type of branching has been reported.[23] Because the nature of the carbohydrate ligand(s) is important for the recognition by the cell surface lectins and consequently for the uptake and routing of the molecules, a set of BODIPY-DCG-04-oligomannose conjugates, bearing oligomannosides that feature natural glycosidic connections (*i.e.* α-(1→3) and α-(1→6) linkages) were designed and synthesized. In this chapter the assembly of three BODIPY-DCG-04-mannose clusters (**2**, **3** and **4**, Figure 1), bearing either a mono-, tri- or heptamannoside targeting entity and their efficacy in labeling of cathepsins in both cell lysates and live cells is described.

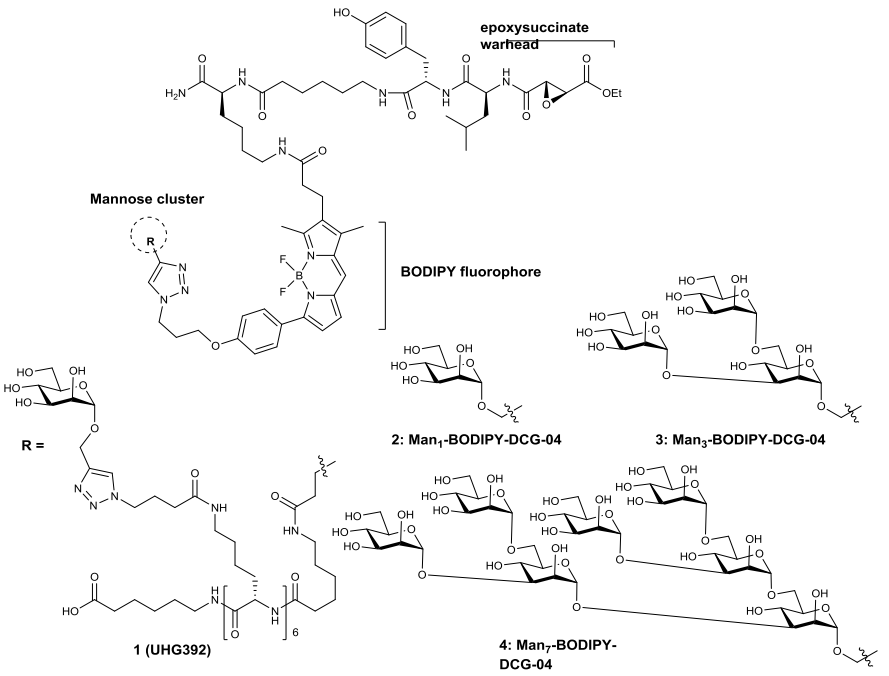


Figure 1: Structures of the known DCG-04 mannose conjugate **1** and the new DCG-04 mannose conjugates **2**, **3** and **4** described in this chapter.

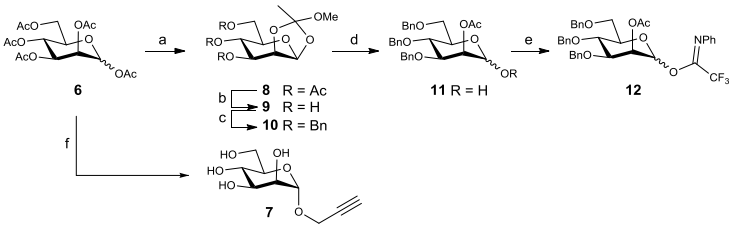
Results and discussion

Synthesis

The projected BODIPY-DCG-04 mannose conjugates **2**, **3** and **4** were assembled by conjugation of the corresponding propargyl mono-, tri- or heptamannosides **7**, **19** and **27** with azide functionalized BODIPY-epoxysuccinate **5** (Scheme 3). The synthesis of monomannoside **7** together with building block **12** and the glycosylation reactions toward propargyl tri- and heptamannoside **19** and **27** are shown in schemes 1 and 2.

Treatment of peracetylated mannose **6** with propargyl alcohol and $BF_3 \cdot Et_2O$ gave monomannoside **7** (Scheme 1).[24] Starting from peracetylated mannose **6**, orthoester **8** was obtained *via* the intermediate formation of a mannosyl iodide, as reported by Adinolfi *et al.*[25] The iodide was intramolecularly substituted to give orthoester **8**, which was deacetylated and subsequently benzylated to give orthoester **10**. Acidic hydrolysis of **10** then yielded hemiacetal **11**[26,27] in 82% over 4 steps. Treatment of this lactol with (*N*-phenyl)trifluoroacetamidoyl chloride in the presence of Cs_2CO_3 afforded (*N*-phenyl)trifluoroimidate donor **12** in 90% yield.

Scheme 1: Syntesis of propargyl mannoside **7** and donor mannoside **12**.



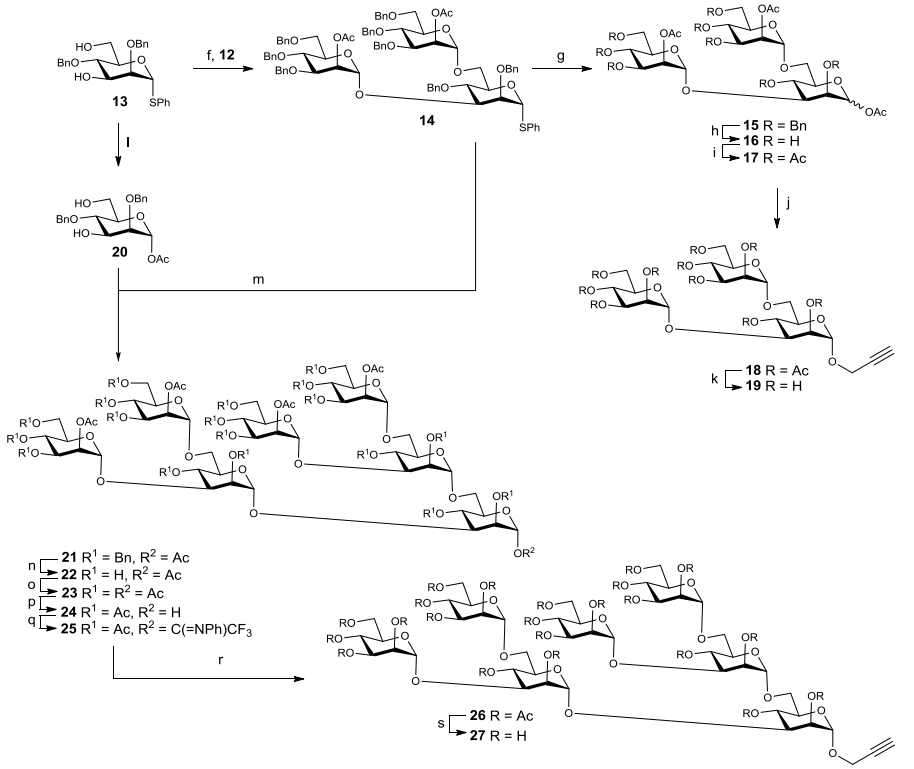
Reagents and conditions (a) i) I_2, Et_3SiH, CH_2Cl_2, reflux; ii) MeOH, 2,6-lutidine, rt; (b) K_2CO_3, MeOH, rt; (c) NaH, BnBr, DMF, 0 °C to rt; (d) DME/H_2O (10:1), *p*TsOH, 82% over 4 steps; (e) Cs_2CO_3, acetone, $(CF_3)C(NPh)Cl$, 90%; (f) propargyl alcohol, $BF_3 \cdot Et_2O$, DCM, rt, 61%.

The construction of key trisaccharide **14**, which was used as a precursor for both the propargyl trimannoside **19** and heptasaccharide **27**, was accomplished by a double glycosylation of known diol **13**[24] using donor **12**

and a catalytic amount of TfOH (Scheme 2). Next trimer **14** was converted into the corresponding anomeric acetate **15** using NIS and AcOH. Removal of all benzyl groups from this trimer required a two-step sequence. The fully protected trisaccharide was first treated with Pd/C and H_2 in a mixture of EtOAc/*t*BuOH/H_2O (1:3:4) and subsequently with Pd/C and H_2 in water to effect removal of all benzyl groups. Peracetylation of the crude trimer **16** yielded **17** in 76% over the two steps. Propargyl alcohol was then condensed with trimannosyl acetate **17** under the agency of $BF_3 \cdot Et_2O$ to provide the fully protected trimer **18**. Global deacetylation under Zemplén conditions yielded the propargyl trimannoside Man_3 **19**.

The heptasaccharide **21** was assembled using trisaccharide donor **14** and acceptor **20**. The latter building block was obtained by treatment of thio mannoside **13**[28] with NIS and AcOH (Scheme 2).[29] This led to the formation of the α-acetate **20** in 40% yield, alongside the generation of its β-anomeric counterpart (37%) and a minor by-product that was characterized as 1,6-anhydro-2,4-di-*O*-benzyl-β-D-mannose (11%). Condensation of both alcohols in acceptor **20** with trisaccharide donor **14** was achieved using the NIS/TfOH promotor couple to yield heptamer **21** as a single product in 62% yield. Hydrogenation of **21** with Pd/C and H_2 in EtOAc/MeOH/H_2O (5:4:1) was followed by a second hydrogenation in MeOH/H_2O (1:1) to give the debenzylated heptamer **22**, which was subjected without further purification to global acetylation. Attempts to introduce the propargyl moiety onto the peracetylated heptamer using $BF_3 \cdot Et_2O$ did not lead to the desired product and therefore a more potent glycosylating agent was used. To this end the anomeric acetyl was chemoselectively deblocked using hydrazine acetate and the liberated alcohol was converted into the (*N*-phenyl)trifluoroimidate donor **25**.

Scheme 2: Synthesis of the propargyl trimannoside Man_3 **19** and propargyl heptamannoside Man_7 **27**.

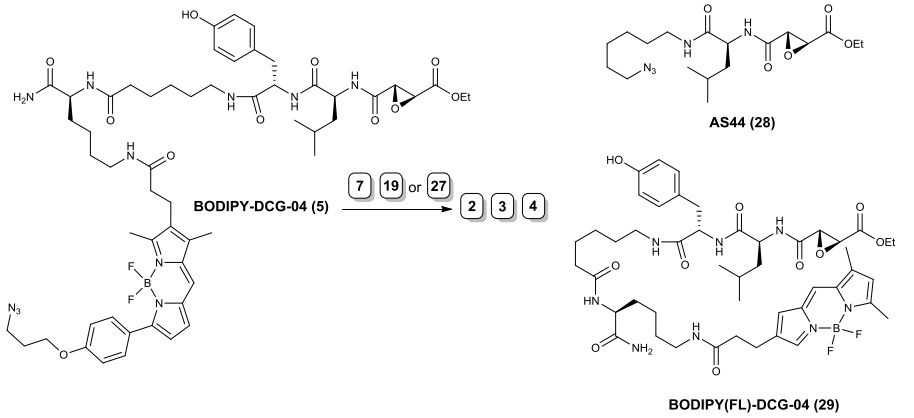


Reagents and conditions: (a) **12**, DCM, TfOH, act. Mol. Siev, -40 °C to rt, 84%; (b) NIS, AcOH, DCE/Et_2O (1:1), 94%; (c) i) Pd/C, H_2, EtOAc/*t*BuOH/H_2O (1:3:4), ii) Pd/C, H_2, H_2O; (d) Ac_2O, pyridine, 0 °C to rt, 76% over 2 steps; (e) propargyl alcohol, $BF_3{\cdot}Et_2O$, DCM, rt, 61%; (f) MeOH, NaOMe, 68%; (g) NIS, DCM/AcOH (1:1), rt, 40%; (h) **14**, NIS, TfOH, act. Mol. Siev., DCM, -40 °C to rt, 62%; (i) Pd/C, H_2, EtOAc/MeOH/H_2O (5:4:1); ii) Pd/C, H_2, MeOH/H_2O (1:1); (j) Ac_2O, pyridine, 0 °C to rt, quantitative yield over two steps; (k) $H_2NNH_2{\cdot}AcOH$, DMF, 0 °C, 79%; (l) $ClC(=NPh)CF_3$, Cs_2CO_3, acetone, quantitative; (m) propargyl alcohol, TfOH, DCM, act. Mol. Siev., -40 °C to 0 °C, 40%; (n) i) NaOMe/MeOH; ii) 0.1M NaOH (aq.), quantitative.

Glycosylation of propargyl alcohol with donor **25** under mild acid catalysis yielded the peracetylated heptamannoside **26** in 40% yield. Deacetylation under standard Zemplén conditions led to the partial removal of the acetyl groups, necessitating an extra saponification step with aqueous 0.1M NaOH to provide the target heptamer Man_7 **27**.

The BODIPY-DCG-04-mannose conjugates were obtained through a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition[30] of azido BODIPY-DCG-04 (**5**) and the propargyl mannosides **7**, **19** and **27** (Scheme 3). After HPLC purification the three target constructs were obtained in 42% (**2**), 24% (**3**) and 32% (**4**) yield respectively.

Scheme 3: Assembly of the mannose-BODIPY-DCG-04 conjugates **2**, **3** and **4** and structures of cathepsin binding probes **5** (BODIPY-DCG-04), **28** (AS44), **29** (azido-DCG-04) and **30** (BODIPY(FL)-DCG-04).



Reagents and conditions: (a) sodium ascorbate, $CuSO_4$, DMF/H_2O (1:1), Man_1-BODIPY-DCG-04 (**2**) 42%, Man_3-BODIPY-DCG-04 (**3**) 24%, Man_7-BODIPY-DCG-04 (**4**) 32%.

Biological evaluation

To investigate labeling of cathepsins by activity-based probes **2**, **3** and **4** their activities were first evaluated in cell lysates. To this end mouse liver lysate was incubated with increasing concentrations of Man_1-BODIPY-DCG-04 (**2**), Man_3-BODIPY-DCG-04 (**3**) and Man_7-BODIPY-DCG-04 (**4**) after which the proteins in the lysates were resolved on SDS-PAGE (Figure 2A). All three mannosyl DCG-04 probes label cathepsins in a concentration-dependent manner, as is evident from Figure 2A. A small difference in gel-shift is apparent for the three different constructs and correlates to their varying molecular weight. A decrease in binding capacity was observed with increasing cluster size suggesting that the steric bulk of the heptamannosyl cluster retards binding and cathepsin inactivation. The diminished binding

efficacy of the larger mannosyl clusters, together with the difference in gel-shift of the labeled proteins indicates that mannosidases present in the cell lysate do not (effectively) trim the probes when bound to the cathepsins or when unbound in the cell extract. Incubation of immature mouse dendritic cell (DC) lysate with the probes showed a similar concentration-dependent binding of cathepsins (Figure 2B).

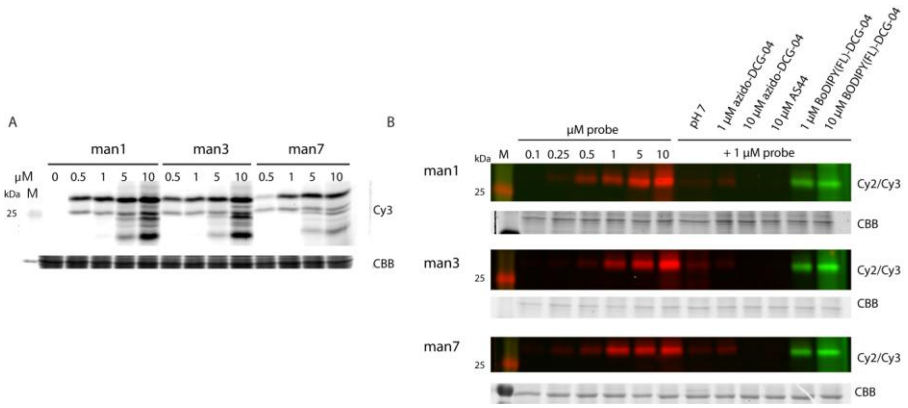


Figure 2. Cathepsin labeling experiments in mouse liver and dendritic cell lysate. Mouse liver lysate (10 µg total protein, A) or immature mouse dendritic cell (DC) lysate (8 µg total protein, B) was incubated (1 h, 37 °C) with increasing concentration of probe **2**, **3** or **4** at pH 5.5 or 1 µM at pH 7. Alternatively, lysates were incubated (1 h, 37 °C) with azido-DCG-04 (1 or 10 µM), AS44 (10 µM) or BODIPY(FL)-DCG-04 (1 or 10 µM), before treatment with probe **2**, **3** or **4** (1 µM, 1 h, 37 °C). Proteins were resolved on 12.5% SDS-PAGE, followed by fluorescence scanning (Cy2 (green): BODIPY(FL), Cy3 (red): BODIPY(TMR) and total protein staining with coomassie brilliant blue (CBB). M: dual color protein molecular weight marker.

In line with the previous findings, changing the pH of the buffer from pH 5.5 (the optimal pH for most cathepsin activity)[18] to pH 7 led to abrogation of cathepsin binding showing that active enzymes are required for labeling. Next a set of competition experiments was performed. The lysates were pre-incubated with different DCG-04 competitors, namely, AS44 **28**[14], azido-DCG-04 **29** and BODIPY(FL)-DCG-04 **30**[18] (See Scheme 3 for the structures of the competitors), followed by incubation with the probes. As

seen in Figure 2B labeling of the cathepsins with the red mannosyl BODIPY-DCG-04 conjugates was effectively prevented leading to either disappearance of the fluorescent bands in the competition experiment with non-fluorescent azido-DCG-04 and AS44, or the appearance of green fluorescent bands in the experiment with the green DCG-04 probe **30**. These competition experiments corroborate that probes **2**, **3** and **4** label active cathepsins.

Next the probes were tested for uptake and binding of cathepsins in living DCs (Figure 3). In line with the results obtained with the cell lysates, a concentration-dependent labeling pattern was observed (Figure 3A, left panel). The most efficient and selective labeling was achieved with the trimannosyl probe **3**, where the monomannosyl compound **2** showed most background fluorescence. Also in these experiments the heptamannoside probe **4** labeled the target cathepsins somewhat less efficient than its trimannoside counterpart **3**. Competition experiments with non-fluorescent cell-permeable azido-DCG-04 probe AS **8** indicated that also in living DCs active cathepsins are labeled by the probes. To test whether uptake of the probes was carbohydrate receptor mediated the DCs were pre-incubated with mannan, a mixture of mannose polysaccharides prior to exposure to the probes. Mannan, the natural substrate, binds to the mannose receptor and thus the uptake is prevented. In doing so, labeling by the tri- and heptamannosyl probes was effectively blocked showing that uptake of these ABPs is receptor dependent. The receptor-mediated uptake and labeling was confirmed by confocal microscopy. Figure 3B shows a clear uptake of Man_3-BODIPY-DCG-04 **3** and Man_7-BODIPY-DCG-04 **4** in DCs but little uptake of Man_1-BODIPY-DCG04 **2** (Figure 3B, left panels). Pre-incubation of the cells with mannan prevented uptake of probes **3** and **4**. Combined, the results indicate that Man_3-BODIPY-DCG-04 **3** and Man_7-BODIPY-DCG-04 **4** are taken up through the intermediacy of a carbohydrate binding receptor, where Man_1-BODIPY-DCG04 **2** can be internalized (at least in part) through a receptor-independent pathway. Receptor mediated internalization is clearly more efficient. Although it has previously been reported that the mannose

receptor can bind monomannosides, in the case at hand it appears that this is not enough for effective internalization of the conjugate.

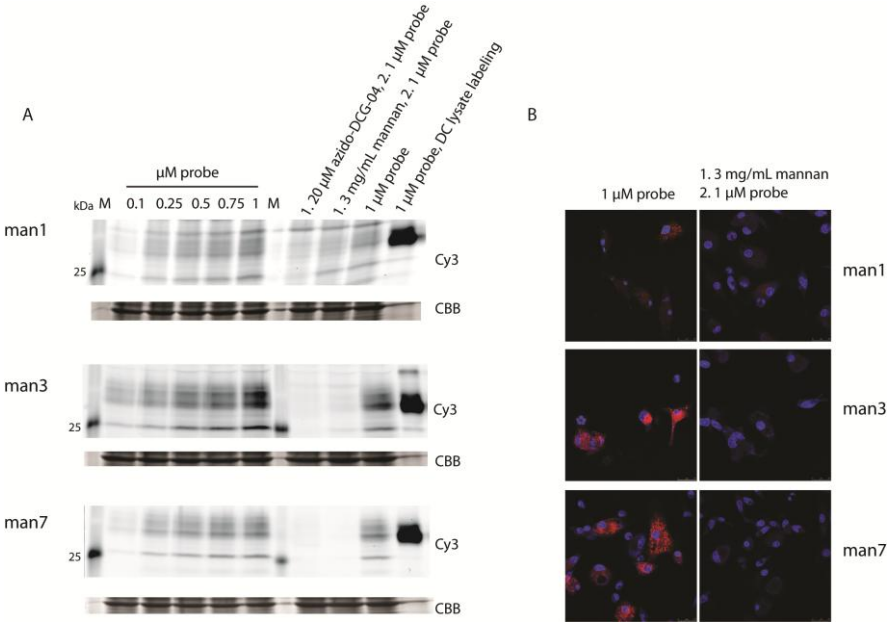


Figure 3. Uptake and cathepsin binding of the probes in live dendritic cells. A) DCs were treated with varying concentrations of **2, 3** or **4** (2 h, 37 ^{o}C) or pre-incubated (1 h, 37 ^{o}C) with azido-DCG-04 (20 μM) or mannan (3 mg/mL), followed by addition of **2, 3** or **4** (1 μM, 2 h, 37 ^{o}C), washed with PBS, lysed and resolved on 12.5% SDS-PAGE. In-gel fluorescence of BODIPY (Cy3) and total protein stain (CBB) are shown. B) Representative confocal microscope images of DCs treated with 1 μM of probes **1, 2** or **3** (left panels) or with mannan (right panels) for 1 h, followed by treatment with the probes. After treatment, cells were washed with PBS, fixed with 4% formaldehyde, nuclei stained with Draq5 and imaged using the Cy3 (λ_{ex} 532 nm) settings for BODIPY (red) and Cy5 (λ_{ex} 635 nm) settings for Draq5 as a nuclear stain (blue).

With respect to our first generation probe (**1**) it appears that DCG-04 labeling with the trimannoside probe is equally efficient. We have however observed a difference in processing of the probes. Where probe **1** seems to be processed by mannosidases in living cells (as judged from the minimal difference in gel-shift for the labeled cathepsins, indicating only a small shift

in molecular size), the current probes are more resistant to the endo/lysosomal action of mannosidases.

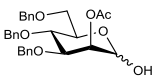
Conclusions

The assembly of three fluorescent cathepsin probes functionalized with different mannosides to investigate the role of these carbohydrate appendages on inhibition efficacy and internalization efficiency has been achieved. The size of the mannose oligosaccharides proved to influence the rate of inhibition, with the largest heptamannoside showing least effective cathepsin labeling in cell lysates at low inhibitor concentrations. The tri- and heptamannoside outcompete the monomannoside probe where it comes to effective uptake in live cells, and the trimannoside-modified DCG04 derivative proved the most effective cathepsin inhibitor in this experimental setup.

Experimental

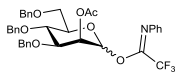
General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under an 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}). Compounds were visualized by UV absorption (λ = 254 nm), staining with 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- and ^{13}C NMR spectra 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, 5µm 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.



2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranoside (11): To a solution of peracetylated mannose **6** (114.5 g, 293.2 mmol) in DCM (750 mL) was added iodine (104.2 g, 410.5 mmol) and triethylsilane (66.3 mL, 410.5 mmol). The reaction mixture was heated till reflux. After 4 h TLC showed complete conversion of the starting material and mixture was cooled to rt. To the reaction mixture was added 2,6-lutidine (140 mL), MeOH (71.2 mL) and the reaction mixture was stirred overnight at rt. The reaction mixture was concentrated *in vacuo*, dissolved in EtOAc, washed with water (1x), 10% $Na_2S_2O_3$ (aq.) (2x), H_2O (3x), brine (2x), dried over Mg_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in MeOH (500 mL), and to the solution was added K_2CO_3 (6.6 g, 48 mmol) and stirred for 4 h at rt. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x). The product was dissolved in DMF (1.0 L) and to the solution was added BnBr (158 mL, 1.32 mol). The reaction mixture was cooled to 0 °C and to the cooled solution was added NaH (60% m/m) (31.7 g, 1.32 mol) in small portions over 6 h. The reaction mixture was

gradually warmed to rt and was stirred overnight at rt. The reaction mixture was cooled to 0 °C and quenched with MeOH. The solvent was removed *in vacuo* and the concentrate was dissolved in Et_2O, washed with H_2O (4x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was dissolved in DME/H_2O (10:1) (1.5 L) and the solution was cooled to 0 °C. To the cooled solution was added *p*TsOH (75 mmol, 14.3 g), after 3 h at 0 °C the reaction was quenched with sat. $NaHCO_3$ (aq.). Brine was added and the organic layer was separated. The product was extracted with DCM (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose **11** as a colourless oil (118.4 g, 240.4 mmol, 82% over 4 steps as an α/β mixture 10:1). Spectroscopic data were in accordance with known literature.[31] Spectroscopic data are reported for the major (α) isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 13H), 7.18 – 7.12 (m, 2H), 5.36 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.19 (s, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 8.2 Hz, 1H), 4.49 (d, *J* = 9.1 Hz, 1H), 4.45 (d, *J* = 10.9 Hz, 1H), 4.09 – 4.05 (m, 1H), 4.03 (dd, *J* = 9.3, 3.2 Hz, 1H), 3.74 (d, *J* = 9.6 Hz, 1H), 3.69 – 3.66 (m, 2H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 138.4, 138.0, 137.9, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 92.5, 77.8, 77.5, 77.1, 76.8, 75.2, 74.7, 73.5, 71.9, 71.2, 69.4, 69.2, 29.8, 21.3.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranoside (12): 

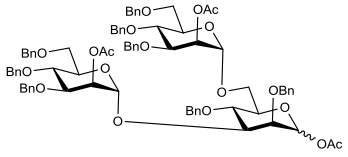
To a solution of mannose **11** (24.6 g, 50 mmol) in acetone (200 mL) was added N-(*p*-anisyl)-2,2,2-trifluoroacetimidoyl chloride (10.4 mL, 68.8 mmol) and cooled to 0 °C. To the cooled solution was added Cs_2CO_3 (20.7 g, 55 mmol) and the reaction mixture was allowed to warm to rt. After 6 h the solids were filtered over celite and the filtrate was concentrated *in vacuo*. Purification by column chromatography yielded trifluoro imidate donor **12** as a yellow oil (29.8 g, 44.9 mmol, 90% as α/β mixture 5:0.2). $[\alpha]_D^{22}$ + 26.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 111.48, 1162.48, 1207.57, 1310.77, 1364.72, 1453.91,

1489.32, 1597.42, 1716.21, 1749.15, 2867.10, 3031.71. Spectroscopic data are reported for the major (α) isomer: ^{1}H NMR (400 MHz, $CDCl_3$, T = 328 °K) δ 7.38 – 7.01 (m, 18H), 6.79 (d, *J* = 7.4 Hz, 2H), 6.20 (s, 1H), 5.47 (dd, *J* = 3.2, 2.0 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.57 (dd, *J* = 11.3 Hz, 2H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.01 (ddd, *J* = 8.3, 3.1, 1.3 Hz, 1H), 3.99 – 3.89 (m, 2H), 3.78 (dd, *J* = 7.8, 3.7 Hz, 1H), 3.72 (dd, *J* = 11.2, 1.7 Hz, 1H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 143.4, 138.5, 138.4, 137.8, 128.9, 128.5, 128.5, 128.3, 128.0, 127.8, 127.8, 127.7, 124.6, 119.6, 77.7, 75.4, 74.5, 74.0, 73.6, 72.4, 68.9, 67.7, 20.9. TLC-MS (*m/z*) 686.7

Phenyl 2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (14): Trifluoro imidate donor **12** (19.9 g, 30 mmol) and acceptor **13** (4.5 g, 10 mmol) were dissolved in DCM (200 mL) and stirred over activated molecular sieves (3Å) at rt for 30 minutes. The solution was cooled to -40 °C and to the cooled solution was added Tf_2O (0.18 mL, 2.0 mmol) and the reaction mixture was gradually allowed to warm to 0 °C. At 0 °C the reaction was quenched with TEA and the mixture was filtered over celite and rinsed with DCM. The organic phase was washed with H_2O and the aqueous phase was extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo*. Purification by column chromatography yielded trimer **14** as a colorless oil (11.76 g, 8.4 mmol, 84%). $[\alpha]_D^{22}$ + 64.6° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 978.54, 1026.58, 1049.14, 1078.97, 1232.74, 1367.72, 1453.88, 1742.59, 2866.77, 3030.51. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.08 (m, 52H), 5.54 (s, 1H), 5.51 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.44 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.22 (d, *J* = 1.9 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 8.8 Hz, 1H), 4.85 (d, *J* = 8.5 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 2.9 Hz, 1H), 4.64 (s, 1H),

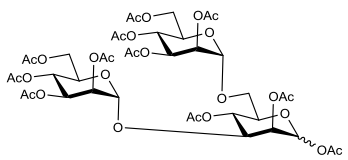
4.63 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 7.8 Hz, 1H), 4.60 (d, *J* = 9.7 Hz, 1H), 4.56 – 4.40 (m, 9H), 4.38 (d, *J* = 11.2 Hz, 1H), 4.22 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.13 (s, 1H), 4.09 (dd, *J* = 9.3, 3.0 Hz, 1H), 4.02 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.96 – 3.86 (m, 5H), 3.83 (d, *J* = 9.4 Hz, 1H), 3.80 – 3.73 (m, 2H), 3.72 – 3.66 (m, 3H), 3.64 (dd, *J* = 11.2, 1.9 Hz, 1H), 3.60 (dd, *J* = 10.7, 1.7 Hz, 1H), 2.13 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.2, 138.7, 138.6, 138.3, 138.0, 137.9, 137.9, 134.8, 131.0, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.3, 100.0, 98.3, 85.0, 79.2, 78.2, 77.9, 77.5, 77.2, 76.8, 75.3, 75.1, 75.0, 74.5, 74.2, 73.7, 73.4, 73.4, 72.4, 72.3, 72.1, 72.0, 71.6, 71.6, 71.6, 69.3, 68.9, 68.8, 68.5, 66.7, 21.3, 21.1. HRMS: $[M+H]^+$ calculated for $C_{84}H_{89}O_{17}S$ 1401.58150, found 1401.58162.

Acetyl 2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α/β-D-mannopyranoside (15) To a suspension of NIS (1.25 g, 5.55 mmol) in DCE/THF (1:1) (27 mL) was added acetic acid (21.2 mL, 370 mmol). To the NIS mixture was added a solution of trimer **14** (5.17 g, 3.7 mmol) in DCE/THF (1:1) (5 mL) and the reaction mixture was stirred overnight at rt. The reaction mixture was diluted with EtOAc, washed with 10% $Na_2S_2O_3$ sol. (aq.) (2x), H_2O, 3x sat (1x). $NaHCO_3$ sol. (aq.) (1x), H_2O (3x), brine (2X), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded trimer **15** as a colorless oil (4.67 g, 3.5 mmol, 94%). $[\alpha]_D^{22}$ + 50.0° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 975.53, 1026.80, 1048.27, 1090.94, 1231.12, 1368.81, 1453.91, 1496.72, 1743.42, 2870.34, 3031.12. ^{1}H NMR (400 MHz, $CDCl_3$) (α/β mixture, 1:0.4) δ 7.41 – 7.08 (m, 56H), 6.18 (d, *J* = 2.0 Hz, 1H), 5.59 (s, 0.4H), 5.50 (ddd, *J* = 8.3, 3.3, 1.9 Hz, 2.8H), 5.20 (d, *J* = 1.8 Hz, 1.4H), 4.96 (d, *J* = 1.9 Hz, 1H), 4.94 (d, *J* = 1.8 Hz, 0.4H), 4.89 (s, 1H), 4.86 (d, *J* = 2.8 Hz, 1.4H), 4.85 – 4.81 (m, 1.4H), 4.79 – 4.71 (m, 1.4H), 4.72 (d, *J*



= 1.7 Hz, 1H), 4.70 (d, *J* = 2.0 Hz, 0.4H), 4.67 (s, 0.4H), 4.66 – 4.63 (m, 2.4H), 4.62-4.60 (m, *J* = 1.9 Hz, 2H), 4.57 (d, *J* = 2.3 Hz, 0.4H), 4.54 (s, 0.4H), 4.51 (s, 0.4H), 4.50 – 4.38 (m, 10H), 4.12 (dd, *J* = 9.6, 3.1 Hz, 1H), 4.02 (ddd, *J* = 8.1, 5.2, 2.7 Hz, 2H), 4.00 – 3.83 (m, 8.4H), 3.84 – 3.78 (m, 1H), 3.78 – 3.65 (m, 6H), 3.64 (s, 0.4H), 3.62 (t, *J* = 2.0 Hz, 0.8H), 3.59 (s, 0.4H), 3.57 (s, 0.4H), 3.51 (ddd, *J* = 8.8, 4.4, 2.0 Hz, 0.4H), 2.35 (s, 1H), 2.15 (s, 3H), 2.14 (s, 1.2H), 2.09 (s, 3H), 2.07 (s, 1.2H), 1.99 (s, 1.2H), 1.95 (s, 3H). ^{13}C NMR (^{100} MHz, $CDCl_3$) δ 170.3, 170.3, 170.2, 170.1, 169.0, 168.9, 138.6, 138.6, 138.5, 138.3, 138.1, 137.9, 137.8, 137.8, 137.6, 129.1, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 125.4, 100.1, 99.7, 98.5, 98.3, 92.8, 90.8, 79.7, 78.1, 78.0, 77.7, 76.5, 76.3, 75.3, 75.2, 75.1, 75.0, 74.7, 74.4, 74.3, 74.2, 74.1, 74.1, 74.0, 73.6, 73.5, 73.5, 73.4, 72.4, 72.2, 72.1, 72.0, 72.0, 71.7, 71.6, 71.5, 71.3, 69.2, 69.0, 68.8, 68.7, 68.6, 68.6, 68.4, 68.3, 66.4, 66.3, 21.3, 21.1, 21.0. HRMS: $[M+H]^+$ calculated for $C_{80}H_{87}O_{19}$ 1351.58361, found 1351.58399.

Acetyl 2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α/β-D-

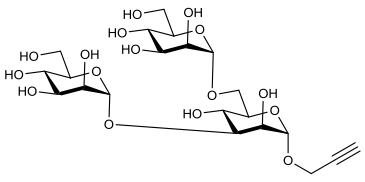


mannopyranoside (17): Trimer **15** (2.5 mmol, 3.38 g) was dissolved in an EtOAc/*t*BuOH/H_2O (1:3:4) mixture (50 mL) and the solution was purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere. After TLC showed complete conversion to a single spot the Pd/C was filtered over a pad of celite, rinsed with MeOH and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed the presence of aromatic signals. The crude was taken up in H_2O (50 mL) and purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere overnight. The Pd/C was filtered over a pad of celite, rinsed with H_2O and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed complete removal of aromatic signals and the crude was co-

evaporated with 1,4-dioxane (3x). The crude was dissolved in pyridine (25 mL) and the solution was cooled to 0 °C. To the cooled solution was added acetic anhydride (2.5 mL) and the reaction mixture was gradually allowed to warm to rt. After complete conversion, the reaction mixture was cooled to 0 °C, quenched with MeOH and concentrated *in vacuo.* The crude product was dissolved in EtOAc and washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (1x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-*O*-acetylated trimer **17** as a colorless oil (1.81 g, 1.9 mmol, 76%). $[\alpha]_D^{22}$ + 40.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 975.27, 1039.52, 1139.46, 1212.30, 1368.79, 1433.73, 1741.84, 2925.08. ^{1}H NMR (300 MHz, $CDCl_3$) (α/β mixture, 1:0.25) δ 6.05 (d, *J* = 1.9 Hz, 1H), 5.81 (s, 0.25H), 5.48 (d, *J* = 3.2 Hz, 0.25H), 5.27 (dd, *J* = 8.8, 2.7 Hz, 4.4H), 5.04 (d, *J* = 2.6 Hz, 2H), 5.02 (s, 0.4H), 4.80 (s, 1.2H), 4.35 – 4.20 (m, 3.4H), 4.19 – 4.00 (m, 7.2H), 3.90 (dq, *J* = 10.0, 3.1 Hz, 1H), 3.75 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.57 (dd, *J* = 10.9, 3.2 Hz, 1H), 2.27 (s, 0.8H), 2.25 (s, 2.6H), 2.18 (s, 4H), 2.16 (d, *J* = 2.1 Hz, 12H), 2.11 (s, 7H), 2.07 (s, 5.5H), 2.05 (s, 3H), 2.00 (s, 4H), 1.99 (s, 4.5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.1, 170.0, 170.0, 169.9, 169.8, 169.6, 99.2, 97.6, 90.4, 74.7, 71.5, 69.9, 69.6, 69.5, 69.3, 69.1, 68.5, 68.2, 67.9, 66.9, 65.8, 62.4, 62.3, 60.4, 20.9, 20.9, 20.8, 20.7, 20.7. HRMS: $[M+H]^+$ calculated for $C_{40}H_{55}O_{27}$ 967.29252, found 967.29269.

Propargyl 2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (18): To a solution of per-*O*-acetylated trimer **17** (29 mg, 30 µmol) in DCM (300 µL) was added a 0.6M propargyl alcohol solution (150 µL, 90 µmol) in DCM and a 0.3M $BF_3{\cdot}Et_2O$ solution (150 µl, 45 µmol) in DCM. The mixture was heated to 50 °C for 6 h after which the reaction mixture was cooled to rt, diluted with EtOAc and quenched with sat. $NaHCO_3$ (aq.). EtOAc was added till the

organic phase was transferred to the top phase. The organic phase was washed with sat. $NaHCO_3$ (2x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-*O*-acetylated propargyl trimer **18** as a white milky oil (17.6 mg, 18.3 µmol, 61%). $[\alpha]_D^{22}$ + 80.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 978.00, 1038.64, 1136.67, 1214.43, 1368.90, 1433.77, 1741.73, 2926.85. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 – 5.17 (m, 7H), 5.04 – 4.98 (m, 3H), 4.82 (d, *J* = 1.8 Hz, 1H), 4.29 – 4.25 (m, 3H), 4.24 (s, 1H), 4.21 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.16 (t, *J* = 2.6 Hz, 1H), 4.13 (d, *J* = 2.4 Hz, 1H), 4.11 (d, *J* = 1.9 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.88 (ddd, *J* = 9.7, 6.6, 2.5 Hz, 1H), 3.78 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.53 (dd, *J* = 10.8, 2.5 Hz, 1H), 2.50 (t, *J* = 2.4 Hz, 1H), 2.23 (s, 3H), 2.16 (s, 3H), 2.14 – 2.13 (m, 9H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (d, *J* = 1.1 Hz, 3H), 1.99 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.8, 170.6, 170.3, 170.2, 170.2, 170.1, 169.9, 169.74, 99.0, 97.4, 96.0, 78.1, 75.7, 74.1, 70.8, 70.2, 70.1, 69.6, 69.5, 69.2, 68.8, 68.4, 68.4, 67.0, 66.1, 66.1, 62.6, 62.5, 54.8, 21.0, 21.0, 20.9, 20.9, 20.9, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{41}H_{55}O_{26}$ 963.29761, found 963.29723.

Propargyl 3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (19):  To a solution of per-*O*-acetylated propargyl trimer **18** (18.3 mg, 17.6 µmol) in MeOH (370 µL) was added a 5 mM sol. NaOMe (370 µL, m1.83 µmol) in MeOH. After complete conversion the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). The solids were filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatography followed by lyophilisation yielded propargyl trimer **19** as a white powder (6.2 mg, 11.3 µmol, 61%). $[\alpha]_D^{22}$ + 114.0° (c = 1.0, MeOH). FT-IR: ν_{max} (neat)/cm^{-1} 981.81, 1042.80, 1131.53, 1363.00, 2490.41, 2929.08, 3285.13. ^{1}H NMR (400 MHz, MeOD) δ 5.06 (s, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.26 (t, *J* = 2.3 Hz,

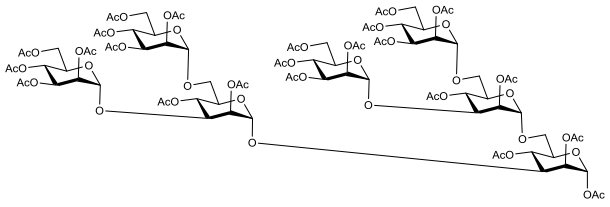
2H), 4.04 (dd, *J* = 3.1, 1.8 Hz, 1H), 3.98 (dd, *J* = 3.3, 1.7 Hz, 1H), 3.94 (dd, *J* = 11.1, 5.2 Hz, 1H), 3.89 – 3.84 (m, 3H), 3.84 – 3.81 (m, 2H), 3.81 – 3.78 (m, 2H), 3.78 – 3.65 (m, 5H), 3.65 – 3.58 (m, 3H), 2.88 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.0, 101.5, 100.2, 80.7, 79.9, 76.2, 74.9, 74.4, 73.8, 72.6, 72.4, 72.1, 72.0, 71.2, 68.7, 68.5, 67.3, 67.1, 62.8, 55.0. HRMS: $[M+H]^+$ calculated for $C_{21}H_{35}O_{16}$ 543.19196, found 543.19224.

Acetyl 2,4-*O*-Benzyl-α-D-mannopyranoside (20a): To a 0 °C cooled solution of NIS (0.25 g, 1.1 mmol) in DCM/AcOH (1:1) (20 mL) was added dropwise a 0.1M solution of thio mannose **13** (10 mL, 1.0 mmol) in DCM. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to rt. After complete consumption of the starting material the reaction was quenched with 10% $Na_2S_2O_3$ (aq.) and the product was extracted with EtOAc (4x). The combined organic phases were washed with H_2O (2x), sat. $NaHCO_3$ (aq.) (3x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded α-*O*-acetyl mannose **20a** as a colourless amorphous solid (0.15 g, 0.4 mmol, 40%). $[\alpha]_D^{22}$ + 14.6° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 1026.60, 1071.89, 1239.98, 1366.52, 1454.28, 1496.96, 1720.48, 2930.75, 3031.24, 3420.07. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 10H), 6.20 (d, *J* = 1.8 Hz, 1H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.0 Hz, 1H), 4.59 (d, *J* = 11.7 Hz, 1H), 3.99 (d, *J* = 7.0 Hz, 1H), 3.83 (dd, *J* = 12.1, 2.6 Hz, 1H), 3.80 – 3.73 (m, 2H), 3.72 – 3.65 (m, 2H), 2.52 (s, 1H), 2.32 (s, 1H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 138.2, 137.25, 128.7, 128.7, 128.3, 128.1, 128.1, 128.0, 91.0, 77.0, 75.6, 75.2, 74.0, 73.1, 71.4, 61.8, 21.0. HRMS: $[M+H]^+$ calculated for $C_{22}H_{27}O_7$ 403.17518, found 403.17527.

Acetyl 2,4-*O* -di-Benzyl-3-*O*-(2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (21): Trimer donor **14** (4.2 g, 3 mmol) and acceptor **20a** (402 mg, 1 mmol) were dissolved in DCM (20 mL) and stirred over activated molecular sieves (3Å) at rt for 30 minutes. To the solution was added NIS (0.74 g, 3.3 mmol) and stirred at rt. After 15 minutes the reaction mixture was cooled to -40 °C and TfOH (0.3 mmol, 27 µL) was added to the mixture. The reaction mixture was gradually warmed to rt and quenched with Et_3N. The mixture was filtered over celite and diluted with DCM. The organic phase was washed with 10% $Na_2S_2O_3$ (aq.) and the aqueous phase was extracted with DCM (5x). The combined organic layers were washed with H_2O (1x), brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH, 1:1) yielded benzylated heptamer **21** as a colourless oil (1.835 g, 0.62 mmol, 62%). $[\alpha]_D^{22}$ + 43.6 (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 977.68, 1026.77, 1051.75, 1232.44, 1368.31, 1453.93, 1496.62, 1742.78, 2868.56, 3032.00. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.07 (m, 90H), 6.17 (d, *J* = 1.8 Hz, 1H), 5.54 – 5.46 (m, 4H), 5.21 (s, 3H), 4.97 (d, *J* = 1.8 Hz, 1H), 4.93 (s, 1H), 4.90 (s, 1H), 4.84 (dt, *J* = 10.5, 5.2 Hz, 5H), 4.75 – 4.69 (m, 3H), 4.64 (d, *J* = 3.8 Hz, 1H), 4.63 – 4.46 (m, 12H), 4.46 – 4.35 (m, 14H), 4.34 (d, *J* = 2.2 Hz, 2H), 4.24 (d, *J* = 12.1 Hz, 1H), 4.15 (ddd, *J* = 18.1, 9.2, 3.0 Hz, 2H), 4.05 (dd, *J* = 9.2, 3.2 Hz, 2H), 3.95 (d, *J* = 8.9 Hz, 2H), 3.93 – 3.82 (m, 10H), 3.83 – 3.71 (m, 3H), 3.71 – 3.46 (m, 15H), 3.39 (dd, *J* = 10.8, 5.9 Hz, 2H), 2.12 (s, 6H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 170.1, 169.0,

138.8, 138.7, 138.6, 138.5, 138.3, 138.3, 138.2, 138.2, 138.2, 138.0, 138.0, 137.9, 137.9, 137.9, 137.8, 137.2, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.2, 127.1, 99.7, 98.5, 98.3, 97.2, 90.7, 78.3, 78.2, 78.0, 77.5, 77.2, 76.8, 76.4, 75.2, 75.1, 74.9, 74.9, 74.6, 74.5, 74.2, 74.1, 74.1, 73.8, 73.45, 73.4, 73.4, 72.3, 72.1, 72.0, 72.0, 71.9, 71.8, 71.8, 71.6, 71.6, 71.3, 71.0, 68.9, 68.8, 68.8, 68.6, 68.3, 68.2, 66.3, 21.2, 21.2, 21.1, 21.1, 21.1.

Acetyl 2,4-*O*-di-acetyl-3-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (23): Benzylated heptamer



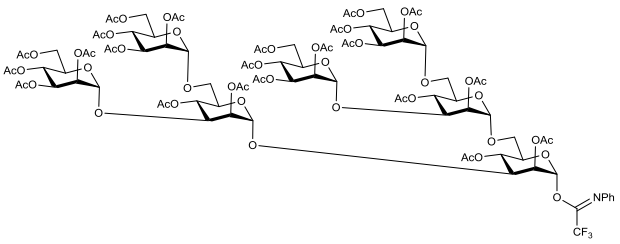
21 (896 mg, 0.3 mmol) was dissolved in EtOAc/MeOH/H_2O (5:4:1) (6 mL) and the solution was purged with argon. To the solution was added cat. Pd/C (10%) and the mixture was stirred overnight at rt under H_2 (g) atmosphere. The Pd/C was filtered over celite, rinsed with methanol and concentrated *in vacuo*. Proton NMR of the crude showed the presence of aromatic signals. The crude was taken up in MeOH/H_2O (1:1) (6 mL) and purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere overnight. the Pd/C was filtered over a pad of celite, rinsed with MeOH and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed complete removal of aromatic signals. The debenzylated intermediate was co-evaporated with pyridine (3x), dissolved in pyridine (10 mL) and the solution was cooled to 0 °C. To the cooled solution was added Ac_2O (1 mL) dropwise and the reaction mixture was

allowed to warm to rt. After complete conversion of the starting material the mixture was cooled to 0 °C and the reaction was quenched with MeOH. The reaction mixture was concentrated *in vacuo* and the crude was dissolved in EtOAc. The organic phase was washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (2x), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-acetylated heptamer **23** as a colourless amorphous solid (635.2 mg, 0.3 mmol, quantitative yield). FT-IR: ν_{max} (neat)/cm^{-1} 976.88, 1038.56, 1084.01, 1138.17, 1213.21, 1369.06, 1432.60, 1742.41, 2935.07. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (d, *J* = 1.9 Hz, 1H), 5.37 – 5.17 (m, 13H), 5.15 (s, 1H), 5.10 – 5.04 (m, 2H), 5.01 (s, 2H), 4.99 (s, 2H), 4.93 (s, 1H), 4.88 – 4.78 (m, 2H), 4.28 (dtd, *J* = 18.4, 10.1, 8.2, 4.0 Hz, 4H), 4.20 – 3.86 (m, 13H), 3.85 – 3.65 (m, 4H), 3.62 – 3.52 (m, 2H), 3.50 (d, *J* = 2.5 Hz, 1H), 2.27 – 1.92 (m, 69H). ^{13}C NMR (^{100} MHz, $CDCl_3$) δ 170.7, 170.6, 170.6, 170.6, 170.5, 170.5, 170.4, 170.4, 170.3, 170.2, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.8, 169.7, 169.7, 169.6, 169.6, 169.6, 169.5, 169.5, 168.4, 168.0, 99.6, 99.2, 99.0, 98.7, 97.4, 97.3, 90.5, 90.5, 77.5, 76.0, 75.6, 75.3, 75.2, 75.0, 74.7, 73.4, 71.4, 70.9, 70.8, 70.7, 70.6, 70.1, 70.0, 69.8, 69.8, 69.6, 69.5, 69.4, 69.4, 69.3, 69.1, 69.0, 69.0, 68.8, 68.7, 68.5, 68.5, 68.4, 67.7, 67.5, 67.5, 67.2, 66.9, 66.3, 66.0, 65.9, 65.8, 65.7, 65.6, 62.3, 62.2, 62.1, 62.0, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5. MALDI: $[M+H]^+$ calculated for $C_{88}H_{119}O_{59}$ 2119.63059, found 2119.63084.

2,4-*O*-di-acetyl-3-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-α/β-D-mannopyranoside (24): To a 0 °C cooled solution of per-acetylated heptamer **23** (0.498 g,

0.235 mmol) in DMF (2.8 mL) was added hydrazine acetate (23.3 mg, 0.259 mmol). The reaction was stirred at 0 °C for 1 h and 30 minutes at rt. After TLC showed complete conversion the reaction was quenched with acetone and the reaction mixture was concentrated *in vacuo*. The crude was dissolved in Et_2O and the organic phase was washed with brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 1-OH acetylated heptamer **24** as a white amorphous solid (0.384 g, 0.186 mmol, 79%). FT-IR: ν_{max} (neat)/cm^{-1} 978.58, 1038.62, 1081.66, 1137.06, 1215.09, 1369.26, 1433.38, 1741.35, 2926.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (dd, *J* = 3.4, 1.6 Hz, 1H), 5.33 – 5.24 (m, 8H), 5.21 (dd, *J* = 10.4, 3.2 Hz, 2H), 5.17 (d, *J* = 3.0 Hz, 2H), 5.11 (t, *J* = 10.1 Hz, 1H), 5.07 – 5.02 (m, 3H), 4.99 (dd, *J* = 4.8, 1.8 Hz, 2H), 4.95 (s, 1H), 4.86 – 4.82 (m, 3H), 4.76 (d, *J* = 5.0 Hz, 1H), 4.37 – 4.22 (m, 4H), 4.18 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.15 – 3.96 (m, 12H), 3.94 (ddd, *J* = 9.7, 5.0, 2.2 Hz, 1H), 3.86 (ddd, *J* = 10.2, 6.0, 2.5 Hz, 1H), 3.76 (tt, *J* = 7.6, 2.7 Hz, 3H), 3.55 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.51 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.47 (d, *J* = 9.5 Hz, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.17 – 2.13 (m, 24H), 2.13 – 2.11 (m, 12H), 2.06 (s, 3H), 2.05 – 2.03 (m, 9H), 2.00 – 1.97 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.9, 170.8, 170.8, 170.7, 170.5, 170.5, 170.5, 170.4, 170.3, 170.2, 170.2, 170.1, 170.1, 167.0, 169.9, 169.9, 169.8, 169.7, 169.7, 99.5, 99.2, 99.0, 97.9, 97.6, 97.4, 91.4, 77.5, 77.2, 76.8, 75.3, 75.0, 74.5, 72.7, 70.9, 70.8, 70.1, 70.0, 69.9, 69.7, 69.6, 69.6, 69.5, 69.4, 69.3, 69.2, 68.6, 68.5, 68.5, 68.4, 68.2, 68.1, 67.7, 67.3, 66.8, 66.7, 66.3, 66.0, 65.7, 62.4, 62.3, 62.2, 62.1, 21.2, 21.1, 21.0, 21.0, 20.9, 20.8, 20.8, 20.7. HRMS: $[M+H]^+$ calculated for $C_{86}H_{117}O_{58}$ 2077.62003, found 2077.62039.

2,4-*O*-di-acetyl-3-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranoside

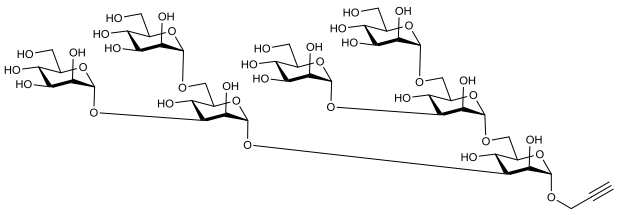


(25): To a solution of 1-OH acetylated heptamer **24** (56.9 mg, 27.4 µmol) in acetone (274 µL) was added a 0.15M *N*-phenyl trifluoroacetimidoyl chloride solution (274 µL, 41.1 µmol) in acetone. To the reaction mixture was added Cs_2CO_3 (15.5 mg, 41.1 µmol) and the mixture was stirred at rt till TLC showed complete conversion of the starting material. The reaction mixture was concentrated *in vacuo* and directly purified without further work up. Purification by column chromatography yielded heptamer imidate donor **25** as a slightly yellow oil (61.6 mg, quantitative yield). FT-IR: ν_{max} (neat)/cm^{-1} 1040.72, 1084.61, 1138.28, 1215.66, 1370.23, 1435.52, 1674.33, 1743.57, 2854.33, 2924.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.5 Hz, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 2H), 5.40 – 5.16 (m, 16H), 5.10 – 5.05 (m, 2H), 5.04 – 4.95 (m, 4H), 4.91 (s, 1H), 4.84 (d, *J* = 1.7 Hz, 1H), 4.81 (s, 1H), 4.36 – 4.22 (m, 4H), 4.22 – 3.88 (m, 13H), 3.87 – 3.79 (m, 1H), 3.79 – 3.70 (m, 3H), 3.60 – 3.46 (m, 3H), 2.25 – 2.01 (m, 54H), 2.01 – 1.95 (m, 9H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.7, 170.7, 170.5, 170.2, 170.1, 170.1, 170.1, 167, 167.0, 169.9, 169.8, 169.8, 169.7, 169.7, 169.5, 143.2, 128.9, 124.7, 119.5, 99.6, 99.4, 99.1, 97.6, 97.6, 75.5, 75.2, 71.9, 70.8, 70.8, 70.0, 69.9, 69.9, 69.8, 69.6, 69.5, 69.5, 69.4, 69.1, 68.7, 68.7, 68.6, 68.6, 67.8, 67.4, 66.8, 66.4, 66.1, 66.0, 65.9, 65.7, 62.5, 62.2, 62.1, 29.8, 21.0, 20.9, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6.

Propargyl 2,4-*O*-di-acetyl-3-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (26): heptamer imidate donor **25** (43.9 mg, 19.5 µmol) was dissolved in a 0.274M propargyl alcohol solution (356 µL, 97.5 µmol) in DCM and the mixture was stirred over activated molecular sieves (3Å) for 30 min. at rt. After 30 min. the mixture was cooled to -40 °C and to the cooled mixture was added a 0.1M TfOH solution (36 µL, 3.9 µmol) in DCM and the reaction mixture was gradually warmed to 0 °C. The reaction was quenched with a 0.1M Et_3N solution (0.1 mL) in DCM and the solution was filtered over celite and concentrated *in vacuo*. Purification by column chromatography yielded per-acetylated propagyl mannose heptamer **25** as a white milky oil (16.5 mg, 7.8 µmol, 40%). FT-IR: ν_{max} (neat)/cm^{-1} 1037.06, 1136.93, 1214.09, 1369.36, 1433.95, 1740.68, 2926.41. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 9.9, 3.5 Hz, 1H), 5.33 – 5.32 (m, 1H), 5.29 – 5.22 (m, 7H), 5.21 (dd, *J* = 10.0, 3.4 Hz, 2H), 5.19 – 5.16 (m, 1H), 5.07 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.05 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.00 – 4.97 (m, 3H), 4.97 – 4.94 (m, 3H), 4.92 – 4.89 (m, 1H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.9 Hz, 1H), 4.32 – 4.23 (m, 6H), 4.17 (td, *J* = 10.0, 3.4 Hz, 2H), 4.14 – 4.09 (m, 2H), 4.08 (s, 1H), 4.07 – 3.99 (m, 4H), 3.97 (dd, *J* = 9.9, 3.4 Hz, 2H), 3.97 – 3.88 (m, 3H), 3.87 (ddd, *J* = 10.3, 5.2, 2.7 Hz, 1H), 3.75 (dq, *J* = 10.8, 5.5 Hz, 3H), 3.71 (d, *J* = 5.6 Hz, 1H), 3.56 (dd, *J* = 11.4, 2.2 Hz, 1H), 3.53 (d, *J* = 2.6 Hz, 1H), 3.52 (d, *J* = 2.6 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.21 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 2.16 – 2.13 (m, 21H), 2.12 – 2.11 (m, 12H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.97 (d, *J* = 1.3 Hz, 6H). ^{13}C

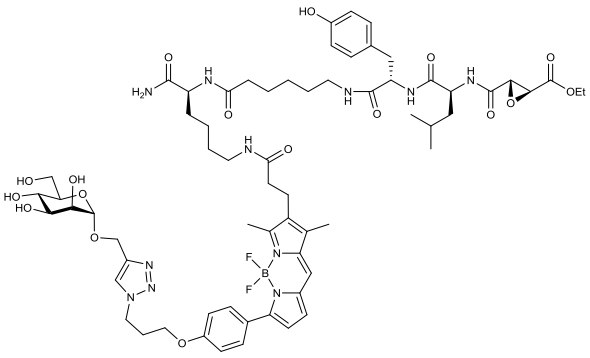
NMR (150 MHz, $CDCl_3$) δ 171.1, 171.0, 170.9, 170.8, 170.8, 170.8, 170.8, 170.8, 170.6, 170.6, 170.6, 170.5, 170.4, 170.3, 170.3, 170.2, 170.2, 170.2, 170.2, 170.1, 170.1, 170.1, 170.0, 170.0, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.8, 169.7, 99.4, 99.2, 97.8, 97.7, 97.3, 96.6, 78.8, 75.6, 75.5, 75.3, 75.0, 71.2, 71.0, 70.9, 70.3, 70.0, 70.0, 69.8, 69.6, 69.6, 69.5, 69.5, 69.5, 69.3, 69.2, 68.8, 68.7, 68.6, 68.6, 68.0, 67.8, 67.6, 66.8, 66.8, 66.4, 66.1, 66.1, 66.0, 66.0, 65.8, 62.5, 62.3, 62.2, 55.0, 21.0, 21.0, 21.0, 21.0, 20.9, 20.9, 20.9, 20.9, 20.8, 20.8, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{89}H_{119}O_{58}$ 2115.63568, found 2115.63581.

Propargyl 3-*O*-(3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (27): To



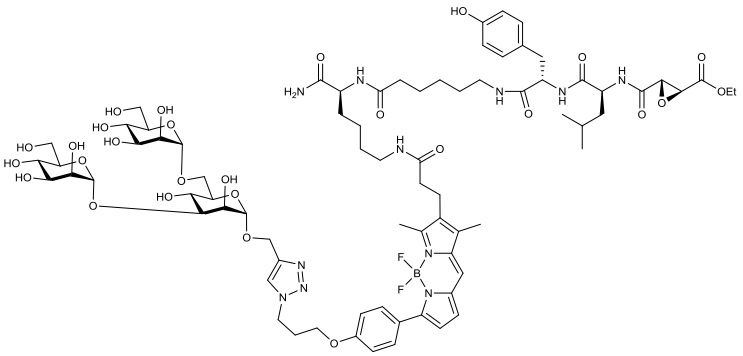
a solution of per-*O*-acetylated propargyl mannose heptamer **26** (16.5 mg, 7.8 µmol) in MeOH (156 µL) was added a 125mM NaOMe (156 µL, 3.9 µmol) solution in MeOH and the reaction was stirred overnight at rt. TLC-MS analysis showed incomplete deacetylation of the starting material and the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). The crude was taken up in H_2O (0.5 mL) and a 0.2M NaOH (aq.) (0.5 mL) solution was added to the solution. The reaction was followed on TLC-MS and after completion the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). Solids were filtered and the filtrate was concentrated *in vacuo*. The product was lyophilized from H_2O without further purification yielding propargyl mannose heptamer **27** as a white powder (9.3 mg, 7.8 µmol, quantitative yield). ^{1}H NMR (600 MHz, D_2O) δ 5.17 (d, *J* = 1.8 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 5.07 (d, *J* = 1.8 Hz, 1H), 5.02 (d, *J* = 1.8 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 1.9 Hz, 1H), δ 4.36 (s, 2H), 4.26 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.16 (dd, *J* = 3.3, 1.8 Hz,

1H), 4.11 (dd, *J* = 3.2, 1.7 Hz, 1H), 4.08 (dt, *J* = 3.6, 1.9 Hz, 2H), 4.03 (dd, *J* = 9.6, 3.5 Hz, 1H), 4.01 – 3.97 (m, 5H), 3.96 – 3.92 (m, 2H), 3.92 – 3.87 (m, 11H), 3.87 – 3.83 (m, 2H), 3.80 – 3.72 (m, 8H), 3.70 (ddd, *J* = 12.6, 7.3, 2.1 Hz, 4H), 3.67 (s, 2H), 3.65 (s, 1H). ^{13}C NMR (150 MHz, D_2O) δ 103.8, 103.7, 103.5, 100.8, 100.7, 100.6, 100.3, 80.1, 79.7, 79.7, 74.70, 74.6, 74.0, 73.9, 73.1, 72.7, 72.2, 71.9, 71.9, 71.7, 71.4, 71.3, 71.3, 71.2, 70.9, 70.9, 70.8, 68.1, 68.1, 68.0, 67.1, 67.1, 66.8, 66.7, 66.6, 66.4, 62.4, 62.2, 56.2. HRMS: $[M+H]^+$ calculated for $C_{45}H_{75}O_{36}$ 1191.40325, found 1191.40308.



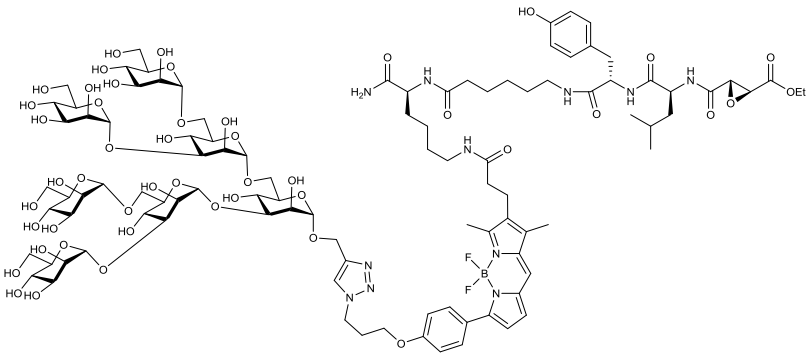
Man$_1$-BODIPY-DCG-04 (2): To a solution of propargyl mannose **7** (1.75 mg, 8 µmol) and BODIPY-DCG-04 **(5)** (8.6 mg, 7.6 µmol) in DMF/H_2O (1:1) (3 mL) was added 0.1 M sodium ascorbate (aq.) (160 µL, 16 µmol) and 0.1M $CuSO_4$ (aq.) (16 µL, 1.6 µmol). The resulting mixture was stirred for 2 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20→ 35% ACN in12') followed by lyophilization from H_2O yielded Man$_1$-BODIPY-DCG-04 (**2**) (4.3 mg, 3.2 µmol, 42%). ^{1}H NMR (600 MHz, $CDCl_3$/MeOD): δ 8.02 (s, 1H), 7.92 (t, *J* = 5.6 Hz, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.76 (t, *J* = 5.6 Hz, 1H), 7.40 (s, 1H), 7.06 (d, *J* = 4.1 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 8.9 Hz, 2H,), 6.69 (d, *J* = 8.5 Hz, 2H, 2), 6.59 (d, *J* = 4.1 Hz, 1H), 4.80 (d, *J* = 12.4 Hz, 1H), 4.67 - 4.60 (m, 3H, CH_2), 4.45 (t, *J* = 7.5 Hz, 1H), 4.40 - 4.36 (m, 1H), 4.30 - 4.19 (m, 3H), 4.08 (t, *J* = 5.8 Hz, 2H), 3.85 (dd, *J* = 11.8, 2.1 Hz, 1H), 3.82 - 3.77 (m, 1H), 3.74 - 3.65 (m, 3H), 3.64 - 3.55 (m, 3H), 3.15 - 3.11 (m, 3H), 3.06 - 3.01 (m, 1H), 2.99 - 2.93 (m, 1H), 2.86 - 2.80 (m, 1H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.50 (s, 3H), 2.42 (p, *J* = 6.5 Hz, 2H), 2.33 (t, *J* = 7.3 Hz,

2H), 2.25 (s, 3H), 2.21 - 2.16 (m, 2H), 1.78 - 1.69 (m, 1H), 1.62 - 1.48 (m, 6H), 1.46 - 1.41 (m, 2H), 1.41 - 1.35 (m, 2H), 1.31 - 1.29 (m, 5H), 1.22 - 1.15 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H,). ^{13}C NMR (150 MHz, $CDCl_3$/MeOD): δ 177.02, 176.02, 174.68, 173.56, 172.96, 168.66, 168.31, 160.68, 160.59, 157.16, 156.37, 145.22, 141.69, 136.49, 135.67, 131.84, 131.81, 131.78, 131.68, 131.30, 129.27, 128.81, 127.14, 125.60, 124.63, 119.12, 116.19, 115.14, 100.70, 74.84, 72.42, 71.93, 68.54, 65.67, 63.19, 62.90, 60.64, 56.36, 54.34, 54.11, 53.33, 53.14, 48.49, 41.56, 40.15, 40.12, 38.14, 36.86, 36.58, 32.64, 30.93, 29.87, 29.74, 27.32, 26.39, 25.80, 24.17, 23.29, 22.02, 21.29, 14.35, 13.28, 9.62. HRMS: $[M+H]^+$ calculated for $C_{65}H_{89}BF_2N_{11}O_{17}$ 1344.64935, found 1344.65139.



Man$_3$-BODIPY-DCG-04 (3): To a solution of propargyl mannoside **19** (2.4 mg, 4.5 µmol) and BODIPY-DCG-04 (**5**) (5.12 mg, 4.5 µmol) in DMF/H_2O (1:1) (2 mL) was added 0.1M sodium ascorbate (aq.) (90 µl, 9 µmol) and 0.1M $CuSO_4$ (aq.) (2.2 µL, 0.22 µmol). The resulting mixture was stirred for 1 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20 → 35% ACN in 12') followed by lyophilization from H_2O yielded Man$_3$-BODIPY-DCG-04 **(3)** (1.8 mg, 1.1 µmol, 24 %) ^{1}H NMR (600 MHz, MeOD): δ 8.08 (s, 1H), 7.88 (d, *J* = 8.9 Hz, 2H,), 7.43 (s, 1H), 7.07 (d, *J* = 4.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 4.1 Hz, 1H), 5.05 (s, 1H), 4.85 - 4.77 (m, 3H), 4.69 - 4.61 (m, 3H), 4.45 (t, *J* = 7.6 Hz, 1H), 4.38 (dd, *J* = 9.3, 5.7 Hz, 1H), 4.29 -

4.23 (m, 3H), 4.09 (t, *J* = 5.9 Hz, 2H), 4.05 (d, *J* = 2.5 Hz, 1H), 3.98 - 3.90 (m, 2H), 3.88 - 3.54 (m, 17H), 3.16 - 3.09 (m, 3H), 3.07 - 2.99 (m, 1H), 2.99 - 2.93 (m, 1H), 2.86 - 2.80 (m, 1H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.42 (p, *J* = 6.5 Hz, 2H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.26 (s, 3H), 2.20 - 2.14 (m, 2H), 1.77 - 1.70 (m, 1H), 1.64 - 1.47 (m, 6H), 1.47 - 1.41 (m, 2H), 1.41 - 1.35 (m, 2H), 1.32 - 1.29 (m, 5H), 1.20 - 1.15 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, MeOD): δ 177.14, 176.13, 174.79, 173.69, 173.08, 168.74, 168.43, 165.61, 163.01, 160.81, 160.67, 157.29, 156.48, 150.29, 145.18, 142.19, 136.59, 135.75, 131.90, 131.77, 131.36, 129.35, 128.91, 127.23, 125.71, 124.73, 119.16, 116.24, 115.22, 103.97, 101.33, 100.79, 80.67, 74.96, 74.39, 73.70, 72.66, 72.46, 72.10, 71.27, 68.80, 68.64, 67.48, 67.14, 65.75, 63.23, 62.91, 60.71, 56.47, 54.38, 54.21, 53.42, 53.19, 41.62, 40.20, 38.19, 36.91, 36.63, 32.71, 31.05, 29.95, 29.82, 27.40, 26.48, 25.88, 24.25, 23.30, 22.02, 21.34, 14.35, 9.59. LC/MS analysis (linear gradient 10% → 90% ACN) t*R*: 6.53 min, ESI-MS (*m/z*): [M + H]+: 1668.40.



Man$_7$-BDP-DCG-04 (4): To a solution of propargyl mannoside **27** (4 mg, 3.4 µmol) and BODIPY-DCG-04 (**5**) (3.8 mg, 3.4 µmol) in DMF/H_2O (1:1) (2 mL) was added 0.1M sodium ascorbate (aq.) (68 µL, 6.8 µmol) and 0.1M $CuSO_4$ (aq.) (6.8 µL, 0.68 µmol). The resulting mixture was stirred for 8 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20 → 35% ACN in 12') followed by lyophilization from H_2O yielded Man$_7$-

BDP-DCG-04 (**4**) (2.5 mg, 1.1 μmol, 32%). ^{1}H NMR (600 MHz, MeOD): δ 8.28 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.95 (s, 1H), 7.90 - 7.85 (m, 2H), 7.79 (t, *J* = 5.6 Hz, 1H), 7.42 (s, 1H), 7.07 (d, *J* = 4.1 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.9 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 4.1 Hz, 1H), 5.11 (s, 1H), 5.07 (s, 1H), 4.99 (s, 1H), 4.81 - 4.64 (m, 8H), 4.50 - 4.43 (m, 1H), 4.41 - 4.36 (m, 1H), 4.31 - 4.23 (m, 3H), 4.19 (d, *J* = 2.8 Hz, 1H), 4.12 - 4.07 (m, 4H), 4.03 - 3.53 (m, 41H), 3.16 - 3.10 (m, 3H), 3.07 - 3.02 (m, 1H), 3.00 - 2.94 (m, 1H), 2.88 - 2.80 (m, 1H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.44 (q, *J* = 6.4 Hz, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.26 (s, 3H), 2.21 - 2.17 (m, 2H), 1.78 - 1.71 (m, 1H), 1.66 - 1.47 (m, 6H), 1.47 - 1.41 (m, 2H), 1.41 - 1.36 (m, 2H), 1.33 - 1.28 (m, 5H), 1.23 - 1.17 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). HRMS: $[M+H]^+$ calculated for $C_{101}H_{149}BF_2N_{11}O_{47}$ 2317.96965, found 2317.97256.

Cell culture of primary cells. Immature dendritic cells were obtained from the bone marrow of C75BL/6 mice and were a gift from the Biopharmaceutical Department (Leiden University). The use of animals was approved by the ethics committee of Leiden University. Mice were sedated, bone marrow of tibiae and femurs was flushed out and washed with PBS. Cells were grown in dendritic cell selection medium (IMDM containing granulocyte-macrophage colony stimulating factor (GM-CSF) (2:1 v/v) containing 8% FCS, penicillin/streptomycin (100 units/mL), glutamax (2 mM) and β-mercaptoethanol (20 μM). Cells were selected for 10 days (37 °C; 5% CO_2) and subcultured every 2-3 days before use in the assays.

Labeling of cathepsins in mouse liver and immature dendritic cell lysate. Lysates (~8-10 μg total protein, determined on a Qubit 2.0 fluorometer, Life Technologies-Invitrogen) in 50 mM sodium citrate pH 5.5 or pH 7 (as indicated), 5 mM DTT, 0.2% CHAPS, and 0.1% Triton X-100, were incubated with the indicated concentration of probe (total volume: 10 μL) for 1 h at 37 °C. For competition experiments, lysates were first incubated with N_3-DCG-04 (1 or 10 μM), AS44 (10 μM) or BODIPY(FL)-DCG-04 (1 or 10

μM) for 1 h, 37 °C, before addition of the probe and continued incubation for 1 h. After treatment, 5x Laemli's sample buffer (including β-mercaptoethanol) was added and the samples were boiled (100 °C, 5 min) and resolved on 12.5% SDS-PAGE. Gels were scanned on a Typhoon 2000 imager (GE Healthcare) using the Cy2 (λ_{ex} 532 nm; λ_{em} 526 nm) and Cy3 (λ_{ex} 532 nm; λ_{em} 580 nm) settings. Total protein loading was determined by staining with Coomassie brilliant blue and subsequent scanning on a BioRad GS800 calibrated densitometer. Image processing was done with ImageJ, representative gels from at least three independent experiments are shown.

Labeling of cathepsins in live immature mouse dendritic cells. Cells were seeded onto tissue-culture coated 24-wells plates (200.000 cells/well, 250 μL medium) and allowed to attach for 2 h (37 °C; 5% CO_2), before addition of inhibitor or probe to the medium. Pre-incubations with N_3-DCG-04 (20 μM) or mannan (3 mg/mL) were conducted for 1 h, followed by addition of compound **2**, **3** or **4** (1 μM) and continued incubation for 2 h. For direct labeling experiments, cells were cultured for 2 h (37 °C; 5% CO_2) in the presence of probes **2**, **3** or **4** (0.1, 0.25, 0.5, 0.75 or 1 μM). After incubation, cells were washed with PBS (2x), lysed (35 μL Invitrogen complete cell extraction buffer) and proteins resolved on 12.5% SDS-PAGE, followed by fluorescence scanning (Cy3 settings) and CBB staining. Image processing was done with ImageJ, representative gels from at least three independent experiments are shown.

Confocal fluorescence microscopy. Experiments were conducted on a Leica TCS SPE confocal microscope, using dsRed filter settings for BODIPY (λ_{ex} 532 nm) and Cy5 settings for Draq5 (λ_{ex} 635 nm). Cells (30-75 x 10^4 cells/well) were seeded onto sterile Labtek II 4- or 8-chamber borosilicate coverglass systems (Fisher Emergo). Dendritic cells were allowed to attach for 2 h before pre-incubation with mannan (3 mg/mL) (1 h, 37 °C, 5% CO_2) and subsequent probe incubation (1 μM, 2 h). Cells were then thoroughly washed (PBS), fixed (4% formaldehyde in PBS), washed

again with PBS, nuclei stained with Draq5 (Thermo Scientific) and imaged. All experiments were performed at least in duplicate.

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