The handle http://hdl.handle.net/1887/37023 holds various files of this Leiden University dissertation.

**Author:** Wong, Chung Sing  
**Title:** The synthesis of mannose-derived bioconjugates and enzyme inhibitors  
**Issue Date:** 2015-12-10
Chapter 2

Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

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. 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 and small synthetic molecules such as folic acid, peptides and carbohydrates. Lectins are carbohydrate-binding receptors involved in a wide variety of cellular recognition and communication processes. 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. Many of the lectins found on these cells are members of the C-type lectin family and these include the mannose receptor (MR), Dectin-1 and 2 as well as DC-SIGN. 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.\textsuperscript{14} DCG-04 was originally reported by Bogyo and co-workers, in a seminal paper,\textsuperscript{15} which, together with the first paper by Cravatt and co-workers on serine hydrolase probes,\textsuperscript{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.\textsuperscript{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\textsuperscript{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.\textsuperscript{19,20} Glycan microarray studies have revealed that DC-SIGN strongly binds high mannose type structures\textsuperscript{21} and available crystal structures of DC-SIGN bound to natural ligands show that a terminal branched trimannose structure, featuring \( \alpha-(1,3) \) and \( \alpha-(1,6) \) mannose branches on a core mannose residue, fits well in the carbohydrate binding
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

site of this signaling receptor. The MR also binds oligomannosides and a preference for the same type of branching has been reported. 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. $\alpha$-(1$\rightarrow$3) and $\alpha$-(1$\rightarrow$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 BF₃·Et₂O 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 11²⁶,²⁷ in 82% over 4 steps. Treatment of this lactol with (N-phenyl)trifluoroacetamidoyl chloride in the presence of Cs₂CO₃ 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) I₂, Et₃SiH, CH₂Cl₂, reflux; ii) MeOH, 2,6-lutidine, rt; (b) K₂CO₃, MeOH, rt; (c) NaH, BnBr, DMF, 0 °C to rt; (d) DME/H₂O (10:1), pTsOH, 82% over 4 steps; (e) Cs₂CO₃, acetone, (CF₃)C(NPh)Cl, 90%; (f) propargyl alcohol, BF₃·Et₂O, 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 13²⁴ using donor 12.
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

and a catalytic amount of TfOH (Scheme 2). Next trimer 14 was converted into the corresponding anomic 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 H₂ in a mixture of EtOAc/tBuOH/H₂O (1:3:4) and subsequently with Pd/C and H₂ 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 BF₃·Et₂O to provide the fully protected trimer 18. Global deacetylation under Zemplén conditions yielded the propargyl trimannoside Man₃ 19.

The heptasaccharide 21 was assembled using trisaccharide donor 14 and acceptor 20. The latter building block was obtained by treatment of thiomannoside 13 with NIS and AcOH (Scheme 2). 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 H₂ in EtOAc/MeOH/H₂O (5:4:1) was followed by a second hydrogenation in MeOH/H₂O (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 BF₃·Et₂O did not lead to the desired product and therefore a more potent glycosylating agent was used. To this end the anemic acetyl was chemoselectively deblocked using hydrazine acetate and the liberated alcohol was converted into the (N-phenyl)trifluoromimidate donor 25.
Scheme 2: Synthesis of the propargyl trimannoside Man₃ 19 and propargyl heptamannoside Man₇ 27.

Reagents and conditions: (a) 12, DCM, TfOH, act. Mol. Siev., -40 °C to rt, 84%; (b) NIS, AcOH, DCE/Et₂O (1:1), 94%; (c) i) Pd/C, H₂, EtOAc/rBuOH/H₂O (1:3:4), ii) Pd/C, H₂, H₂O; (d) Ac₂O, pyridine, 0 °C to rt, 76% over 2 steps; (e) propargyl alcohol, BF₃·Et₂O, DCM, rt, 61%; (f) MeOH, NaOMe, 68%; (g) NIS, DCM/MeOH (1:1), rt, 40%; (h) 14, NIS, TfOH, act. Mol. Siev., DCM, -40 °C to rt, 62%; (i) Pd/C, H₂, EtOAc/MeOH/H₂O (5:4:1); ii) Pd/C, H₂, MeOH/H₂O (1:1); (j) Ac₂O, pyridine, 0 °C to rt, quantitative yield over two steps; (k) H₂NNH₂·AcOH, DMF, 0 °C, 79%; (l) CI(C=NH)CF₃, Cs₂CO₃, 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 Man₇ 27.
The BODIPY-DCG-04-mannose conjugates were obtained through a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azido BODIPY-DCG-04 (5) and the propargyl mannoses 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$_2$O (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)\textsuperscript{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 \textsuperscript{28}, azido-DCG-04 \textsuperscript{29} and BODIPY(FL)-DCG-04 \textsuperscript{30}(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 Man₃-BODIPY-DCG-04 3 and Man₇-BODIPY-DCG-04 4 in DCs but little uptake of Man₁-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 Man₃-BODIPY-DCG-04 3 and Man₇-BODIPY-DCG-04 4 are taken up through the intermediacy of a carbohydrate binding receptor, where Man₁-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 °C) or pre-incubated (1 h, 37 °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 °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$_2$O$_5$ and stored over activated 3 Å molecular sieves under an argon atmosphere. Propargyl alcohol was distilled over K$_2$CO$_3$ 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 ($\lambda = 254$ nm), staining with 20% H$_2$SO$_4$ in EtOH or with a solution of
(NH₄)₆Mo₇O₂₄·4H₂O (25 g/l) in 10% H₂SO₄ in H₂O followed by charring at +/- 140 °C. ¹H- and ¹³C 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 C₁₈ (Gemini, 4.6 mm x 50 mm, 5µm particle size, Phenomenex) analytical column and buffers A: H₂O, 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% Na₂S₂O₃ (aq.) (2x), H₂O (3x), brine (2x), dried over Mg₂SO₄, filtered and concentrated in vacuo. The residue was dissolved in MeOH (500 mL), and to the solution was added K₂CO₃ (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
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

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 \textit{in vacuo} and the concentrate was dissolved in Et\textsubscript{2}O, washed with H\textsubscript{2}O (4x), brine (2x), dried over MgSO\textsubscript{4}, filtered and concentrated \textit{in vacuo}. The crude was dissolved in DME/H\textsubscript{2}O (10:1) (1.5 L) and the solution was cooled to 0 °C. To the cooled solution was added ρTsOH (75 mmol, 14.3 g), after 3 h at 0 °C the reaction was quenched with sat. NaHCO\textsubscript{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\textsubscript{4}, filtered and concentrated \textit{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.\textsuperscript{31} Spectroscopic data are reported for the major (α) isomer: \textit{1}H NMR (400 MHz, CDCl\textsubscript{3}) δ 7.36 – 7.23 (m, 13H), 7.18 – 7.12 (m, 2H), 5.36 (dd, \textit{J} = 3.3, 1.9 Hz, 1H), 5.19 (s, 1H), 4.85 (d, \textit{J} = 10.8 Hz, 1H), 4.69 (d, \textit{J} = 11.2 Hz, 1H), 4.59 (d, \textit{J} = 12.1 Hz, 1H), 4.52 (d, \textit{J} = 8.2 Hz, 1H), 4.49 (d, \textit{J} = 9.1 Hz, 1H), 4.45 (d, \textit{J} = 10.9 Hz, 1H), 4.09 – 4.05 (m, 1H), 4.03 (dd, \textit{J} = 9.3, 3.2 Hz, 1H), 3.74 (d, \textit{J} = 9.6 Hz, 1H), 3.69 – 3.66 (m, 2H), 2.14 (s, 3H). \textit{13}C NMR (100 MHz, CDCl\textsubscript{3}) δ 170.6, 138.4, 138.0, 137.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 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\textsubscript{2}CO\textsubscript{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 \textit{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). [α]\textsubscript{D}\textsuperscript{22} + 26.4° (c = 1.0, DCM). FT-IR: \textit{v}_{\text{max}} (neat)/cm\textsuperscript{-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: ¹H NMR (400 MHz, CDCl₃, 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 = 11.2, 1.7 Hz, 1H), 2.11 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 169.9, 143.4, 138.5, 138.4, 137.8, 128.9, 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-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 Tf₂O (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 H₂O and the aqueous phase was extracted with DCM (4x). The combined organic layers were dried over MgSO₄, filtered, concentrated in vacuo. Purification by column chromatography yielded trimer 14 as a colorless oil (11.76 g, 8.4 mmol, 84%). [α]D²² + 64.6° (c = 1.0, DCM). FT-IR: νmax (neat)/cm⁻¹ 978.54, 1026.58, 1049.14, 1078.97, 1232.74, 1367.72, 1453.88, 1742.59, 2866.77, 3030.51. ¹H NMR (400 MHz, CDCl₃) 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). 13C NMR (100 MHz, CDCl3) 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, 128.4, 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, 69.3, 68.9, 68.8, 68.5, 66.7, 21.3, 21.1. HRMS: [M+H]+ calculated for C84H89O17S 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% Na2S2O3 sol. (aq.) (2x), H2O, 3x sat (1x). NaHCO3 sol. (aq.) (1x), H2O (3x), brine (2X), dried over MgSO4, filtered and concentrated in vacuo. Purification by column chromatography yielded trimer 15 as a colorless oil (4.67 g, 3.5 mmol, 94%). [α]D22 + 50.0° (c = 1.0, DCM). FT-IR: νmax (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. 1H NMR (400 MHz, CDCl3) (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, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 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/tBuOH/H$_2$O (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$_2$O (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$_2$O 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. NaHCO₃ (aq.) (1x), H₂O (3x), brine (2x), dried over MgSO₄, 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%). [α]D⁺²² + 40.4° (c = 1.0, DCM). FT-IR: νmax (neat)/cm⁻¹ 975.27, 1039.52, 1139.46, 1212.30, 1368.79, 1433.73, 1741.84, 2925.08. ¹H NMR (300 MHz, CDCl₃) (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). ¹³C NMR (75 MHz, CDCl₃) 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 C₄₀H₅₅O₂₇ 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 BF₃·Et₂O 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. NaHCO₃ (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$_2$O (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%). $[\alpha]_D^{22} + 80.4^\circ$ (c = 1.0, DCM). FT-IR: $v_{\text{max}}$ (neat)/cm$^{-1}$ 978.00, 1038.64, 1136.67, 1214.43, 1368.90, 1433.77, 1714.73, 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%). $[\alpha]_D^{22} + 114.0^\circ$ (c = 1.0, MeOH). FT-IR: $v_{\text{max}}$ (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) $\delta$ 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$_2$S$_2$O$_3$ (aq.) and the product was extracted with EtOAc (4x). The combined organic phases were washed with H$_2$O (2x), sat. NaHCO$_3$ (aq.) (3x), H$_2$O (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%). $[\alpha]_{D}^{22} + 14.6^\circ$ (c = 1.0, DCM). FT-IR: $v_{\text{max}}$ (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$_3$) $\delta$ 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$_3$) $\delta$ 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-\(\alpha\)-D-mannopyranosyl)-6-O-(2-O-acetyl-3,4,6-O-tri-benzyl-\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-D-mannopyranosyl)-6-O-(2,4-O-di-benzyl-3-O-(2-O-acetyl-3,4,6-O-tri-benzyl-\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-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 (3 Å) 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 \(\text{Et}_3\text{N}\). The mixture was filtered over celite and diluted with DCM. The organic phase was washed with 10% \(\text{Na}_2\text{S}_2\text{O}_3\) (aq.) and the aqueous phase was extracted with DCM (5x). The combined organic layers were washed with \(\text{H}_2\text{O}\) (1x), brine (1x), dried over MgSO\(_4\), filtered and concentrated \textit{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: \(v_{\text{max}}\) (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\(_3\)) \(\delta\) 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\(_3\)) \(\delta\) 170.3, 170.2, 170.1, 170.1, 169.0, 169.0,
138.8, 138.7, 138.6, 138.5, 138.3, 138.2, 138.2, 138.0, 138.0, 137.9, 137.9, 137.8, 137.2, 128.7, 128.6, 128.5, 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, 127.7, 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.

**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)-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$_2$O (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$_2$O (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$_2$O (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. NaHCO₃ (aq.) (2x), H₂O (3x), brine (3x), dried over MgSO₄, 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: v_max (neat)/cm⁻¹ 976.88, 1038.56, 1084.01, 1138.17, 1213.21, 1369.06, 1432.60, 1742.41, 2935.07. ¹H NMR (400 MHz, CDCl₃) δ 5.98 (d, J = 1.9 Hz, 1H), 5.37 – 5.17 (m, 13H), 5.15 (s, 1H), 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). ¹³C NMR (100 MHz, CDCl₃) δ 170.7, 170.6, 170.5, 170.4, 170.3, 170.0, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.7, 169.9, 169.6, 169.6, 169.6, 169.5, 168.0, 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, 69.6, 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, 62.0, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5. MALDI: [M+H]+ calculated for C₈₈H₁₁₉O₅₉ 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,
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

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 Et₂O and the organic phase was washed with brine (3x), dried over MgSO₄, 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: v_max (neat)/cm⁻¹ 978.58, 1038.62, 1081.66, 1137.06, 1215.09, 1369.26, 1433.38, 1741.35, 2926.28. ¹H NMR (400 MHz, CDCl₃) 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, δ = 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). ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 170.9, 170.8, 170.8, 170.7, 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 C₈₆H₁₁₇O₅₈ 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)-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)-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, 41.1 µmol) in acetone. To the reaction mixture was added Cs₂CO₃ (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: νmax (neat)/cm⁻¹ 1040.72, 1084.61, 1138.28, 1215.66, 1370.23, 1435.52, 1674.33, 1743.57, 2854.33, 2924.27. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C NMR (100 MHz, CDCl₃) 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, 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.
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

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)-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 Et₃N solution (0.1 mL) in DCM and the solution was filtered over celite and concentrated in vacuo. Purification by column chromatography yielded per-acetylated propargyl mannose heptamer 25 as a white milky oil (16.5 mg, 7.8 µmol, 40%). FT-IR: νₘₐₓ (neat)/cm⁻¹ 1037.06, 1136.93, 1214.09, 1369.36, 1433.95, 1740.68, 2926.41. ¹H NMR (600 MHz, CDCl₃) 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 = 3.4, 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). ¹³C
NMR (150 MHz, CDCl$_3$) $\delta$ 171.1, 171.0, 170.9, 170.8, 170.8, 170.8, 170.6, 170.6, 170.5, 170.4, 170.3, 170.3, 170.2, 170.2, 170.1, 170.1, 170.0, 170.0, 170.0, 170.0, 169.9, 169.9, 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, 66.4, 66.1, 66.1, 66.0, 65.8, 62.5, 62.3, 62.2, 55.0, 21.0, 21.0, 21.0, 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-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$_2$O (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$_2$O 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$_2$O) $\delta$ 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), $\delta$ 4.36 (s, 2H), 4.26 (dd, $J = 3.3, 1.8$ Hz, 1H), 4.16 (dd, $J = 3.3, 1.8$ Hz,
Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates

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\(_2\)O) 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 \(\mu\)mol) and BODIPY-DCG-04 (5) (8.6 mg, 7.6 \(\mu\)mol) in DMF/H\(_2\)O (1:1) (3 mL) was added 0.1 M sodium ascorbate (aq.) (160 \(\mu\)L, 16 \(\mu\)mol) and 0.1M CuSO\(_4\) (aq.) (16 \(\mu\)L, 1.6 \(\mu\)mol). 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\(_4\)OAc, B: linear gradient 20\(\rightarrow\) 35% ACN in12’) followed by lyophilization from H\(_2\)O yielded Man\(_1\)-BODIPY-DCG-04 (2) (4.3 mg, 3.2 \(\mu\)mol, 42%). \(^1\)H NMR (600 MHz, CDCl\(_3\)/MeOD): δ 8.02 (s, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.86 (t, J = 5.6 Hz, 1H), 7.40 (s, 1H), 7.06 (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), 2.19 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.84 (s, 3H), 1.80 (s, 3H), 1.76 (s, 3H), 1.72 (s, 3H), 1.69 (s, 3H), 1.65 (s, 3H), 1.61 (s, 3H), 1.57 (s, 3H), 1.53 (s, 3H), 1.49 (s, 3H), 1.45 (s, 3H), 1.41 (s, 3H), 1.37 (s, 3H), 1.33 (s, 3H), 1.29 (s, 3H), 1.25 (s, 3H), 1.21 (s, 3H), 1.17 (s, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 0.97 (s, 3H), 0.93 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.81 (s, 3H), 0.77 (s, 3H), 0.73 (s, 3H), 0.69 (s, 3H), 0.65 (s, 3H), 0.61 (s, 3H), 0.57 (s, 3H), 0.53 (s, 3H), 0.49 (s, 3H), 0.45 (s, 3H), 0.41 (s, 3H), 0.37 (s, 3H), 0.33 (s, 3H), 0.29 (s, 3H), 0.25 (s, 3H), 0.21 (s, 3H), 0.17 (s, 3H), 0.13 (s, 3H), 0.09 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H), 0.00 (s, 3H), 0.00 (s, 3H).
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$_2$N$_{11}$O$_{17}$ 1344.64935, found 1344.65139.

**Man$_3$-BODIPY-DCG-04 (3):** To a solution of propargyl mannioside 19 (2.4 mg, 4.5 µmol) and BODIPY-DCG-04 (5) (5.12 mg, 4.5 µmol) in DMF/H$_2$O (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$_4$OAc, B: linear gradient 20 → 35% ACN in 12’) followed by lyophilization from H$_2$O 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% $\rightarrow$ 90% ACN) tR: 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$_2$O (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$_4$OAc, B: linear gradient 20 $\rightarrow$ 35% ACN in 12') followed by lyophilization from H$_2$O 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, 4H), 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 $C_{101}H_{146}BF_2N_{11}O_{47}$ 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<sub>ex</sub> 532 nm; λ<sub>em</sub> 526 nm) and Cy3 (λ<sub>ex</sub> 532 nm; λ<sub>em</sub> 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% CO₂), before addition of inhibitor or probe to the medium. Pre-incubations with N₃-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% CO₂) 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<sub>ex</sub> 532 nm) and Cy5 settings for Draq5 (λ<sub>ex</sub> 635 nm). Cells (30-75 x 10⁴ 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% CO₂) 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.

References

Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates


