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The modular synthesis of multivalent functionalised glycodendrons for the detection of lectins including DC-SIGN†

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Glycodendrons are excellent tools for the biological evaluation of lectins. Herein, we present the expedient and efficient synthesis of a versatile second generation dendron scaffold using double exponential growth methodology. The dendron scaffold can be rapidly functionalised, as illustrated by the conjugation of the dendron scaffold to biotin or a fluorescent probe, followed by oxyamine glycan conjugation to the glycan of choice. The use of a fluorescent Lewis[X] glycodendron for the detection of the C-type lectin DC-SIGN on macrophages is then demonstrated.

Introduction

Glycoconjugates, such as glycolipids and glycoproteins, are essential to all forms of life. These fundamental cellular constituents are involved in a variety of biological processes, such as host cell recognition, inflammation, cell signalling and proliferation,[1,2] with the carbohydrate portion of the glycoconjugate often interacting with lectins (carbohydrate binding proteins) that are expressed on the surface of cells. Lectins, however, generally have a weak affinity for their carbohydrate ligands and therefore require multivalent interactions in order to induce a biological response.[3] Thus, to study carbohydrate–lectin binding, the glycan epitope is typically conjugated to a substrate that allows for multivalent presentation.[4] To this end, dendrimers and dendrons have both been used for the multivalent presentation of glycans. One advantage of the use of a glycodendron is that its core can be functionalised with reporter groups (*e.g.*, fluorescent groups, biotin) or with toxins, which can allow for the detection, isolation or elimination of cells that expresses the target lectin.

Given the application of glycodendrons for the study of carbohydrate–lectin interactions, we became interested in developing a highly efficient synthesis of a dendron scaffold that could be readily functionalised with the molecular probe of choice and with glycans using our recently developed oxyamine linker methodology.[5,6] This oxyamine methodology is not only highly efficient and allows for the conjugation of naturally occurring (isolated) glycans, but it also leads to the formation of β-linked GlcNAc residues, which are commonly found conjugated to proteins in the form of *N*-glycans.[7] To this end, we proposed that the target glycodendron **1** could be prepared *via* peptide coupling of the carboxy-functionalised dendron **2** to the amine-functionalised neoglycoside prepared *via* conjugation of glycan **3** to 3-(methoxyamino)propan-1-amine (**4**) (Scheme 1). The dendron scaffold **2** would in turn contain the molecular probe of choice. The oxyamine ligation methodology has not been used for the conjugation of multiple copies of a glycan to a molecular scaffold, so we first envisioned synthesizing a glycodendron containing GlcNAc as proof-of-concept, before extending our methodology to include the glycan Lewis[X]. Lewis[X] is a ligand for the C-type lectin DC-SIGN [also known as Cluster of Differentiation 209 (CD209)],[8] which is expressed on the cell wall of several cells types,[9–11] with alternatively-activated ('M2-like') macrophages expressing higher levels of DC-SIGN compared to their classically-activated ('M1-like') counterparts.[12] Moreover, DC-SIGN can be used by the human immunodeficiency virus (HIV), Ebola, hepatitis C, and non-viral pathogens, such as *Mycobacterium tuberculosis*, to infect host cells.[13,14] Accordingly, DC-SIGN is an attractive target for glycodendron/glycodendrimer therapeutics.[15–18]
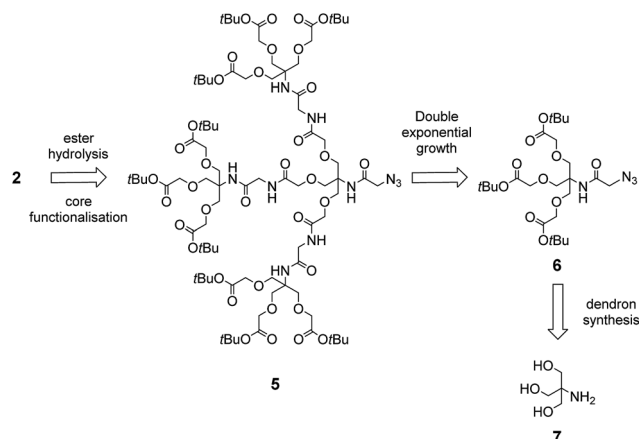
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Scheme 1 Assembly of glycodendrons using an amine-functionalised oxyamine linker.
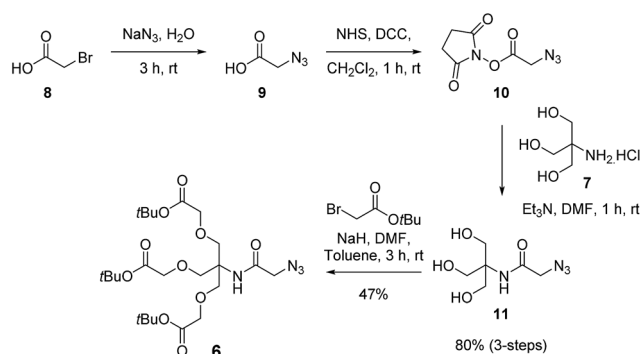
Scheme 2 Retrosynthesis of the dendron scaffold.

To synthesise the dendron scaffold **2** we proposed a double exponential growth strategy.[19] For the double exponential growth methodology, a protected first-generation dendron is synthesised and then converted to a second-generation dendron in a parallel synthesis. This strategy has merit since it allows for the easy purification of the first-generation dendron, minimises the number of steps to assemble larger dendrons, and therefore facilitates the rapid assembly of second-, third- and fourth-generation dendrons.[20] Depending on the functional groups at the periphery of the dendron, a variety of strategies can then be employed for the conjugation of glycans, including copper-catalysed 'click' reactions,[21] and reductive amination.[22] We envisioned that dendron **2** could be obtained from precursor **5** *via* Staudinger reduction of the azide in **5** to an amine and subsequent peptide coupling with a probe equipped with a carboxylic acid, followed by hydrolysis of the terminal *t*-butyl esters groups (Scheme 2). The protected dendron **5** could in turn be prepared from the first generation dendron **6** *via* double exponential growth methodology. Finally, it was envisioned that dendron **6** could be synthesised in two steps from the highly versatile branching unit tris(hydroxymethyl)aminomethane (TRIS, **7**)[23] using amide formation and tri-*O*-alkylation with *tert*-butyl bromoacetate.
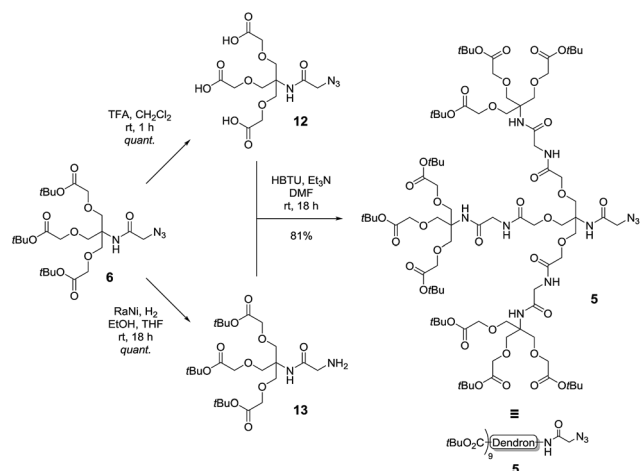
Results

The synthesis of the first generation dendron commenced with azide substitution of bromoacetic acid (**8**) to give azide derivative **9**, which in turn was converted into the activated ester **10** *via* a DCC-mediated coupling with *N*-hydroxysuccinimide (Scheme 3). Conjugation of **10** with excess TRIS (**7**) then gave triol **11** in 80% yield over three steps. With the triol in hand, alkylation using NaH and *tert*-butyl bromoacetate in DMF then gave the first generation dendron **6**. Here it was determined that the rapid addition of NaH to a mixture of triol **11** and *tert*-butyl bromoacetate in DMF : toluene (1 : 1) at room temperature minimised the formation of partially alkylated intermediates and afforded dendron **6** in 47% yield. While this alkylation yield may appear modest, the remaining steps for the synthesis of our
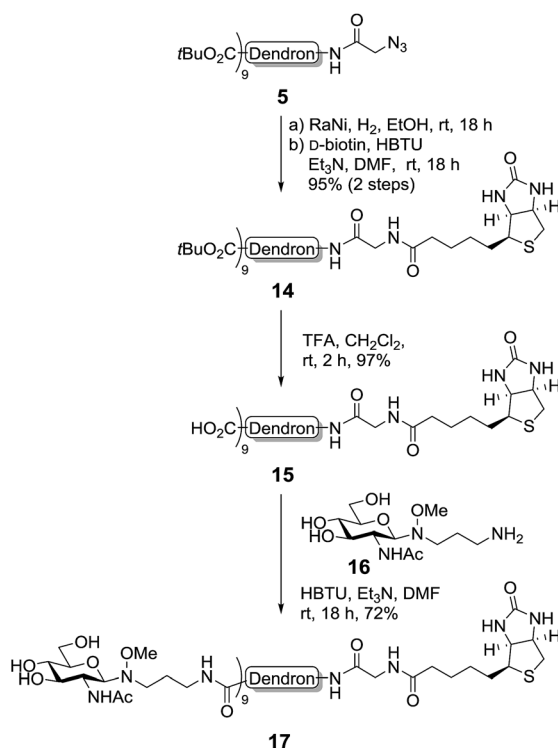
target dendron scaffold **5** are very efficient (*vide infra*). Moreover, similar yields have been reported for the tri-*O*-alkylation of TRIS using analogous substrates, as illustrated by Dupuy *et al.* who reported a 53% yield for the tri-*O*-alkyl-ation of *N,N*-diphenyl-TRIS with *tert*-butyl bromoacetate using a two-phase system,[24] while Newkome *et al.* prepared a tri-acid functionalised dendron core in two-steps *via* the Michael addition of TRIS to acrylonitrile followed by nitrile hydrolysis to give the ethyl esters in 54% over two steps.[25] It should also be noted that our route was readily amenable to synthesis on the gram scale, and following purification of dendron **6** *via* silica gel chromatography followed by reverse phase (C$_{18}$ beads) chromatography, the first-generation dendron **6** was obtained in excellent purity.

With the first generation dendron in hand, the synthesis of the second-generation dendron was then explored using the double exponential growth approach. Accordingly, the first generation dendron **6** was divided into two batches for conversion into the two reactive substrates (Scheme 4). Deprotection of the *tert*-butyl esters of **6** *via* the agency of TFA/CH$_2$Cl$_2$ (1/1, v/v) gave the tri-valent acid **12** in quantitative yield, while azide reduction of **6**, using RANEY® nickel mediated hydrogenation, afforded primary amine **13**, again in quantitative yield. Next, *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium

Scheme 3 Synthesis of the first generation dendron **8**.

Scheme 4 Synthesis of second generation dendron **5** using a double exponential growth coupling strategy.

hexafluorophosphate (HBTU)-mediated coupling of tri-acid **12** with amine **13** was undertaken to give the second-generation dendron **5** in 81% yield and in >95% purity following silica gel chromatography followed by reverse phase (C₁₈ beads) chromatography. Again, these reactions could be performed on the gram scale.
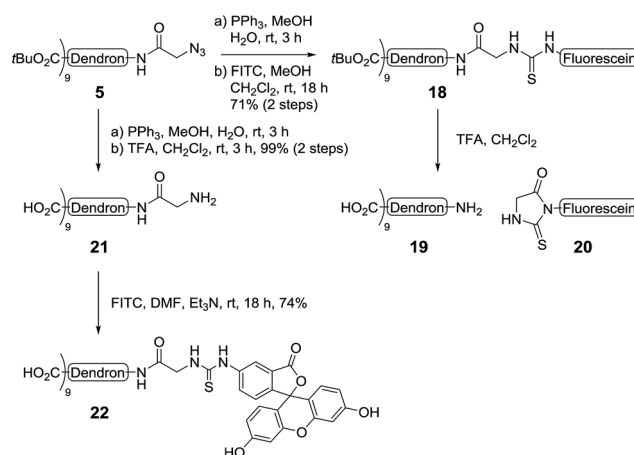
To demonstrate the versatility of our synthetic methodology, we then sought to functionalise the dendron scaffold **5** with either biotin or a fluorescent group. First, proof-of-concept was established *via* the synthesis of a second-generation biotin-functionalised dendron scaffold containing GlcNAc (Scheme 5). To this end, the azide in dendron **5** was converted into an amide *via* RANEY® nickel hydrogenation and the crude product coupled to D-biotin using an HBTU-mediated peptide ligation to give the biotinylated dendron **14** in 95% yield over two steps (Scheme 5). Removal of the *tert*-butyl esters using TFA in CH₂Cl₂ then occurred smoothly to afford carboxy dendron **15** in excellent yield, which was subsequently conjugated to oxyamine glycoside **16**, itself prepared in 81% yield *via* the conjugation of GlcNAc to 3-(methoxyamino)propanyl-amine hydrochloride **4** according to previously published procedures.[5] To facilitate the conjugation, excess oxyamine-functionalised glycan **16** (2 equiv. per carboxyl group) was used and the reaction mixture was stirred at room temperature overnight. Dialysis (cellulose ester dialysis membrane, 500 Da molecular weight cut-off) for 48 hours with a 0.1 M Na₂HPO₄ buffer at pH 7.5 to prevent oxyamine linker hydrolysis,[26] followed by lyophilisation and reverse phase chromatography (C₈) then gave the biotinlyated glycodendron **17** in very good yield (72%).
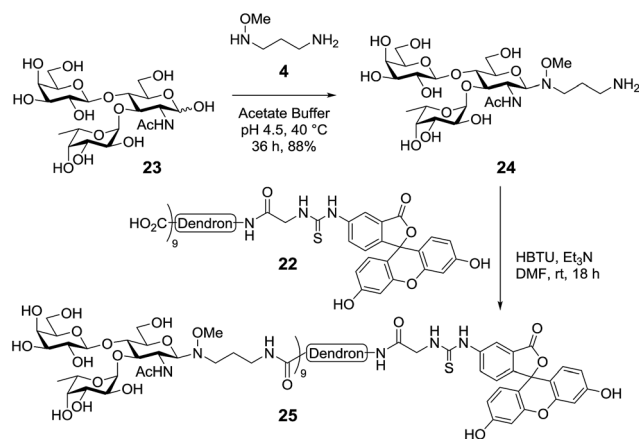
To synthesise the Lewis[X] glycodendron, a similar reaction route to that employed for the biotinylation of the dendrimer core was undertaken. Accordingly, the azide-group in the *tert*-butyl ester-functionalised dendron **5** was first reduced to the corresponding amine and then coupled to fluorescein isothiocyanate (FITC) to yield fluorescein derivative **18** in good yield (71%, 2 steps, Scheme 6). Treatment of **18** with TFA however, resulted in the Edman degradation[27] products **19** and **20**. To prevent this reaction, the core dendron **5** was first converted into the corresponding amine *via* Staudinger reduction and the *tert*-butyl esters were then removed *via* treatment with TFA to give the amine-functionalised carboxy dendron **21** in excellent yield (99%, 2 steps). Next, conjugation with FITC under basic conditions (NEt₃ in DMF) gave the target fluorescent dendron **22**, also in very good (74%) yield. The fluorescent dendron was initially obtained as the triethylamine salt after size exclusion chromatography, however, as this salt hampered ligation to the neoglycoconjugates, ion exchange (Dowex-H⁺) was performed to obtain the dendron as the nona-valent acid.

For the assembly of the target Lewis[X] glycodendron, Lewis[X] (**23**)[28] was condensed with amine-functionalised oxyamine linker **4**[10] (Scheme 7). When the reaction was performed at room temperature, only a small amount of glycoconjugate was observed, however, heating the reaction at 40 °C for 36 hours saw complete conversion to the desired oxyamine-linked *N*-glycan. Purification by size exclusion chromatography (BioGel P2) then allowed for the isolation of the target oxyamine-linked glycan as the ammonium formate salt. As the presence of this salt could lead to the formation of formamide by-products in the subsequent peptide coupling reaction, the oxyamine-linked glycan was treated with ion exchange resin (Dowex OH⁻) to give the free amine **24** in excellent yield (88%). Next, conjugation with the FITC-labelled second-generation dendron **22** was performed using an HBTU-mediated peptide coupling reaction. Here, the order of addition of reagents was found to be important with the fluorescent dendron **22** being dissolved in freshly distilled DMF and then added to the Lewis[X] glycan **23** followed by the addition of HBTU and Et₃N, as the glycan itself had poor solubility in DMF alone, though completely dissolved after the addition of HBTU

Scheme 5 Functionalisation of the second-generation dendron with biotin and GlcNAc.

Scheme 6 Synthesis of fluorescent dendron.

Scheme 7 Synthesis of the Lewis[X] functionalized glycodendron *via* conjugation of Lewis[X] to the fluorescein-derived second-generation dendron using the bi-functional oxyamine linker.

and Et$_3$N. The reaction mixture was then stirred at room temperature overnight, upon which analysis by HRMS indicated that the desired nona-valent glycodendron was formed (m/z for $[C_{285}H_{476}N_{36}O_{168}S]^{4+}$ calcd: 1781.7393, obsd: 1781.7380). Purification of the glycodendron using size exclusion chromatography (Sephadex CM C-25, 0.1 M aq. NH$_4$HCO$_2$) then allowed for the isolation of the fluorescent Lewis[X]-functionalised glycodendron **25** in 51% yield.

To demonstrate the potential of glycodendron **25** as a chemical tool, we then assessed whether the glycodendron

Fig. 1 The expression of DC-SIGN on THP-1 + PMA + IL-4 cells measured with (A) anti-DC-SIGN antibody and (B) unglycosylated dendron 21 (negative control) and the fluorescent Lewis[X] glycodendron 27. (C) The co-staining with anti-DC-SIGN antibody does not affect glycodendron binding to THP-1 + PMA + IL-4 cells, single staining with 100 μM glycodendron (green), co-staining of the anti-DC-SIGN antibody with 100 μM glycodendron (red); (D) the expression of DC-SIGN visualised with glycodendron and anti-DC-SIGN antibody on THP-1 + PMA + IL-4 cells, using single staining with the anti-DC-SIGN antibody (blue), co-staining of the antibody with 100 μM glycodendron (red) and unstained cells (purple).

could be used to detect DC-SIGN on the human macrophage cell line THP-1. To this end, human THP-1 cells were converted to the M2-like phenotype *via* treatment with phorbol-12-myristate-13-acetate (PMA) and interleukin (IL)-4,[29] and the presence of DC-SIGN on the cells confirmed through use of an anti-DC-SIGN antibody (Fig. 1A). Glycodendron **25** was then made up in a stock-solution of 0.66 mg mL^{-1} (0.1 μM) and assessed for its ability to detect DC-SIGN on the THP-1 + PMA + IL-4 cells, with the unglycosylated dendron **22** being used as a negative control (Fig. 1B). As illustrated, both the anti-DC-SIGN antibody and the glycodendron **25** bound DC-SIGN in a concentration dependent manner (Fig. 1A and B). The unglycosylated dendron **22** did not show binding to the macrophages at low μM concentrations, with only the highest concentration tested (100 μM) giving a slight increase in mean fluorescence due to non-specific binding. Next, co-staining of the glycodendron with the anti-DC-SIGN antibody and **25** was conducted (Fig. 1C and D). Here, it is important to note that the co-staining with the anti-body did not interfere with the glycan-mediated binding.
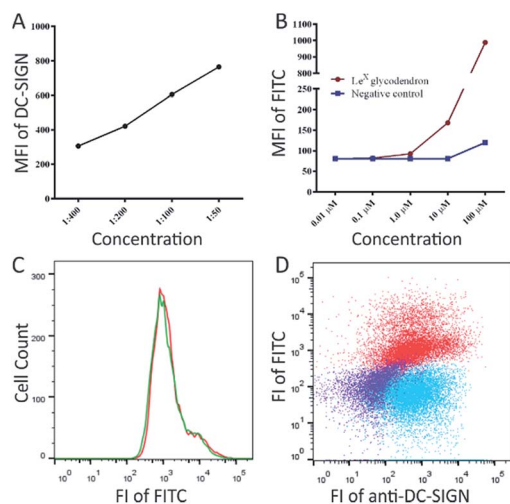
Conclusions

In conclusion, we have presented the design and synthesis of a dendron scaffold that can be readily functionalised at both its core and periphery. The synthesis of the second-generation dendron scaffold **5** was achieved in six linear steps and in an excellent overall yield of 30%. Moreover, the ease of functionalisation of the dendron core was demonstrated *via* its rapid and efficient conversion to both biotinylated and fluorescently labelled derivatives. The use of oxyamine ligation methodology to efficiently conjugate multiple glycans to a dendron scaffold was presented *via* the conjugation of GlcNAc to the biotinylated dendron scaffold and the conjugation of Lewis[X] to a fluorescently labelled dendron scaffold, whereby the fluorescently-labelled Lewis[X] glycodendron was used to detect DC-SIGN on macrophages. Given the efficiency and versatility of our procedures, we envision undertaking future carbohydrate–lectin binding studies employing glycodendron **25** and related glycodendrons in due course.

Experimental

General procedures

Prior to use, THF was distilled from sodium and benzophenone, CH$_2$Cl$_2$ was distilled from P$_2$O$_5$, DMF was distilled from BaO, Et$_3$N was distilled from KOH. All other reagents were used as received. 3-(Methoxyamino)propanyl-amine hydrochloride **4**,[5] oxyamine-linked GlcNAc **16**,[5] and Lewis[X] (**23**)[27] were synthesised as previously described. All solvents were removed by evaporation under reduced pressure. Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV$_{254}$) with detection by UV-absorption (short wave UV – 254 nm; long wave UV – 366 nm), by dipping in 10% H$_2$SO$_4$ in EtOH followed by charring at ~150 °C, by dipping in I$_2$ in silica, or by dipping into a solution of ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40-

63 micron). AccuBOND II ODS-C$_{18}$ (Agilent) was used for reverse phase chromatography. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) sampling accessory and are reported in wave numbers (cm^{-1}). Nuclear magnetic resonance spectra were recorded at 20 °C in D$_2$O, CD$_3$OD, CDCl$_3$, or pyridine-d$_5$ using either a Varian INOVA operating at 500 MHz or Varian VNMRS operating at 600 MHz. Chemical shifts are given in ppm (δ) relative to solvent residues. NMR peak assignments were made using COSY, HSQC and HMBC experiments.

N-Hydroxysuccinimidyl azidoacetate (10). To a solution of bromoacetic acid **8** (15.1 g, 0.11 mol) in water (30 mL), NaN$_3$ (30.0 g, 0.43 mmol) was added and the reaction mixture was stirred at r.t. for 3 h. The reaction mixture was diluted with 6 M aq. HCl (50 mL) and extracted with Et$_2$O (2 × 100 mL). The organic layers were dried with MgSO$_4$, filtered and concentrated *in vacuo*, to give azide **9** as a colourless oil (10.7 g, 0.11 mmol, 99%). *The crude reaction product was used in the esterification reaction without further purification.* To a solution of crude 2-azido acetic acid **9** (9.70 g, 96.0 mmol) in dry CH$_2$Cl$_2$ (100 mL), N-hydroxysuccinamide (13.2 g, 115 mmol) and N,N'-dicyclo-hexylcarbodiimide (17.8 mL, 115 mmol) were added at 0 °C and the reaction mixture was stirred at r.t. for 18 h. The reaction mixture was filtered and concentrated *in vacuo*. To the crude residue, EtOAc (150 mL) was added and the solids were removed by filtration and the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE/EtOAc, 75/25 → 25/75, v/v) and crystallised from CH$_2$Cl$_2$/PE to yield title compound **10** as a white solid (17.9 g, 90.2 mmol, 94%). R_f = 0.25 (PE/EtOAc, 1/1, v/v); mp 110–115 °C; IR (film) 2995, 2935, 2110, 1818, 1781, 1727, 1428, 1416, 1369, 1278, 1199, 1087 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.24 (s, 2H, CH$_2$–N$_3$), 2.88 (s, 4H, 2 × CH$_2$C=O); ^{13}C NMR (125 MHz, CDCl$_3$) δ 168.6 (2 × N–C=O), 164.3 (O–C=O), 48.1 (CH$_2$–N$_3$), 25.7 (2 × αCH$_2$).

N-Azidoacetyl-1,1,1-tris(hydroxymethyl)aminomethane (11). To a solution of succinimidyl ester **12** (8.83 g, 44.6 mmol) in freshly distilled DMF (100 mL), TrisRIS(hydroxymethyl)amino-methane **7** (26.9 g, 222 mmol) and Et$_3$N (0.6 mL, 4.5 mmol) were added and the reaction mixture was stirred at r.t. for 18 h. The reaction mixture was concentrated *in vacuo*, then co-evaporated with H$_2$O to remove traces of DMF. The residue was purified by silica gel column chromatography (dry-loading, PE : EtOAc, 50/50 → 0/100, v/v) yielded the title compound **11** as a white foam (7.84 g, 38.4 mmol, 86%). R_f = 0.50 (CH$_2$Cl$_2$/EtOH/MeOH/NH$_3$ (aq. 33%), 5/2/2/1, v/v/v/v); IR (film) 3359, 2979, 2946, 2890, 2113, 1739, 1650, 1540, 1454, 1367, 1282, 1229, 1217, 1053 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 4.00 (s, 2H, CH$_2$–N$_3$), 3.76 (s, 6H, 3 × CH$_2$–O); ^{13}C NMR (125 MHz, D$_2$O) δ 170.4 (C=O amide), 62.1 (C$_q$ tris) 60.1 (3 × CH$_2$–O), 51.9 (CH$_2$–N$_3$); HRMS(ESI) m/z calcd for [C$_6$H$_{13}$N$_4$O$_4$]$^+$: 205.0931, obsd: 205.0930.

N-Azidoacetyl-1,1,1-tris(tert-butyloxycarbonylmethyloxymethyl)aminomethane (6). To a solution of triol **11** (366 mg, 1.79 mmol) in DMF (9 mL) and toluene (9 mL), tert-butyl bromoacetate (1.06 mL, 7.16 mmol) and NaH (286 mg, 7.16 mmol) were added and

the reaction mixture was stirred at r.t. for 4 h. The reaction mixture was quenched by the addition of ice water (20 mL) and was then extracted with Et$_2$O (2 × 100 mL). The organic layers were dried with MgSO$_4$, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (PE : EtOAc, 85/15 → 75/25, v/v) and reverse phase column chromatography (C$_{18}$, H$_2$O/MeOH, 50/50 → 20/80, v/v) yielded tri-alkylated **6** as a white foam (464 mg, 0.85 mmol, 47%). R_f = 0.44 (PE/EtOAc, 70/30, v/v); IR (film) 3316, 2979, 2935, 2107, 1745, 1684, 1536, 1473, 1428, 1393, 1368, 1229, 1158, 1119, 1037, 971, 915, 845, 731, 647 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.86 (s, 1H, NH), 3.95 (s, 6H, 3 × CH$_2$C=O), 3.92 (s, 6H, 3 × CH$_2$–O), 3.85 (s, 2H, CH$_2$–N$_3$), 1.45 (s, 27H, CH$_3$ tBu); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.2 (3 × C=O ester), 167.5 (1 × C=O amide), 82.0 (3 × C$_q$ tBu), 70.1 (3 × CH$_2$–O), 68.9 (3 × CH$_2$C=O), 60.1 (C$_q$ tris), 52.9 (CH$_2$–N$_3$), 28.2 (9 × CH$_3$ tBu); HRMS(ESI) m/z calcd for [C$_{24}$H$_{43}$N$_4$O$_{10}$]$^+$: 547.2974, obsd: 547.2981.

N-Azidoacetyl-1,1,1-tris(carboxymethyloxymethyl)amino-methane (12). To a solution of dendron **6** (508 mg, 0.93 mmol) in CH$_2$Cl$_2$ (5 mL), freshly distilled trifluoroacetic acid (5 mL) was added and the reaction mixture was stirred at r.t. for 2 h. The reaction mixture was then concentrated *in vacuo* and coevaporated with CH$_2$Cl$_2$ (10 mL). The residue was purified by reverse phase column chromatography (C$_{18}$, H$_2$O/MeOH, 100/0 → 70/30, v/v) yielded acid **12** as a colourless oil. R_f = 0.20 (CH$_2$Cl$_2$/EtOH/MeOH/NH$_3$ (aq. 33%), 5/2/2/1, v/v/v/v); IR (film) 3418, 2930, 2114, 1727, 1661, 1547, 1427, 1249, 1121 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 4.20 (s, 6H, 3 × CH$_2$C=O), 3.98 (s, 6H, 3 × CH$_2$–O), 3.90 (s, 2H, CH$_2$–N$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 174.3 (3 × C=O), 170.2 (1 × C=O amide), 69.6 (3 × CH$_2$–O), 68.1 (3 × CH$_2$C=O), 59.9 (C$_q$ tris), 52.0 (CH$_2$–N$_3$); HRMS(ESI) m/z calcd for [C$_{12}$H$_{19}$N$_4$O$_{10}$Na]$^+$: 401.0915, obsd: 401.0920.

N-Glycyl-1,1,1-tris(tert-butyloxycarbonylmethyloxymethyl)-aminomethane (13). To a solution of dendron **6** (220 mg, 0.40 mmol) in ethanol (1 mL) and THF (1 mL), activated RANEY® nickel (120 mg) was added and hydrogen gas was then bubbled through the reaction mixture at r.t. for 18 h. The reaction mixture was filtered, washed with sat. NaCl (aq.) (50 mL) containing 1 M NaOH (1 mL), extracted with CH$_2$Cl$_2$ (2 × 50 mL) and the combined organic layers were dried, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/MeOH, 100/0 → 85/15, v/v) yielded amine **6** as a colourless oil (207 mg, 0.40 mmol, 99%). R_f = 0.09 (EtOAc/MeOH, 90/10, v/v); IR (film) 3336, 2978, 3933, 1745, 1674, 1520, 1368, 1300, 1229, 1159, 1121, 1044, 847 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.82 (s, 1H, NH), 3.92 (s, 6H, 3 × CH$_2$C=O), 3.88 (s, 6H, 3 × CH$_2$–O), 3.30 (s, 2H, CH$_2$–NH$_2$), 2.07 (bs, 2H, NH$_2$) 1.42 (s, 27H, 9 × CH$_3$ tBu); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.2 (1 × C=O amide), 170.3 (3 × C=O ester), 81.8 (3 × C$_q$ tBu), 70.2 (3 × CH$_2$–O), 69.0 (3 × CH$_2$C=O), 59.5 (C$_q$ tris), 45.6 (CH$_2$–NH$_2$), 28.2 (9 × CH$_3$ tBu); HRMS(ESI) m/z calcd for [C$_{24}$H$_{45}$N$_2$O$_{10}$Na]$^+$: 543.2888, obsd: 543.2891.

N-Azidoacetyl-1,1,1-tris(1,1,1-tris[tert-butyloxycarbonylmethyl-oxymethyl]methylamidocarbonylmethylamidocarbonylmethyloxymethyl)aminomethane (5). First generation tri-acid **12** (118 mg, 0.31 mmol) and primary amine **13** (728 mg, 1.40 mmol) were co-

evaporated with DMF (2 × 5 mL). To the mixture DMF (3.1 mL), HBTU (590 mg, 1.56 mmol) and NEt$_3$ (0.65 mL, 4.67 mmol) were added and the reaction mixture was stirred at r.t. for 14 h. The reaction mixture was diluted with Et$_2$O (50 mL) and washed with 0.1 M HCl (aq.) (2 × 50 mL). The combined water layers were extracted with Et$_2$O (2 × 50 mL) and the combined organic layers were dried with MgSO$_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (PE/ EtOAc, 75/25 → 0/100, v/v; followed by EtOAc/MeOH 100/0 → 95/5, v/v) and reverse phase column chromatography (C$_{18}$, H$_2$O/ MeOH, 50/50 → 0/100, v/v) yielded second generation dendron **5** as a colourless oil (475 mg, 0.25 mmol, 81%). R_f = 0.48 (EtOAc/ MeOH, 95/5, v/v); IR (film) 3316, 3970, 2934, 2107, 1745, 1670, 1526, 1368, 1231, 1159, 1120, 917, 846, 731 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 7.62 (s, 3H, 3 × NH-TRIS), 7.44 (s, 1H, 1 × NH-TRIS), 7.24 (t, 3H, J_{NH,CH_2} = 5.0 Hz, 3 × NH gly), 4.06 (s, 6H, 3 × CH$_2$C=O), 4.01 (d, 6H, $J_{CH_2,NH}$ = 4.5 Hz, 3 × CH$_2$–N), 3.98 (s, 2H, CH$_2$–N$_3$), 3.95 (s, 18H, 9 × CH$_2$C=O), 3.93 (s, 6H, 3 × CH$_2$–O), 3.89 (s, 18H, 9 × CH$_2$–O), 1.45 (s, 54H, 18 × CH$_3$ t-Bu); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.3 (9 × C=O ester), 169.6 (3 × C=O amide), 168.5 (3 × C=O amide), 168.2 (1 × C=O amide), 82.0 (C$_q$ tBu), 70.7 (3 × CH$_2$–O), 70.6 (3 × CH$_2$C=O), 70.2 (9 × CH$_2$–O), 68.9 (9 × CH$_2$C=O), 60.0 (3 × C$_q$ tris), 59.8 (1 × C$_q$ tris) 52.5 (CH$_2$–N$_3$), 42.2 (3 × CH$_2$–NH), 28.3 (27 × CH$_3$ tBu); HRMS(ESI) m/z calcd for [C$_{84}$H$_{146}$N$_{10}$O$_{37}$]$^{2+}$: 943.4920, obsd: 943.4929.

N-(2-ᴅ-Biotinylamido-acetyl)-1,1,1-tris(1,1,1-tris[*tert*-butyl-oxy-carbonylmethyloxymethyl]methylamidocarbonyl-methylamido-carbonylmethyloxymethyl)aminomethane (14). To a solution of second generation dendron **5** (139 mg, 73.7 μmol) in ethanol (1 mL) and THF (1 mL), activated RANEY® nickel (100 mg) was added and hydrogen gas was bubbled through the reaction mixture at r.t. for 20 h. The reaction mixture was filtered, washed with sat. aq. NaCl (50 mL) containing 1 M NaOH (1 mL), extracted with CH$_2$Cl$_2$ (2 × 50 mL) and concentrated in vacuo. The resulting oil was then passed through a silica gel plug (EtOAc/MeOH, 100/0 → 85/15, v/v) to yield crude amine as a colourless oil, which was then used without further purification. HRMS(ESI) m/z calcd for [C$_{84}$H$_{148}$N$_8$O$_{37}$]$^{2+}$: 930.4967, obsd: 930.4960. To the crude second generation dendron amine in DMF (0.73 mL), ᴅ-biotin (27.0 mg, 110 μmol), HBTU (55.9 mg, 147 μmol) and NEt$_3$ (25 μL) were added and the reaction mixture was stirred at r.t. for 18 h. The reaction was diluted with Et$_2$O (50 mL) and washed with sat. aq. NaCl (2 × 50 mL). The combined water layers were extracted with Et$_2$O (2 × 50 mL) and the combined organic layers were dried with MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography (CH$_2$Cl$_2$/MeOH, 100/0 → 90/10, v/v) and reverse phase column chromatography (C$_{18}$, H$_2$O/MeOH, 50/50 → 0/100, v/v) yielded second-generation dendron **14** as a colourless oil (146 mg, 70.1 μmol, 95%). R_f = 0.50 (CH$_2$Cl$_2$/MeOH, 90/10, v/v); IR (film) 3309, 3064, 2979, 2933, 1744, 1668, 1527, 1368, 1230, 1158, 1118, 1035, 846, 733 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.66 (s, 3H, 3 × NH-TRIS), 7.64 (t, 1H, $J_{CH_2,NH}$ = 5.7 Hz, NH-gly), 7.57 (s, 1H, NH-TRIS), 7.54 (t, 3H, $J_{CH_2,NH}$ = 5.1 Hz, 3 × NH-gly), 6.30 (s, 1H, NH biotin), 5.65 (s, 1H, NH biotin), 4.45 (dd, 1H, $J_{7,8a}$ = 5.3 Hz, $J_{6,7}$ = 7.0 Hz, H7-biotin), 4.27 (dd, 1H, $J_{5,6}$ = 5.1 Hz, $J_{6,7}$ = 7.0 Hz, H6-biotin), 4.00 (s, 6H, 3 × CH$_2$O), 3.97

(t, 6H, $J_{CH_2,NH}$ = 4.3 Hz, 3 × CH$_2$N), 3.92 (s, 18H, 9 × CH$_2$O), 3.88–3.80 (m, 26H, 9 × CH$_2$C=O, 3 × CH$_2$C=O, CH$_2$N), 3.08 (dt, 1H, $J_{5,6}$ = 4.8 Hz, $J_{4,5}$ = 7.0 Hz, H5-biotin), 2.85 (dd, 1H, $J_{7,8a}$ = 5.3 Hz, $J_{8a,8b}$ = 13.0 Hz, H8a-biotin), 2.69 (d, 1H, $J_{8a,8b}$ = 12.8 Hz, H8b-biotin), 2.28–2.16 (m, 2H, CH$_2$-1-biotin), 1.71–1.50 (m, 6H, CH$_2$-2, CH$_2$-3, CH$_2$-4-biotin), 1.41 (s, 81H, 27 × CH$_3$ tBu); ^{13}C NMR (125 MHz, CDCl$_3$) δ 174.4 (C=O biotin), 170.6 (C=O dendron), 170.1 (9 × C=O), 170.0 (3 × C=O), 169.0 (3 × C=O), 163.9 (C=O urea biotin), 81.9 (9 × C$_q$ tBu), 70.6 (3 × CH$_2$C=O), 70.4 (3 × CH$_2$–O), 70.0 (9 × CH$_2$–O), 68.8 (9 × CH$_2$C=O), 61.8 (C6-biotin), 60.1 (C7-biotin), 59.9 (3 × C$_q$ tris), 59.5 (1 × C$_q$ tris), 55.5 (C5-biotin), 43.7 (1 × CH$_2$–N), 42.2 (3 × CH$_2$–N), 40.3 (C8-biotin), 35.2 (C1-biotin), 28.2 (C2-biotin), 28.0 (27 × CH$_3$), 27.9 (C4-biotin), 25.3 (C3-biotin); HRMS(ESI) m/z calcd for [C$_{94}$H$_{162}$N$_{10}$O$_{39}$S]$^{2+}$: 1043.5355, obsd: 1043.5351.

N-(2-ᴅ-Biotinylamidoacetyl)-1,1,1-tris(1,1,1-tris[carboxymethyl-oxymethyl]methylamidocarbonylmethylamidocarbonyl-methyloxy-methyl)aminomethane (15). To a solution of second-generation dendron **14** (12.0 mg, 5.8 μmol) in CH$_2$Cl$_2$ (1 mL), freshly distilled trifluoroacetic acid (1 mL) was added and the reaction mixture was stirred at r.t. for 3 h. The crude reaction mixture was concentrated in vacuo and coevaporated with water (10 × 1 mL). Purification by reverse phase column chromatography (C$_{18}$, H$_2$O/ MeOH, 100/0 → 70/30, v/v) yielded nona-acid **15** (8.8 mg, 5.6 mmol, 97%). R_f = 0.1 (EtOAc/MeOH, 80/20, v/v); IR (film) 3360, 3005, 2990, 2978, 2948, 1721, 1656, 1552, 1472, 1431, 1343, 1245, 1200, 1123, 1032, 1018, 979 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 4.57 (dd, 1H, $J_{7,8a}$ = 5.1 Hz, $J_{6,7}$ = 7.5 Hz, H7-biotin), 4.39 (dd, 1H, $J_{5,6}$ = 4.6, $J_{6,7}$ = 7.5 Hz, H6-biotin), 4.18 (s, 18H, 9 × CH$_2$CO), 4.12 (s, 6H, 3 × CH$_2$CO), 3.95 (s, 6H, 3 × CH$_2$NH), 3.88 (s, 18H, 9 × CH$_2$O), 3.86 (s, 6H, 3 × CH$_2$O), 3.28 (dt, 1H, $J_{5,6}$ = 5.3 Hz, $J_{4,5}$ = 8.0 Hz, H5-biotin), 2.96 (dd, 1H, $J_{7,8a}$ = 5.0 Hz, $J_{8a,8b}$ = 13.0 Hz, H8a-biotin), 2.74 (d, 1H, $J_{8a,8b}$ = 13.0 Hz, H8b-biotin), 2.30 (t, 2H, $J_{1,2}$ = 7.2 Hz, CH$_2$-1-biotin), 1.74–1.50 (m, 4H, CH$_2$-4-biotin, CH$_2$-2-biotin), 1.39 (p, 2H, $J_{2,3}$ = $J_{3,4}$ = 7.4 Hz, CH$_2$-3-biotin); ^{13}C NMR (125 MHz, CDCl$_3$) δ 77.1 (C=O biotin), 174.2 (9 × C=O), 172.8 (3 × C=O), 171.3 (1 × C=O), 170.7 (3 × C=O), 165.2 (C=O urea biotin), 70.0 (3 × CH$_2$C=O), 69.8 (3 × CH$_2$–O), 69.7 (9 × CH$_2$–O), 68.0 (9 × CH$_2$C=O), 62.0 (C6-biotin), 60.2 (C7-biotin), 59.8 (3 × C$_q$ tris), 59.6 (1 × C$_q$ tris), 55.2 (C5-biotin), 43.0 (1 × CH$_2$–N), 42.4 (3 × CH$_2$–N), 39.6 (C8-biotin), 35.1 (C1-biotin), 27.9 (C2-biotin), 27.6 (C4-biotin), 25.0 (C3-biotin); HRMS(ESI) m/z calcd for [C$_{58}$H$_{90}$N$_{10}$O$_{39}$S]$^{2+}$: 791.2538, obsd: 791.2536.

N-(2-ᴅ-Biotinylamido-acetyl)-1,1,1-tris(1,1,1-tris[3-(N-(2-acet-amido-2-deoxy-β-ᴅ-glucopyranosyl)-O-methyl-hydroxylamine) propylamidocarbonylmethyloxymethyl]methyl-amidocarbonyl-methylamidocarbonylmethyloxy-methyl)aminomethane (17). Second-generation dendron nona-acid **15** (1.5 mg, 0.95 μmol) and GlcNAc-amine **16** (5.3 mg, 17.1 μmol) were co-evaporated with freshly distilled DMF (2 × 2 mL). To the mixture DMF (0.2 mL) was added and the reaction mixture was half concentrated under reduced pressure to remove traces of Et$_2$NH. To the reaction mixture, HBTU (13.7 mg, 36 μmol) and distilled NEt$_3$ (25 μL) were added and the reaction mixture was stirred at r.t. for 14 h. The reaction mixture was diluted with water (2 mL) and purified using a dialysis in a Na$_2$HPO$_4$ solution (1 g L^{-1}), to remove the conjugation byproducts. The water

was replaced twice a day for 4 days. The dendron was then purified using reverse phase column chromatography (C$_8$, H$_2$O/MeOH, 100/0 → 70/30, v/v) to give glycodendron **17** as a colourless oil (2.8 mg, 0.68 μmol, 72%). ^{1}H NMR (600 MHz, D$_2$O) δ 4.58 (dd, 1H, $J_{7,8a}$ = 5.1 Hz, $J_{6,7}$ = 7.7 Hz, H7-biotin), 4.40 (dd, 1H, $J_{5,6}$ = 4.7 Hz, $J_{6,7}$ = 7.7 Hz, H6-biotin), 4.32 (d, 9H, $J_{1,2}$ = 9.7 Hz, 9 × H-1 GlcNAc), 4.12 (s, 6H, 3 × CH$_2$C=O dendron), 4.07 (s, 18H, 9 × CH$_2$C=O dendron), 3.96 (s, 6H, 3 × CH$_2$–N dendron), 3.92–3.83 (m, 37H, 1 × CH$_2$N dendron, 9 × H-2 GlcNAc, 9 × H-6a GlcNAc, 9 × CH$_2$-dendron), 3.72 (dd, 9H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 12.4 Hz, 9 × H-6b GlcNAc), 3.51 (dd, 9H, $J_{2,3}$ = 8.8 Hz, $J_{3,4}$ = 9.8 Hz, 9 × H-3 GlcNAc), 3.48 (s, 27H, 9 × NOCH$_3$), 3.43–3.28 (m, 28H, 9 × CH$_2$a-3 linker, 9 × H-4 GlcNAc, 9 × H-5 GlcNAc, H5-biotin), 3.24 (dt, 9H, $J_{2,3}$ = 7.2 Hz, $J_{3a,3b}$ = 13.4 Hz, 9 × CH$_2$b-3 linker), 3.01–2.87 (m, 19H, 9 × CH$_2$-1 linker, H8a-biotin), 2.75 (d, 1H, $J_{8a,8b}$ = 12.9 Hz, H8b-biotin), 2.30 (t, 2H, $J_{1,2}$ = 7.3 Hz, CH$_2$-1-biotin), 2.03 (s, 27H, 9 × N–Ac), 1.88–1.78 (m, 1H, H4a-biotin), 1.77–1.69 (m, 18H, 9 × CH$_2$-2 linker), 1.69–1.58 (m, 1H, H4a-biotin) 1.58–1.50 (m, 2H, CH$_2$-2-biotin), 1.44–1.36 (m, 2H, CH$_2$-3-biotin); HRMS(ESI) m/z calcd for [C$_{166}$H$_{298}$N$_{37}$O$_{84}$S]$^{3+}$: 1395.6641, obsd: 1395.6648.

N-Glycyl-1,1,1-tris(1,1,1-tris[carboxy-methyloxymethyl]methyl-amidocarbonylmethylamidocarbonylmethyloxymethyl)amino-methane trifluoroacetic acid (21). To a solution dendron **5** (60.5 mg, 31.8 μmol) in MeOH/H$_2$O/CH$_2$Cl$_2$ (3 mL, 3/1/1, v/v/v), triphenylphosphine (25.0 mg, 95.4 μmol) was added and the reaction mixture was stirred at r.t. for 18 h. The crude reaction mixture was concentrated in vacuo and co-evaporated with CH$_2$Cl$_2$ (2 × 10 mL). The residue was dissolved in CH$_2$Cl$_2$ (3 mL), TFA (3 mL) was added and the reaction mixture was stirred at r.t. for 3 h. The solvent was evaporated by an argon stream, H$_2$O (10 mL) was added, and the phosphine salts were removed via filtration, and the resulting residue was concentrated in vacuo to give crude amino acid **21**. (45.9 mg, 31.6 μmol, 99%). R_f = 0.05 (tBuOH/AcOH/H$_2$O, 4/1/1, v/v/v); IR (film) 3344, 3003, 2981, 2950, 2937, 2930, 1718, 1651, 1543, 1473, 1458, 1434, 1241, 1199, 1120, 1032, 952 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 4.06 (s, 18H, 9 × CH$_2$C=O), 4.00 (s, 6H, 3 × CH$_2$C=O), 3.83 (s, 6H, 3 × CH$_2$N), 3.77 (s, 6H, 3 × CH$_2$O), 3.75 (s, 18H, 9 × CH$_2$O), 3.71 (s, 2H, 1 × CH$_2$NH$_2$); ^{13}C NMR (125 MHz, D$_2$O) δ 174.1 (9 × C=O CO$_2$H), 172.6 (3 × C=ONH), 170.6 (3 × C=O), 166.7 (1 × C=O), 162.8, 162.5, 162.2, 161.9 (C=O TFA), 119.4, 117.1, 114.8, 112.5 (CF$_3$ TFA), 69.83, 69.77 (3 × CH$_2$O, 3 × CH$_2$C=O), 69.6 (9 × CH$_2$O), 67.9 (9 × CH$_2$C=O), 59.9 (1 × C$_q$ tris), 59.7 (3 × C$_q$ tris), 42.3 (3 × CH$_2$NH), 40.6 (1 × CH$_2$NH$_2$); HRMS(ESI) m/z calcd for [C$_{48}$H$_{76}$N$_8$O$_{37}$]$^{2+}$: 678.2150, obsd: 678.2136.

N-[N-([(3′,6′-Dihydroxy-3-oxospiro[isobenzofuran-1(3H),9′-(9H)xanthen]-5-yl]amino-thioxomethyl)glycyl]-1,1,1-tris(1,1,1-tris[carboxymethyloxymethyl]methylamido-carbonylmethylamido-carbonylmethyloxymethyl)aminomethane (22). To a solution of dendron **21** (25.1 mg, 17.3 μmol) in DMF (3 mL), fluorescein isothiocyanate isomer I (7.4 mg, 19.0 μmol) and Et$_3$N (1 mL) were added and the reaction mixture was stirred at r.t. for 18 h. The crude reaction mixture was concentrated in vacuo. The residue was purified with size exclusion chromatography (LH-

20, CH$_2$Cl$_2$/MeOH, 50/50, v/v) and concentrated in vacuo to give the trimethylamine salt of dendron **22**. The dendron was co-evaporated with H$_2$O and purified using ion exchange chromatography (Dowex H$^+$, elute with H$_2$O) to give dendron **22** (22.3 mg, 12.8 mmol, 74%) as a yellow oil. R_f = 0.05 (tBuOH/AcOH/H$_2$O, 4/1/1, v/v/v); IR (film) 3463, 3366, 3074, 3015, 2970, 2946, 1738, 1656, 1548, 1436, 1366, 1229, 1216, 1206, 1119, 1051, 1032, 897 cm^{-1}; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.22 (s, 1H, H-3), 8.13–7.98 (m, NH), 7.94 (s, 3 × NH), 7.88 (s, 1 × NH), 7.84 (d, 1H, $J_{5,6}$ = 8.8 Hz, H-5), 7.17 (d, 1H, $J_{5,6}$ = 8.8 Hz, H-6), 6.78–6.63 (m, 4H, 2 × H-13, 2 × H-10), 6.58 (d, 2H, $J_{9,10}$ = 8.2 Hz, 2 × H-11), 4.35 (s, 2H, 1 × CH$_2$NH), 4.20–4.02 (bs, 24H, 3 × CH$_2$C=O, 9 × CH$_2$C=O), 4.02–3.92 (m, 12H, 3 × CH$_2$O, 3 × CH$_2$NH), 3.92–3.86 (m, 18H, 9 × CH$_2$O); ^{13}C NMR (125 MHz, CD$_3$OD) δ 183.5 (C=S), 174.5 (9 × C=O), 173.0 (3 × C=O), 172.0 (1 × C=O glyc), 171.4 (3 × C=O glyc), 171.2 (C=O C-1), 161.5, 154.2 (C-12, C14), 149.7 (C-7), 142.2 (C-2/4/8), 132.1 (C-5), 130.4 (C-13), 130.3 (C-2/4/8), 128.9 (C-2/4/8), 125.8 (C-6), 120.3 (C-3), 113.8 (C-11), 111.5 (C-9), 103.6 (C-10), 71.4 (3 × CH$_2$–O), 71.0 (9 × CH$_2$O), 69.3 (3 × CH$_2$C=O), 69.2 (9 × CH$_2$C=O), 61.3 (3 × C$_q$ tris), 61.2 (1 × C$_q$ tris), 48.9 (1 × CH$_2$NH), 43.5 (3 × CH$_2$NH); HRMS(ESI) m/z calcd for [C$_{69}$H$_{87}$N$_9$O$_{42}$S]$^{2+}$: 872.7329, obsd: 872.7335.

N-(2-Acetamido-2-deoxy-3-O-(α-L-fuco-pyranosyl)-4-O-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-N-(3-aminopropyl)-O-methylhydroxylamine (24). To a solution of Lewis^X **23** (5.6 mg, 10.6 μmol), in a AcOH/NH$_4$OAc buffer (0.5 mL, 2 M, freshly prepared, pH 4.5), 3-(methoxyamino)propanyl-amine hydrochloride **4** (15.9 mg, 113.2 μmol) was added and the reaction mixture was stirred at 40 °C for 35 h. The crude mixture was directly loaded onto a size exclusion column (Bio-Gel P-2, 1200 × 18 mm) and eluted with 0.1 M aq. NH$_4$HCO$_2$. Lyophilisation of the product fractions afforded neoglycoside **24** (5.7 mg, 88%). R_f = 0.10 (CH$_2$Cl$_2$/EtOH/MeOH/NH$_3$ (aq. 35%), 5/2/2/1, v/v/v/v); $\alpha_D^{19.5}$ = −9.3 (c = 0.2, MeOH); IR (film) 3341, 2925, 2852, 17 117, 1647, 1586, 1466, 1451, 1415, 1380, 1350, 1302, 1233, 1193, 1085, 1026, 968, 917 cm^{-1}; ^{1}H NMR (500 MHz, D$_2$O) δ 5.12 (d, 1H, $J_{1''',2'''}$ = 3.9 Hz, H-1′′′), 4.84 (q, 1H, $J_{5''',6'''}$ = 6.7 Hz, H-5′′′), 4.49–4.42 (m, 1H, H-1′, H-1″), 4.05 (m, 2H, H-2′, H-6a′), 3.93–3.81 (m, 5H, H-3′′′, H-4′′′, H-4′, H-3′, H-6b′), 3.80 (d, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 2.9 Hz, H-4′′′), 3.76 (m, 3H, H-6a″, H-6b″, H-2″), 3.64 (dd, 1H, $J_{3'',4''}$ = 3.2 Hz, $J_{2'',3''}$ = 9.9 Hz, H-3″), 3.60–3.55 (m, 1H, H-5″), 3.54–3.46 (m, 1H, H-5″), 3.54–3.46 (m, 5H, OCH$_3$, H-5′, H-2″), 3.11–2.92 (m, 4H, CH$_2$-1, CH$_2$-3), 2.02 (s, 3H, CH$_3$ Ac), 1.95 (m, 2H, CH$_2$-2), 1.17 (d, 3H, $J_{5''',6'''}$ = 6.7 Hz, H-6′′′); ^{13}C NMR (125 MHz, D$_2$O) 170.5 (C=O), 101.8 (C-1″), 98.7 (C-1′′′), 90.5 (C-1′), 76.9 (C-5′), 76.1 (C-3′), 74.8 (C-5″), 73.2 (C-4′), 72.4 (C-3′′′), 71.8 (C-4″), 70.9 (C-2″), 69.1 (C-3′′′), 68.3 (C-4″), 67.6 (C-2′′′), 66.7 (C-5′′′), 61.4 (C-6″), 60.9 (OCH$_3$), 59.7 (C-6′), 52.5 (C-2′), 47.2 (C-1), 37.5 (C-3), 24.5 (C-2), 22.1 (CH$_3$ Ac), 15.2 (C-6′′′); HRMS(ESI) m/z calcd for [C$_{24}$H$_{46}$N$_3$O$_{15}$]$^+$: 616.2923, obsd: 616.2938.

N-[N-([(3′,6′-Dihydroxy-3-oxospiro[isobenzofuran-1(3H),9′-(9H)xanthen]-5-yl]amino-thioxomethyl)glycyl]-1,1,1-tris(1,1,1-tris[3-(N-(2-acetamido-2-deoxy-3-O-(α-L-fuco-pyranosyl)-4-O-(β-D-galactopyranosyl)-β-D-gluco-pyranosyl)-N-(3-aminopropyl)-O-)-O-

methyl-hydroxylamine)propylamidocarbonylmethyloxymethyl] methylamidocarbonyl-methylamidocarbonylmethyloxy-methyl) aminomethane (25). To the oxyamine-functionalised Lewis^X trisaccharide 24 (6 mg, 9.8 μmol), a solution of FITC labelled second generation dendron 22 (0.94 mg, 0.54 μmol) in freshly distilled and half-concentrated DMF (250 μL) was added, followed by the addition of HBTU (5.6 mg, 15 μmol) and Et$_3$N (10 μL, 72 μmol). The reaction mixture was stirred at r.t. for 18 h. The crude mixture was then diluted with H$_2$O (1 mL) and purified by size exclusion chromatography (Sephadex CM C-25, 0.1 M aq. NH$_4$HCO$_2$). Lyophilisation of the product afforded fluorescent glycodendron 25 (1.9 mg, 0.28 μmol, 51%) as a yellow foam. UV-VIS: (H$_2$O), $\lambda_{Abs,max}$ = 495 nm; ^{1}H NMR (600 MHz, D$_2$O) δ 8.05 (bs, 1H), 7.88–7.85 (m, 1H), 7.73–7.70 (m, 1H), 7.66–7.64 (m, 1H), 7.38–7.22 (m, 4H), 6.85–6.58 (m, 5H), 5.12 (d, 9H, J = 3.9 Hz), 4.84 (q, 9H, J = 6.7 Hz), 4.49–4.42 (m, 18H), 4.41–4.35 (m, 18H), 4.20–3.55 (m, 171H), 3.54–3.46 (m, 45H), 3.40–3.16 (m, 27H), 3.03–2.88 (m, 18H), 2.08–1.99 (m, 27H), 1.82–1.65 (m, 18H), 1.17 (d, 27H); HRMS(ESI) m/z calcd for [C$_{285}$H$_{476}$N$_{36}$O$_{168}$S]$^{4+}$: 1781.7393, obsd: 1781.7380.

THP-1 derived macrophage differentiation

The THP-1 acute monocytic lymphoma cell line was cultured in RPMI 1640 medium supplemented with 10% Fetal Calf Serum (FCS), 1% Glutamax and 1% Penstrep, and seeded 2.5 × 10^5 cells per mL. Differentiation of the THP-1 cells was induced via the treatment with PMA (50 ng mL^{-1}, 48 h.) followed by addition of IL-4 (20 ng mL^{-1}, 24 h) to obtian 'M2-like' Mφs according to the procedures of Puig-Kröger et al.[29] For subsequent analysis, the differentiated cells were detached from the tissue culture plates by incubating the cells in PBS on ice.

Flow cytometry

The macrophages were stained by incubating for 1 hour at 4 °C with either FITC-labelled glycodendron 25, negative control (non-glycosylated dendron 22) or anti-DC-SIGN antibody, washed twice with PBS, and DC-SIGN expression measured by flow cytometry. The fluorescent Lewis^X glycodendron and the negative control were used in various concentrations (10^{-1}, 10^1, 10^2, 10^3, 10^4 and 10^5 nM, diluted in 10% FCS), where the human anti-DC-SIGN antibody (phycoerythrin-labelled) positive control was diluted by a factor 2 (1/50 → 1/1600, v/v).

Conflicts of interest

There are no conflicts to declare.

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