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Synthesis of cross-reactive carbohydrate determinants fragments as tools for in vitro allergy diagnosis

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ABSTRACT

Four biotinylated tri and tetrasaccharide fragments of plant and invertebrate *N*-glycans were synthesized using methyl *tert*-butyl phenyl (MBP) thioglycosides donors in order to evaluate their involvement in cross-allergies as cross-reactive carbohydrate determinants (CCDs). Various levels of reactivity to anti-bee and anti-HRP antibodies and with sera from allergic patients were observed when the conjugates were coated on streptavidin microplates. The results showed the potential utility of these xylosylated and fucosylated oligosaccharide fragments in determining CCD antibody epitopes.

1. Introduction

Health problems due to extreme allergic responses are a serious and growing health issue in the society at large. The increased prevalence of this type of disease and the aggravation of the cross-allergy phenomenon (allergy to apparently unrelated allergens) require the identification of the allergen structures involved. Cross-linking of cell-bound IgE on mast cells or basophils by allergenic glycoproteins causes the release of histamine and other mediators, which results in allergic symptoms. Allergen epitopes are not only peptides, but also can be carbohydrate moieties, called carbohydrate cross-reactive determinants (CCDs), which have been found in different allergens from plants (pollens, food, latex) or animals (insect venoms, seafood). *N*-Glycans containing α 1,3 fucose and β 1,2 xylose branches, specifically found in *N*-glycans from plants and invertebrate animals are most frequently recognised as CCD epitopes. This high degree of cross-reactivity has been explained by the conserved structure of *N*-glycans in plants and invertebrates, sharing several sequences that are not found in mammalian *N*-glycans (Fig. 1).

As it has been reported that both α 1,3 fucose and β 1,2 xylose moieties were involved in IgE binding,[1] the synthesis of xylosylated and fucosylated *N*-glycan fragments were required in order to study the respective involvement of these two features. The fragments described therein were prepared in a biotinylated form, as previously done with *Candida albicans* cell wall oligomannosides.[2] The aim of this work is to determine the minimal epitopes of these CCDs, and thus to prepare standardised tools for diagnosis and follow-up of patients' treatment. Carbohydrate epitopes are usually of limited size (a tri- or tetrasaccharide) and thus we decided to focus on tri- and tetrasaccharidic fragments containing either xylose or fucose (Fig. 2). Compound **1** corresponds to a xylosylated fragment of both M0X and M0XF^3, compound **2** is a xylosylated fragment of both MMX and MMXF^3, compound **3** is the fucosylated core region of MMF^3, M0XF^3 and MMXF^3, compound **4** is the fucosylated core of MMF^{3}F^6. These structures were then subject to preliminary immunogenic evaluations.

2. Results

2.1. Chemical synthesis

The preparation of protected trisaccharides intermediates **5** and **6** (Fig. 3) was communicated recently by our group,[3] using the efficient thioglycosides **7**, **8** and **9**, obtained from the odourless 2-methyl-5-*tert*-buthylthiophenol. The *N*-glycan fragments herein synthesized were coupled to the previously described biotin derivative **10**.[2]

In the 1990's, A. Lipták and J.F.G. Vliegenthart[4] had previously explored the synthesis of xylosylated and fucosylated *N*-glycans

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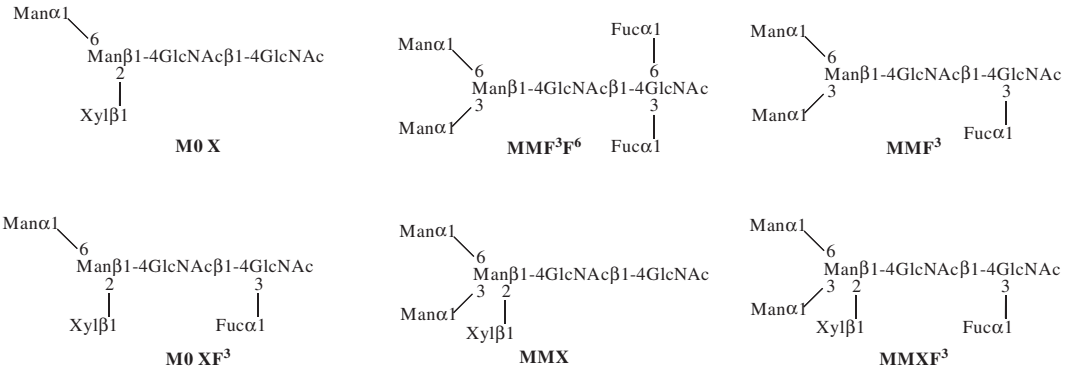


Figure 1. Structures of some of the most common CCDs.

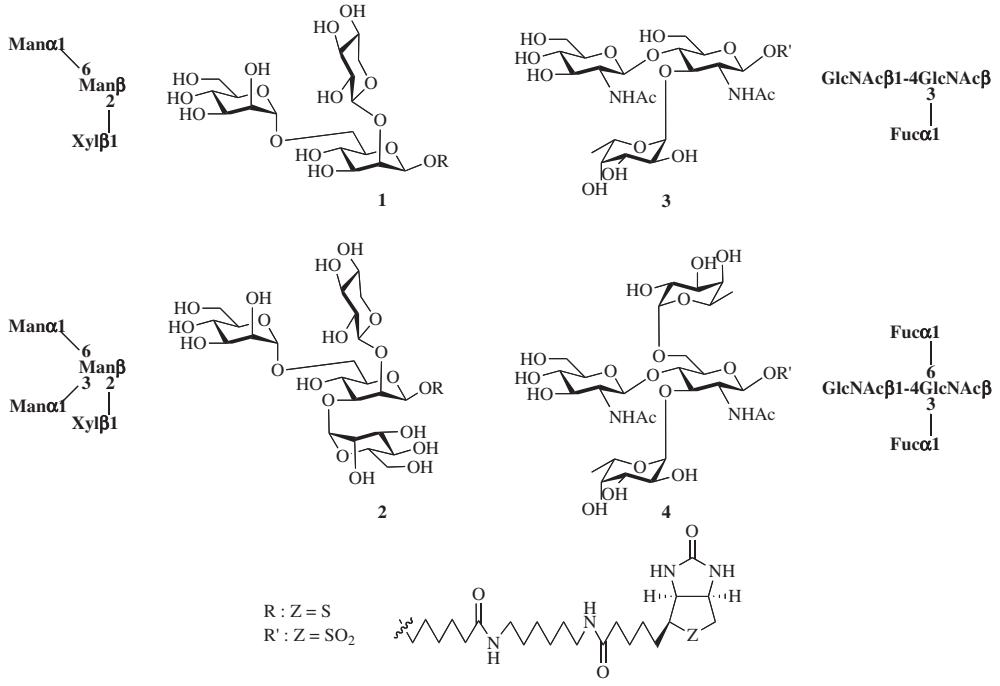


Figure 2. Synthetic targets.

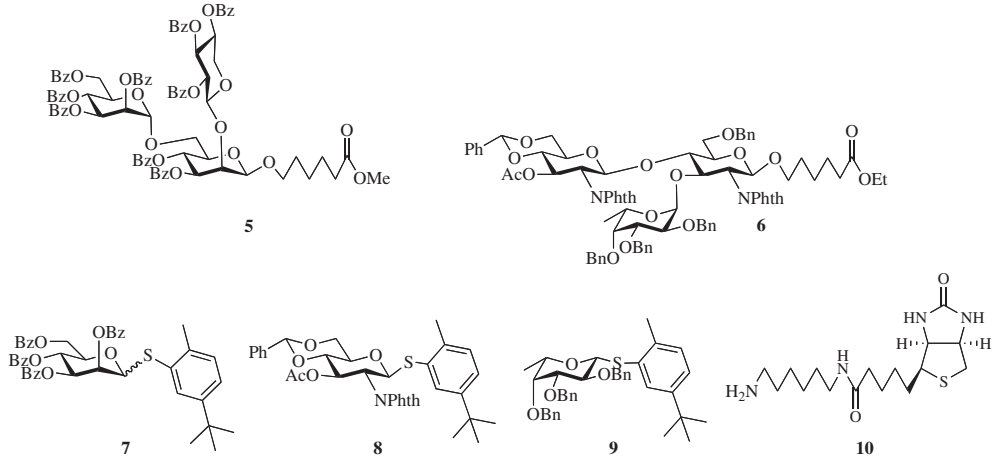


Figure 3. Starting blocks: trisaccharides **5** and **6**; thioglycosides **7**, **8** and **9** and biotin block **10**.[3]

and described the difficulties encountered during the construction of these structures. Unfortunately, there have been no reports on the biological evaluation of these structures following their syntheses.

2.2. Synthesis of biotinylated xylosylated fragments

The synthesis of the xylosylated tetrasaccharide (Scheme 1) began with the acetalisation of known **11**[3] to give **12**. In a first approach, the regioselective opening of **12** was attempted in order to obtain the diol **13** which after dimannosylation would have led to the tetrasaccharide **17** more directly. Likely due to the presence of a free hydroxyl in position 3, the selective reduction step[5] was unsuccessful. Alternatively compound **12** was first mannosylated at position 3 in an excellent yield to give trisaccharide **14**. The reduction of benzylidene was then achieved in a good yield to give two regioisomers **15** and **16** (in an 8:2 ratio). Compound **15** was finally mannosylated to provide **17** in a moderate yield (46%) with a reduced efficiency attributed to the steric hindrance generated by the benzoylated mannoside and xyloside present on the upper face of the β mannoside of this highly branched structure.

Trisaccharide **5** and tetrasaccharide **17** were then deprotected and acetylated to give acids **19** and **23**. The hydrolysis (Water/THF) of the mixed anhydride formed during the acetylation of **18** and **21** was found to be important in order to ensure the coupling reaction with **10**. After coupling of **19** and **23** to the biotinylating agent **10**, the acetate protecting groups were removed to finally give **1** and **2**, respectively (Schemes 2 and 3).

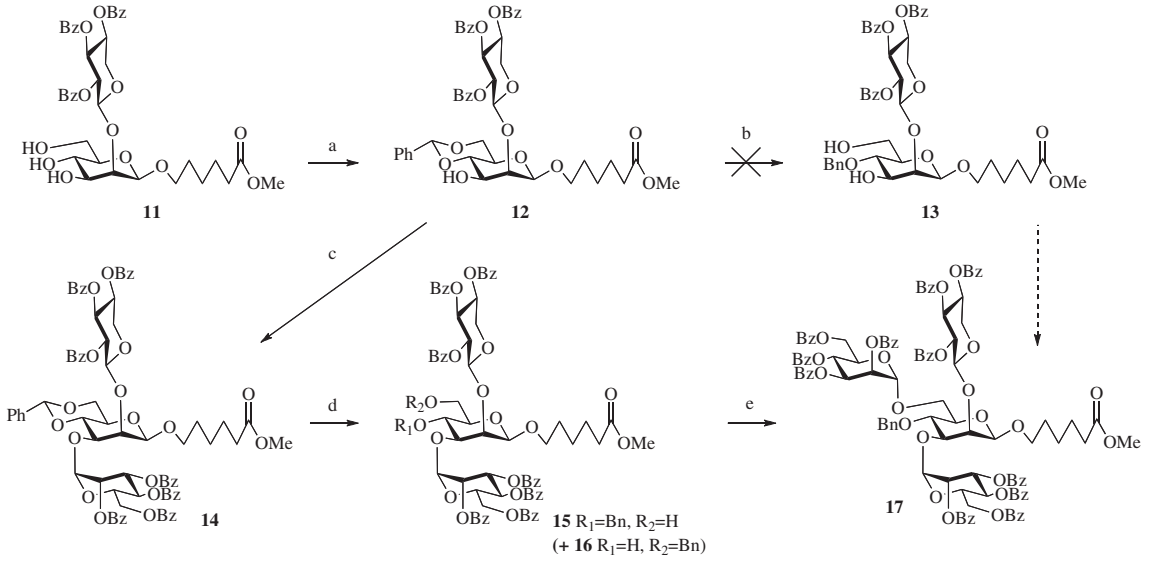
2.3. Synthesis of biotinylated fucosylated fragments

The synthesis of fucosylated fragments was undertaken next. In our first approach, we planned to introduce the two fucoses in one step onto the glucosamine moiety and then complete the synthesis by the glycosylation of the second glucosamine unit (Scheme 4).

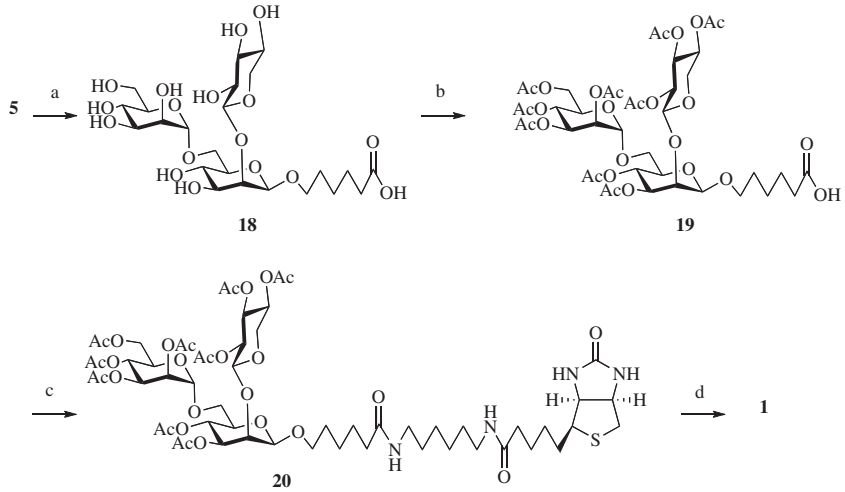
This scheme proved unsuccessful as the regioselective opening of **26** (prepared from **25**[3]) failed to give any expected diol despite the use of different reduction systems such as DIBAL-H,[6] $TMSCl/NaBH_3CN$,[7] $Et_3SiH/PhBCl_2$[5] and $NEt_3BH_3/AlCl_3$.[8] We had already contemplated an alternative route, because—as described from the work of Lipták and co-workers and more recently from Crich's studies[9]—the glycosylation of position 4 of glucosamine derivatives can be difficult; therefore, the preparation of a chitobiose intermediate during the early steps was examined as a potential approach to circumvent these problems. Using this plan, a regioselective glycosylation between the glucosaminyl donor **30** and acceptor **27** (obtained after selective protection of **25**) was attempted (Scheme 5). The yield of this glycosylation was low and suffered from a poor regioselectivity, in contrast with other related glycosylations.[10] It was then clear that protection of the 3-hydroxyl was necessary for an efficient glycosylation and *tert*-butyl dimethyl silyl (TBS) was chosen at this point to provide this (Scheme 6).

To begin the new strategy, ethyl-6-hydroxyhexanoate was glycosylated with previously described intermediate **31**[3] in good yield to give **32**. After quantitative benzylidene reduction and protection of position 6 as a TBS ether, **34** was obtained and was glycosylated with donor **8** to provide the chitobiose derivative **35** in a satisfactory yield (61%). The removal of the two TBS groups (**35**+**36**) was achieved using NH_4F in DMF[11] as other conditions were not effective: TBAF/THF at room temperature was found to not be reactive enough, and either warming the reaction to 70 °C or using acetic acid in combination led to the cleavage of the acetate groups. The fucosylation of diol **36** by fucosyl donor **9** was then examined under various conditions. In dichloromethane, a mixture of tetrasaccharides **37** and **38** was obtained in a 57% yield, with fucose in position 3 appearing only as an α-linkage, while the fucosylation of position 6 had low stereoselectivity (α/β, 3:1). The addition of diethyl ether to dichloromethane (1:1 mixture) strongly improved the yield (91%) and slightly improved the stereoselectivity (α/β: 3.5:1) (Scheme 6). Compounds **37** and **38** were then used as a mixture of isomers in the next steps to eventually recover compound **43** in a 53% yield.

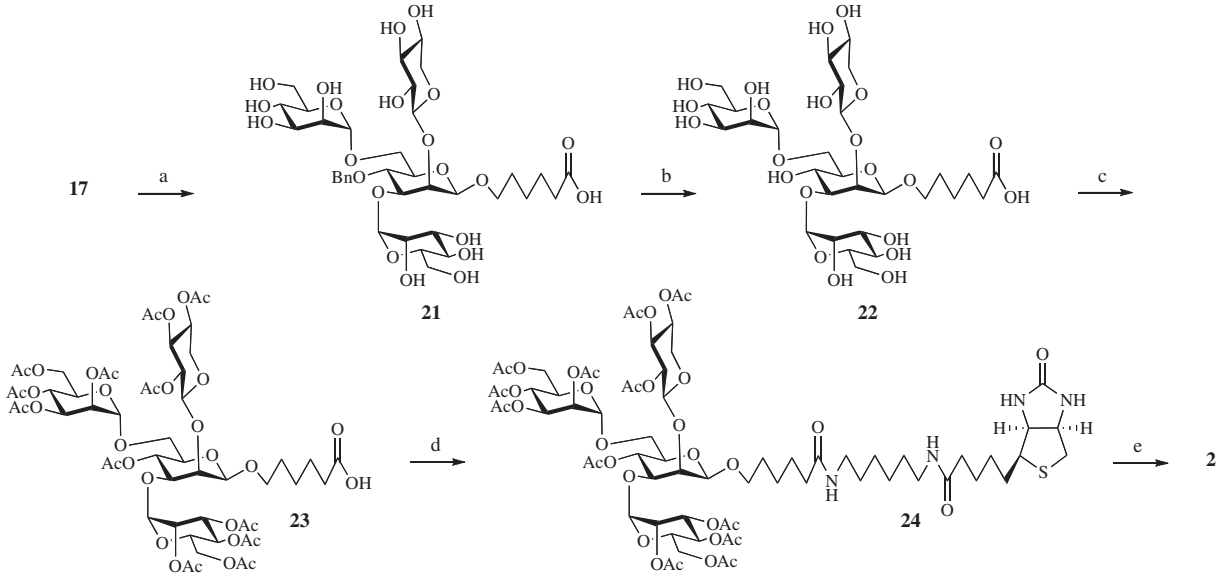
As the presence of non-participating benzyl protecting groups was necessary to achieve α-fucosylation in good selectivity, we chose to convert the biotin thioether function to a sulfone function. We have used this strategy previously[2] as the biotin does not interfere with the palladium hydrogenolysis catalyst when oxidised to sulfone, and biotin sulfone still presents a strong affinity to streptavidin. Phthalimido groups were removed and the amino functions were acetylated to give **39** and **43**. Ethyl esters were saponified and the acids were then coupled to **10** to give the biotin conjugates **40** and **44**. Oxidation to sulfones **42** and **45** (using *meta*-chloroperbenzoic acid), followed by hydrogenolysis of these latter, afforded the final conjugates **3** and **4** (Scheme 7).



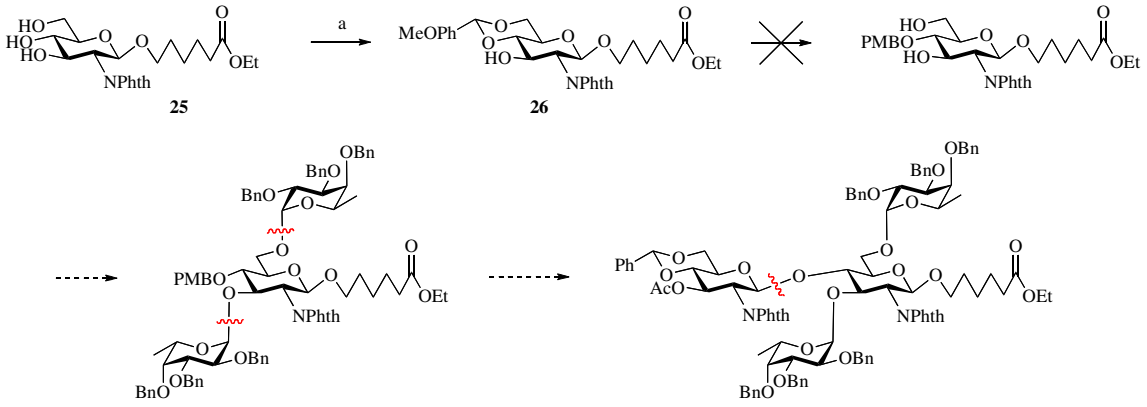
Scheme 1. Synthesis of **17**. Reagents and conditions: (a) dimethyl benzaldehyde acetal, CSA, CH_3CN, 89%; (b) Et_3SiH, $PhBCl_2$, 4 Å molecular sieves, CH_2Cl_2; (c) **7**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 95%; (d) Et_3SiH, $PhBCl_2$, 4 Å molecular sieves, CH_2Cl_2, −78 °C 88%, **15**:**16**, 8:2 ratio; (e) **7**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 46%.



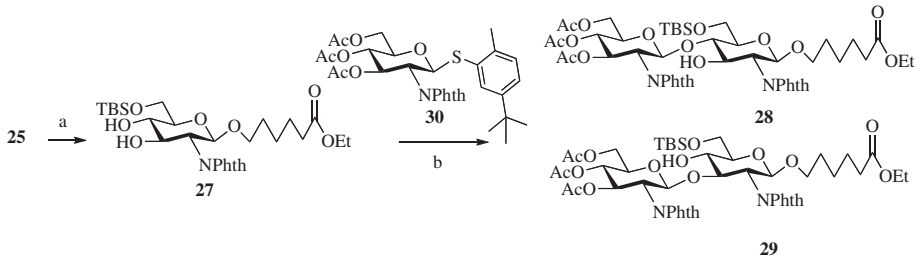
Scheme 2. Synthesis of **1**. Reagents and conditions: (a) MeONa, MeOH, then NaOH, H_2O, 70 °C, 98%; (b) Ac_2O, pyridine then H_2O/THF (v/v: 1:2); (c) **10**, EDC, DMAP, DMF, 60 °C (49% two steps); (d) MeONa, MeOH, 94%.



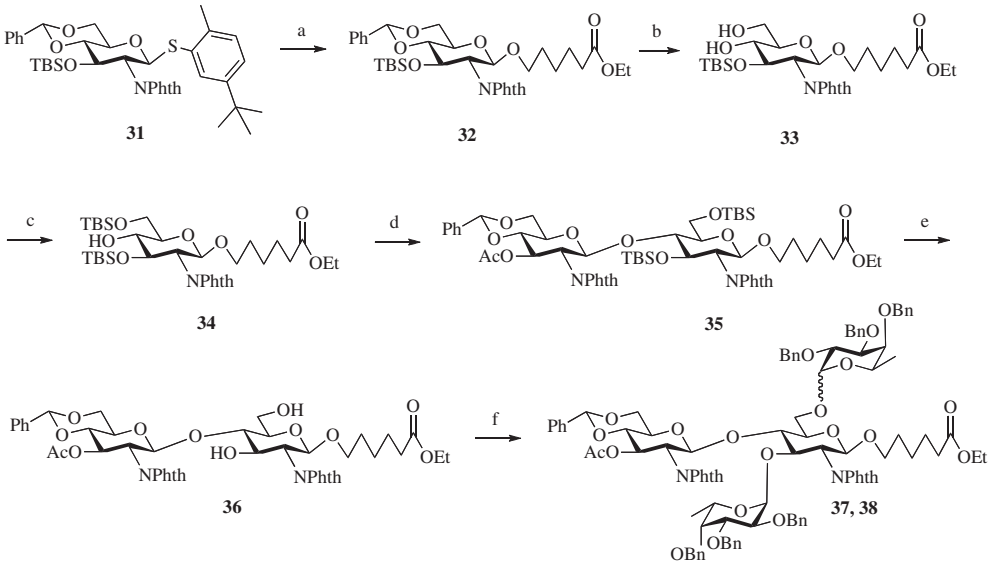
Scheme 3. Synthesis of **2**. Reagents and conditions: (a) MeONa, MeOH, then NaOH, H_2O; (b) H_2, Pd black, $Pd(OH)_2$/C; (c) Ac_2O, pyridine; then H_2O/THF; (d) **10**, EDC, DMAP, DMF, 70 °C, 30% overall yield; (e) MeONa, MeOH, 62%.



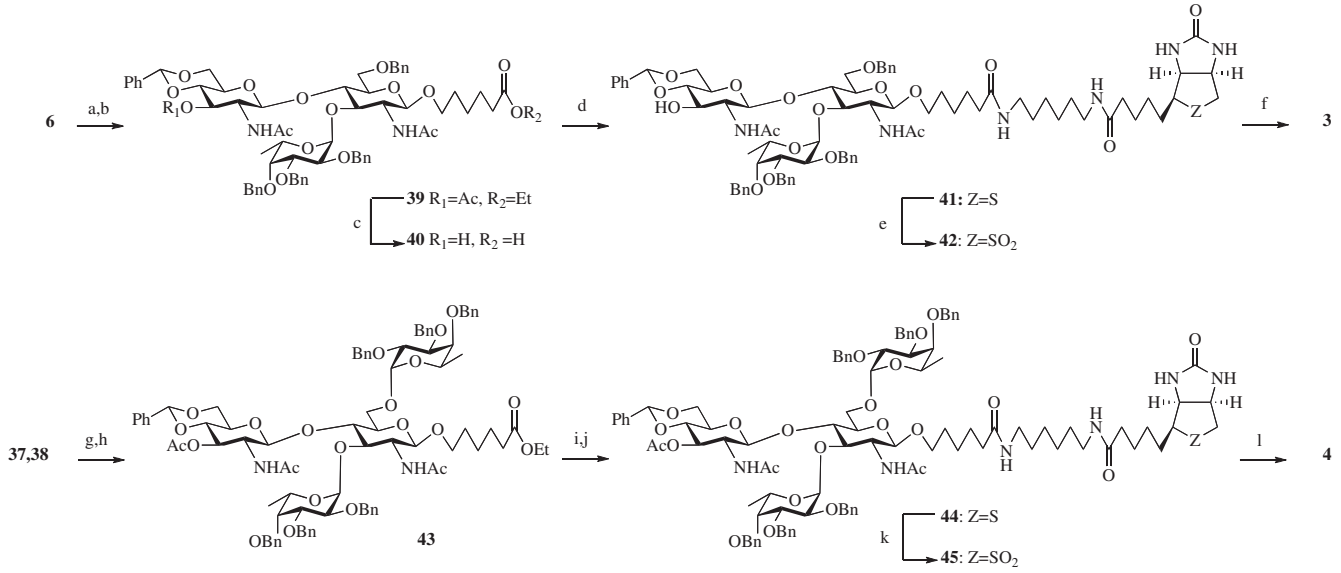
Scheme 4. Reagents and conditions: Benzaldehyde dimethyl acetal, CSA, CH_3CN, 81%.



Scheme 5. Reagents and conditions: (a) TBSCl, pyridine, DMAP, 56%; (b) NBS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 21%, (**28**/**29**: 2:1).



Scheme 6. Reagents and conditions: (a) ethyl 6-hydroxyhexanoate, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 90%; (b) H_2, Pd/C, AcOEt, 100%; (c) TBSCl, DMAP, pyridine, 90%; (d) **8**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 61%; (e) NH_4F, DMF, 100 °C, 80%; (f) **9**, NIS, TfOH, 4 Å molecular sieves, Et_2O/CH_2Cl_2, 91%, (β/α: 1:3.5).



Scheme 7. Synthesis of **3** and **4**. Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$, EtOH, 90 °C; (b) Ac_2O, pyridine, 89% (two steps); (c) aq 10 M NaOH, THF, 75 °C, 100%; (d) **10**, DMAP, EDC, DMF, 70 °C, 54%; (e) mCPBA, CH_2Cl_2, 84%; (f) H_2, Pd/C, methanol, H_2O, 87%; (g) $N_2H_4{\cdot}H_2O$, EtOH, 90 °C; (h) Ac_2O, pyridine 58%; (i) aq 10 M NaOH, THF, 75 °C; (j) **10**, EDC, DMAP, DMF, 70 °C, 51%; (k) mCPBA, CH_2Cl_2, 84%; (l) H_2, Pd/C, methanol, H_2O, 100%.

2.4. Strain in branched chitobiosides: Analysis of the X-ray structure

An X-ray structure of crystalline **39** could be obtained (see Fig. 4) and an unexpected conformation could be evidently seen. The first glucosamine was distorted and adopts an unusual $^{O}S_{4}$ conformation. The conformation adopted by the core glucosamine could be explained by the steric hindrance induced by the adjacent fucosyl and glucosaminyl moieties (bearing bulky protecting groups), respectively, on position 3 and 4.

Figure 5 obtained from the crystal structure of **39**, clearly depicts an anti parallel conformation of position 3 and 4 of the core glucosamine. By analogy this gives for compound **6** a possible explanation for the poor reactivity or accessibility of the position 4 of the core glucosamine during our attempted glycosylations.

2.5. Biological evaluation

2.5.1. Reactivity with anti-bee venom and anti-horseradish peroxidase

As a first evaluation of the synthesized conjugates (**1–4**), an ELISA using anti-bee venom and anti-horseradish peroxidase rabbit antibodies was performed (Fig. 6) as these antisera are known to cross-react with fucosylated and/or xylosylated *N*-glycans.[12] The results showed that the xylose-containing conjugates (compounds **1** and **2**) bind particularly well to anti-HRP, but that the fucose-containing ones bind only weakly (the difucosylated compound **4** binds weakly to anti-bee venom, whereas both compounds **3** and **4**, respectively, bound more significantly to anti-HRP). The weaker binding to the fucosylated samples was expected from previous data with exoglycosidase-treated *N*-glycans;[12]

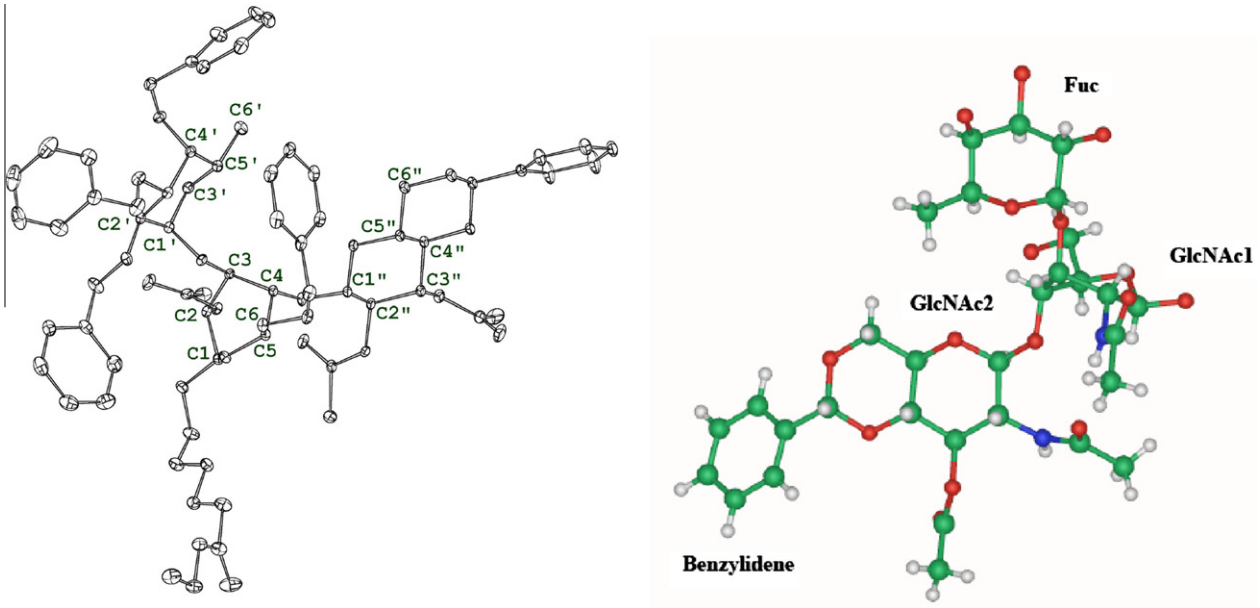


Figure 4. X-ray structure of **39** (left), **39** represented without the benzyl groups and aglycon (right).

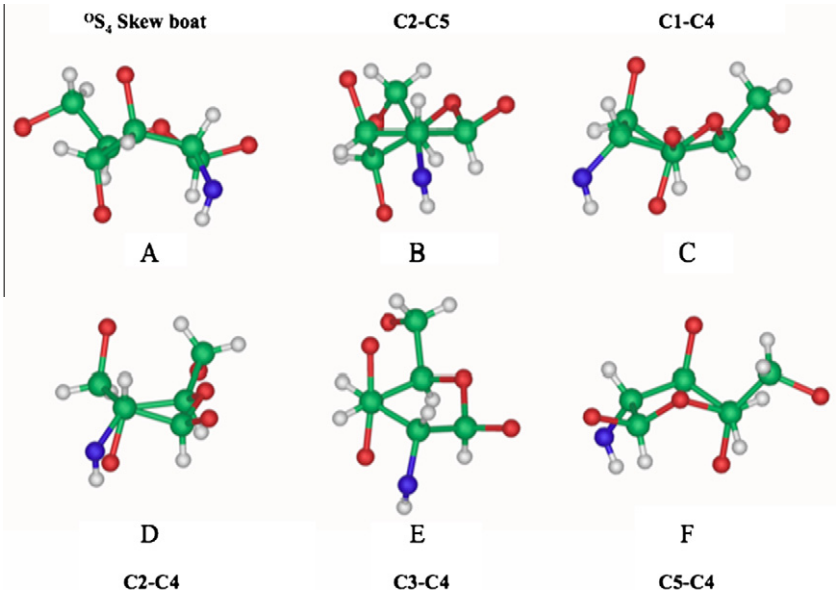


Figure 5. $^{O}S_{4}$ conformational observations of the core glucosamine (from crystal structure of **39**). (A) Side view, (B) in C2–C5 axis, (C) in C1–C4 axis, (D) in C2–C4 axis, (E) in C3–C4 axis, (F) in C5–C4 axis. The analysis were made with iMol version 0.40 Copyright © 2002–2007 Piotr Rotkiewicz.

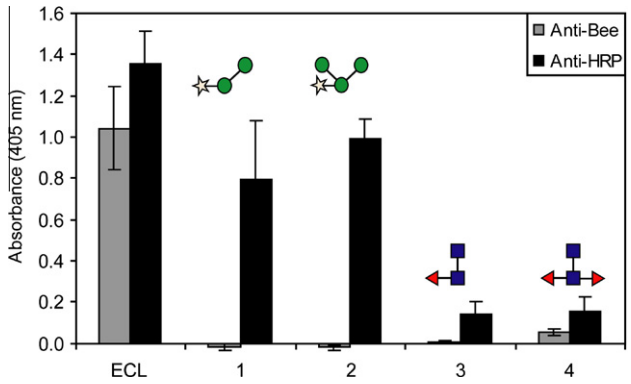


Figure 6. ELISA with anti-bee venom and anti-horseradish peroxidase antisera. Microtitre wells were coated with streptavidin followed by biotinylated *Erythrina cristagalli* lectin (ECL, as a positive control) or compounds **1** (M0X fragment), **2** (MMX fragment), **3** (monofucosylated F^3 core fragment) and **4** (difucosylated F^3F^6 core fragment) at 5 μg/mL. 'Blank corrected' results of the average of two independent duplicate assays are presented (total $n = 4$, with standard deviations); structures of the oligosaccharide fragments are shown according to the nomenclature of the Consortium for Functional Glycomics. BSA-M0XF^3 was used to coat one set of lanes as a positive control, whereas biotinylated ECL was shown not to bind BSA-blocked plates unless streptavidin was used to coat the wells (data not shown).

biotinylated *Erythrina cristagalli* lectin was used as a positive control since this plant lectin contains MMXF-type glycans.[13]

2.5.2. Reactivity with patients' sera

Fourteen sera (1–14) from multiple pollen sensitised patients' containing IgE antibodies reactive towards bromelain, as detected by ImmunoCAP, were selected for the study (Fig. 7). With exception of serum 9 and serum 11, all sera also contained IgE cross-reacting with HRP and *E. cristagalli* lectin (ECL). Controls consisted of sera from two non atopic subjects. The synthetic glycoconjugates were then tested for IgE binding in an ELISA assay. Three out of fourteen sera displayed additional IgE binding to xylosylated compound **2**, two to xylosylated compound **3** and two to fucosylated compounds **3** and **4**. Testing streptavidin as negative control gave no results in any of the sera tested. This weak reactivity with patients' sera was rather unexpected and may indicate that longer fragments are required for a better IgE binding.

3. Conclusion

Four biotinylated *N*-glycan fragments potentially involved in cross-allergies have been synthesized using exclusively MBP thioglycosides donors. The results highlighted the difficulty of constructing branched oligosaccharides in particular chitobiosides. When the biotinylated *N*-glycan fragments were coated on streptavidin microplates, various levels of reactivity to anti-carbohydrate antibodies and patients' sera were observed. These preliminary studies showed the potential utility of xylosylated and fucosylated oligosaccharide fragments in determining CDD antibody epitopes.

4. Experimental part

4.1. Immunological evaluation

4.1.1. IgG ELISA

Microtitre plates (MaxiSorp Immuno Plate, Nalge Nunc International, Roskilde, Denmark) were coated with streptavidin (5 μg/mL) in sodium carbonate buffer, except for where BSA-M0XF^3 was applied as the sole antigen. The plates were blocked with BSA to prevent non-specific interactions. Streptavidin-coated wells were then incubated with compounds **1**, **2**, **3** or **4** or with biotinylated *E. cristagalli* lectin at 5 μg/mL. Then rabbit anti-bee venom (1:3000) or rabbit anti-HRP (1:10,000) were applied. Finally, alkaline-phosphatase-conjugated anti-rabbit antiserum (1:10,000) was used and *p*-nitrophenyl-phosphate was the chromogenic substrate (results in terms of absorbance at 405 nm after an enzymatic reaction time of 15 min at 37 °C are shown).

4.1.2. Patients' sera

Fourteen sera (1–14) from multiple pollen sensitised patients' containing IgE antibodies directed against bromelain, as detected by ImmunoCAP, have been selected for the study. With exception of patients' sera Nos. 9 and 11, all sera also contained HRP-specific IgE. Controls consisted of sera from two non atopic subjects.

4.1.3. IgE ELISA

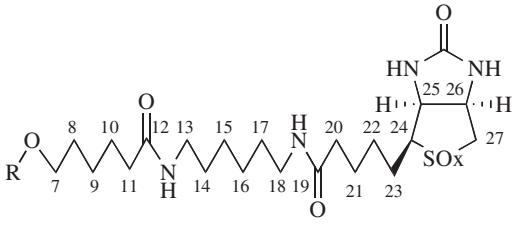
Microtitre plates, pre-coated as appropriate with streptavidin, were coated with 0.5 μg antigen (biotinylated compounds 1, 2, 3 or 4 with biotinylated *E.* cristagalli lectin and HRP as positive controls and streptavidin as negative control) per well overnight at 4 °C. After blocking with Tris-buffered saline (TBS), 0.05% (v/v) Tween 20 and 3% (w/v) milk powder, 1:7 diluted sera were applied onto the coated plates and incubated overnight at 4 °C. After washing, the plates were incubated with a 1:1000 diluted alkaline phosphatase-conjugated mouse anti-human IgE antibody (BD Pharmingen, San Diego, CA, USA) for 2 h at room temperature. Colour development was performed using 0.1% (w/v) disodium *p*-nitrophenyl phosphate substrate (Sigma–Aldrich, Steinheim, Germany) and the optical density (OD) was measured at 405 nm (550 nm as reference wavelength) after 30 min. Sera of two non-allergic subjects were used as negative controls and OD values were counted positive when they exceeded the mean OD of the negative controls by more than three standard deviations.

4.2. Chemical synthesis

4.2.1. General procedures

All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting points were determined in capillary tubes in a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 digital polarimeter at 22 ± 3 °C. Compound purity was checked by TLC on Silica Gel 60 F_{254} (E. Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica Gel 60 (E. Merck). ^{1}H NMR spectra were recorded with Brüker AM 250, AM 400 instruments. Chemical ionisation and FAB mass spectrometry were recorded with Jeol MS700: CI (gas: ammonia); FAB (matrix: NBA, NaI).

Chain numbering of biotin:



4.2.2. 5-Carboxypentyl 2-*O*-(β-D-xylopyranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside biotin conjugate (1)

To a mixture of **18** (0.341 g, 0.579 mmol) and DMAP (0.028 g, 0.229 mmol, 0.4 equiv) in anhydrous pyridine, was added under argon acetic anhydride (2.50 mL, 26.47 mmol, 45 equiv). The mixture was stirred overnight at room temperature and concentrated. A

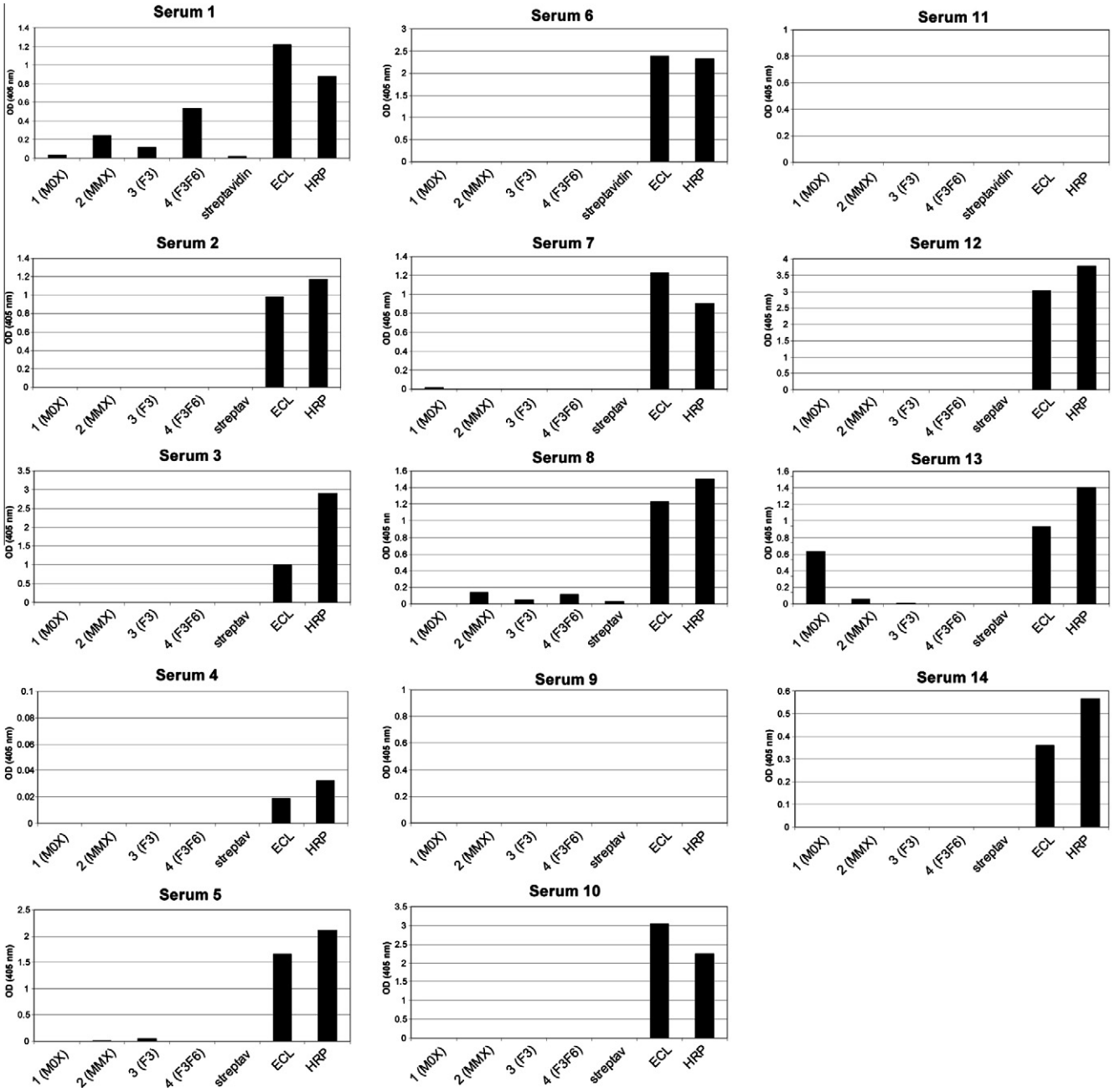


Figure 7. IgE ELISA with selected human sera. Reactivity to compounds **1**–**4** or ECL on streptavidin-coated microtitre plates was tested similarly as for the IgG reactivity; HRP was introduced as a second positive control.

solution of the residue was stirred in THF (4 mL) and water (1 mL) until disappearance of the mixed anhydride (TLC control: CH_2Cl_2/methanol: 9:1). A solution of the residues **19** and **10** (0.396 g, 1.158 mmol, 2 equiv), DMAP (0.084 g, 0.694 mmol, 1.2 equiv) and EDC (0.165 g, 0.868 mmol, 1.5 equiv) in anhydrous DMF (8 mL) was stirred overnight at 70 °C. Another portion of EDC (0.055 g, 0.289 mmol, 0.5 equiv) was added to complete the reaction and after 2 h, the solution was concentrated. The residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 95:5) to give 0.366 g of **20** (49%) as a syrup. R_f: 0.43 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −24 (*c* 0.3, $CHCl_3$). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{57}H_{86}O_{27}N_4SNa$ 1313.5097, found 1313.5099. Compound **20** (0.360 g, 0.279 mmol) was dissolved in methanol (6 mL) and sodium (0.032 g, 1.391 mmol, 5 equiv) was added. The solution was stirred at room temperature overnight and neutralised (IR 120 H^+), filtered and concentrated. The residue was purified on a G15 column to give, after lyophilisation, 240 mg of **1** (94%) as a white powder. $[\alpha]_D^{25}$ +0.3 (*c* 5, H_2O/MeOH (1:1)). ^1H NMR (400 MHz, D_2O) δ: 4.79 (s, 1H, $1H_1$), 4.64 (s, 1H, $1H_1$), 4.51 (m, 1H, H_{26}), 4.41 (d, 1H, $J_{H1'-H2'}$ = 7.2 Hz, $H_{1'}$), 4.32 (m, 1H, H_{25}), 4.04 (s, 1H, H_2), 3.89–3.64 (m, 8H, $1H_2$, $1H_7$), 3.60–3.48 (m, 6H), 3.43 (m, 1H), 3.36–3.13 (m, 4H), 3.07 (m, 4H, H_{13}, H_{18}), 2.89 (m, 1H, $1H_{27}$), 2.68 (d, 1H, J_{gem} = 13.0 Hz, $1H_{27}$), 2.13 (m, 4H, H_{11}, H_{20}), 1.66–1.23 (m, 20H, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}). ^{13}C NMR (100 MHz, D_2O) δ: 176.9 (CONH), 176.7 (CONH), 165.5 (CO urea), 104.7 ($C_{1'}$), 100.5 ($1C_1$), 99.9 ($1C_1$), 78.9 ($1C_2$), 75.8 ($C_{3'}$), 74.8, 73.7 ($C_{2'}$), 73.0, 72.5, 70.9 ($1C_2$), 70.2 ($1C_6$), 70.2, 69.6, 67.5, 67.0, 66.1 ($1C_6$), 65.5 ($C_{5'}$), 62.4 (C_{25}), 61.2 (C_7), 60.6 (C_{26}), 55.8 (C_{24}), 40.1 (C_{27}), 39.5 (C_{13}, C_{18}), 36.1 (C_{11} or

C_{20}), 35.9 (C_{11} or C_{20}), 28.8, 28.7, 28.6, 28.3, 28.1, 26.1, 25.6, 25.6, 25.1, 24.3. MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{39}H_{68}O_{18}N_4SNa$ 935.4147, found 935.4131.

4.2.3. 5-Carboxypentyl 2-*O*-(β-D-xylopyranosyl)-3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside biotin conjugate (2)

Compound **17** (0.300 g, 0.150 mmol) was dissolved in THF (2 mL) and methanol (5 mL) and sodium (0.034 g, 1.500 mmol, 10 equiv) was added. The mixture was degassed with stirring and NaOH (10 M, 0.3 mL, 3.000 mmol, 20 equiv) was added. The mixture was stirred overnight at room temperature and was then neutralised (IR 120 H^+), filtered and concentrated. The crude product **21** was dissolved in methanol (10 mL) and water (HPLC grade, 2 mL) and Pd Black (20 mg) and $Pd(OH)_2/C$ (20%, 50 mg) were added. Vacuum and H_2 were alternated and the mixture was stirred at room temperature under H_2 overnight. The mixture was filtered off through Celite and concentrated. The residue was dissolved in water and filtered through a 0.45 μm syringe filter and concentrated. The residue **22** was dissolved in anhydrous pyridine (4 mL) and DMAP (22 mg), acetic anhydride (0.7 mL) were added under argon. The mixture was stirred for 2 days at room temperature. Concentrated and the residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude product **23** was dissolved in anhydrous DMF (6 mL) and were added under argon: **10** (0.256 g, 0.750 mmol, 5 equiv), DMAP (0.020 g, 0.160 mmol, 1.1 equiv) and EDC (0.057 g, 0.300 mmol, 2 equiv). The solution was stirred for 3 h at 70 °C. EDC (0.057 g, 0.300 mmol, 2 equiv) was added to complete the reaction and after over night stirring, concentrated. The residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 95:5) to give 70 mg of **24** (30% over 4 steps) as a syrup. **24** (0.067 mg, 0.042 mmol) was dissolved in methanol (5 mL) and sodium (0.030 g, 1.304 mmol, 30 equiv) was added. The mixture was degassed with stirring and was stirred overnight. The mixture was neutralised (IR 120 H^+), filtered and concentrated. The residue was purified on a G15 column to give, after lyophilisation, 28 mg of **2** (62%) as a white powder. $[\alpha]_D^{25}$ +19 (*c* 1.5, MeOH/H_2O (1:1)). ^1H NMR (400 MHz, D_2O) δ: 5.04 (s, 1H, $1H_1$), 4.79 (s, 1H, $1H_1$), 4.66 (s, 1H, $1H_1$), 4.50 (dd, 1H, $J_{H26-H27}$ = 5.0 Hz, $J_{H26-H25}$ = 7.8 Hz, H_{26}), 4.39 (d, 1H, $J_{H1'-H2'}$ = 7.4 Hz, $H_{1'}$), 4.32 (dd, 1H, $J_{H25-H24}$ = 4.4 Hz, H_{25}), 4.08 (s, 1H, $1H_2$), 3.94–3.45 (m, 21H), 3.36 (m, 2H), 3.22 (m, 1H, H_{24}), 3.14 (t, 1H, *J* = 11.0 Hz, $1H_6$), 3.10–3.05 (m, 4H, H_{13}, H_{18}), 2.89 (dd, 1H, J_{gem} = 13.0 Hz, H_{27}), 2.68 (d, 1H, J_{gem} = 13.0 Hz, H_{27}), 2.14 (m, 4H, H_{11}, H_{20}), 1.65–1.20 (m, 20H, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}). ^{13}C NMR (100 MHz, D_2O) δ: 177.0 (CO NH), 176.6 (CO NH), 165.6 (CO urea), 105.6 ($C_{1'}$), 102.3 ($1C_1$), 100.4 ($1C_1$), 99.8 ($1C_1$), 80.2, 77.7 (2C), 75.8, 74.6, 73.9, 73.7, 73.0, 70.9, 70.6, 70.4, 70.3 (C_7), 70.2, 69.6, 67.1, 67.0, 65.7 ($1C_6$), 65.3 ($1C_6$), 62.4 (C_{25}), 61.4 ($C_{5'}$, 1C), 61.2 ($C_{5'}$, C_6), 60.6 (C_{26}), 55.8 (C_{24}), 40.0 (C_{27}), 39.5 (C_{13}, C_{18}), 36.1 (C_{11} or C_{20}), 35.8 (C_{11} or C_{20}), 28.0–24.0 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}, C_{23}). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{45}H_{78}O_{23}N_4SNa$ 1097.4675, found 1097.4645.

4.2.4. 5-Carboxypentyl 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-(α-L-fucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside oxidised biotin conjugate (3)

To a solution of **42** (0.100 mg, 0.067 mmol) in methanol (4 mL) and water (HPLC grade, 1 mL) was added Pd/C (10%) (50 mg, 0.5 g per g of substrate). Vacuum and H_2 were alternated and then the mixture was stirred at room temperature under H_2 overnight. The mixture was filtered off through Celite and concentrated. The residue was dissolved in water (HPLC grade) and washed with CH_2Cl_2 (3 times). The aqueous layer was evaporated and the residue was purified on a G15 column to give, after lyophilisation, 60 mg of **3** (87%) as a white powder. $[\alpha]_D^{25}$ −26 (*c* 2, MeOH/H_2O (1:1)). ^1H NMR (400 MHz, D_2O) δ: 5.00 (d, 1H, $J_{H1'-H2'}$ = 4.0 Hz, $H_{1'}$), 4.74–4.65 (m, 3H, H_{25}, H_{26}, $H_{5'}$), 4.46 (d, 1H, J_{H1-H2} = 8.4 Hz, $1H_1$), 4.41 (d, 1H, J_{H1-H2} = 8.0 Hz, $1H_1$), 3.91–3.73 (m, 8H), 3.70–3.61 (m, 3H), 3.56–3.34 (m, 7H), 3.29 (d, 1H, J_{gem} = 14.9 Hz, H_{27}), 3.19–3.07 (m, 5H), 2.21 (t, 2H, H_{11} or H_{20}), 2.15 (t, 2H, H_{11} or H_{20}), 1.98 (s, 3H, CH_3 NHAc), 1.94 (s, 3H, CH_3 NHAc), 1.88–1.81 (m, 1H), 1.75–1.57 (m, 3H), 1.57–1.41 (m, 10H), 1.30–1.21 (m, 6H), 1.19 (d, 3H, $J_{H6'-H5'}$ = 6.6 Hz, $1H_{6'}$). ^{13}C NMR (100 MHz, D_2O) δ: 177.0 (CONH), 176.7 (CONH), 174.9 (CONH), 174.5 (CONH), 164.4 (CO urea), 101.3 ($1C_1$), 100.7 ($1C_1$), 98.8 ($C_{1'}$), 76.3, 75.7, 75.3, 74.0, 73.9, 72.4, 71.1, 70.7 (C_7), 69.5, 68.0, 67.0 ($C_{5'}$), 61.9 ($1C_6$), 60.7 (C_{24}), 60.3 ($1C_6$), 56.1 ($1C_2$), 56.0 ($1C_2$), 54.5 (C_{25} or C_{26}), 54.1 (C_{27}), 50.1 (C_{25} or C_{26}), 39.6 (C_{13} or C_{18}), 39.5 (C_{13} or C_{18}), 36.1 (C_{11} or C_{20}), 35.8 (C_{11} or C_{20}), 28.6 (2C), 28.5, 26.0 (2C), 25.5 (2C), 25.4, 25.0, 22.6 (CH_3 NHAc), 22.4 (CH_3 NHAc), 21.2, 15.8 ($C_{6'}$). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{44}H_{76}N_6O_{20}SNa$ 1063.4732, found 1063.4768.

4.2.5. 5-Carboxypentyl 3,6-di-*O*-(α-L-fucopyranosyl)-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside oxidised biotin conjuguate (4)

To a solution of **45** (0.190 g, 0.104 mmol) in methanol (4 mL) and water (HPLC grade, 1 mL) was added Pd/C (10%) (95 mg, 0.5 g per g of substrate). Vacuum and H_2 were alternated and the mixture was stirred at room temperature under H_2 overnight. The mixture was filtered off through Celite and concentrated. The residue was dissolved in water (HPLC grade) and washed with CH_2Cl_2 (3 times). The aqueous layer was evaporated and the residue was purified on a G15 column to give, after lyophilisation, 123 mg of **4** (100%) as a white powder. $[\alpha]_D^{25}$ −55 (*c* 2, MeOH/H_2O (1:1)). ^1H NMR (400 MHz, D_2O) δ: 5.02 (d, 1H, J_{H1-H2} = 3.9 Hz, $1H_1$ fucose), 4.84 (d, 1H, J_{H1-H2} = 3.8 Hz, $1H_1$ fucose), 4.70–4.61 (m, 3H, H_{25}, H_{26}, $1H_5$ fucose), 4.55 (d, 1H, J_{H1-H2} = 8.4 Hz, $1H_1$), 4.38 (d, 1H, J_{H1-H2} = 8.0 Hz, $1H_1$), 4.05 (q, 1H, J_{H5-H6} = 6.6 Hz, $1H_5$ fucose), 3.92 (t, 1H, *J* = 9.0 Hz, 1H), 3.87–3.80 (m, 4H), 3.78–3.58 (m, 9H), 3.52–3.23 (m, 8H), 3.15–3.04 (m, 5H, H_{13}, H_{18}, 1H), 2.17 (t, 2H, *J* = 7.2 Hz, H_{11} or H_{20}), 2.12 (t, 2H, *J* = 7.3 Hz, H_{11} or H_{20}), 1.94 (s, CH_3 NHAc), 1.91 (s, CH_3 NHAc), 1.85–1.77 (m, 1H, $1H_{23}$), 1.69–1.53 (m, 3H), 1.52–1.39 (m, 10H), 1.27–1.20 (m, 6H), 1.17 (d, 3H, J_{H6-H5} = 6.6 Hz, CH_3 fucose), 1.12 (d, 3H, J_{H6-H5} = 6.6 Hz, CH_3 fucose). ^{13}C NMR (100 MHz, D_2O) δ: 176.9 (CO NH), 176.6 (CO NH), 174.9 (CO NH), 174.4 (CO NH), 164.4 (CO urea), 101.2 ($1C_1$), 100.4 ($1C_1$), 99.3 ($1C_1$ fucose), 98.9 ($1C_1$ fucose), 76.2, 75.3, 74.3, 73.9, 73.6, 72.4, 72.2, 71.1, 70.4 (C_7), 69.9, 69.5, 68.5, 68.0, 67.1, 67.0, 66.4 ($1C_6$), 62.0 ($1C_6$), 60.7 (C_{24}), 56.2 ($1C_2$), 56.0 ($1C_2$), 54.5 (C_{25} or C_{26}), 54.1 (C_{27}), 50.0 (C_{25} or C_{26}), 39.5 (C_{12}, C_{18}), 36.0 (C_{11} or C_{20}), 35.7 (C_{11} or C_{20}), 28.6, 28.5 (2C), 26.0, 25.5 (3C), 25.0, 22.6 (CH_3 NHAc), 22.5 (CH_3 NHAc), 21.3 (C_{23}), 15.8 ($1C_6$ fucose), 15.7 ($1C_6$ fucose). MS FAB+-MS *m/z* $[M]^-$ calcd for $C_{50}H_{85}N_6O_{24}Na$ 1185.5, found 1185.7.

4.2.6. 5-Carboxymethylpentyl 2-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-4,6-*O*-benzylidene-β-D-mannopyranoside (12)

Compound **11** (0.560 g, 0.744 mmol) was dissolved in anhydrous acetonitrile (5 mL). Dimethyl benzaldehyde acetal (0.224 mL, 1.489 mmol, 2 equiv), camphorsulfonic acid (0.051 g, 0.223 mmol, 0.3 equiv) were added under argon and the mixture was stirred at room temperature overnight. A saturated $NaHCO_3$ solution was added and the product was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7:3–5:5) to give 0.558 g of **12** (89%) as a white solid. R_f: 0.19 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −77 (*c* 2, $CHCl_3$). Mp:

146–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07–8.00 (m, 6H, H Ar), 7.60–7.35 (m, 14H, H Ar), 5.83 (t, 1H, $J_{H3'-H2'} = J_{H3'-H4'} = 7.4$ Hz, $H_{3'}$), 5.38–5.34 (m, 2H, H benzylidene, $H_{4'}$), 5.29 (d, 1H, $H_{1'}$), 5.01 (dd, 1H, $J_{H2'-H1'} = 5.6$ Hz, $H_{2'}$), 4.64 (dd, 1H, $J_{H5'-H4'} = 12.1$ Hz, $J_{H5'-H6'} = 4.4$ Hz, $1H_{5'}$), 4.45 (s, 1H, H_1), 4.22 (d, 1H, $J_{H2-H3} = 3.2$ Hz, H_2), 4.13 (m, 1H, $1H_6$), 3.84–3.67 (m, 6H, H_3, H_4, $1H_{5'}$, CH_3 OMe), 3.59–3.52 (m, 2H, $1H_6$, $1H_7$), 3.30 (td, 1H, $J_{gem} = 9.4$ Hz, $J_{H7-H8} = 6.8$ Hz, $1H_7$), 3.23 (td, 1H, $J_{H5-H4} = 9.7$ Hz, $J_{H5-H6} = 4.8$ Hz, H_5), 2.94 (d, 1H, $J_{OH-H3} = 8.0$ Hz, OH), 2.28 (t, 2H, $J_{H11-H10} = 7.4$ Hz, H_{11}), 1.59 (m, 2H, H_{10}), 1.46 (m, 2H, H_8), 1.27 (m, 2H, H_9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4 (CO COOMe), 165.9 (CO Bz), 165.7 (CO Bz), 165.5 (CO Bz), 137.0–126.0 (C Ar), 102.3 (C benzylidene), 101.5 (C_1), 100.7 ($C_{1'}$), 79.4 (C_4), 77.3 (C_2), 70.5 ($C_{2'}$), 70.5 ($C_{3'}$), 70.4 (C_3), 70.0 (C_7), 69.6 ($C_{4'}$), 68.8 (C_6), 67.5 (C_5), 61.7 ($C_{5'}$), 51.9 (CH_3 OMe), 34.2 (C_{11}), 29.4 (C_8), 25.9 (C_9), 25.0 (C_{10}). MS FAB+-HRMS *m/z* [M+Na]$^+$ calcd for $C_{46}H_{48}O_{15}Na$ 863.2891, found 863.2871.

4.2.7. 5-Carboxymethylpentyl 2-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-4,6-*O*-benzylidene-β-D-mannopyranoside (14)

To a mixture of **12** (0.320 g, 0.318 mmol), **7** (0.290 g, 0.382 mmol, 1.2 equiv) and 4 Å molecular sieves (500 mg) in anhydrous CH_2Cl_2 (4 mL), were added under argon: NIS (0.143 g, 0.636 mmol, 2 equiv) and TfOH (0.003 mL, 0.031 mmol, 0.1 equiv). After 20 min the mixture was filtered, extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8:2) to give 430 mg of **14** (95%) as a white solid. R_f: 0.38 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −72 (*c* 1, $CHCl_3$). Mp: 102–103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.36 (d, 2H, *J* = 7.1 Hz, H Ar), 8.15–8.10 (m, 6H, H Ar), 8.06 (d, 2H, *J* = 7.1 Hz, H Ar), 8.01 (d, 2H, *J* = 7.1 Hz, H Ar), 7.86 (d, 2H, *J* = 7.2 Hz, H Ar), 7.70–7.25 (m, 26H, H Ar), 6.24–6.17 (m, 2H, $H_{3''}$, $H_{4''}$), 6.01 (t, 1H, $J_{H2''-H1''} = 2.0$ Hz, $H_{2''}$), 5.71 (t, 1H, $J_{H3'-H2'} = 3.4$ Hz, $H_{3'}$), 5.66–5.63 (m, 3H, $H_{1'}$, $H_{2'}$, $H_{1''}$), 5.57 (d, 1H, *J* = 2.6 Hz, $H_{4'}$), 5.35 (dd, 1H, $J_{gem} = 12.5$ Hz, $J_{H5'-H4'} = 1.8$ Hz, $1H_{5'}$), 4.85 (m, 2H, $1H_{6''}$, H benzylidene), 4.73 (m, 1H, $1H_{5''}$), 4.56 (dd, 1H, $J_{gem} = 12.1$ Hz, $J_{H6''-H5''} = 4.7$ Hz, $1H_{6''}$), 4.38 (m, 2H, H_1, H_2), 4.32 (dd, 1H, $J_{gem} = 12.5$ Hz, $J_{H5'-H4'} = 1.5$ Hz, $H_{5'}$), 4.16 (dd, 1H, $J_{gem} = 10.3$ Hz, $J_{H6-H5} = 4.8$ Hz, $1H_6$), 4.06 (dd, 1H, $J_{H3-H4} = 10.0$ Hz, $J_{H3-H2} = 3.1$ Hz, H_3), 3.81 (m, 2H, H_4, $1H_7$), 3.65 (s, 3H, CH_3 OMe), 3.44 (t, 1H, $J_{H6-H5} = J_{gem} = 10.3$ Hz, $1H_6$), 3.32 (td, 1H, $J_{gem} = 9.1$ Hz, $J_{H7-H8} = 6.4$ Hz, $1H_7$), 3.19 (td, 1H, $J_{H5-H4} = 9.6$ Hz, H_5), 2.18 (t, 2H, $J_{H11-H10} = 7.4$ Hz, H_{11}), 1.60–1.49 (m, 4H, H_8, H_{10}), 1.38 (m, 2H, H_9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4 (CO ester), 166.4–165.0 (Cq Ar), 137.3–126.2 (C Ar), 102.0 (C_1), 101.0 (C benzylidene), 99.4 ($C_{1'}$ or $C_{1''}$), 98.6 ($C_{1'}$ or $C_{1''}$), 78.3 (C_4), 75.7 (C_3), 75.7 (C_2), 70.6 ($C_{2''}$), 70.1 (C_7), 69.9 ($C_{5''}$), 69.8 ($C_{3''}$), 68.9 (C_6), 68.5 ($C_{4'}$), 67.6 (C_5, $C_{2'}$), 67.5 ($C_{4''}$), 67.2 ($C_{3'}$), 63.3 ($C_{6''}$), 59.7 ($C_{5'}$), 51.8 (CH_3 OMe), 34.1 (C_{11}), 29.5 (C_8 or C_{10}), 26.0 (C_9), 25.0 (C_8 or C_{10}). MS FAB+-HRMS *m/z* [M+Na]$^+$ calcd for $C_{80}H_{74}O_{124}Na$ 1441.4468, found 1441.4496.

4.2.8. 5-Carboxymethylpentyl 2-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-4-*O*-benzyl-β-D-mannopyranoside (15) and 5-carboxymethylpentyl 2-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-6-*O*-benzyl-β-D-mannopyranoside (16)

To a mixture of **14** (0.890 g, 0.627 mmol), 4 Å molecular sieves (1 g), triethylsilane (0.303 mL, 1.881 mmol, 3 equiv) in anhydrous CH_2Cl_2 (10 mL) was added under argon at −78 °C, dichlorophenylborane (0.277 mL, 2.131 mmol, 3.4 equiv). The mixture was stirred for 15 min and triethylamine (4 mL) and methanol (4 mL) were added dropwise. The mixture was allowed to warm up to room temperature, filtered and concentrated. The crude product was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7:3) to give 0.792 g (88%) of a syrup containing two regioisomers (NMR determination: 8:2: **15**/**16**). R_f: 0.54 (cyclohexane/EtOAc: 5:5). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12–7.25 (m, 40H, H Ar), 6.20 (m, 2H, $H_{3''}$, $H_{4''}$), 5.96 (m, 1H, $H_{2''}$), 5.91 (t, 1H, $J_{H3'-H2'} = J_{H3'-H4'} = 7.7$ Hz, $H_{3'}$), 5.74 (dd, 1H, $J_{H2'-H1'} = 5.8$ Hz, $H_{2'}$), 5.65 (ddd, 1H, $J_{H4'-H5'a} = 4.4$ Hz, $J_{H4'-H5'b} = 11.7$ Hz, $H_{4'}$), 5.46 (d, 1H, $J_{H1''-H2''} = 1.4$ Hz, $H_{1''}$), 5.35 (d, 1H, $H_{1'}$), 5.01 (m, 1H, $H_{5''}$), 4.96 (d, 1H, $J_{gem} = 10.6$ Hz, CHPh), 4.85 (dd, 1H, $J_{gem} = 12.1$ Hz, $J_{H6''-H5''} = 2.9$ Hz, $1H_{6''}$), 4.77 (dd, 1H, $J_{gem} = 12.1$ Hz, $J_{H5'-H4'} = 4.4$ Hz, $1H_{5'}$), 4.68 (d, 1H, CHPh), 4.55 (dd, 1H, $J_{H6''-H5''} = 4.5$ Hz, $1H_{6''}$), 4.31 (s, 1H, H_1), 4.24 (d, 1H, $J_{H2-H1} = 2.8$ Hz, H_2), 4.08 (t, 1H, $J_{H4-H3} = J_{H4-H5} = 9.5$ Hz, H_4), 3.90 (m, 1H, $H_{5'}$), 3.84–3.81 (m, 2H, H_3, $1H_6$), 3.70 (s, 3H, OMe), 3.61 (m, 1H, $1H_6$), 3.36 (m, 1H, $1H_7$), 3.21 (m, 1H, H_5), 3.10 (m, 1H, $1H_7$), 2.59 (s, 1H, OH (C_6)), 2.30 (t, 2H, *J* = 7.8 Hz, H_{11}), 1.61–1.19 (m, 6H, H_8, H_9, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.5 (CO COOMe), 166.0–165.0 (Cq Ar), 136.0–128.0 (C Ar), 101.4 ($C_{1'}$), 100.9 (C_1), 100.6 ($C_{1''}$), 81.4 (C_3), 77.2 (C_2), 76.2 (CH_2Ph), 76.1 (C_5), 74.6 (C_4), 71.5 ($C_{2'}$), 71.2 ($C_{3'}$), 71.0 ($C_{2''}$), 70.2 ($C_{4'}$, $C_{3''}$), 70.0 (C_7), 69.6 ($C_{5''}$), 67.9 ($C_{4''}$), 63.6 ($C_{5'}$), 62.2 (C_6, $C_{6''}$), 51.9 (CH_3 OMe), 34.2 (C_{11}), 29.4 (C_8), 25.8 (C_9), 25.0 (C_{10}). MS FAB+-HRMS *m/z* [M+Na]$^+$ calcd for $C_{80}H_{76}O_{24}Na$ 1443.4624, found 1443.4628.

4.2.9. 5-Carboxymethylpentyl 2-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-4-*O*-benzyl-β-D-mannopyranoside (17)

To a mixture of **15** (0.090 g, 0.063 mmol), **7** (0.057 g, 0.075 mmol, 1.2 equiv) and 4 Å molecular sieves (100 mg) in anhydrous CH_2Cl_2 (2 mL), were added under argon: NIS (0.028 g, 0.126 mmol, 2 equiv) and TfOH (1 μL, 0.010 mmol, 0.17 equiv). The mixture was allowed too stir for 20 min and was then filtered, extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7:3) to give 58 mg of **17** (46%) as a white solid. R_f: 0.24 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −59 (*c* 2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16–7.80 (m, 22H, H Ar), 7.70–7.25 (m, 38H, H Ar), 6.20 (t, 1H, $J_{H4''-H3''} = J_{H4''-H5''} = 10.2$ Hz, $H_{4''}$), 6.18 (m, 1H, $H_{3''}$), 6.13 (t, 1H, $J_{H4'''-H3'''} = J_{H4'''-H5'''} = 10.1$ Hz, $H_{4'''}$), 5.91 (m, 1H, $H_{2''}$), 5.86 (dd, 1H, $J_{H3'''-H2'''} = 3.2$ Hz, $J_{H3'''-H4'''} = 10.1$ Hz, $H_{3'''}$), 5.79 (t, 1H, $J_{H3'-H2'} = J_{H3'-H4'} = 6.3$ Hz, $H_{3'}$), 5.72 (dd, 1H, $J_{H2'-H1'} = 4.6$ Hz, $H_{2'}$), 5.63 (dd, 1H, $J_{H2'''-H1'''} = 1.8$ Hz, $J_{H2'''-H3'''} = 3.2$ Hz, $H_{2'''}$), 5.58 (dt, 1H, $J_{H4'-H5'a} = 3.9$ Hz, $J_{H4'-H5'b} = J_{H4'-H3'} = 6.3$ Hz, $H_{4'}$), 5.48 (d, 1H, $H_{1'}$), 5.42 (d, 1H, $J_{H1''-H2''} = 1.3$ Hz, $H_{1''}$), 4.99 (m, 1H, $H_{5''}$), 4.93–4.84 (m, 4H, $H_{1'''}$, $1H_{6''}$, $1H_{5'}$, CHPh), 4.69 (dd, 1H, $J_{gem} = 12.4$ Hz, $J_{H6'''-H5'''} = 2.3$ Hz, $1H_{6'''}$), 4.57–4.49 (2dd, 2H, $1H_{6'''}$, $1H_{6''}$), 4.45–4.40 (m, 2H, $H_{5'''}$, CHPh), 4.38 (s, 1H, H_1), 4.29 (d, 1H, $J_{H2-H3} = 2.8$ Hz, H_2), 4.03 (dd, 1H, $J_{gem} = 12.2$ Hz, $J_{H5'-H4'} = 6.0$ Hz, $1H_{5'}$), 3.82–3.76 (m, 2H, H_3, $1H_7$), 3.70 (d, 1H, $J_{gem} = 9.5$ Hz, $1H_6$), 3.62 (s, 3H, OMe), 3.58 (t, 1H, $J_{H4-H3} = J_{H4-H5} = 9.5$ Hz, H_4), 3.50–3.39 (m, 2H, H_5, $1H_6$), 3.34 (td, 1H, *J* = 6.9 Hz and 6.8 Hz, $1H_7$), 2.16 (t, 2H, *J* = 7.4 Hz, H_{11}), 1.76–1.20 (m, 6H, H_8, H_9, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.4 (CO COOMe), 166–165 (Cq Ar), 133–128 (C Ar), 100.8 (C_1), 100.5 ($C_{1''}$), 100.3 ($C_{1'}$), 97.7 ($C_{1'''}$), 81.6 (C_3), 76.5 (C_2), 76.0 (CH_2Ph), 75.8 (C_4), 75.3 (C_5), 70.9 ($C_{2''}$), 70.7 ($C_{2'''}$), 70.4 ($C_{3'''}$), 70.2 ($C_{3'}$, $C_{3''}$), 70.0 ($C_{2'}$, C_7), 69.8 ($C_{5''}$), 69.5 ($C_{4'}$), 69.3 ($C_{5'''}$), 68.2 (C_6), 67.6 ($C_{4''}$), 67.1 ($C_{4'''}$), 63.5 ($C_{6''}$), 62.9 ($C_{6'''}$), 61.4 ($C_{5'}$), 51.7 (CH_3 OMe), 34.1 (C_{11}), 29.6 (C_8), 26.0 (C_9), 25.1 (C_{10}). MS FAB+-HRMS *m/z* [M+Na]$^+$ calcd for $C_{114}H_{102}O_{33}Na$ 2021.6201, found 2021.6230.

4.2.10. 5-Carboxypentyl 2-*O*-(β-D-xylopyranosyl)-6-*O*-(α-D-mannopyranosyl)-β-D-mannopyranoside (18)

Compound **5** (1.00 g, 0.650 mmol) was dissolved in anhydrous methanol (20 mL) and sodium (0.100 g, 4.347 mmol, 6.7 equiv)

was added. The mixture was stirred overnight at rt. A NaOH solution (10 M, 3.00 mL, 30 mmol, 46 equiv) was added and the mixture was heated at 70 °C overnight. The cooled mixture was neutralised (IR 120 H^+), filtered and concentrated. The residue was purified on a G15 column to give, after lyophilisation, 375 mg of **18** (98%) as a white powder. $[\alpha]_D^{25}$ −19 (*c* 1, D_2O). ^1H NMR (400 MHz, D_2O) δ: 4.75 (s, 1H, $1H_1$), 4.61 (s, 1H, $1H_1$), 4.37 (d, 1H, $J_{H1'-H2'}$ = 7.5 Hz, $H_{1'}$), 4.02 (d, 1H, J_{H2-H3} = 2.5 Hz, $1H_2$), 3.85 (m, 1H, $1H_2$), 3.83–3.60 (m, 7H, $1H_3$, $1H_7$, 5H), 3.53–3.46 (m, 6H, $1H_3$, $H_{4'}$, $1H_7$, 3H), 3.44–3.36 (m, 1H), 3.30 (t, 1H, $J_{H3'-H2'}$ = $J_{H3'-H4'}$ = 8.9 Hz, $H_{3'}$), 3.23 (dd, 1H, $H_{2'}$), 3.12 (t, 1H, J_{gem} = J_{H6-H5} = 10.9 Hz, $1H_6$), 2.27 (t, 2H, $J_{H11-H10}$ = 7.3 Hz, H_{11}), 1.53–1.46 (m, 4H, H_8, H_{10}), 1.30–1.23 (m, 2H, H_9). ^{13}C NMR (100 MHz, D_2O) δ: 179.4 (CO COOH), 104.6 ($C_{1'}$), 100.5 ($1C_1$), 99.8 ($1C_1$), 78.8 ($1C_2$), 75.7 ($C_{3'}$), 74.7, 73.6 ($C_{2'}$), 72.9, 72.4, 70.8, 70.2 ($1C_2$), 70.1 ($C_{5'}$), 69.5, 67.5, 66.9, 66.1 (C_7), 65.4 ($1C_6$), 61.2 ($1C_6$), 34.0 (C_{11}), 28.7 (C_8 or C_{10}), 25.1 (C_9), 24.2 (C_8 or C_{10}). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{23}H_{44}O_{17}N$ 606.2609, found 606.2584.

4.2.11. 5-Carboxyethylpentyl 4,6-*O*-*para*-methoxybenzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (26)

Compound **25** (1.452 g, 3.215 mmol) was dissolved in anhydrous acetonitrile (40 mL) under argon. To the solution were added successively: dimethyl *para*-methoxybenzaldehyde acetal (1.11 mL, 6.430 mmol, 2 equiv) and camphorsulfonic acid (0.223 g, 0.964 mmol, 0.3 equiv). After 2 h, a saturated $NaHCO_3$ solution was added and the product was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7:3) to give 1.495 g of **26** (81%) as a white foam. R_f: 0.15 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −35 (*c* 2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.86 (dd, 2H, *J* = 3.1 Hz, *J* = 5.5 Hz, H Ar Phth), 7.72 (dd, 2H, *J* = 3.0 Hz, *J* = 5.2 Hz, H Ar Phth), 7.42 (m, 2H, H Ar), 6.90 (m, 2H, H Ar), 5.53 (s, 1H, H benzylidene), 5.23 (d, 1H, J_{H1-H2} = 8.4 Hz, H_1), 4.59 (m, 1H, H_3), 4.36 (dd, 1H, J_{gem} = 10.3 Hz, J_{H6-H5} = 4.3 Hz, $1H_6$), 4.21 (dd, 1H, J_{H2-H3} = 10.4 Hz, H_2), 4.06 (q, 2H, *J* = 7.0 Hz, CH_2 OEt), 3.84–3.78 (m, 5H, OMe, $1H_6$, $1H_7$), 3.63–3.53 (m, 2H, H_4, H_5), 3.43 (td, 1H, J_{gem} = 9.8 Hz, *J* = 6.4 Hz, $1H_7$), 3.05 (d, 1H, *J* = 3.8 Hz, OH (C_3)), 1.99 (m, 2H, H_{11}), 1.50–1.33 (m, 4H, H_8, H_{10}), 1.22 (t, 3H, *J* = 7.0 Hz, CH_3 OEt), 1.17–1.06 (m, 2H, H_9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3 (CO ester), 160.1 (CO Phth), 134.0–113.0 (C Ar), 101.6 (C benzylidene), 98.7 (C_1), 82.0 (C_4), 69.5 (C_7), 68.5 (C_6), 68.4 (C_3), 66.0 (C_5), 60.0 (CH_2 OEt), 56.6 (C_2), 55.1 (OMe), 33.8 (C_{11}), 28.8 (C_8 or C_{10}), 25.1 (C_9), 24.2 (C_8 or C_{10}), 14.0 (CH_3 OEt). MS DCI+-HRMS *m/z* $[M+1]^+$ calcd for $C_{30}H_{36}NO_{10}$ 570.2339, found 570.2346.

4.2.12. 5-Carboxyethylpentyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (27)

To a solution of 5-carboxyethylpentyl 2-deoxy-2-phthalimido-β-D-glucopyranoside **25** (0.557 g, 1.235 mmol) in anhydrous pyridine (10 mL) were added under argon: DMAP (0.015 g, 0.123 mmol, 0.1 equiv) and TBSCl (0.204 g, 1.358 mmol, 1.1 equiv). The mixture was allowed to stir overnight. The solvent was evaporated and the product was extracted with CH_2Cl_2, washed with HCl (1 M) and neutralised with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 95:5) to give 393 mg of **27** (56%) as syrup. R_f: 0.39 (cyclohexane/EtOAc: 95:5). $[\alpha]_D^{25}$ −26 (*c* 20, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ: 7.70–7.56 (m, 4H, H Phth), 5.03 (d, 1H, J_{H1-H2} = 8.3 Hz), 3.92 (m, 1H), 3.95–3.80 (m, 5H, CH_2 OEt, 3H), 3.64 (m, 1H, $1H_7$), 3.50–3.26 (m, 3H, $1H_7$), 1.85 (dt, 2H, *J* = 2.6 Hz, *J* = 7.5 Hz, H_{11}), 1.28 (m, 4H, H_8, H_{10}), 1.09 (t, 3H, *J* = 7.1 Hz, CH_3 OEt), 1.00 (m, 2H, H_9), 0.79 (s, 9H, CH_3 *t*Bu TBS), 0.00 (s, 3H, CH_3 TBS), −1.77 (s, 3H, CH_3 TBS).

4.2.13. 5-Carboxyethylpentyl 4-*O*-(3,4,6-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (28) and 5-carboxyethylpentyl 3-*O*-(3,4,6-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (29)

To a mixture of **27** (0.190 g, 0.336 mmol), **30** (0.241 g, 0.403 mmol, 1.2 equiv) and 4 Å molecular sieves (0.2 g) in anhydrous CH_2Cl_2 (4 mL), were added under argon: NBS (0.150 g, 0.840 mmol, 2.5 equiv) and TfOH (0.015 mL, 0.168 mmol, 0.5 equiv). After 20 min the mixture was filtered, extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc/acetone: 5:4:1) to give 42 mg of **28** (14%) as a syrup R_f: 0.44 (cyclohexane/EtOAc/acetone: 5:4:1), $[\alpha]_D^{25}$ −4 (*c* 2, $CHCl_3$) and 22 mg of **29** (7%) as a syrup R_f: 0.32 (cyclohexane/EtOAc/acetone: 5:4:1), $[\alpha]_D^{25}$ +13 (*c* 1, $CHCl_3$). The regioselectivity was demonstrated by addition of trichloroacetyl isocyanate and ^1H NMR analysis: **28**: ^1H NMR (400 MHz, $CDCl_3$) δ: 7.89–7.73 (m, 8H, H Ar), 5.85 (dd, 1H, J_{H3-H4} = 9.0 Hz, J_{H3-H2} = 10.6 Hz, H_3), 5.50 (d, 1H, J_{H1-H2} = 8.5 Hz, H_1), 5.14 (d, 1H, $J_{H1'-H2'}$ = 8.5 Hz, $H_{1'}$), 5.09 (dd, 1H, J_{H4-H5} = 9.9 Hz, H_4), 4.36 (m, 2H, H_2, $H_{3'}$), 4.17 (m, 2H, $2H_6$), 4.09–4.04 (m, 3H, CH_2 OEt, $H_{2'}$), 3.99 (m, 1H, H_5), 3.73–3.61 (m, 2H, $H_{4'}$, $1H_7$), 3.47 (dd, 1H, J_{gem} = 11.5 Hz, $J_{H6'-H5'}$ = 1.4 Hz, $1H_{6'}$), 3.36 (m, 2H, $H_{5'}$, $1H_7$), 3.28 (dd, $J_{H6'-H5'}$ = 4.4 Hz, $1H_{6'}$), 2.04 (m, 5H, CH_3 OAc, H_{11}), 1.93 (s, 3H, CH_3 OAc), 1.85 (s, 3H, CH_3 OAc), 1.42 (m, 4H, H_8, H_{10}), 1.22 (t, 3H, *J* = 7.2 Hz, CH_3 OEt), 1.12 (m, 2H, H_9), 0.81 (s, 9H, *t*Bu), −0.11 (s, 3H, CH_3 TBS), −0.15 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.4 (CO ester), 170.4 (CO OAc), 169.9 (CO OAc), 169.4 (CO OAc), 134.6–123.0 (C Ar), 98.6 (C_1), 97.5 ($C_{1'}$), 81.7 ($C_{4'}$), 74.3 ($C_{5'}$), 71.6 (C_5), 70.2 (C_3), 69.9 ($C_{3'}$), 68.8 (C_4), 68.7 (C_7), 62.0 (C_6), 61.4 ($C_{6'}$), 60.0 (CH_2 OEt), 55.9 ($C_{2'}$), 54.5 (C_2), 33.9 (C_{11}), 28.8 (C_8 or C_{10}), 25.7 (*t*Bu), 25.3 (C_9), 24.4 (C_8 or C_{10}), 20.5 (CH_3 OAc), 20.3 (CH_3 OAc), 20.2 (CH_3 OAc), 14.1 (CH_3 OEt), −5.3 (CH_3 TBS), −5.4 (CH_3 TBS). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{48}H_{62}N_2O_{18}SiNa$ 1005.3665, found 1005.3679. Compound **29**: ^1H NMR (400 MHz, $CDCl_3$) δ: 7.70–7.00 (m, 8H, H Ar), 5.58 (dd, 1H, J_{H3-H2} = 10.7 Hz, J_{H3-H4} = 9.0 Hz, H_3), 5.43 (d, 1H, J_{H1-H2} = 8.4 Hz, H_1), 5.11 (dd, 1H, J_{H4-H5} = 9.0 Hz, H_4), 4.87 (d, 1H, $J_{H1'-H2'}$ = 8.5 Hz, $H_{1'}$), 4.58 (dd, 1H, $J_{H3'-H4'}$ = 8.0 Hz, $J_{H3'-H2'}$ = 10.7 Hz, $H_{3'}$), 4.32 (dd, 1H, H_2), 4.25 (m, 2H, $2H_6$), 4.09–4.01 (m, 4H, CH_2 OEt, $H_{2'}$, $1H_{6'}$), 3.97 (ddd, 1H, J_{H5-H6} = 10.3 Hz, J_{H5-H6} = 4.7 Hz, H_5), 3.84 (dd, 1H, J_{gem} = 11.3 Hz, $J_{H6'-H5'}$ = 5.8 Hz, $1H_{6'}$), 3.71 (dt, 1H, J_{gem} = 9.8 Hz, *J* = 6.1 Hz, $1H_7$), 3.56 (dd, 1H, $J_{H4'-H5'}$ = 8.0 Hz, $H_{4'}$), 3.45 (m, 1H, $H_{5'}$), 3.25 (m, 1H, $1H_7$), 2.16 (s, 3H, CH_3 OAc), 2.02 (s, 3H, CH_3 OAc), 1.89 (m, 2H, H_{11}), 1.75 (s, 3H, CH_3 OAc), 1.71 (s, 1H, OH (C_4)), 1.36–1.20 (m, 7H, CH_3 OEt, H_8, H_{10}), 0.95–0.87 (m, 11H, *t*Bu, H_9), 0.10 (s, 3H, CH_3 TBS), 0.09 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3 (CO ester), 170.5 (CO OAc), 169.8 (CO OAc), 169.3 (CO OAc), 134.0–123.1 (C Ar), 98.1 (C_1), 97.7 ($C_{1'}$), 82.0 ($C_{3'}$), 76.4 ($C_{5'}$), 71.8 (C_5), 70.2 (C_3), 69.9 ($C_{4'}$), 68.8 (C_7), 68.6 (C_4), 63.0 ($C_{6'}$), 61.8 (C_6), 60.0 (CH_2 OEt), 55.1 ($C_{2'}$), 54.4 (C_2), 33.9 (C_{11}), 28.7 (C_8 or C_{10}), 25.8 (*t*Bu), 25.1 (C_9), 24.2 (C_8 or C_{10}), 20.5–20.1 (CH_3 OAc), −5.22 (CH_3 TBS), −5.25 (CH_3 TBS). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{48}H_{62}N_2O_{18}SiNa$ 1005.3665, found 1005.3676.

4.2.14. 5-Carboxyethylpentyl 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (32)

To a mixture of **31** (1.000 g, 1.483 mmol), ethyl 6-hydroxy-hexanoate (0.362 mL, 2.225 mmol, 1.5 equiv) and 4 Å molecular sieves (4 g) in anhydrous CH_2Cl_2 (15 mL) were added under argon: NIS (0.667 g, 2.966 mmol, 2 equiv) and TfOH (0.026 mL, 0.296 mmol, 0.2 equiv). The mixture was stirred for 3 h and was filtered, extracted with CH_2Cl_2 and washed with saturated $Na_2S_2O_3$ and $NaHCO_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and

concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8:2) to give 0.873 g of **32** (90%) as a white solid. R_f: 0.34 (cyclohexane/EtOAc: 8:2). $[\alpha]_D^{25}$ −22 (*c* 4, $CHCl_3$). Mp: 90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90–7.30 (m, 9H, H Ar), 5.56 (s, 1H, H benzylidene), 5.24 (d, 1H, J_{H1-H2} = 8.5 Hz, H_1), 4.65 (dd, 1H, J_{H3-H4} = 8.4 Hz, J_{H3-H2} = 10.2 Hz, H_3), 4.40 (dd, 1H, J_{gem} = 10.3 Hz, J_{H6-H5} = 4.6 Hz, $1H_6$), 4.24 (dd, 1H, H_2), 4.09 (q, 2H, *J* = 7.0 Hz, CH_2 OEt), 3.83 (m, 2H, $1H_6$, $1H_7$), 3.66 (td, 1H, J_{H5-H4} = 9.4 Hz, H_5), 3.59 (dd, 1H, H_4), 3.44 (m, 1H, $1H_7$), 2.01 (m, 2H, H_{11}), 1.50–1.38 (m, 4H, H_8, H_{10}), 1.24 (t, 3H, CH_3 OEt), 1.18–1.10 (m, 2H, H_9), 0.63 (s, 9H, *t*Bu TBS), −0.08 (s, 3H, CH_3 TBS), −0.24 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3 (CO ester), 137.0–126.0 (C Ar), 101.8 (C benzylidene), 98.7 (C_1), 82.7 (C_4), 69.5 (C_3), 69.5 (C_7), 68.7 (C_6), 66.2 (C_5), 60.0 (CH_2 OEt), 57.7 (C_2), 33.9 (C_{11}), 28.9 (C_8 or C_{10}), 25.3 (*t*Bu TBS), 25.2 (C_9), 24.3 (C_8 or C_{10}), 17.6 (Cq *t*Bu TBS), 14.1 (CH_3 OEt), −4.2 (CH_3 TBS), −5.4 (CH_3 TBS). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{35}H_{51}N_2O_9Si$ 671.3364, found 671.3356.

4.2.15. 5-Carboxyethylpentyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (33)

To a solution of **32** (0.775 g, 1.185 mmol) in ethyl acetate (9 mL) was added Pd/C (10%) (387 mg, 0.5 g per g of substrate). Vacuum and H_2 were alternated and then the mixture was stirred at room temperature under H_2 for 3 h. The mixture was filtered off through Celite and concentrated to give 670 mg of pure **33** (100%) as a syrup. R_f: 0.39 (cyclohexane/EtOAc: 5:5). $[\alpha]_D^{25}$ −1 (*c* 6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84–7.72 (m, 4H, H Ar), 5.16 (d, 1H, J_{H1-H2} = 8.5 Hz, H_1), 4.40 (dd, 1H, J_{H3-H4} = 8.4 Hz, J_{H3-H2} = 10.4 Hz, H_3), 4.09 (dd, 1H, H_2), 4.05 (q, 2H, *J* = 7.1 Hz, CH_2 OEt), 3.95 (dd, 1H, J_{gem} = 11.8 Hz, J_{H6-H5} = 3.3 Hz, $1H_6$), 3.87 (dd, 1H, J_{H6-H5} = 4.2 Hz, $1H_6$), 3.77 (td, 1H, J_{gem} = 9.8 Hz, J_{H7-H8} = 6.2 Hz, $1H_7$), 3.62 (m, 1H, H_4), 3.50 (m, 1H, H_5), 3.40 (td, 1H, $J_{H7'-H8}$ = 6.4 Hz, $1H_7$), 3.08 (d, 1H, J_{OH-H4} = 3.7 Hz, OH), 1.97 (m, 2H, H_{11}), 1.39 (m, 4H, H_8, H_{10}), 1.20 (t, 3H, CH_3 OEt), 1.07 (m, 2H, H_9), 0.67 (s, 9H, *t*Bu TBS), 0.05 (s, 3H, CH_3 TBS), −0.27 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.4 (CO ester), 134–122 (C Ar), 98.1 (C_1), 75.2 (C_5), 72.6 (C_3), 72.4 (C_4), 69.2 (C_7), 62.2 (C_6), 60.0 (CH_2 OEt), 57.1 (C_2), 33.8 (C_{11}), 28.8 (C_8 or C_{10}), 25.4 (*t*Bu TBS), 25.1 (C_9), 24.2 (C_8 or C_{10}), 17.6 (Cq *t*Bu TBS), 14.0 (CH_3 OEt), −4.1 (CH_3 TBS), −5.2 (CH_3 TBS). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{28}H_{47}N_2O_9Si$ 583.3051, found 583.3043.

4.2.16. 5-Carboxyethylpentyl 3,6-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (34)

To a mixture of **33** (0.554 g, 0.980 mmol), DMAP (0.012 g, 0.098 mmol, 0.1 equiv) in anhydrous pyridine (3 mL) was added under argon TBSCl (0.441 g, 2.941 mmol, 3 equiv). The mixture turned cloudy and was stirred overnight. Concentrated and the product was extracted with CH_2Cl_2, washed with HCl (1 M) and neutralised with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8:2) to give 603 mg of **34** (90%) as syrup. R_f: 0.60 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −9 (*c* 3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.8–7.7 (m, 4H, H Ar), 5.08 (d, 1H, J_{H1-H2} = 8.6 Hz, H_1), 4.35 (dd, 1H, J_{H3-H4} = 7.9 Hz, J_{H3-H2} = 10.5 Hz, H_3), 4.04 (dd, 1H, H_2), 4.00 (q, 2H, *J* = 7.2 Hz, CH_2 OEt), 3.91 (dd, 1H, J_{gem} = 10.6 Hz, J_{H6-H5} = 4.9 Hz, $1H_6$), 3.85 (dd, 1H, J_{H6-H5} = 5.3 Hz, $1H_6$), 3.71 (td, 1H, J_{gem} = 9.8 Hz, J_{H7-H8} = 6.2 Hz, $1H_7$), 3.50 (td, 1H, J_{H4-H5} = J_{H4-H3} = 7.9 Hz, J_{H4-OH} = 2.3 Hz, H_4), 3.44 (m, 1H, H_5), 3.33 (m, 1H, $1H_7$), 3.15 (d, 1H, OH), 1.91 (m, 2H, H_{11}), 1.34 (m, 4H, H_8, H_{10}), 1.15 (t, 3H, CH_3 OEt), 1.04 (m, 2H, H_9), 0.86 (s, 9H, *t*Bu TBS), 0.60 (s, 9H, *t*Bu TBS), 0.06 (s, 3H, CH_3 TBS), 0.06 (s, 3H, CH_3 TBS), 0.01 (s, 3H, CH_3 TBS), −0.28 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.0 (CO ester), 168.0 (CO NPhth), 167.0 (CO NPhth), 133.0–122.0 (C Ar), 97.8 (C_1), 74.6 (C_4), 74.2 (C_5), 72.5 (C_3), 68.7 (C_7), 64.6 (C_6), 59.8 (CH_2 OEt), 56.8 (C_2), 33.7 (C_{11}), 28.7 (C_8 or C_{10}), 25.6 (*t*Bu TBS), 25.3 (*t*Bu TBS), 25.1 (C_9), 24.2 (C_8 or C_{10}), 18.0 (Cq *t*Bu TBS), 17.5 (Cq *t*Bu TBS), 14.0 (CH_3 OEt), −4.2 (CH_3 TBS), −5.4 (CH_3 TBS), −5.6 ($2CH_3$ TBS). MS DCI+-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{34}H_{61}N_2O_9Si_2$ 697.3916, found 697.3922.

4.2.17. 5-Carboxyethylpentyl 4-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-3,6-di-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (35)

To a mixture of **34** (0.177 g, 0.260 mmol), **8** (0.234 g, 0.390 mmol, 1.5 equiv) and 4 Å molecular sieves (200 mg) in anhydrous CH_2Cl_2 (3 mL), was added under argon NIS (0.667 g, 2.966 mmol, 2.0 equiv). The mixture was stirred, cooled down to −25 °C and TfOH (0.002 mL, 0.026 mmol, 0.1 equiv) was added. The mixture was allowed to warm up to 0 °C over 1 h and was then filtered, extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8:2) to give 0.178 g of **35** (61%) as a white solid. R_f: 0.29 (cyclohexane/EtOAc: 7:3). $[\alpha]_D^{25}$ −30 (*c* 1.5, $CHCl_3$). Mp: 87–88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92–7.35 (m, 13H, H Ar), 5.97 (dd, 1H, *J* = 9.5 Hz, *J* = 10.0 Hz, $H_{3'}$), 5.67 (d, 1H, $J_{H1'-H2'}$ = 8.4 Hz, $H_{1'}$), 5.55 (s, 1H, H benzylidene), 5.04 (d, 1H, J_{H1-H2} = 8.5 Hz, H_1), 4.42 (m, 2H, $H_{2'}$, $1H_6$), 4.28 (dd, 1H, J_{H3-H4} = 8.8 Hz, J_{H3-H2} = 10.0 Hz, H_3), 4.06 (m, 4H, CH_2 OEt, H_2, H_4), 3.82 (t, 1H, *J* = 9.9 Hz, $1H_6$), 3.73 (m, 3H, $1H_6$, $H_{4'}$, $H_{5'}$), 3.64 (td, 1H, J_{gem} = 9.8 Hz, J_{H7-H8} = 6.3 Hz, $1H_7$), 3.57 (dd, 1H, J_{gem} = 12.1 Hz, J_{H6-H5} = 2.5 Hz, $1H_6$), 3.31 (td, 1H, $J_{H7'-H8}$ = 6.3 Hz, $1H_7$), 3.11 (d, 1H, *J* = 9.4 Hz, H_5), 2.00 (td, 2H, J_{gem} = 7.4 Hz, $J_{H11-H10}$ = 4.6 Hz, H_{11}), 1.89 (s, 3H, CH_3 OAc), 1.38 (m, 4H, H_8, H_{10}), 1.22 (t, 3H, *J* = 7.0 Hz, CH_3 OEt), 1.08 (m, 2H, H_9), 1.01 (s, 9H, *t*Bu TBS), 0.79 (s, 9H, *t*Bu TBS), 0.25 (s, 3H, CH_3 TBS), 0.18 (s, 3H, CH_3 TBS), 0.15 (s, 3H, CH_3 TBS), −0.37 (s, 3H, CH_3 TBS). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 176.0 (CO), 173.0 (CO), 169.0 (CO), 167.0 (2CO), 134.0–123.0 (C Ar), 101.4 (C benzylidene), 97.8 (C_1), 96.6 ($C_{1'}$), 79.3 ($C_{4'}$), 74.9 (C_5), 74.1 (C_4), 70.7 (C_3), 69.7 ($C_{3'}$), 68.5 ($C_{6'}$), 68.1 (C_7), 65.9 ($C_{5'}$), 61.2 (C_6), 59.9 (CH_2 OEt), 57.5 (C_2), 55.1 ($C_{2'}$), 33.8 (C_{11}), 28.8 (C_8 or C_{10}), 25.7 (2*t*Bu TBS, CH_3 OEt), 25.2 (C_9), 24.2 (C_8 or C_{10}), 20.4 (CH_3 OAc), 18.2 (Cq *t*Bu TBS), 17.5 (Cq *t*Bu TBS), −3.0 (CH_3 TBS), −5.0 (CH_3 TBS), −5.2 (CH_3 TBS), −5.3 (CH_3 TBS). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{57}H_{76}N_2O_{16}Si_2Na$ 1123.4631, found 1123.4657.

4.2.18. 5-Carboxyethylpentyl 4-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (36)

To a solution of **35** (1.000 g, 0.908 mmol) in anhydrous DMF (10 mL) was added under argon ammonium fluoride (0.672 g, 18.16 mmol, 20 equiv). The mixture was stirred and heated at 100 °C overnight. Concentrated and the residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 5:5) to give a 0.666 g of **36** (80%) as white solid. R_f: 0.25 (cyclohexane/EtOAc: 5:5). Mp: 225–226 °C. $[\alpha]_D^{25}$ −26 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93–7.73 (m, 8H, H Ar), 7.45–7.41 (m, 2H, H Ar), 7.35 (m, 3H, H Ar), 5.91 (t, 1H, $J_{H3'-H2'}$ = $J_{H3'-H4'}$ = 9.1 Hz, $H_{3'}$), 5.63 (d, 1H, $J_{H1'-H2'}$ = 7.7 Hz, $H_{1'}$), 5.52 (s, 1H, H benzylidene), 5.15 (d, 1H, J_{H1-H2} = 7.5 Hz, H_1), 4.43–4.32 (m, 3H, $H_{2'}$, $1H_6$, H_3), 4.12–4.02 (m, 3H, CH_2 OEt, H_2), 3.96 (s, 1H, OH C_3), 3.89–3.69 (m, 5H, $H_{4'}$, H_4, $1H_6$, $H_{5'}$, $1H_7$), 3.46–3.34 (m, 3H, H_5, $1H_6$, $1H_7$), 3.20 (m,

1H, $1H_6$), 1.98 (m, 2H, H_{11}), 1.90 (s, 3H, CH_3 OAc), 1.80 (br s, 1H, OH C_6), 1.43–1.33 (m, 4H, H_8, H_{10}), 1.20 (t, 3H, CH_3 OEt), 1.16–1.07 (m, 2H, H_9). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.9 (CO ester), 170.4 (CO Phth), 136.0–126.0 (C Ar), 102.1 (C benzylidene), 100.0 ($C_{1'}$), 98.6 (C_1), 81.8 (C_4 or $C_{4'}$), 79.0 (C_4 or $C_{4'}$), 74.4 (C_5), 70.1 ($C_{3'}$), 69.9 (C_3), 69.8 (C_7), 68.5 ($1C_6$), 66.6 (C_5), 61.1 ($1C_6$), 60.5 (CH_2 OEt), 56.3 (C_2), 55.6 ($C_{2'}$), 34.3 (C_{11}), 29.3 (C_8 or C_{10}), 25.6 (C_9), 24.7 (C_8 or C_{10}), 20.9 (CH_3 OAc), 14.6 (CH_3 OEt). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{45}H_{48}N_2O_{16}Na$ 895.2902, found 895.2883.

4.2.19. 5-Carboxyethylpentyl 3,6-di-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside mixture (37,38)

To a mixture of **35** (0.100 g, 0.114 mmol), **9** (0.163 g, 0.273 mmol, 2.4 equiv) and 4 Å molecular sieves (300 mg) in anhydrous CH_2Cl_2 (1.5 mL) and anhydrous Et_2O (1.5 mL), were added under argon: NIS (0.102 g, 0.456 mmol, 4 equiv) and TfOH (1 μL, 0.011 mmol, 0.1 equiv). The mixture was stirred for 5 min at room temperature and was then filtered, extracted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7:3) to give 177 mg of a syrup containing **37** and **38** (91%) (α/β: 3.5:1 estimated by ^{1}H NMR). R_f: 0.67 (cyclohexane/EtOAc: 5:5).

4.2.20. 5-Carboxyethylpentyl 4-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (39)

Compound **6** (0.650 g, 0.471 mmol) was dissolved in ethanol (10 mL) and hydrazine monohydrate (0.230 mL, 4.713 mmol, 10 equiv) was added. The mixture was stirred at 90 °C overnight. The mixture was allowed to cool down to room temperature, filtered and concentrated. The residue was dissolved in anhydrous pyridine (5 mL) and acetic anhydride (2 mL) was added under argon. The mixture was stirred at room temperature overnight. Concentrated and the crude product was extracted with CH_2Cl_2, washed with an HCl solution (1 M) and neutralised with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 5:5) to give 0.507 g of **39** (89%) as a syrup. R_f: 0.62 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −90 (*c* 6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46–7.25 (m, 25H, H Ar), 6.73 (d, 1H, J_{NH-H2} = 9.0 Hz, NHAc), 5.99 (d, 1H, J_{NH-H2} = 9.1 Hz, NHAc), 5.48 (s, 1H, H benzylidene), 5.26 (d, 1H, $J_{H1'-H2'}$ = 3.4 Hz, $H_{1'}$), 5.14 (t, 1H, $J_{H3''-H2''} = J_{H3''-H4''}$ = 9.9 Hz, $H_{3''}$), 5.00 (d, 1H, J_{gem} = 11.6 Hz, CHPh), 4.88–4.83 (2d, 2H, J_{gem} = 12.1 Hz, J_{gem} = 11.5 Hz, 2CHPh), 4.77–4.68 (m, 3H, 3CHPh), 4.50–4.42 (m, 4H, H_1, $H_{1''}$, 2CHPh), 4.22 (dd, 1H, J_{gem} = 10.4 Hz, J_{H6-H5} = 4.8 Hz, $1H_6$), 4.15–4.06 (m, 5H, H_2, $H_{2'}$, $H_{2''}$, CH_2 OEt), 3.95–3.89 (m, 3H, $H_{5'}$, 2H), 3.84–3.64 (m, 7H, $3H_6$, $1H_7$, $H_{4''}$, 2H), 3.58 (s, 1H), 3.40–3.34 (m, 2H, $1H_7$, 1H), 2.25 (t, 2H, $J_{H11-H10}$ = 7.6 Hz, H_{11}), 2.07 (s, 3H, CH_3 OAc), 2.02 (s, 3H, NHAc), 1.94 (s, 3H, NHAc), 1.61–1.48 (m, 4H, H_{10}, H_8), 1.30 (m, 2H, H_9), 1.25 (t, 3H, *J* = 7.1 Hz, CH_3 OEt), 1.10 (d, 3H, $J_{H6'-H5'}$ = 6.3 Hz, $1H_{6'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.1 (CO), 172.0 (CO), 171.4 (CO), 170.5 (CO), 139.4 (Cq Ar), 139.1 (Cq Ar), 139.0 (Cq Ar), 138.3 (Cq Ar), 137.2 (Cq Ar), 128.0–126.0 (C Ar), 101.8 (C benzylidene), 100.9 ($1C_1$), 100.4 ($1C_1$), 96.6 ($C_{1'}$), 79.3, 78.5 ($C_{4''}$), 78.0, 76.8 ($C_{2'}$), 75.1 (CH_2Ph), 74.8, 74.6, 73.8 (CH_2Ph), 73.4 (CH_2Ph), 73.2, 72.8 (CH_2Ph), 71.7 ($C_{3''}$), 70.1 ($1C_6$), 69.2 (C_7), 68.8 ($1C_6$), 67.2, 66.7, 60.6 (CH_2 OEt), 55.0 ($1C_2$), 51.5 ($1C_2$), 34.6 (C_{11}), 29.5 (C_8 or C_{10}), 25.8 (C_9), 25.0 (C_8 or C_{10}), 23.8 (CH_3 NHAc), 23.5 (CH_3 NHAc), 21.3 (CH_3 OAc), 17.1 ($C_{6'}$), 14.6 (CH_3 OEt). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{67}H_{82}O_{18}N_2Na$ 1225.5460, found 1225.5458.

4.2.21. 5-Carboxypentyl 4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (40)

Compound **39** (0.500 g, 0.415 mmol) was dissolved in THF (5 mL) and NaOH (10 M, 500 μL, 4.150 mmol, 10 equiv) was added. The mixture was stirred at 75 °C overnight. The mixture was allowed to cool down to room temperature, neutralised (IR 120 H^+), filtered and concentrated to give 0.470 g of **40** (100%) as a syrup. R_f: 0.47 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −88 (*c* 2.5, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.24 (m, 1H, NHAc), 7.95 (m, 1H, NHAc), 7.50–7.25 (m, 27H, H Ar), 5.43 (s, 1H, H benzylidene), 5.35 (d, 1H, $J_{H1'-H2'}$ = 3.6 Hz, $H_{1'}$), 5.00–4.77 (m, 3H), 4.69–4.56 (m, 5H, $1H_1$, 4CHPh), 4.43 (d, 1H, *J* = 7.2 Hz, $1H_1$), 4.35 (m, 1H, $H_{5'}$), 4.15 (m, 1H, $1H_6$), 4.10–3.99 (m, 3H, $H_{2'}$, $1H_2$, 1H), 3.94–3.90 (m, 2H, $1H_3$, 1H), 3.84–3.74 (m, 5H, $1H_2$, $2H_6$, 2H), 3.66 (m, 1H), 3.56 (t, 1H, *J* = 10.0 Hz, $1H_6$), 3.45–3.36 (m, 3H, $1H_3$, $1H_4$, 1H), 3.32 (m, 1H), 3.20 (dd, 1H, *J* = 5.0 Hz, *J* = 12.0 Hz, $1H_5$), 2.27 (t, 2H, *J* = 7.4 Hz, H_{11}), 2.01 (s, 3H, CH_3 NHAc), 2.00 (s, 3H, CH_3 NHAc), 1.58 (m, 4H, H_8, H_{10}), 1.37 (m, 2H, H_9, 1H), 1.21 (d, 3H, $J_{H6'-H5'}$ = 6.5 Hz, $1H_{6'}$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.8 (CO), 139.4–126.0 (C Ar), 101.8 (C benzylidene), 101.4 ($1C_1$), 100.8 ($1C_1$), 96.1 ($C_{1'}$), 81.9, 78.8, 78.7, 76.2, 75.7, 75.2 (CH_2Ph), 74.9, 73.6, 73.1 (CH_2Ph), 72.7 (CH_2Ph), 72.2 (CH_2Ph), 71.1, 69.3 ($1C_6$ and C_7), 68.6 ($1C_6$), 66.9 ($C_{5'}$), 66.5 ($1C_5$), 57.1 ($1C_2$), 54.8 ($1C_2$), 34.0 (C_{11}), 29.3 (C_8 or C_{10}), 25.6 (C_9), 24.8 (C_8 or C_{10}), 22.2 (CH_3 NHAc), 22.1 (CH_3 NHAc), 16.1 ($C_{6'}$). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{63}H_{76}N_2O_{17}Na$ 1155.5042, found 1155.5156.

4.2.22. 5-Carboxypentyl 4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside biotin conjugate (41)

Compound **40** (0.400 g, 0.353 mmol) was dissolved in anhydrous DMF (5 mL) under argon with **10** (0.241 g, 0.706 mmol, 2 equiv), DMAP (0.051 g, 0.423 mmol, 1.2 equiv) and EDC (0.134 g, 0.706 mmol, 2 equiv). The solution was stirred for 3 h at 70 °C. EDC (0.067 g, 0.353 mmol, 1 equiv) was added to complete the reaction and after 2 h, concentrated. The residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 9:1) to give 0.282 g of **41** (54%) as a syrup. R_f: 0.33 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −49 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51–7.20 (m, 26H, H Ar, NHAc), 7.03 (d, 1H, *J* = 8.5 Hz, NHAc), 6.46 (t, 1H, *J* = 5.5 Hz, NH), 6.41 (t, 1H, *J* = 5.2 Hz, NH), 6.29 (s, 1H, NH urea), 5.54 (s, 1H, NH urea), 5.47 (s, 1H, H benzylidene), 5.28 (d, 1H, $J_{H1'-H2'}$ = 3.2 Hz, $H_{1'}$), 4.98 (d, 1H, J_{gem} = 11.4 Hz, CHPh), 4.82 (m, 2H, 2CHPh), 4.71–4.64 (m, 3H, 3CHPh), 4.53–4.48 (m, 3H, $2H_1$, CHPh), 4.40 (d, 1H, J_{gem} = 11.6 Hz, CHPh), 4.29 (m, 1H), 4.22 (m, 2H), 4.05 (m, 2H), 3.95 (m, 3H), 3.80–3.75 (m, 6H), 3.61 (t, 1H, *J* = 9.0 Hz), 3.55 (s, 1H), 3.50 (t, 1H, *J* = 8.1 Hz), 3.39–3.30 (m, 3H), 3.24–3.15 (m, 4H), 3.06 (m, 1H), 2.77 (dd, 1H, J_{gem} = 12.7 Hz, $J_{H27-H26}$ = 4.2 Hz, H_{27}), 2.61 (d, 1H, J_{gem} = 12.7 Hz, $1H_{27}$), 2.15 (m, 4H, H_{11}, H_{20}), 1.95 (s, 3H, CH_3 NHAc), 1.94 (s, 3H, CH_3 NHAc), 1.65–1.27 (m, 20H, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}), 1.08 (d, 3H, $J_{H6'-H5'}$ = 6.1 Hz, CH_3 $H_{6'}$). ^{13}C NMR (100 MHz, $CHCl_3$) δ: 173.9 (CO), 173.7 (CO), 172.8 (CO), 170.7 (CO), 164.2 (CO), 139.3–137.5 (Cq Ar), 128.0–126.0 (C Ar), 102.2 (C benzylidene), 101.0 ($1C_1$), 98.8 ($1C_1$), 96.5 ($C_{1'}$), 82.0, 79.2 (2C), 78.2, 76.5, 75.4, 75.2 (CH_2Ph), 75.0, 73.8 (CH_2Ph), 73.4 (CH_2Ph), 72.8 (CH_2Ph), 71.5, 70.9 ($1C_6$), 69.3 ($1C_6$), 68.8 (C_7), 67.1, 66.6 (2C), 62.1 (C_{25} or C_{26}), 60.4 (C_{25} or C_{26}), 57.4, 55.9 (C_{24}), 40.9 (C_{27}), 39.3 (C_{12} or C_{18}), 39.3 (C_{12} or C_{18}), 37.0 (C_{11} or C_{20}), 35.9 (C_{11} or C_{20}), 28.0–25.0 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}, C_{23}), 23.8 (CH_3 NHAc),

23.0 (CH_3 NHAc), 17.1 ($C_{6'}$). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{79}H_{104}N_6O_{18}SNa$ 1479.7026, found 1479.7040.

4.2.23. 5-Carboxypentyl 4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside oxidised biotin conjugate (42)

To a solution of **41** (0.019 g, 0.013 mmol) in anhydrous CH_2Cl_2 (0.5 mL), was added mCPBA (0.007 g, 0.039 mmol, 3 equiv) under argon. The mixture was stirred at room temperature overnight. The mixture was washed with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 9:1) to give 16 mg of **42** (84%) as a syrup. R_f: 0.26 (CH_2Cl_2/MeOH: 9:1). $[\alpha]_D^{25}$ −50 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, CD_3OD) δ: 7.47–7.29 (m, 25H, H Ar), 5.43 (s, 1H, H benzylidene), 5.38 (d, 1H, $J_{H1'-H2'}$ = 3.5 Hz, $H_{1'}$), 4.96 (d, 1H, J_{gem} = 11.2 Hz, CHPh), 4.88–4.81 (m, 3H, 3CHPh), 4.69–4.48 (m, 7H, $1H_1$, 4CHPh, H_{25}, H_{26}), 4.43–4.38 (m, 2H, $1H_1$, $H_{5'}$), 4.13 (dd, 1H, J_{gem} = 10.3 Hz, J_{H6-H5} = 4.9 Hz, $1H_6$), 4.08–3.99 (m, 3H, $1H_2$, 2H), 3.95–3.91 (m, 2H), 3.84–3.74 (m, 6H, $1H_2$, $2H_6$, $1H_7$, 2H), 3.66 (m, 1H), 3.54 (t, 1H, J_{gem} = 10.2 Hz, $1H_6$), 3.43 (m, 2H, $1H_7$, 1H), 3.36–3.28 (m, 2H, $1H_{27}$, 1H), 3.21–3.09 (m, 7H, H_{13}, H_{18}, $1H_{27}$, H_{24}, 1H), 2.23–2.15 (m, 4H, H_{11}, H_{20}), 2.02 (s, 3H, CH_3 NHAc), 2.01 (s, 3H, CH_3 NHAc), 1.87 (m, 1H, $1H_{23}$), 1.77–1.48 (m, 13H), 1.40 (m, 6H), 1.24 (d, 3H, $J_{H6'-H5'}$ = 6.4 Hz, $1H_{6'}$). ^{13}C NMR (100 MHz, CD_3OD) δ: 175.0 (CO), 174.7 (CO), 172.7 (CO), 172.0 (CO), 163.6 (CO urea), 139.5 (Cq Ar), 139.2 (Cq Ar), 138.8 (Cq Ar), 138.7 (Cq Ar), 137.9 (Cq Ar), 128–126 (C Ar), 101.8 (C benzylidene), 101.5 ($1C_1$), 100.8 ($1C_1$), 96.1 ($C_{1'}$), 81.9, 78.8 (2C), 76.1, 75.6, 75.3 (CH_2Ph), 74.8, 73.6, 73.0 (CH_2Ph), 72.7 (CH_2Ph), 72.2 (CH_2Ph), 71.1, 69.4 (C_7), 69.1 ($1C_6$), 68.7 ($1C_6$), 66.9, 66.5, 60.8 (C_{24}), 57.1 ($1C_5$), 55.0 ($1C_5$), 54.6 (C_{25} or C_{26}), 54.2 (C_{27}), 50.0 (C_{25} or C_{26}), 39.2 (C_{13} and C_{18}), 36.1 (C_{11} or C_{20}), 35.6 (C_{11} or C_{20}), 29.3–25.7 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}), 22.4 (CH_3 NHAc), 22.3 (CH_3 NHAc), 21.6 (C_{23}), 16.2 ($C_{6'}$). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{79}H_{104}N_6O_{20}SNa$ 1511.6924, found 1511.6882.

4.2.24. 5-Carboxyethylpentyl 3,6-di-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside (43)

Compounds **37** and **38** (1.030 g, 0.604 mmol) were dissolved in ethanol (15 mL), THF (5 mL) and hydrazine monohydrate (0.293 mL, 6.040 mmol, 10 equiv) was added. The solution was stirred at 90 °C overnight. The mixture was allowed to cool down to room temperature, filtered and concentrated. The residue was dissolved in anhydrous pyridine (10 mL) and acetic anhydride (1.2 mL) was added under argon. The mixture was stirred at room temperature overnight. Concentrated and the crude product was extracted with CH_2Cl_2, washed with an HCl solution (1 M) and neutralised with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (EtOAc) and the diastereoisomers were separated by column chromatography on silica gel (CH_2Cl_2/EtOAc: 5:5) to give 540 mg of **43** (58%) as a syrup. R_f: 0.40 (EtOAc). $[\alpha]_D^{25}$ −65 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.33 (m, 35H, H Ar), 6.47 (br s, 1H, NHAc), 6.22 (br s, 1H, NHAc), 5.48 (s, 1H, H benzylidene), 5.35 (d, 1H, J_{H1-H2} = 2.4 Hz, $1H_1$ fucose), 5.20 (t, 1H, J_{H3-H2} = J_{H3-H4} = 8.7 Hz, $H_{3'}$), 5.03 (m, 2H, 2CHPh), 4.95–4.65 (m, 12H, 10CHPh, $2H_1$), 4.43 (d, 1H, J_{H1-H2} = 5.6 Hz, $1H_1$ fucose), 4.35 (dd, 1H, *J* = 4.5 Hz, *J* = 10.3 Hz), 4.29 (m, 1H), 4.19–4.12 (m, 5H, CH_2 OEt, $1H_2$ fucose, 2H), 4.03–3.98 (m, 7H, $1H_5$ fucose, 6H), 3.88 (m, 1H), 3.78–3.53 (m, 7H, $1H_7$, 6H), 3.36 (m, 1H, $1H_7$), 2.28 (t, 2H, $J_{H11-H10}$ = 7.5 Hz, H_{11}), 2.08 (s, 3H, CH_3 OAc), 1.98 (CH_3 NHAc), 1.94 (CH_3 NHAc), 1.65–1.48 (m, 4H, H_8, H_{10}), 1.36–1.26 (m, 8H, H_9, $1H_6$ fucose, CH_3 (OEt), 1.13 (d, 3H, J_{H6-H5} = 6.3 Hz, $1H_6$ fucose). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.1 (CO COOEt), 171.6 (CO), 170.9 (CO), 170.5 (CO), 139.0–137.0 (Cq Ar), 129.0–126.0 (C Ar), 101.9 (C benzylidene), 101.3 ($1C_1$ fucose), 100.6 ($1C_1$), 98.7 ($1C_1$), 97.4 ($1C_1$ fucose), 80.2, 79.9, 79.1, 78.2, 77.8, 77.2, 76.0, 75.8, 75.4 (CH_2Ph), 75.3 (CH_2Ph), 74.7, 74.4, 74.1 (CH_2Ph), 73.7 (CH_2Ph), 73.4 (CH_2Ph), 73.2 (CH_2Ph), 72.0, 69.2 (C_7), 69.1 ($1C_6$), 67.4 ($1C_6$), 67.3, 67.0, 66.6, 60.6 (CH_2 OEt), 55.7 ($1C_2$), 54.2 ($1C_2$), 34.6 (C_{11}), 29.4 (C_8 or C_{10}), 25.9 (C_9), 25.0 (C_8 or C_{10}), 23.8 (CH_3 NHAc), 23.7 (CH_3 NHAc), 21.3 (CH_3 OAc), 17.4 ($1C_6$ fucose), 17.6 ($1C_6$ fucose), 14.7 (CH_3 OEt). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{87}H_{104}N_2O_{22}Na$ 1551.6978, found 1551.6990.

4.2.25. 5-Carboxypentyl 3,6-di-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside biotin conjuguate (44)

Compound **43** (0.466 g, 0.304 mmol) was dissolved in THF (3 mL) and NaOH (10 M, 300 μL, 3.040 mmol, 10 equiv) was added. The solution was stirred at 75 °C overnight. The mixture was allowed to cool down to room temperature, neutralised (IR 120 H^+), filtered and concentrated. The crude product was dissolved in anhydrous DMF (6 mL) under argon with **10** (0.205 g, 0.608 mmol, 2 equiv), DMAP (0.043 g, 0.360 mmol, 1.2 equiv) and EDC (0.086 g, 0.450 mmol, 1.5 equiv). The mixture was stirred for 1 h at 70 °C. EDC (0.028 g, 0.150 mmol, 0.5 equiv) was added to complete the reaction and after 3 h, concentrated. The residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 9:1) to give 0.273 g of **44** (51%) as a syrup. R_f: 0.24 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −53 (*c* 7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.43–7.23 (m, 35H, H Ar), 6.64 (s, 1H, NHCO), 6.52 (s, 1H, NHCO), 6.32 (s, 1H, NH urea), 5.80 (s, 1H, NH urea), 5.44 (s, 1H, H benzylidene), 5.36 (s, 1H, $1H_1$ fucose), 5.01–4.57 (m, 14H, $1H_1$, $1H_1$ fucose, 12H CHPh), 4.43 (d, 1H, J_{H1-H2} = 5.4 Hz, $1H_1$), 4.30–3.35 (m, 24H), 3.17 (m, 4H, H_{13}, H_{18}), 3.00 (m, 1H, H_{24}), 2.68 (m, 1H, $1H_{27}$), 2.56 (d, 1H, J_{gem} = 12.4 Hz, $1H_{27}$), 2.15 (m, 4H, H_{11}, H_{20}), 1.98 (s, 3H, CH_3 NHAc), 1.94 (s, 3H, CH_3 NHAc), 1.75–1.09 (m, 26H, J_{H6-H5} = 5.8 Hz, J_{H6-H5} = 6.1 Hz, $2H_6$ fucose, H_8, H_9, H_{10}, H_{14}, H_{15}, H_{16}, H_{17}, H_{21}, H_{22}, H_{23}). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{99}H_{126}N_6O_{22}SNa$ 1805.8544, found 1805.8570.

4.2.26. 5-Carboxypentyl 3,6-di-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside oxidised biotin conjuguate (45)

To a solution of **44** (0.230 g, 0.128 mmol) in anhydrous CH_2Cl_2 (5 mL), was added mCPBA (0.066 g, 0.386 mmol, 3 equiv) under argon. The mixture was stirred at room temperature overnight. The mixture was washed with a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol: 8:2) to give 195 mg of **45** (84%) as a white foam. R_f: 0.07 (CH_2Cl_2/methanol: 9:1). $[\alpha]_D^{25}$ −54 (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, CD_3OD) δ: 7.42–7.18 (m, 35H, HAr), 5.45 (d, 1H, J_{H1-H2} = 2.9 Hz, $1H_1$ fucose), 5.39 (s, 1H, H benzylidene), 5.01 (m, 2H, $1H_1$, 1CHPh), 4.96 (d, 1H, J_{H1-H2} = 3.0 Hz, $1H_1$ fucose), 4.93–4.65 (m, 10H, $1H_5$ fucose, 9CHPh), 4.63–4.59 (m, 2H, 2CHPh), 4.48 (m, 2H, H_{25}, H_{26}), 4.36 (d, 1H, *J* = 7.5 Hz), 4.19–3.86 (m, 12H, $3H_6$, $1H_5$ fucose, 8H), 3.82–3.72 (m, 6H, $1H_6$, $1H_7$, 4H), 3.50–3.38 (m, 3H, $1H_7$, 2H), 3.28 (dd, 1H, $J_{H27-H26}$ = 5.8 Hz, J_{gem} = 14.2 Hz, $1H_{27}$), 3.18–3.13 (m, 5H, H_{24}, H_{13}, H_{18}), 3.09 (d, 1H, $1H_{27}$), 2.23–2.15 (m, 4H, H_{11}, H_{20}), 2.02 (s, CH_3 NHAc), 2.00 (s, CH_3 NHAc), 1.86 (m, 1H, $1H_{23}$), 1.76–1.49 (m, 13H), 1.35–1.22 (m, 10H, $1H_6$ fucose, 7H), 1.11 (d,

3H, J_{H6-H5} = 6.3 Hz, $1H_6$ fucose). ^{13}C NMR (100 MHz, CD_3OD) δ: 174.9 (CO), 174.6 (CO), 172.6 (CO), 172.1 (CO), 163.6 (CO urea), 139.6–138.0 (Cq Ar), 129.0–126.5 (C Ar), 101.9 (C benzylidene), 101.6 ($1C_1$), 100.7 ($1C_1$), 97.4 ($1C_1$ fucose), 96.3 ($1C_1$ fucose), 82.4, 79.4, 79.0, 78.8 (2C), 76.6, 75.8, 75.4 (CH_2Ph), 75.2 (CH_2Ph), 74.6, 74.2, 73.5, 73.0 ($2CH_2Ph$), 72.8 (CH_2Ph), 72.3 (CH_2Ph), 71.4, 69.1 (C_7), 69.0 ($1C_6$), 67.0, 66.8, 66.4, 65.3 ($1C_6$), 60.8 (C_{24}), 57.0, 56.1, 54.6 (C_{25} or C_{26}), 54.2 (C_{27}), 50.0 (C_{25} or C_{26}), 39.2 (C_{13}, C_{18}), 36.1 (C_{11} or C_{20}), 35.6 (C_{11} or C_{20}), 29.0–25.0 (C_8, C_9, C_{10}, C_{14}, C_{15}, C_{16}, C_{17}, C_{21}, C_{22}), 22.6 (CH_3 NHAc), 22.1 (CH_3 NHAc), 21.6 (C_{23}), 16.4 ($1C_6$ fucose), 16.0 ($1C_6$ fucose). MS FAB+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{99}H_{126}N_6O_{24}SNa$ 1837.8441, found 1837.8444.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.001.

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