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Synthesis of Le^x-neoglycoconjugate to study carbohydrate–carbohydrate associations and its intramolecular interaction

Jesús M. de la Fuente and Soledad Penadés*

Grupo de Carbohidrato, Instituto de Investigaciones Químicas, CSIC, Isla de la Cartuja, Américo Vespucio s/n, 41092 Sevilla, Spain

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Abstract—A straightforward synthetic strategy for the preparation of the Le^x neoglycoconjugate (11,11′-dithio bis[undecanyl-β-D-galactopyranosyl-(1→4)-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside]) and methyl Le^x glycoside starting from the same trisaccharide donor is reported. This donor represents a very useful precursor for the construction of Le^x derivatives. The Le^x neoglycoconjugate has allowed the preparation of polyvalent gold glyconanoparticles and self-assembled monolayers on gold. ^{1}H NMR of the Le^x neoglycoconjugates suggests an intramolecular Le^x–Le^x *cis*-interaction in water, which is destroyed by addition of methanol.

1. Introduction

The Lewis X (Le^x) antigen is the terminal trisaccharide moiety of numerous cell surface glycolipids and glycoproteins which are involved in selectin-mediated cell–cell adhesion and recognition processes.[1] In a mouse model of pre-implantation embryo, differential expression of 'stage-specific embryonic antigen 1' (SSEA-1) was observed at the 8–16 cell (morula) stage, which correlates approximately in time with the onset of compaction, and declines rapidly after compaction, being restricted to the inner cell mass of the blastocyst.[2] SSEA-1 was identified as Lewis X trisaccharide.[3,4] Thus, the involvement of the Le^x antigen in compaction, the first cell adhesion event of ontogenic development, was proposed.[5] Based on the observation that a homotypic Le^x–Le^x interaction[5] may play a crucial role in morula compaction process, Hakomori et al.[6] have advocated the concept of Ca^{2+}-mediated carbohydrate–carbohydrate interaction as a new biological mechanism for cell adhesion and recognition.[7] Characteristic features of this interaction are strong dependency on divalent cations, high specificity and low affinity. Although the existence of this interaction has now been accepted, the mechanism has not yet been clarified owing to the difficulty of analysing weak affinity interactions. The understanding and control of interactions between carbohydrates face two main challenges. One has its origin in the low affinity that characterizes these interactions. Nature overcomes this problem by a polyvalent presentation of ligands and receptors at the cell surface.[8] The other problem arises from the difficulty in obtaining chemically well-defined glycoconjugates from natural sources. Attempts to identify and quantify carbohydrate–carbohydrate interactions in solution with monomeric ligands were unsuccessful.[9] Multivalent carbohydrate presentation using liposomes,[10,11] polymers[12] and neoglycoproteins[13] has recently been used to obtain information on this interaction. Polyvalence seems to be mandatory to increase the degree of this interaction.

Our laboratory is developing an integrated methodology to obtain chemical tools for studying the Ca^{2+}-mediated carbohydrate self-interactions (Fig. 1). The elusive nature of these interactions makes it necessary to input data from different methodologies to obtain a complete picture of the mechanisms involved. Our integrated approach is based on self-assembled monolayers (SAM), by which neoglycoconjugates of natural significant oligosaccharides are attached to two- (2D) or three- (3D) dimensional gold surfaces, creating highly polyvalent arrays of carbohydrates with well-defined chemical composition. The 2D self-assembled monolay-

* Corresponding author. Tel.: +34 954489561; fax: +34 954460565; e-mail: penades@cica.es

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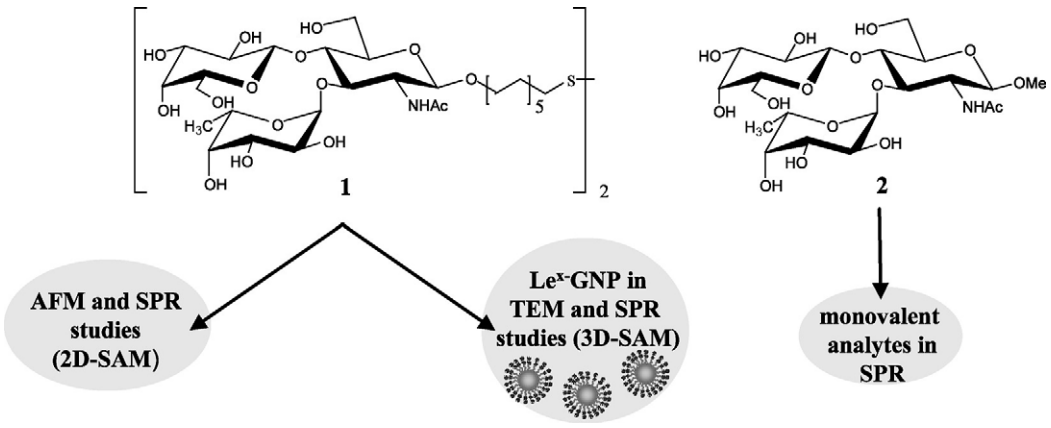


Figure 1. Le^x-neoglycoconjugate **1** and methyl Le^x glycoside **2** and their uses as polyvalent (SAMs) and monovalent analytes.

ers on gold is a strategy that has already been used by different groups to study carbohydrate–protein interactions.[14,15] Our 3D polyvalent system is based on gold nanoclusters functionalised with carbohydrate antigens (glyconanoparticles, GNP).[16,17] Following this strategy we have demonstrated, by using transmission electron microscopy (TEM), the selective ability of the Le^x determinant for self-recognition in calcium-containing aqueous solutions.[16] Well defined 2D-SAM of Le^x determinant have also been used to determine, by an atomic force microscopy (AFM) study, the adhesion forces between individual Le^x molecules.[18] Furthermore, the combination of SAMs of alkanothiolates on gold presenting Le^x epitopes as the substrate, gold glyconanoparticles as the analyte and detection by surface plasmon resonance (SPR) has allowed us to quantify the kinetics of the putative Ca^{2+}-mediated Le^x–Le^x self-interaction.[19]

We report herein the total synthesis of Le^x neoglycoconjugate **1**, the molecule used in our previous studies, by an efficient and stereospecific route based on previously reported two-stage activation procedure for oligosaccharide construction.[20,21] The synthesis of the trisaccharide Le^x has represented some of the most complex synthetic targets.[22–27] The neoglycoconjugate **1** was synthesized with a linker ending in a thiol group, to allow us to attach it to gold surfaces for the preparation of polyvalent glyconanoparticles of Le^x (3D-SAM),[16,17] and self-assembled monolayers (2D-SAM).[18,19] Self assembled monolayers of alkanethiolates on gold are structurally well-organized substrates that provide excellent model surfaces for studies in biology.[14,15,28] The methyl Le^x glycoside **2** was also prepared as a monovalent ligand.

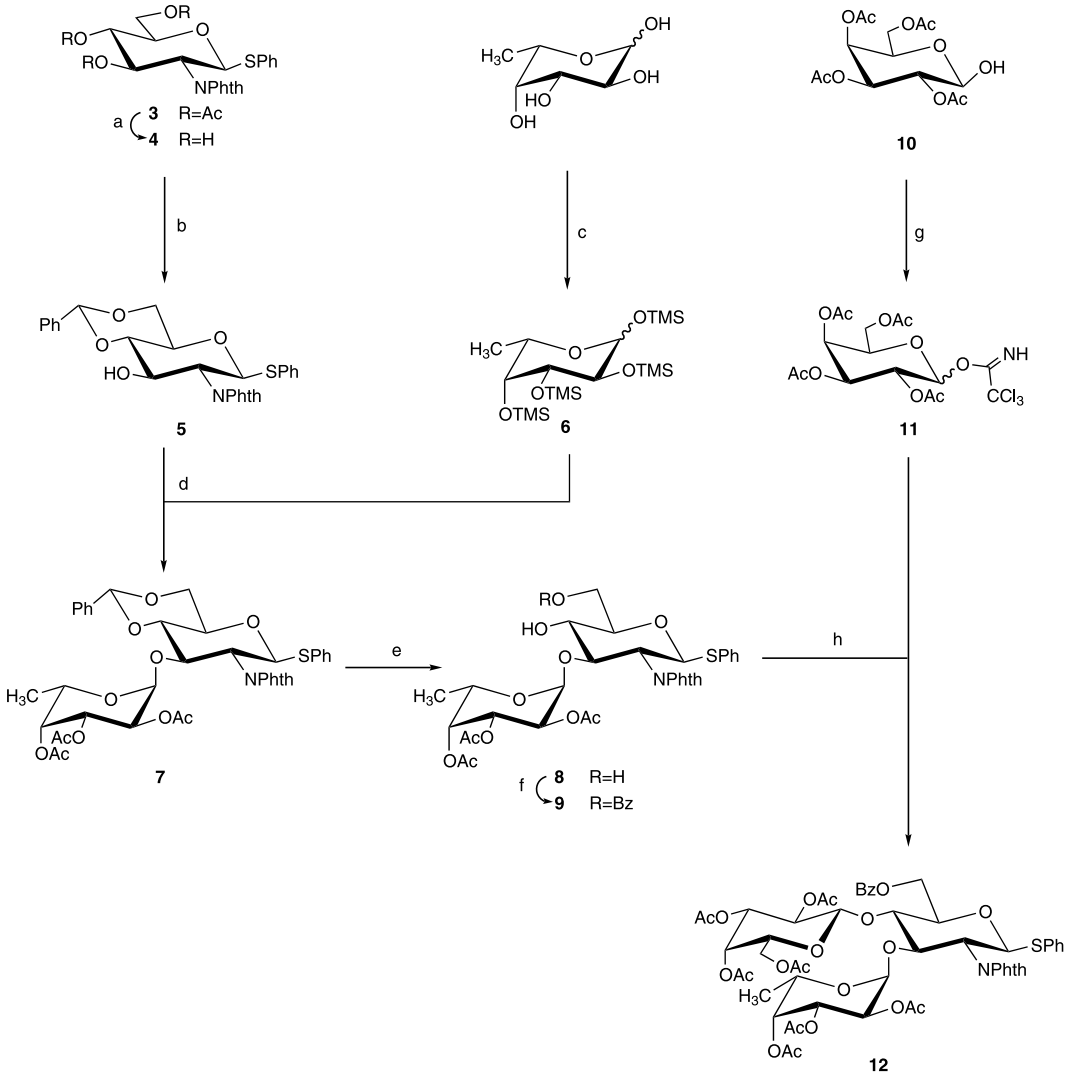
2. Results and discussion

The chemical synthesis of 11,11′-dithio bis[undecanyl-β-D-galactopyranosyl-(1→4)-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside] **1** and methyl β-D-galactopyranosyl-(1→4)-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside **2** is shown in Schemes 1 and 2. Both molecules have been obtained from the trisaccharide intermediate **12** using the thiophenyl glycosylation method.[29]

For the preparation of **12** a previous successful strategy for construction of Le^x epitopes[20,21]—attachment of the fucosyl residue to the 3-hydroxy group of the glucosamine moiety followed by introduction of the galactosyl residue to the 4-hydroxy group of the disaccharide obtained in the first glycosylation reaction—was envisaged.

To this end, D-glucosamine was transformed into the known *N*-phthalimide (Phth) protected thiophenyl (SPh) peracetylated derivative **3** (Scheme 1).[30–32] In order to block glycosylation at the 4- and 6-positions, de-*O*-acetylation with MeONa–MeOH[33] to give **4**, followed by 4,6-*O*-benzylidenation with benzaldehyde dimethyl acetal[34] were performed providing the desired starting material **5** in good yield. For the fucosylation of **5**, a method described by Hindsgaul at al.[35] was chosen because of the simplicity of preparation of the donor **6** and the unique chromatographic properties of the reaction products, which permit facile separation of the desired glycoside and recovery of the unreacted acceptor on silica gel. Reaction of L-fucopyranose with chlorotrimethylsilane (TMS-Cl) and triethylamine (TEA) in *N*,*N*-dimethylformamide (DMF) gave **6**.[35] Reaction of **6** with iodotrimethylsilane (TMS-I) in CH_2Cl_2 resulted in the quantitative formation of the iodo-fucopyranoside that was not isolated. The protected disaccharide **7** was prepared by addition of **6** and 2,6-di-*tert*-butylpyridine to the iodo-fucopyranoside, deprotection of the TMS groups using MeOH and subsequent acetylation, obtaining a good yield (80%) in four steps.

Deprotection of the benzylidene groups in **7** with ethanethiol using $BF_3{\cdot}Et_2O$ as catalyst dramatically accelerated the reaction to form **8**. Selective benzoyl-protection[36] of the primary alcohol group with benzoyl cyanide and a catalytic amount of triethylamine at −40°C gave the desired 4-*O*-unprotected, 6-*O*-benzoyl-protected disaccharide **9** in good yields (95%). The galactosylation of **9** with the already described galacto-



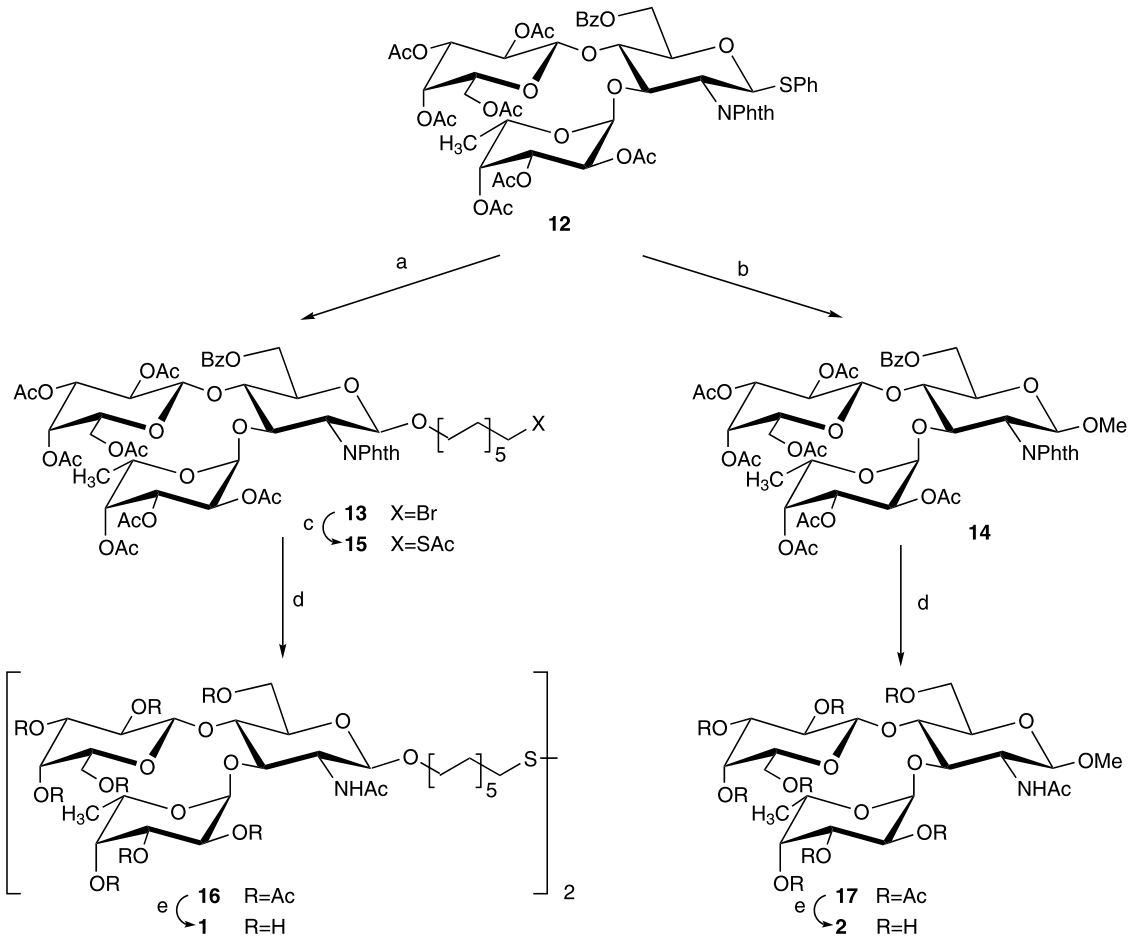
Scheme 1. *Reagents and conditions*: (a) MeOH/MeONa, quantitative; (b) benzylidene dimethyl acetal, PTSA cat.; CH_3CN, 84%; (c) ClTMS, TEA, DMF, 90%; (d) i. TMS-I, CH_2Cl_2; ii. **5**, 2,6-di-*tert*-butylpyridine; iii. MeOH; iv. Ac_2O, py, 80%, four steps; (e) EtSH, $BF_3{\cdot}Et_2O$ cat., CH_2Cl_2, 83%; (f) BzCN, CH_3CN, NEt_3 cat., −40°C, 95%; (g) Cl_3CCN, Cs_2CO_3, CH_2Cl_2, 95%; (h) **9**, TMSOTf (0.1 equiv.), Et_2O:CH_2Cl_2 (5:1), 0°C, 80%.

syl donor **11**,[37] was carried out at 0°C using TMSOTf (0.1 equiv.) as promoter, and ether:CH_2Cl_2 (5:1) as solvent,[20] thus affording the desired protected Le^X-trisaccharide building block **12** in 80% yield.

Trisaccharide **12** is the donor in the synthesis of neoglycoconjugate **1** as well as methyl glycoside **2**. A standard protocol for glycosylation in CH_2Cl_2 in the presence of NIS and triflic acid at −60°C was used.[29] As acceptor, 11-bromo undecanol and MeOH were chosen (Scheme 2) to obtain **13** and **14**, respectively, in very good yields. Replacement of the bromide group in **13** by the thioacetate group could be carried out with KSAc, and $(NBu^t)_4I$ in butanone, yielding the corresponding neoglyconjugate **15** in quantitative yield.

Removal of the acyl and phthalimide groups in **14** and **15** by treatment with ethylendiamine in 2-buthanol at 90°C was quite straightforward.[38] Peracetylation of the deprotected trisaccharides afforded **16** and **17**. Using Zemplén conditions,[39] the unprotected neoglycolipid-Le^x **1** and methyl Le^x glycoside **2** were obtained in high overall yields. The β-anomeric protons of both compounds could be assigned by ^1H NMR spectroscopy, thus confirming the configurations at the anomeric centers.

Conjugates **1** and **2** are water and methanol soluble. Neoglycolipid **1** was isolated as a disulfide derivative. A significant fact is the difference between the ^1H NMR spectra of **1** in D_2O and in CD_3OD (Fig. 2). The spectra of **1** in D_2O (Fig. 2a) shows a broadening for all signals similar to that observed in the spectrum of Le^x-glyconanoparticles (Fig. 2e). In the case of glyconanoparticles, this line broadening can be attributed to the slowly rotating macromolecules in solution. However, in the case of **1**, an intramolecular *cis*-interaction between the Le^x molecules in water can be suggested as being responsible for the broadening. The intramolecular nature of this interaction is supported by the persis-



Scheme 2. *Reagents and conditions*: (a) 11-Bromo-undecanol, NIS, TfOH, CH_2Cl_2, –60°C, 80%; (b) NIS, TfOH, CH_2Cl_2; MeOH, –60°C, 98%; (c) SAcK, NBu_4I cat., butanone, 60°C quantitative; (d) i. ethylenediamine, 2-butanol; ii. Ac_2O, py, 80–60%; (e) MeOH, MeONa, 90%.

tence of the signal broadening even at highly diluted water solution. Addition of increasing amounts of CD_3OD to the D_2O solution of **1** abolished this self-interaction. Some well-resolved signals appear already in CD_3OD/D_2O (1:1) solution (Fig. 2b) and in 65% CD_3OD/D_2O solution all signals are well-resolved in the spectrum, indicating that hydrophobic interactions are involved (Fig. 2c). The tendency of the Le^x disulfide **1** to interact intramolecularly in water cannot exclusively be attributed to the hydrophobicity of the aliphatic chain, but rather to specific interactions between carbohydrate moieties. This is supported by the lack of broadening in the carbohydrate signals of the corresponding *lactose*-disulfide neoglycoconjugate observed in the ^1H NMR spectrum in water (Fig. 2f).[40] In this case the disulfide bond adopts the usual *trans* conformation keeping the lactose moieties in *trans* position.

In conclusion, we have shown that the thiophenyl trisaccharide **12** is a very good precursor for the construction of the Le^x neoglycoconjugate and methyl Le^x glycoside. In addition, the tendency of Le^x trisaccharide to self-interact in a *cis*-intramolecular manner has been suggested by NMR spectroscopy, supporting the capacity of this antigen to establish homotypic carbohydrate–carbohydrate interactions.

3. Experimental

3.1. General procedures

3.1.1. Materials and methods. TLC analysis was performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulphuric acid/ethanol (1/9, v/v) followed by heating at over 200°C. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm; 0.2–0.063 mm; 0.040–0.015 mm; Merck). Optical rotations were determined with a Perkin–Elmer 341 polarimeter. ^1H and ^{13}C NMR spectra were acquired on Bruker DPX-300, DRX-400 and DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to D_2O. Elemental analyses were performed with a Leco CHNS-932 apparatus, after drying analytical samples over phosphorous pentoxide for 24 h. Mass spectra were recorded with a MALDI-TOF GSG System spectrometer. Samples of the products were dissolved in MeOH at mM concentration and 2,5-dihidroxybenzoic acid was used as matrix.

3.1.2. Titration with CD_3OD. A D_2O solution of **1** (21 mM) was prepared. CD_3OD (1 mL) was added in portions via microsyringe (5×20 μL, 2×50 μL, 8×100

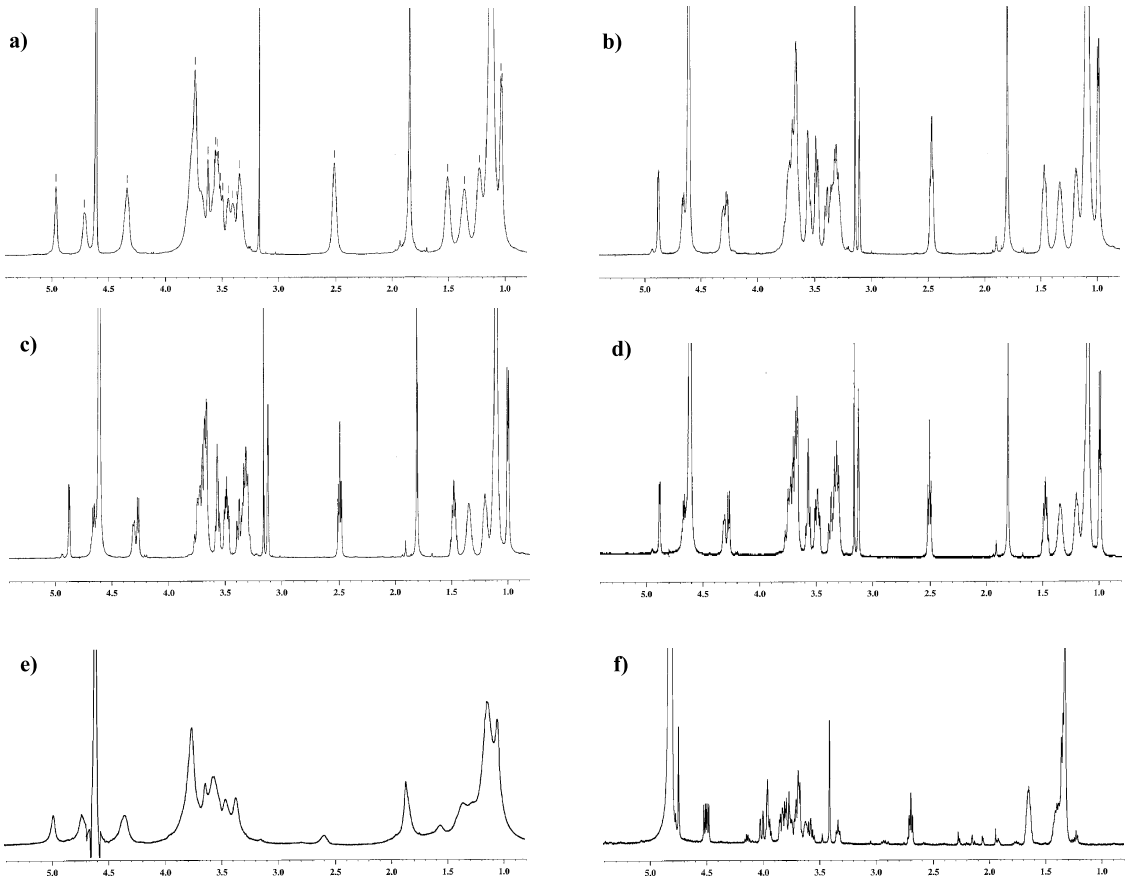


Figure 2. ^{1}H NMR spectra of: (a) **1** in D_2O; (b) **1** in CD_3OD/D_2O (1:1); (c) **1** in 65% CD_3OD/D_2O; (d) **1** in CD_3OD; (e) glyconanoparticles of **1** in D_2O; (f) *lactose*-neoglycoconjugate in D_2O.

μL). The ^{1}H NMR spectrum of each solution was recorded, and the signal broadening was observed. The temperature was kept constant at 298±1 K.

3.2. Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 3

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[30,31] (8.63 g, 18.1 mmol, 1 equiv.) was dissolved in dry dichloromethane (80 mL), and to this solution thiophenol (2.2 mL, 21.7 mmol, 1.2 equiv.) and boron trifluoride etherate (11.5 mL, 90.5 mmol, 5 equiv.) were added. When the reaction was complete (TLC, AcOEt:hexane, 1:1), the solution was washed with saturated aqueous sodium hydrogencarbonate and then with water before being dried with anhydrous sodium sulfate. Removal of the solvent afforded a light yellow syrup which crystallized on treatment with ethanol to afford **3** as a yellow solid (6.52 g, 69%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90–7.70 (m, 4H, NPhth), 7.50–7.20 (m, 5H, SPh), 5.82 (t, 1H, $J=9.6$ Hz), 5.74 (d, 1H, $J=10.5$ Hz, H-1), 5.16 (t, 1H, $J=9.8$ Hz), 4.37 (t, 1H, $J=10.2$ Hz), 4.31 (dd, 1H, $J=12.0$, 4.8 Hz), 4.23 (dd, 1H, $J=12.3$, 2.3 Hz), 4.00–3.90 (m, 1H, H-5), 2.13, 2.06, 1.84 (s, 9H, 3 OAc).

3.3. Phenyl 2-deoxy-2-phthalimide-1-thio-β-D-glucopyranoside, 4

Compound **3** (6.51 g, 12.35 mmol, 1 equiv.) was added to a solution of MeONa in MeOH (1N, 90 mL) and the mixture was stirred at room temperature for 3 h. When the reaction was complete (TLC, AcOEt:hexane, 1:1), amberlite IR-120 (H^+) was added until the medium was neutral. The mixture was filtered and concentrated to give **4** as an amorphous solid (5.24 g, quantitative); ^{1}H NMR (300 MHz, CD_3OD): δ 8.00–7.80 (m, 4H, NPhth), 7.50–7.20 (m, 5H, SPh), 5.59 (d, 1H, $J=10.5$ Hz, H-1), 4.27 (dd, 1H, $J=10.2$, 8.1 Hz), 4.11 (t, 1H, $J=10.2$ Hz), 3.94 (m, 1H), 3.76 (dd, 1H, $J=11.7$, 5.1 Hz), 3.50–3.30 (m, 2H).

3.4. Phenyl 4,5-benzyliden-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 5

Compound **4** (5.15 g, 12.84 mmol, 1 equiv.) was dissolved in acetonitrile (100 mL), and to this solution was added benzaldehyde dimethyl acetal (3.86 mL, 25.69 mmol, 2 equiv.) and a catalytic amount of *p*-toluensulfonic acid. The reaction was kept under an argon atmosphere and room temperature for 2 h. When the reaction was complete (TLC, AcOEt:hexane, 1:1), tri-

ethylamine was added until neutral medium. The solution was concentrated and flash chromatography of the residue (AcOEt:hexane, 1:2) gave **5** as a white solid (5.18 g, 86%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00–7.70 (m, 4H, NPhth), 7.60–7.20 (m, 10H, SPh, CH-Ph), 5.72 (d, 1H, $J=10.5$ Hz, H-1β), 5.59 (s, 1H, CH-Ph), 4.67 (td, 1H, $J=9.6$, 3.1 Hz, H-2), 4.44 (dd, 1H, $J=10.2$, 4.2 Hz), 4.36 (t, 1H, $J=10.2$ Hz), 3.86 (t, 1H, $J=9.9$ Hz), 3.75 (td, 1H, $J=9.4$, 4.5 Hz), 3.64 (t, 1H, $J=9.0$ Hz), 2.45 (d, 1H, $J=3.0$ Hz, OH); MALDI-TOF: calcd for $C_{27}H_{23}NO_6S$ 489.1, found m/z 512.2 ([M+Na]$^+$), 528.9 ([M+K]$^+$). Elemental analysis: found C, 66.22; H, 4.99; N, 2.72; calcd for $C_{27}H_{23}NO_6S$ (489.1) C, 66.26; H, 4.70; N, 2.86%.

3.5. Pertrimethylsilyl-fucose, 6

L-Fucose (1 g, 6.09 mmol, 1 equiv.) and triethylamine (4.4 mL, 31.55 mmol, 5.18 equiv.) were dissolved in dry DMF (30 mL). Chloride trimethylsilyl (4 mL, 31.55 mmol, 5.18 equiv.) was added at 0°C, and the reaction was stirred at room temperature for 4 h (TLC, AcOEt:hexane, 1:1). The solution was diluted with pentane (100 mL), and extracted with cooled water (3×30 mL). The organic layer was dried (anhydrous sodium sulfate), filtered and concentrated to give **6** as a colourless syrup (2.48 g, 90%).

3.6. Phenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-4,5-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 7

To a solution of **6** (0.65 g, 1.45 mmol, 3 equiv.) in dry dichloromethane (10 mL), ITMS (0.21 mL, 1.45 mmol, 3 equiv.) was added at room temperature and stirred for 20 min. A solution of **5** (0.26 g, 0.53 mmol, 1.1 equiv.) and 2,6-di-*tert*-butylpyridine (0.33 mL, 1.45 mmol, 3 equiv.) in dry dichloromethane (15 mL) was added and the resulting mixture was stirred at room temperature. When the reaction was complete (TLC, AcOEt:hexane, 1:1), methanol (30 mL) was added, and the solution was stirred for 30 min. The mixture was neutralized with triethylamine, and concentrated to give a syrup. Acetic anhydride (0.8 mL) and pyridine (1.6 mL) were added under cooling to the residue. The mixture was stirred at room temperature and then poured into ice-water. The emulsion was extracted with AcOEt (3×20 mL). The extract was dried and concentrated. The reaction was followed by TLC (AcOEt:hexane, 2:1, 3 elution). Flash chromatography of the residue (AcOEt:hexane, 1:4) gave **7** as an amorphous solid (0.32 g, 80% for four steps); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90–7.70 (m, 4H, NPhth), 7.50–7.10 (m, 10H, SPh, CH-Ph), 5.57 (s, 1H, CH-Ph), 5.57 (d, 1H, $J=10.5$ Hz, H-1), 5.24 (dd, 1H, $J=10.8$, 2.7 Hz), 5.01 (d, 1H, $J=2.7$ Hz), 4.90–4.70 (m, 3H), 4.50–4.40 (m, 2H), 4.33 (m, 1H, H-5′), 3.90–3.70 (m, 3H), 2.01, 2.00, 1.92 (s, 9H, 3 OAc), 0.49 (d, 3H, $J=6.3$ Hz, H-6′); MALDI-TOF: calcd for $C_{39}H_{39}NO_{13}S$ 761.1, found m/z 784.4 ([M+Na]$^+$), 800.7 ([M+K]$^+$). Elemental analysis: found C, 59.19; H, 5.14; N, 1.98; calcd for $C_{39}H_{39}NO_{13}S\cdot 2H_2O$ (797.1) C, 58.72; H, 5.40; N, 1.76%.

3.7. Phenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 8

Compound **7** (1.49 g, 1.95 mmol, 1 equiv.) was dissolved in dry dichloromethane (100 mL). EtSH (0.8 mL, 10.5 mmol, 5 equiv.) and a catalytic amount of $BF_3\cdot Et_2O$ were added. The solution was stirred at room temperature. When the reaction was complete (TLC, AcOEt:hexane, 1:1), the solution was washed with a saturated aqueous sodium hydrogencarbonate solution and then with water before being dried with anhydrous sodium sulfate. The mixture was concentrated and flash chromatography of the residue (AcOEt:hexane, 1:2) gave **8** as a syrup (1.09 g, 83%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00–7.70 (m, 4H, NPhth), 7.50–7.10 (m, 5H, SPh), 5.59 (d, 1H, $J=9.6$ Hz, H-1), 5.29 (d, 1H, $J=1.8$ Hz, H-4′), 5.20–5.00 (m, 3H, H-1′, H-2′, H-3′), 4.50 (m, 1H, H-5′), 4.50–4.30 (m, 3H, H-2), 3.99 (dd, 1H, $J=11.7$, 2.55 Hz, H-6a), 3.87 (dd, 1H, $J=11.9$, 3.9 Hz, H-6b), 3.80–3.50 (m, 2H, H-5), 2.12, 2.05, 1.89 (s, 9H, 3 OAc), 1.16 (d, 3H, $J=6.6$ Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.3, 170.0, 134.7, 132.9, 132.0, 129.4, 128.6, 124.6, 123.8, 97.9, 83.8, 82.7, 79.7, 71.4, 71.2, 67.8, 66.6, 63.0, 60.8, 54.1, 31.4, 21.0, 19.8, 16.3; MALDI-TOF: calcd for $C_{32}H_{35}NO_{13}S$ 673.1, found m/z 695.9 ([M+Na]$^+$), 712.2 ([M+K]$^+$). Elemental analysis: found C, 55.77; H, 5.25; N, 1.98%; calcd for $C_{32}H_{35}NO_{13}S\cdot 1H_2O$ (691.1) C, 55.56; H, 5.35; N, 1.98%.

3.8. Phenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→3)-6-*O*-benzoyl-2-deoxy-2-phthalimide-1-thio-β-D-glucopyranoside, 9

Compound **8** (1.13 g, 1.68 mmol, 1 equiv.) was dissolved in dry acetonitrile (75 mL) under an argon atmosphere at −40°C. Benzoyl cyanide (0.22 mL, 1.85 mmol, 1.1 equiv.) and a catalytic amount of triethylamine were added. The reaction was stirred at −40°C. The reaction was monitored by TLC (AcOEt:hexane, 1:1). Methanol was added three times to remove generated HCN and the mixture was concentrated. Flash chromatography of the residue (AcOEt:hexane, 1:2) gave **9** as an white solid (1.24 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15–7.00 (m, 14H, SPh, Bz, NPhth), 5.55 (d, 1H, $J=10.2$ Hz, H-1), 5.27 (bs, 1H, H-4′), 5.16 (dd, 1H, $J=10.5$, 3.0 Hz, H-3′), 5.10–5.00 (m, 2H, H-1′, H-2′), 4.80–4.60 (m, 2H, H-6), 4.50 (m, 1H, H-5′), 4.43–4.30 (m, 2H, H-3, H-2), 4.25 (s, 1H, OH), 3.90–3.80 (m, 1H, H-5), 3.70–3.50 (m, 1H, H-4), 2.09, 2.04, 1.87 (s, 9H, 3 OAc), 1.11 (d, 3H, $J=6.6$ Hz, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 170.3, 170.0, 167.1, 134.8, 133.7, 133.1, 132.1, 130.3, 130.2, 129.2, 128.9, 128.4, 124.5, 97.8, 83.8, 82.1, 71.2, 70.9, 67.9, 67.8, 66.5, 64.3, 54.1, 31.4, 21.0, 19.9, 16.1; MALDI-TOF: calcd for $C_{39}H_{39}NO_{14}S$ 777.1, found m/z 800.4 ([M+Na]$^+$), 817.0 ([M+K]$^+$). Elemental analysis: found C, 58.95; H, 5.39; N, 1.83; calcd for $C_{39}H_{39}NO_{14}S\cdot 1H_2O$ (795.1) C, 58.86; H, 5.16; N, 1.76%.

3.9. 2,3,4,6-Tetra-*O*-acetyl-D-galactopyranose, 10

D-Galactose pentaacetate (5 g, 12.8 mmol, 1 equiv.) was dissolved in DMF (50 mL). Hydrazine acetate (1.42 g, 15.4 mmol, 1.2 equiv.) was added, and was stirred at 50°C for 45 min. The reaction was monitored by TLC (hexane:EtOAc, 1:1). The mixture was diluted with ethyl acetate (50 mL) and washed with brine. The organic layer was dried over anhydride sodium sulfate, filtered and concentrated to give **10** as a white solid (4 g, 90%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.50–5.30 (m, 3H), 5.20–5.00 (m, 2H), 4.69 (d, 0.4H, $J=6.6$ Hz, H-1β), 4.43 (m, 1H, H-4α), 4.10–4.00 (m, 3H, H-5α), 4.00–3.90 (m, 0.4H, H-5β), 3.00–2.70 (m, 1H), 2.11, 2.06, 2.01, 1.96 (s, 12H, 4 OAc).

3.10. Trichloroacetimidate 2,3,4,6-tetra-*O*-acetyl-D-galactopyranoside, 11

Compound **10** (9.52 g, 27.4 mmol, 1 equiv.) was dissolved in dry dichloromethane (100 mL) under an argon atmosphere and treated with Cl_3CCN (27.5 mL, 274 mmol, 10 equiv.) and cesium carbonate (9.82 g, 30.14 mmol, 1.1 equiv.) and the mixture was stirred at room temperature. When the reaction was complete (TLC, AcOEt:hexane, 1:1) the mixture was filtered over Celite, and was concentrated to give **11** as an amorphous solid (12.82 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (s, 1H, NH), 6.69 (d, 1H, $J=3.3$ Hz, H-1α), 5.77 (dd, 1H, $J=3$, 1.2 Hz, H-4), 5.63 (dd, 1H, $J=9.3$, 3.0 Hz), 4.79 (t, 1H, $J=6.0$ Hz), 4.60–4.40 (m, 2H), 3.46 (m, 1H), 2.79, 2.67, 2.67, 2.66 (s, 12H, 4 OAc).

3.11. Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 12

A mixture of **9** (3.47 g, 4.47 mmol, 1 equiv.) and **11** (6.61 g, 13.41 mmol, 3 equiv.) was dissolved in ether:dichloromethane (5:1 v/v, 120 mL) at 0°C. TMSOTf (0.08 mL, 0.45 mmol, 0.1 equiv.) was added and the mixture was stirred at 0°C. The reaction was monitored by TLC (AcOEt:hexane, 1:2, 3 elutions). More donor **11** solution in ether (6.61 g, 13.41 mmol, 3 equiv.) was added when this disappeared in the reaction. Triethylamine was added, the mixture was concentrated. Flash chromatography of the residue (AcOEt:hexane, 1:4) gave **12** as an amorphous solid (3.96 g, 80%); $[\alpha]_D^{23}$ −25.3 ($c=1$, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.03–8.01 (m, 2H, Bz), 7.83–7.75 (m, 4H, NPhth), 7.62–7.49 (m, 3H, Bz), 7.27–6.98 (m, 5H, SPh), 5.44 (d, 1H, $J=10.5$ Hz, H-1), 5.34 (m, 2H), 5.10 (m, 2H, H-2″), 4.93 (m, 4H, H-6a, H-5′, H-1′), 4.81 (dd, 1H, $J=11.0$, 4.0 Hz.), 4.76 (t, 1H, $J=9.5$ Hz, H-3), 4.62 (d, 1H, $J=8$ Hz, H-1″), 4.53 (m, 1H), 4.34 (m, 2H, H-2, H-6b), 4.25 (m, 1H), 3.99 (t, 1H, $J=9.5$ Hz, H-4), 3.84 (m, 1H, H-5), 3.77 (t, 1H, $J=7.0$ Hz), 2.14, 2.08, 2.05, 2.04, 2.03, 2.01, 1.93 (s, 21H, 7 OAc), 1.18 (d, 3H, $J=6.5$ Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.6, 170.5, 170.4, 169.9, 169.7, 169.0, 165.7, 134.5, 133.6, 132.8, 129.7, 128.0, 123.8, 100.7, 95.4, 84.1, 77.4, 75.5, 72.4, 71.4, 71.2, 71.1, 70.7, 68.9, 68.1, 67.6, 66.7, 64.4, 62.3, 60.7, 60.4, 55.4, 20.8, 20.8, 20.6, 20.5, 20.5, 15.9; MALDI-TOF: calcd for $C_{53}H_{57}NO_{23}S$ 1106.1, found m/z 1107.1 ($[M+H]^+$). Elemental analysis: found C, 54.24; H, 5.01; N, 1.25; calcd for $C_{53}H_{57}NO_{23}S\cdot 3H_2O$ (1160.1) C, 53.94; H, 5.34; N, 1.19%.

3.12. 11-Bromo-undecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 13

To a solution of **12** (50 mg, 0.045 mmol, 1 equiv.), and 11-bromo-undecanol (20 μL, 0.09 mmol, 2 equiv.) in dry dichloromethane (5 mL) 4 Å molecular sieves (50 mg) was added and the mixture was stirred at room temperature for 1 h and then cooled to −60°C. NIS (41 mg, 0.18 mmol, 4 equiv.) and TfOH (4.5 μL, 0.045 mmol, 1 equiv.) were added to the reaction mixture and the stirring was continued at −60°C. The course of the reaction was monitored by TLC (AcOEt:hexane, 1:2). The solution was neutralized with triethylamine and a white precipitate appeared. The precipitate was filtered off and washed with dichloromethane. The filtrate and washings were combined, and the solution was washed with $Na_2S_2O_3$ (1 M) and water, dried (anhydrous sodium sulfate) and concentrated. Flash chromatography of the residue (AcOEt:hexane, 1:3) gave **13** as an amorphous solid (44 mg, 80%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.00 (m, 2H, Bz), 7.85–7.68 (m, 4H, NPhth), 7.65–7.50 (m, 3H, Bz), 5.40–5.30 (m, 2H, H-4′), 5.20–4.85 (m, 7H, H-1, H-1′, H-5′), 4.80–4.75 (m, 2H), 4.67 (d, 1H, $J=8.1$ Hz, H-1″), 4.55 (m, 1H), 4.36 (dd, 1H, $J=12.3$ Hz, H-2), 4.30–4.20 (m, 2H), 4.12–4.00 (m, 1H), 3.80–3.68 (m, 3H, CHO), 3.39 (t, 1H, $J=6.9$ Hz, CHBr), 3.29 (m, 1H, CHO), 3.17 (t, 1H, $J=6.9$ Hz, CHBr), 2.16, 2.11, 2.08, 2.05, 2.04, 1.95, 1.91 (s, 21H, 7 OAc), 1.50–1.70 (m, 21H, CH_2CH_2, H-6′).

3.13. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside, 14

A solution of **12** (0.5 g, 0.45 mmol, 1 equiv.) in methanol (1.5 mL, 45 mmol, 100 equiv.) and dry dichloromethane (20 mL) was cooled to −60°C. To the reaction mixture NIS (0.31 g, 1.35 mmol, 3 equiv.) and TfOH (45 μL, 0.45 mmol, 1 equiv.) were added and stirring was continued at −60°C. When the reaction was finished (TLC, AcOEt:hexane, 2:1), the solution was neutralized with triethylamine. The solution was washed with $Na_2S_2O_3$ (1 M) and water, dried (anhydrous sodium sulfate) and concentrated. Flash chromatography of the residue (AcOEt:hexane, 1:1) gave **14** as an amorphous solid (0.46 g, quantitative); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.00 (m, 2H, Bz), 7.90–7.70 (m, 4H, NPhth), 7.70–7.40 (m, 3H, Bz), 5.35 (m, 2H), 5.20–5.08 (m, 2H), 5.01–4.95 (m, 3H, H-1), 4.90 (dd, 2H, $J=9.9$, 3.6 Hz), 4.83–4.72 (m, 2H), 4.67 (d, 1H, $J=8.4$ Hz, H-1″), 4.55 (m, 1H), 4.36 (dd, 1H, $J=12.2$,

3.7 Hz), 4.29–4.22 (m, 2H), 4.05 (m, 1H), 3.81–3.74 (m, 2H, H-5), 3.33 (s, 3H, OCH_3), 2.17, 2.12, 2.08, 2.05, 2.05, 2.03, 1.95 (s, 21H, 7 OAc), 1.21 (d, 3H, $J=6.3$ Hz, H-6′).

3.14. 11-Thioacetyl-undecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-6-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, 15

Compound **13** (0.04 g, 0.036 mmol, 1 equiv.), SAcK (0.01 g, 0.108 mmol, 3 equiv.) and a catalytic amount of NBu_4I were dissolved in butanone (5 mL), and stirred for 3 h at 60°C. When the reaction was complete (TLC, AcOEt:hexane, 1:1) the emulsion was extracted with AcOEt (3×20 mL) and the extract washed with water (20 mL). The extract was dried and concentrated. Flash chromatography of the residue (AcOEt:hexane, 1:2) gave **15** as an amorphous solid (46 mg, quantitative); $[\alpha]_D^{23}$ −16.6 ($c=1$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.00 (m, 2H, Bz), 7.90–7.70 (m, 4H, NPhth), 7.70–7.50 (m, 3H, Bz), 5.40–5.30 (m, 2H), 5.21–5.09 (m, 2H), 5.05 (d, 1H, $J=8.7$ Hz, H-1), 5.02–4.93 (m, 3H, H-1′), 4.88 (dd, 1H, $J=10.5$, 3.6 Hz), 4.83–4.74 (m, 2H), 4.66 (d, 1H, $J=8.1$ Hz, H-1″), 4.57 (dd, 1H, $J=11.4$, 6.6 Hz), 4.36 (dd, 1H, $J=12.3$, 3.9 Hz), 4.34 (m, 2H), 4.09 (t, 1H, $J=9.3$ Hz), 3.80–3.70 (m, 3H, H-5, CHO), 3.28 (m, 1H, CHO), 2.85 (t, 2H, $J=7.2$ Hz, CH_2S), 2.32, 2.18, 2.12, 2.09, 2.07, 2.05, 1.96, 1.92 (s, 24H, 8 OAc), 1.60–0.70 (m, 21H, CH_2CH_2, H-6′); ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.9, 171.2, 170.9, 170.8, 170.4, 170.2, 169.5, 166.1, 152.5, 134.8, 134.0, 131.9, 130.8, 130.1, 129.8, 129.2, 123.9, 100.9, 98.6, 95.6, 75.7, 73.5, 71.8, 71.7, 71.5, 70.3, 70.1, 69.3, 68.6, 68.2, 67.1, 64.6, 62.6, 61.1, 56.8, 31.3, 31.1, 29.9, 29.7, 29.5, 29.4, 26.8, 21.2, 21.1, 16.1; MALDI-TOF: calcd for $C_{60}H_{77}NO_{25}S$ 1243.1, found m/z 1266.2 ($[M+Na]^+$), 1281.6 ($[M+K]^+$). Elemental analysis: found C, 56.48; H, 6.08; N, 1.12; calcd for $C_{60}H_{77}NO_{25}S\cdot 1H_2O$ (1261.1) C, 57.10; H, 6.26; N, 1.11%.

3.15. 11,11′-Dithio bis[undecyl(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-(6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside], 16

Compound **15** (0.44 g, 0.35 mmol, 1 equiv.) was dissolved in 2-buthanol (60 mL). Ethilendiamine (10.5 mL, 156 mmol, 300 equiv.) was added. The reaction was stirred for 18 h at 90°C. When the reaction was complete (TLC, MeOH), the solvent was removed. Methanol was added three times and each time the mixture was re-concentrated. A mixture of acetic anhydride (20 mL) and dry pyridine (40 mL) was added with cooling. The mixture was kept for 24 h at room temperature. The solvent was removed, and the product was purified by silica gel column (AcOEt) to give **16** as a white solid (0.44 g, 80%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.51 (d, 1H, $J=8.5$ Hz, NH), 5.40 (m, 2H, H-1′, H-4″), 5.35 (d, 1H, $J=3.0$ Hz, H-4′), 5.18 (dd, 1H, $J=11.0$, 3.5 Hz, H-3′), 5.07 (dd, 1H, $J=10.5$, 8.5 Hz, H-2″), 4.97 (m, 2H, H-2′, H-3″), 4.84 (m, 1H, H-5′), 4.58 (m, 2H, H-1, H-6b), 4.46 (m, 2H, H-1″, H-6b″), 4.26 (dd, 1H, $J=11.5$, 7.5 Hz, H-6″a), 4.09 (m, 2H, H-6a, H-3), 3.85 (t, 1H, $J=7.0$ Hz, H-5″), 3.79 (t, 1H, $J=8.5$ Hz, H-4), 3.74 (m, 1H, CHO), 3.64 (m, 1H, H-2), 3.52 (m, 1H, H-5), 3.38 (m, 1H, CHO), 2.65 (t, 2H, $J=7.0$ Hz, CH_2S), 2.17, 2.12, 2.11, 2.08, 2.05, 2.04, 1.95, 1.94, 1.94 (s, 27H, 9 Ac), 1.67–1.22 (m, 18H, CH_2CH_2), 1.19 (d, 3H, $J=6.5$ Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.8, 170.6, 170.5, 170.4, 170.3, 170.0, 169.8, 169.2, 100.5, 100.0, 95.3, 74.6, 73.2, 72.8, 71.4, 71.1, 70.9, 69.6, 69.0, 68.4, 68.1, 66.7, 64.3, 62.1, 60.8, 56.1, 39.2, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 29.2, 28.5, 25.9, 23.4, 21.0, 20.9, 20.8, 20.7, 20.6, 20.6, 20.5, 15.9; MALDI-TOF: calcd for $C_{94}H_{144}N_2O_{46}S_2$ 2100.1, found m/z 2123.1 ($[M+Na]^+$), 2138.2 ($[M+K]^+$).

3.16. Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-(1→3)-6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside, 17

Compound **14** (0.46 g, 0.44 mmol, 1 equiv.) was dissolved in 2-buthanol (20 mL). Ethylenediamine (8.8 mL, 132 mmol, 300 equiv.) was added. The reaction was stirred for 18 h at 90°C. When the reaction was complete (TLC, MeOH), the solvent was removed. Methanol was added three times to remove the excess ethylenediamine and the mixture was concentrated. A mixture of acetic anhydride (15 mL) and dry pyridine (30 mL) was added under cooling. The mixture was kept for 24 h at room temperature. The solvent was removed, and the product was purified by silica gel column (AcOEt) to give **17** (0.23 g, 60%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.66 (d, 1H, $J=9.0$ Hz, NH), 5.39 (m, 2H, H-1′), 5.33 (d, 1H, $J=3$ Hz, H-4′), 5.18 (dd, 1H, $J=11.0$, 3.0 Hz, H-3′), 5.08 (t, 1H, $J=9.0$ Hz), 4.99 (m, 2H, H-2′, H-5″), 4.70 (m, 1H, H-5′), 4.57 (dd, 1H, $J=12.0$, 3.5 Hz, H.6b), 4.51 (d, 1H, $J=6.5$ Hz, H-1), 4.45 (d, 1H, $J=8.0$ Hz, H-1″), 4.41 (m, 1H, H-4″), 4.25 (dd, 1H, $J=11.0$, 7.5 Hz, H-2″), 4.19 (dd, 1H, $J=11.5$, 5.0 Hz, H-6a), 4.09 (t, 1H, $J=7.5$ Hz, H-3), 3.86 (t, 1H, $J=7.0$ Hz, H-3″), 3.82 (t, 1H, $J=7.5$ Hz, H-4), 3.77 (m, 1H, H-2), 3.59 (m, 1H, H-5), 3.40 (s, 3H, OCH_3), 2.17, 2.12, 2.11, 2.07, 2.05, 1.97, 1.96, 1.94 (s, 24H, 8 OAc), 1.63 (s, 3H, NHAc), 1.18 (d, 3H, $J=6.5$ Hz, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.9, 171.0, 170.7, 170.6, 170.4, 170.0, 169.9, 169.4, 137.9, 129.0, 128.2, 125.3, 101.0, 100.4, 94.9, 74.3, 72.7, 72.5, 71.3, 71.1, 70.8, 68.9, 68.2, 68.0, 66.7, 64.4, 62.4, 60.8, 56.6, 23.4, 21.5, 20.9, 20.9, 20.8, 20.7, 20.6, 20.5, 15.9; MALDI-TOF: calcd for $C_{37}H_{53}NO_{23}$ 879.0, found m/z 902.4 ($[M+Na]^+$), 918.8 ($[M+K]^+$). Elemental analysis: found C, 49.76; H, 5.98; N, 1.81; calcd for $C_{37}H_{53}NO_{23}\cdot 1H_2O$ (897) C, 49.50; H, 6.13; N, 1.56%.

3.17. 11,11′-Dithio bis[undecyl β-galactopyranosyl-(1→4)-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside], 1

Compound **16** (0.44 g, 0.21 mmol, 1 equiv.) was added to a solution of MeONa in MeOH (0.1 N, 20 mL) and it was stirred at room temperature for 12 h. When the reaction was finished (TLC, MeOH), amberlite IR-120 (H^+) was added until neutral medium. The mixture was

filtered and concentrated to give **1** as an amorphous solid (0.27 g, 91%); $[\alpha]_D^{23}$ −65.4 ($c=1$, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.00 (d, 1H, $J=4.0$ Hz, H-1′), 4.80 (m, 1H, H-5′), 4.42 (m, 2H, H-1, H-1″), 4.00–3.80 (m, 7H), 3.80–3.67 (m, 3H), 3.67–3.55 (m, 2H), 3.50–3.35 (m, 5H), 2.65 (t, 2H, $J=7.2$ Hz, CH_2S), 1.93 (s, 3H, NHAc), 1.70–1.20 (m, 18H, CH_2CH_2), 1.15 (d, 3H, $J=6.5$ Hz, H-6′); ^{13}C NMR (75 MHz, CD_3OD): δ 173.7, 103.8, 102.4, 100.4, 77.4, 76.6, 75.2, 74.9, 73.7, 72.8, 71.2, 70.7, 69.9, 67.7, 62.8, 61.4, 57.5, 39.8, 30.8, 30.7, 30.5, 30.3, 30.2, 29.4, 27.2, 24.2, 23.1, 16.6; MALDI-TOF: calcd for $C_{62}H_{112}N_2O_{30}S_2$ 1428.2, found m/z 1451.6 ([M+Na]$^+$), 1467.3 ([M+K]$^+$), 1305.3 ([M−Fuc+Na]$^+$). Elemental analysis: found C, 50.11; H, 7.68; N, 2.02; calcd for $C_{62}H_{112}N_2O_{30}S_2\cdot 3H_2O$ (1482.2) C, 50.20; H, 7.96; N, 1.89%.

3.18. Methyl β-D-galactopyranosyl-(1→4)-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside, 2

Compound **17** (0.21 g, 0.24 mmol, 1 equiv.) was added to a solution of MeONa in MeOH (0.1N, 20 mL) and the mixture was stirred at room temperature for 12 h. When the reaction was complete (TLC MeOH), amberlite IR-120 (H$^+$) was added until neutral medium. The mixture was filtered and concentrated to give **2** as an amorphous solid (0.12 g, 92%); $[\alpha]_D^{23}$ −74.5 ($c=1$, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 5.03 (d, 1H, $J=3.5$ Hz, H-1′), 4.76 (m, 1H, H-5′), 4.40 (d, 1H $J=7.5$ Hz, H-1), 4.38 (d, 1H, $J=8.0$ Hz, H-1″), 3.94 (m, 1H), 3.90–3.75 (m, 6H), 3.72 (m, 1H), 3.70–3.58 (m, 4H), 3.57 (d, 1H, $J=3.5$ Hz), 3.53 (m, 2H), 3.43 (s, 3H, OCH_3), 1.95 (s, 3H, NHAc), 1.10 (d, 3H, $J=7.0$ Hz, H-6′); ^{13}C NMR (75 MHz, CD_3OD): δ 173.9, 103.9, 103.2, 100.4, 77.4, 76.7, 76.6, 75.2, 74.9, 73.7, 72.7, 71.2, 70.0, 67.7, 62.8, 61.3, 57.3, 57.0, 23.0, 16.6; MALDI-TOF: calcd for $C_{21}H_{37}NO_{15}$ 543.0, found 565.9 ([M+Na]$^+$), 581.9 ([M+K]$^+$). Elemental analysis: found C, 44.82; H, 6.83; N, 2.35; calcd for $C_{21}H_{37}NO_{15}\cdot H_2O$ (561) C, 44.92; H, 6.95; N, 2.50%.

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