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Synthesis of a Lewis b hexasaccharide thioglycoside donor and its use towards an extended mucin core Tn heptasaccharide structure and a photoreactive biotinylated serine linked hexasaccharide†

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Investigation into *Heliobacter pylori* binding to Lewis b (Le^b) antigens through the blood group antigen binding adhesion protein (BabA) requires structurally well-defined tools. A Le^b hexasaccharide thioglycoside donor was chemically prepared through a linear approach starting from D-lactose. This donor can be used to attach reducing end linkers providing a range of options for conjugation techniques or to further extend the oligosaccharide structure. To evaluate its efficiency as a donor, it was coupled to a 6-OH GalNAc acceptor, producing an extended Le^b-containing Tn mucin core structure in 84% yield, and to L-serine in 72% yield. The latter compound was subsequently functionalized with a photolabile diazirine linker and biotin, creating a Le^b hexasaccharide structure–function tool suitable for lectin tagging interaction studies. This donor opens a wide range of possibilities for conjugation of Le^b structures to produce a variety of chemical biology tools to assist in the study of these interactions.

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Introduction

Heliobacter pylori infections of the gastric epithelium can lead to gastric inflammation and may progress to gastroduodenal diseases such as peptic ulceration and gastric adenocarcinoma.[1–5] *H. pylori* binds to Lewis b-antigen (Le^b) structures present in the gastric epithelium. This binding is mediated by its blood group antigen binding adhesion protein (BabA).[6–8] Chemically defined structures have proved to be an invaluable tool for probing the binding of *H. pylori* BabA to Le^b antigens.[8–11] In our first synthesis of the Le^b hexasaccharide, we constructed and utilized a Le^b tetrasaccharide thioglycoside donor that was coupled to an azide-propyl lactose acceptor to afford the hexasaccharide.[12] However, the donor was prone to elimination rather than glycosylation reaction. More effective routes to the hexasaccharide were developed using a linear synthesis of the Le^b hexasaccharide starting from the same lactose acceptor;[13–15] these syntheses are high-yielding and can be performed on a large scale but give little flexibility for changes at the reducing end of the molecule. Therefore, a more adaptable approach involving a Le^b hexasaccharide donor was investigated, allowing the construction of a variety of Le^b-containing chemical biology tools. Two earlier examples of Le^b hexasaccharide donors have been reported, a fluoride donor by Sato *et al.*[16] and a 1,2-epoxide donor by Danishefsky *et al.*[17] However, a very low yield[16] and no stereoselectivity[17] were experienced in initial glycosylations with these two donors limiting their use in further applications. Considering our earlier good experiences with large block thioglycoside donors,[18–22] a Le^b hexasaccharide thioglycoside **1** (Fig. 1) was designed as the target donor.

Results and discussion

Apart from the orthogonal glycosylations required, the major difference from our earlier syntheses was the design and syn-

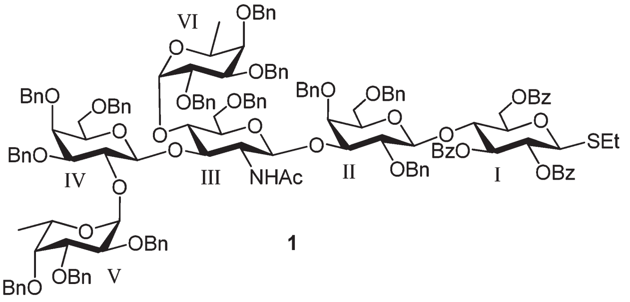

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Fig. 1 Target Le^b hexasaccharide thioglycoside donor.

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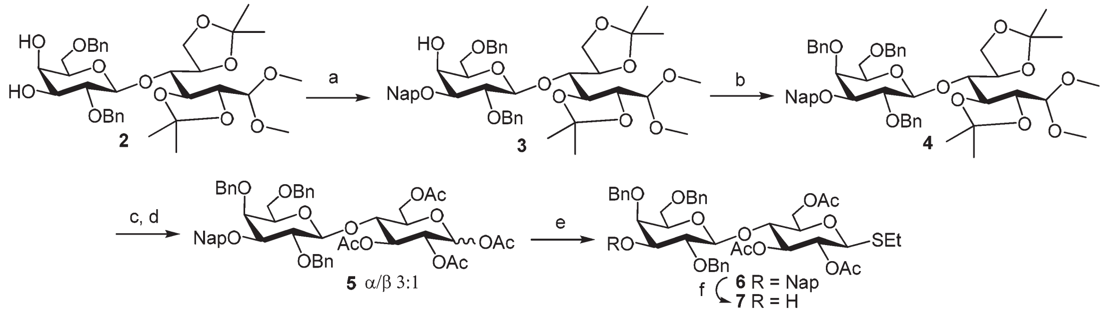
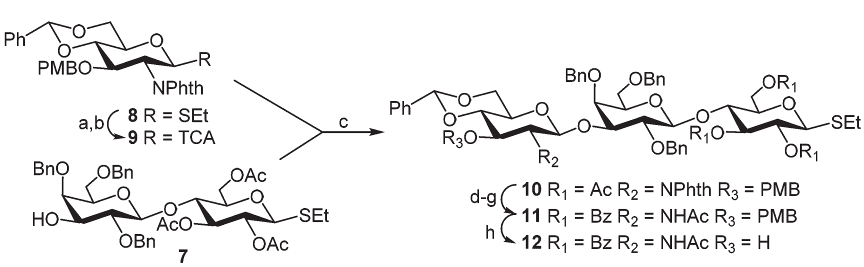
thesis of the lactose acceptor, in particular, its protecting group pattern. Benzyl protecting groups were used on the galactose residue to ensure good reactivity of the 3′-OH acceptor, and acyl protecting groups on the glucose residue, to ensure β-selectivity in the glycosylations with the hexasaccharide donor **1**. This protecting group pattern could be introduced starting from known compound **2** (Scheme 1).[23,24] The Catelani group published procedure to **2** can effectively be performed on a large scale, the triisopropylidenelactose intermediate has been synthesised on a 250 kg scale[25] and we synthesized compound **2** on a 30 g scale. Regioselective introduction of a naphtylmethyl ether (Nap) in the 3′-position using tin activation (→**3**) followed by benzylation afforded compound **4**, with the desired protecting group pattern in the galactose moiety. Acidic acetal hydrolysis followed by acetylation gave compound **5** with an acylated glucose. Due to the presence of the benzyl protecting groups, the Lewis acid-promoted formation of the ethyl thiodisaccharide **6**, had to be monitored very carefully by TLC (R_f 0.44 in toluene : EtOAc 4 : 1, v/v) to avoid decomposition. Attempts to use TMSOTf for the introduction of the thioethyl group were not successful, but with BF_3-etherate as promoter compound **6** was obtained in an 81% yield based on recovered starting material predominantly consisting of the less reactive α-acetate. The Nap group of disaccharide **6** was then cleaved using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give lactose acceptor **7** in a 79% yield.

An orthogonal glycosylation strategy was needed to build the hexasaccharide without activating the reducing end thioglycoside donor. It was decided to use trichloroacetimidate (TCA) donors for the introduction of the glucosamine and galactose residues and glycosyl bromides with halide-assisted glycosylation conditions for the introduction of the two fucose moieties, the latter being reproducibly high yielding in earlier synthesis.[13–15] When performing orthogonal glycosylations with thioglycoside acceptors, especially with non-bulky thio aglycons, there is always the risk of intramolecular aglycon transfer, the extent is hard to predict and very much dependent on the acceptor structure and of the thioaglycone used.[26] In this synthesis we found that acceptor **7**, although being an ethyl thiosaccharide, showed no tendency at all for aglycon transfer reactions.

For the introduction of the glucosamine moiety a suitable donor with a β-directing participating group and orthogonal protecting groups at the 3- and 4-position was needed to be able to selectively glycosylate these positions at a later stage, *N*-phthalimido derivative **8**[27,28] (Scheme 2) was selected. Compound **8** possesses an orthogonal *para*-methoxybenzyl group (PMB) at the 3-position, and a benzylidene acetal in the 4,6-positions, which can be selectively opened to free the 4-position at a later stage. The thioethyl donor **8** was converted into its trichloroacetimidate **9** by hydrolysis of the thioethyl group using *N*-iodosuccinimide (NIS), followed by formation of



Scheme 1 Reagents and conditions: (a) dibutyltin oxide, tetra-butyl ammonium bromide, 2-(bromomethyl)naphthalene, toluene, reflux, 81%; (b) NaH, BnBr, 0 °C → rt, 89%; (c) 80% AcOH, 70 °C; (d) sodium acetate, Ac_2O, reflux, 83% (over 2 steps, α/β mixture (3 : 1)); (e) EtSH, $BF_3{\cdot}OEt_2$, $ClCH_2CH_2Cl$, 0 °C, 81% (based on recovered starting material); (f) DDQ, CH_2Cl_2–MeOH 4 : 1, rt, 79%.



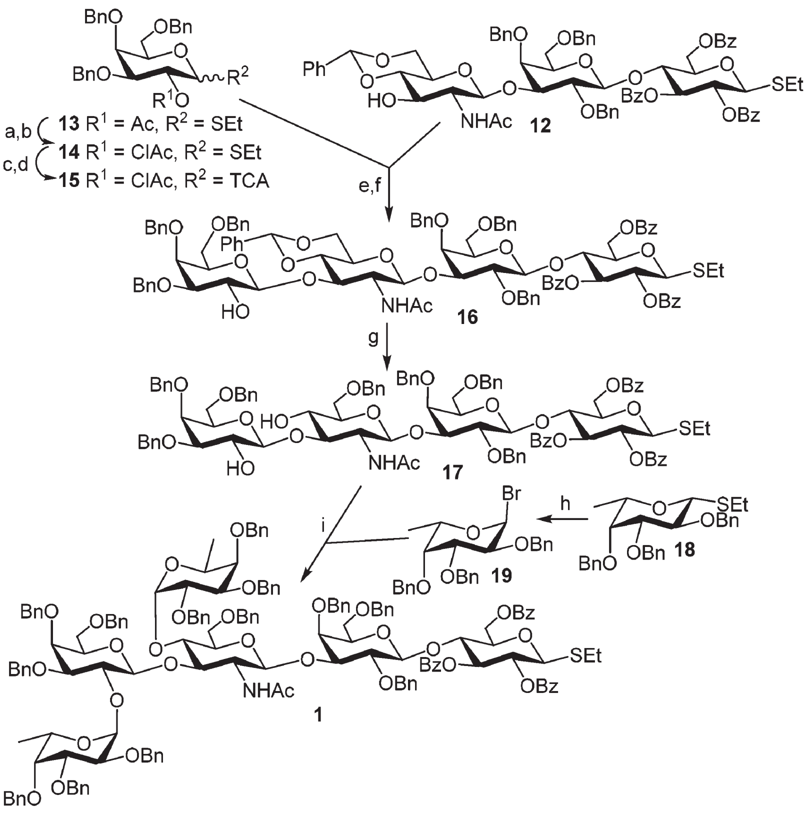
Scheme 2 Reagents and conditions: (a) NIS, CH_3CN–H_2O 10 : 1, rt; (b) Cl_3CCN, DBU, $ClCH_2CH_2Cl$, 0 °C, 92% (over 2 steps); (c) TMSOTf, CH_2Cl_2, −20 °C, 78%; (d) sodium methoxide, MeOH, rt; (e) EtOH, hydrazine hydrate, reflux; (f) Ac_2O, MeOH, rt.; (g) BzCl, pyridine, 0 °C, 83% (over 4 steps); (h) DDQ, CH_2Cl_2/iso-butanol (4 : 1), 78%.

the imidate with trichloroacetonitrile and 1,8-diazabicyclo (5.4.0)undec-7-ene (DBU) (92% over 2 steps after silica gel column chromatography).

Donor **9** was employed in a TMSOTf-catalyzed glycosylation reaction with acceptor **7** affording the desired trisaccharide **10** in a 78% yield. Due to earlier findings, in which the removal of the phthalimido group in a fully assembled hexasaccharide was problematic and low yielding,[13] we decided to remove this protecting group and introduce the acetamide moiety prior to further glycosylation steps, despite being aware of problematic reports on the use of acetamido-containing acceptors.[29–31] The acetyl groups in trisaccharide **10** were removed using Zemplén conditions and the phthalimido group cleaved using hydrazine hydrate in ethanol. The amino group was then chemoselectively acetylated with acetic anhydride in methanol followed by benzoylation of the hydroxyl groups to give compound **11** in a yield of 83% over 4 steps. The benzoyl groups were preferred over acetyl protecting groups to enhance lipophilicity and therefore solubility of subsequent intermediates but also to improve glycosylation properties of the target hexasaccharide donor.[32] In contrast to the removal of the Nap group in compound **6**, the removal of the PMB group to obtain derivative **12** was challenging due to persistent side reactions resulting in poor yields when carrying out the reaction with DDQ in CH_2Cl_2–MeOH 4 : 1. By varying the solvent mixture to CH_2Cl_2–isobutanol, yields were significantly improved and trisaccharide acceptor **12** obtained in 78% yield.[33]

For the introduction of the galactose moiety, a donor with an orthogonal β-selective participating group at the 2-position was needed. Since benzoyl groups were present in trisaccharide **12**, and it was known that removal of a $2'''$-*O*-acetyl group in a lacto-*N*-tetraose (LNT) tetrasaccharide requires unusually strong basic conditions,[15] we chose a chloroacetyl group to protect this position (Scheme 3). The acetyl group of the known galactose derivative **13**[34] was removed using Zemplén conditions and replaced with a chloroacetyl group (→**14**, 88%) followed by conversion into the corresponding trichloroacetimidate donor **15**. This time, NBS was used to hydrolyse the thioethyl group whereafter treatment with DBU and trichloroacetonitrile afforded compound **15** as a α/β mixture (10 : 1). It was possible to separate the anomers using silica gel chromatography. However, the high reactivity of the compound[35] resulting in a rather low yield of 47% after chromatography.

In the next step, donor **15** was employed in a TMSOTf-catalyzed glycosylation reaction with trisaccharide acceptor **12** (Scheme 3). The reaction had to be monitored carefully by TLC (toluene : EtOAc 1 : 1) as an intermediate (possibly the orthoester) was initially formed which was slowly converted into the

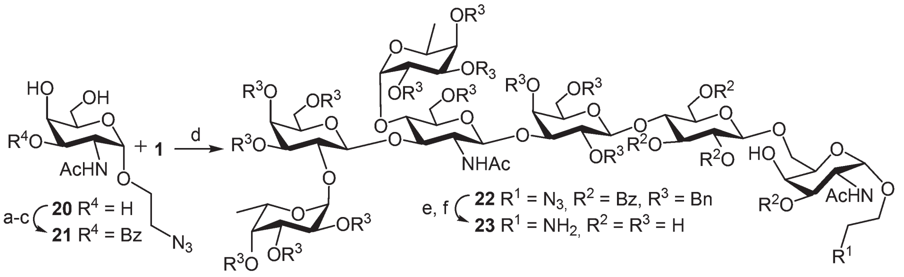


Scheme 3 Reagents and conditions: (a) sodium methoxide, MeOH, rt, 99%; (b) chloroacetyl chloride, CH_2Cl_2–pyridine 14 : 1, 0 °C → rt, 89%; (c) NBS, CH_3CN–H_2O 10 : 1, rt; (d) trichloroacetonitrile, DBU, $ClCH_2CH_2Cl$, 0 °C, 47% (over 2 steps, α/β mixture (10 : 1)). (e) TMSOTf, CH_2Cl_2, −30 °C → −5 °C; (f) lutidine, thiourea, MeOH, 70 °C, 47% (over 2 steps); (g) $NaCNBH_3$, HCl (1 M in Et_2O), 71%; (h) Br_2, CH_2Cl_2, rt; (i) tetraethylammonium bromide, CH_2Cl_2–DMF 3.5 : 1, rt, 89%.

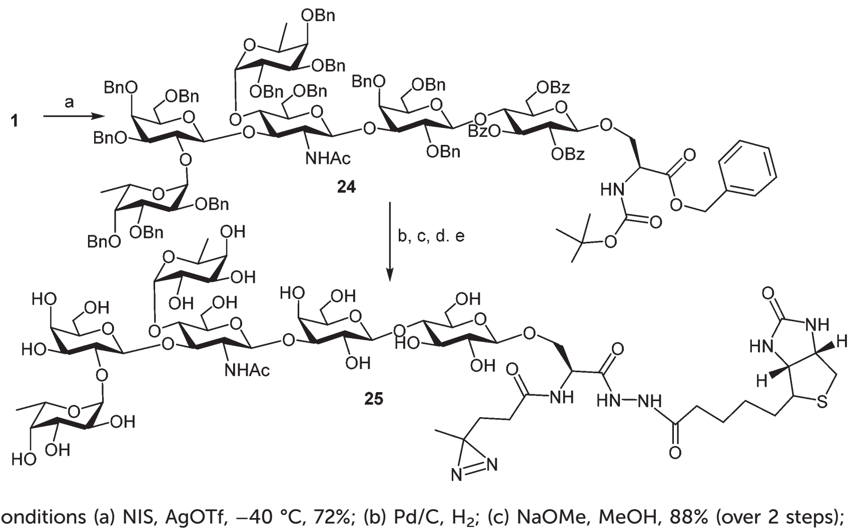
desired tetrasaccharide. Due to difficulties in the isolation of the product, the crudely worked-up reaction mixture was directly treated with thiourea and lutidine in methanol to remove the chloroacetyl group affording the 2‴-OH tetrasaccharide **16** (47% over two steps). The benzylidene group of the GlcNAc moiety of compound **16** was then selectively opened to free the 4″-position using $NaCNBH_3$, affording tetrasaccharide 2‴,4″-diol acceptor **17**. In the final step towards the hexasaccharide donor, fucose thioglycoside **18**[36] was converted into its bromide **19** and then directly employed in a completely α-selective halide-assisted glycosylation with tetrasaccharide acceptor **17** to give the target Lewis b donor **1** in an 89% yield. In summary, hexasaccharide donor **1** (600 mg) was synthesized from compound **2** in a ten step sequence and in a 15% overall yield.

The donor properties of hexasaccharide donor **1** were then investigated. We were interested in Lewis b extended mucin core structures so GalNAc derivative **21** was chosen as a trial acceptor. Acceptor **21** was prepared from compound **20**[37] *via* introduction of a benzylidene group at the 4,6-positions, followed by subsequent benzoylation and final removal of the acetal (Scheme 4). Glycosylation of the 4,6-diol acceptor **21** with hexasaccharide donor **1**, using NIS and AgOTf as promoters, afforded exclusively the β-(1 → 6)-linked heptasaccharide **22** in an 84% yield. Heptasaccharide **22** was then deprotected, the benzoyl groups were removed under Zemplén conditions, followed by hydrogenolysis of the benzyl and azido groups to afford globally deprotected heptasaccharide **23** in a 92% yield over the 2 steps.

We were further interested in photo-active tools to enable cross-linking of Lewis b-structures to neighbouring interacting structures. Hexasaccharide donor **1** was therefore glycosylated with Boc-Ser-OBn using the NIS/AgOTf promotor system producing the serine linked hexasaccharide **24** in 72% yield (Scheme 5). Hydrogenolysis with Pd/C and H_2 followed by saponification using Zemplén conditions produced the globally deprotected compound (88% over 2 steps) which was carried through as crude material to the following two-step bifunctionalising process. A photolabile diazirine linker was introduced to the free amine of the serine residue using an activated *N*-hydroxysuccinimide ester of the corresponding diazirine linker, subsequently the carboxylic acid group of the



Scheme 4 Reagents and conditions: (a) benzaldehyde dimethylacetal, *p*-TsOH·H_2O, DMF, rt; (b) benzoyl chloride, pyridine, rt; (c) AcOH (80% in H_2O), 85 °C, 34% (over 3 steps); (d) AgOTf, NIS, CH_2Cl_2, −40 °C → 0 °C, 84%; (e) sodium methoxide, MeOH, rt; (f) H_2 (5 bar), Pd/C, HCl (1 N in H_2O), THF–EtOH–H_2O, 92% (over 2 steps).



Scheme 5 Reagents and conditions (a) NIS, AgOTf, −40 °C, 72%; (b) Pd/C, H_2; (c) NaOMe, MeOH, 88% (over 2 steps); (d) *N*-succinimidyl-diazirine (SDA), PBS buffer; (e) HOBt, EDCl, biotin hydrazate.

serine was coupled to biotin hydrazate, using HOBt and EDCI as coupling reagents, producing the photoreactive biotinylated hexasaccharide **25**.

Conclusions

We have successfully synthesized a novel Le^b block donor *via* a linear approach using a lactose acceptor that can be produced on a large scale and two new selectively protected trichloroacetimidate donors. The synthesized Le^b hexasaccharide block donor gave high yields and complete stereoselectivity in a glycosylation reaction to obtain a Le^b-extended Tn mucin core structure. Coupling of the donor to serine and additional extension to a bifunctionalised diazirine-biotin linked hexasaccharide further showed the versatility of the donor and its potential use in carbohydrate–protein structure–function interaction elucidation. This donor opens a wide range of possibilities for conjugation of Le^b structures to produce alternative targets.

Experimental

General methods

Please see ESI† for NMR spectra and experimental for compounds **3–7**, **9**, **14–15** and **21**.

General methods

The $^1H/^{13}C$ NMR spectra (δ in ppm, relative to TMS in $CDCl_3$ and relative to solvent peak in D_2O) were recorded with Varian spectrometers (400/100 MHz, 500/125 MHz or 600/150 MHz) at 25 °C. Assignments were aided by ^1H–^1H and ^1H–^{13}C correlation experiments. HRMS spectra were recorded on a micromass LCT instrument from Waters. Optical rotations were measured on a PerkinElmer polarimeter with a Na lamp (589 nm) at 20 °C and are not corrected. TLC was carried out on precoated 60 F254 silica gel alumina plates (Merck) using UV-light, H_2SO_4 (10% in ethanol) and/or ninhydrin-solution (ninhydrin/CH_3COOH/ethanol [0.3 : 3 : 100 w/v/v]. Flash chromatography was performed on silica gel (Apollo scientific, pore size 60 Å, particle size 40–63 μm) or *via* pre-packed columns (Biotage AB, particle size 50 μm) on a Biotage SP4 system. Size exclusion chromatography was performed using Biogel P2 (Biorad, <45 μm bead size, 100–1800 MW) with H_2O-*n*-butanol (99 : 1) as eluent.

Ethyl (4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-2-deoxy-2-phthalamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (10). Acceptor **7** (1.90 g, 2.43 mmol) and donor **9** (2.41 g, 3.64 mmol) were dissolved in CH_2Cl_2 (50 mL), MS 4 Å were added and the mixture was stirred for 15 min. The suspension was cooled to −20 °C, TMSOTf (14 μL, 0.08 mmol) added and the mixture stirred for 30 min. After completion of the reaction Et_3N (50 μL) was added and the mixture concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (toluene–EtOAc) to afford **10** (2.43 g, 78%) as a colourless solid. R_f 0.45 (toluene–EtOAc 4 : 1); $[\alpha]_D^{20}$ −1.7° (*c* 1.33, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.70–7.63 (m, 1H, Ar), 7.56–7.50 (m, 3H, Ar), 7.43–7.26 (m, 14H, Ar), 7.19–7.10 (m, 4H, Ar), 6.90–6.84 (m, 4H, Ar) and 6.36–6.31 (m, 2H, Ar), 5.62 (s, 1H, C*H*Ph), 5.46 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^{III}), 5.00–4.91 (m, 2H), 4.88–4.82 (m, 1H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.52–4.35 (m, 7H), 4.29–4.20 (m, 3H), 4.07 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1^{II}), 4.04–3.98 (m, 2H), 3.88–3.75 (m, 4H), 3.70–3.59 (m, 3H), 3.57 (s, 3H, $C_6H_4OCH_3$), 3.54–3.44 (m, 3H), 3.37 (dd, *J* = 9.8, 7.6 Hz, 1H), 3.11 (ddd, *J* = 9.8, *J* = 5.4, *J* = 1.7 Hz, 1H), 2.63–2.50 (m, 2H, CH_2CH_3), 2.00, 1.88 and 1.78 (9H, $COCH_3$), 1.17 (t, *J* = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.2 and 169.6 ($COCH_3$), 167.37 (NCO(Phth)), 158.8, 138.9, 138.5, 137.8, 137.3, 133.5, 131.2, 130.1, 129.5, 129.1, 128.5, 128.3, 128.2, 127.9, 127.9, 127.7, 127.4, 126.7, 126.3, 126.1, 123.1 and 113.3 (Ar), 102.5 (C-1^{II}), 101.4 (*C*HPh), 100.1 (C-1^{III}), 83.2 (C-1^{I}), 83.1 (2C, C-4^{III}, C-3^{II}), 79.0 (C-2^{II}), 77.1 (C-5^{I} (HSQC)), 76.1 (C-4^{II}), 74.8 (CH_2Ph), 74.2 (2C, CH_2Ph, C-3^{III}), 73.8 (2C, CH_2PhOCH_3, C-4^{I}), 73.5 (2C, C-3^{I}, CH_2Ph), 73.4 (C-5^{II}), 69.8 (C-2^{I}), 68.8 (C-6^{III}), 68.6 (C-6^{II}), 65.9 (C-5^{III}), 62.3 (C-6^{I}), 56.4 (C-2^{III}), 54.9 ($PhOCH_3$), 24.1 (CH_2CH_3), 20.8, 20.7, 20.6 ($COCH_3$), 14.9 (CH_2CH_3); ES-HRMS calcd for $C_{70}H_{75}NO_{20}S$ $[Na]^+$ 1304.4501 found 1304.4436.

Ethyl (4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (11). A solution of sodium methoxide (1 M in MeOH) was added dropwise to a solution of compound **10** (4.90 g, 3.82 mmol) in dry MeOH (200 mL) and the mixture stirred for 3 h. After completion of the reaction (TLC toluene–EtOAc 1 : 1) the solution was neutralised with Dowex 50 W+ ion exchange resin, filtered, concentrated under reduced pressure and directly used for the next step. The residue was dissolved in EtOH (150 mL), hydrazine hydrate (5.54 mL, 114 mmol) added and the mixture refluxed for 36 h. After completion of the reaction the solvent was evaporated and the residue co-evaporated with toluene (2 × 30 mL). The crude material was dissolved in EtOAc (150 mL), washed with saturated aq $NaHCO_3$ (100 mL) and the aqueous layer extracted with EtOAc (2 × 50 mL). The combined organic fractions were dried over $MgSO_4$, filtered, concentrated under reduced pressure, and the crude was dried *in vacuo*. The residue was dissolved in MeOH (150 mL) and the resulting solution cooled to 0 °C before acetic anhydride (0.467 mL, 4.95 mmol) was added. The reaction mixture was stirred for 3 h while allowing to warm to room temperature. Then the mixture was concentrated, co-evaporated with toluene (3 × 20 mL) and dried *in vacuo*. The crude material was dissolved in pyridine (40 mL), benzoyl chloride (1.55 mL, 13.3 mmol) added at 0 °C and the mixture stirred for 2 h. After completion of the reaction, MeOH (2 mL) was added and the mixture concentrated and co-evapoarted with toluene. The residue was dissolved in CH_2Cl_2 (200 mL), washed with saturated aq $NaHCO_3$ (100 mL) and the organic layer dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography on silica gel (toluene–EtOAc) gave **11**

(4.36 g, 83%) as a colourless solid. R_f 0.62 (toluene–EtOAc 2 : 1); $[\alpha]_D^{20}$ +2.7° (c 1.79, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) 8.01–7.81 (m, 6H, Ar), 7.60–7.08 (m, 31H, Ar), 6.82–6.76 (m, 2H, Ar), 5.68–5.61 (m, 1H), 5.53 (s, 1H, C*H*Ph), 5.46–5.40 (m, 1H), 4.91–4.84 (m, 2H), 4.77 (d, J = 11.8 Hz, 1H), 4.73–4.66 (m, 4H), 4.61 (d, J = 12.3 Hz, 1H), 4.47 (d, J = 11.5 Hz, 1H), 4.42 (dd, J = 12.1, J = 5.2 Hz, 1H), 4.35–4.26 (m, 3H), 4.15–4.06 (m, 3H), 3.80–3.49 (m, 11H), 3.44–3.37 (m, 1H), 3.31–3.26 (m, 1H), 2.91 (dd, J = 9.0, J = 5.1 Hz, 1H), 2.85–2.79 (m, 1H), 2.73–2.59 (m, 2H, CH_2CH_3), 1.46 (s, 3H, $NCOCH_3$), 1.19 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9 ($NCOCH_3$), 165.9, 165.7 and 165.4 (*C*OPh), 159.2, 139.2, 138.8, 138.0, 137.4, 133.2, 133.1, 132.6, 130.5, 130.3, 129.9, 129.8, 129.6, 129.3, 129.0, 128.5, 128.4, 128.3, 128.0, 127.8, 127.6, 127.5, 127.1, 126.6, 126.1 and 113.7 (Ar), 103.1 (C-1^{II}), 101.9 (C-1^{III}), 101.2 (*C*HPh), 83.6 (C-1^{I}), 82.4 (C-4^{III}), 80.7 (C-3^{II}), 79.9 (C-2^{II}), 77.6 (C-5^{I}), 77.0 (C-3^{III} (HSQC)) 75.6 (C-4^{II}), 75.3 (C-4^{I}), 74.9 (CH_2Ph), 74.4 (CH_2Ph), 74.2 (C-3^{I}), 73.6 (CH_2PhOCH_3), 73.1 (2C, CH_2Ph, C-5^{II}), 70.6 (C-2^{I}), 68.8 (C-6^{II}), 67.7 (C-6^{III}), 65.9 (C-5^{III}), 63.0 (C-6^{I}), 56.7 (C-2^{III}), 55.3 ($PhOCH_3$), 24.3 (CH_2CH_3), 23.1 ($NCOCH_3$), 14.9 (CH_2CH_3); ES-HRMS calcd for $C_{79}H_{81}NO_{19}S$ $[Na]^+$ 1402.5021 found 1402.4957.

Ethyl (4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (12). DDQ (0.438 g, 1.93 mmol) was added to a solution of **11** (0.761 g, 0.511 mmol) in dry CH_2Cl_2 (12 mL) and isobutanol (3 mL) at 0 °C and the mixture stirred for 2 h while allowing to warm to room temperature. The solvent was diluted with CH_2Cl_2 (50 mL) and washed with saturated aq $NaHCO_3$ (2 × 100 mL) and the organic layer dried over $MgSO_4$, filtered and concentrated. Flash chromatography (toluene–EtOAc) afforded **12** (0.539 g, 78%) as a white solid. R_f 0.38 (toluene–EtOAc 1 : 1); $[\alpha]_D^{20}$ −10.5° (c 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01–7.92 (m, 4H), 7.90–7.85 (m, 2H, Ar), 7.60–7.54 (m, 1H, Ar), 7.53–7.26 (m, 21H, Ar), 7.23–7.10 (m, 7H, Ar), 5.71–5.63 (m, 1H), 5.53 (s, 1H), 5.49–5.43 (m, 1H), 5.30 (d, J = 5.2 Hz, 1H), 5.08 (d, J = 12.7 Hz, 1H), 4.81–4.74 (m, 1H), 4.68 (m, 5H), 4.45 (dd, J = 12.1, J = 5.1 Hz, 1H), 4.38–4.28 (m, 3H), 4.19–4.09 (m, 3H), 3.84–3.77 (m, 1H), 3.77–3.68 (m, 4H), 3.62–3.49 (m, 3H), 3.40 (m, 1H), 3.36–3.30 (m, 1H), 2.97 (dd, J = 9.0, J = 5.3 Hz, 1H), 2.86–2.80 (m, 1H), 2.75–2.61 (m, 2H, CH_2CH_3), 1.48 (s, 3H, $COCH_3$), 1.20 (t, J = 7.4 Hz, 3H, CH_2CH_3) (OH was not observed); ^{13}C NMR (125 MHz, $CDCl_3$) 172.4 ($NCOCH_3$), 165.9, 165.8 and 165.3 (*C*OPh), 138.9, 138.3, 137.8, 137.0, 133.3, 132.6, 130.2, 129.9, 129.7, 129.6, 129.3, 129.1, 128.8, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.3, 126.4 and 126.1 (Ar), 103.0 (C-1^{II}), 102.3 (C-1^{III}), 101.9 (*C*HPh), 83.6 (C-1^{I}), 81.5 (C-4^{III}), 81.1 (C-3^{II}), 79.6 (C-2^{II}), 77.5 (C-5^{I}), 75.7 (C-4^{II}), 75.0 (C-4^{I}), 74.6 (CH_2Ph), 74.5 (CH_2Ph), 74.1 (C-3^{I}), 73.2 (2C, CH_2Ph, C-5^{II}), 72.7 (C-3^{III}), 70.5 (C-2^{I}), 68.5 (C-6^{III}), 67.5 (C-6^{II}), 66.3 (C-5^{III}), 63.1 (C-6^{I}), 59.1 (C-2^{III}), 24.3 (CH_2CH_3), 22.9 ($NHCOCH_3$), 14.9 (CH_2CH_3); ES-HRMS calcd for $C_{71}H_{73}NO_{18}S$ $[Na]^+$ 1282.4446 found 1282.4392.

Ethyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-(4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (16). Acceptor **12** (1.095 g, 0.869 mmol) and imidate **15** (0.875 g, 1.30 mmol) were dissolved in CH_2Cl_2 (43.5 mL), molecular sieves 4 Å were added and the mixture was stirred for 15 min. The mixture was then cooled to −30 °C and TMSOTf (55 µL, 0.30 mmol) added, and the reaction mixture stirred for 3 h while allowing to warm to −5 °C. The reaction was quenched by addition of Et_3N (0.05 mL), and the mixture filtered through a pad of Celite and concentrated. Purification by flash chromatography (toluene–EtOAc) afforded a mixture of the tetrasaccharide together with inseparable impurities (1.188 g) and recovered acceptor **12** (0.090 g). 2,6-Lutidine (1.60 mL, 13.4 mmol) and thiourea (1.02 g, 13.4 mmol) were subsequently added to a solution of the obtained crude material (1.188 g) in MeOH (100 mL) and the mixture was stirred at 70 °C overnight. When all starting material was consumed the mixture was concentrated, dissolved in CH_2Cl_2 (50 mL) and subsequently washed with 1 N aq HCl (30 mL) and saturated aq $NaHCO_3$ (30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Flash chromatography (toluene–EtOAc) afforded **16** (0.640 g, 47%) over 2 steps (based on recovered starting material in the first step) as a colourless solid. R_f 0.36 (toluene–EtOAc 2 : 1); $[\alpha]_D^{20}$ −9.6° (c 1.88, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.01–7.92 (m, 4H, Ar), 7.89–7.83 (m, 2H, Ar), 7.58–7.53 (m, 1H, Ar), 7.52–7.08 (m, 43H, Ar), 5.67–5.61 (m, 1H), 5.50 (s, 1H), 5.48–5.40 (m, 2H), 4.90 (d, J = 11.5 Hz, 1H), 5.67–5.61 (m, 1H), 5.50 (s, 1H), 5.48–5.40 (m, 2H), 4.90 (d, J = 11.5 Hz, 1H), 4.86–4.77 (m, 3H), 4.70 (d, $J_{1,2}$ = 9.9 Hz, 1H, H-1^{I}), 4.66 (m, 2H), 4.59 (m, 3H), 4.40 (dd, J = 12.0, J = 5.3 Hz, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.33–4.24 (m, 4H), 4.22 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^{IV}), 4.14–4.04 (m, 3H), 4.01–3.95 (m, 1H), 3.91–3.87 (m, 1H), 3.86–3.84 (m, 1H), 3.74 (m, 1H), 3.71–3.63 (m, 4H), 3.60–3.54 (m, 2H), 3.52 (dd, J = 10.7, J = 5.5 Hz, 1H), 3.49–3.44 (m, 1H), 3.39 (dd, J = 9.7, J = 4.9 Hz, 1H), 3.35 (dd, J = 8.4, J = 4.9 Hz, 1H), 3.31–3.25 (m, 2H), 2.96–2.75 (m, 3H), 2.73–2.59 (m, 2H, SCH_2CH_3), 1.53 (s, J = 11.6 Hz, 3H, $NCOCH_3$), 1.18 (t, J = 7.4 Hz, 3H, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0 ($NCOCH_3$), 164.8, 164.7 and 164.3 (*C*OPh), 138.2, 137.8, 137.7, 137.5, 137.0, 136.7, 135.8, 132.2, 132.1, 131.5, 129.3, 128.8, 128.6, 128.3, 128.1, 127.4, 127.3, 127.2, 127.0, 126.9, 126.8, 126.7, 126.6, 126.5, 126.4, 126.1, 125.8 and 125.2 (Ar), 102.2 (C-1^{IV}), 102.1 (C-1^{II}), 101.6 (C-1^{III}), 100.6 (*C*HPh), 82.5 (C-1^{I}), 80.5 (C-3^{IV}), 80.0 (C-3^{II}), 79.2 (C-4^{III}), 78.6 (C-2^{II}), 76.6 (C-5^{I}), 74.8 (C-3^{III}), 74.6 (C-4^{II}), 74.3 (C-4^{I}), 73.9 (CH_2Ph), 73.7 (CH_2Ph), 73.4 (CH_2Ph), 73.2 (C-3^{I}), 72.7 (C-5^{IV}), 72.4 (2C, C-4^{IV}, CH_2Ph), 72.1 (2C, CH_2Ph, C-5^{II}), 71.6 (CH_2Ph), 69.5 (2C, C-2^{I}, C-2^{IV}), 67.7 (C-6^{III}), 67.3 (C-6^{IV}), 66.7 (C-6^{II}), 65.2 (C-5^{III}), 62.0 (C-6^{I}), 55.2 (C-2^{III}), 23.2 (CH_2CH_3), 22.2 ($NCOCH_3$), 13.9 (CH_2CH_3); ES-HRMS calcd for $C_{98}H_{101}NO_{23}S$ $[Na]^+$ 1714.6383 found 1714.6360.

Ethyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-(6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (17). A solution of HCl (1 M in Et_2O) was added drop-wise to a mixture **16** (0.170 g, 0.100 mmol) and $NaCNBH_3$ (158 mg, 2.51 mmol) in dry THF (6 mL) until a pH 1–2 was obtained. The reaction mixture was stirred for 2 h and frequently monitored by TLC. The mixture was neutralized with Et_3N and filtered through a Celite pad. Concentration and co-evaporation with MeOH (3 × 10 mL), followed by flash chromatography (toluene–EtOAc) afforded **17** (0.121 g, 71%) as a colourless solid. R_f 0.60 (toluene–EtOAc 1 : 1); $[\alpha]_D^{20}$ +4.1° (*c* 0.38, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) *δ* 7.99–7.92 (m, 4H, Ar), 7.89–7.84 (m, 2H, Ar), 7.56–7.52 (m, 1H, Ar), 7.52–7.48 (m, 1H, Ar), 7.41–7.10 (m, 42H, Ar), 5.68–5.63 (m, 1H), 5.46–5.40 (m, 1H), 5.06 (d, *J* = 8.2 Hz, 1H, N*H*COCH$_3$), 4.95 (d, *J* = 12.4 Hz, 1H), 4.87 (d, *J* = 11.6 Hz, 1H), 4.81–4.75 (m, 2H), 4.73–4.63 (m, 5H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.49–4.46 (m, 2H), 4.44–4.39 (m, 2H), 4.37–4.30 (m, 4H), 4.13–4.05 (m, 3H), 4.01 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1$^{\mathrm{IV}}$), 3.92–3.88 (m, 2H), 3.83–3.75 (m, 4H), 3.68–3.63 (m, 1H), 3.62–3.53 (m, 4H), 3.48–3.42 (m, 3H), 3.40 (dd, *J* = 8.8, *J* = 5.2 Hz, 1H), 3.34 (dd, *J* = 9.8, *J* = 2.9 Hz, 1H), 3.32–3.28 (m, 1H), 2.93–2.92 (m, 1H), 2.90 (dd, *J* = 9.0, *J* = 5.4 Hz, 1H), 2.80 (dd, *J* = 8.9, *J* = 7.8 Hz, 1H), 2.72–2.60 (m, 2H, SCH_2CH_3), 1.48 (s, 3H, $NCOCH_3$), 1.20–1.16 (m, 3H, SCH_2CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 171.7 (NH*C*OCH$_3$), 165.9, 165.8 and 165.3 (*C*OPh), 139.1, 138.9, 138.4, 138.3, 138.2, 138.0, 137.5, 130.2, 129.9, 129.8, 129.6, 129.3, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.1 and 126.2 (Ar), 104.8 (C-1$^{\mathrm{IV}}$), 103.0 (C-1$^{\mathrm{II}}$), 101.6 (C-1$^{\mathrm{III}}$), 87.1 (C-3$^{\mathrm{III}}$), 83.5 (C-1$^{\mathrm{I}}$), 81.4 (2C, C-3$^{\mathrm{II}}$, C-3$^{\mathrm{IV}}$), 79.7 (C-2$^{\mathrm{II}}$), 77.6 (C-5$^{\mathrm{I}}$), 75.7 (C-4$^{\mathrm{II}}$), 75.3 (C-4$^{\mathrm{III}}$), 75.2 (C-4$^{\mathrm{I}}$), 74.7 (*C*H$_2$Ph), 74.6 (*C*H$_2$Ph), 74.5 (*C*H$_2$Ph), 74.1 (2C, C-3$^{\mathrm{I}}$, C-5$^{\mathrm{IV}}$), 73.7 (*C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 73.3 (2C, C-4$^{\mathrm{IV}}$, C-5$^{\mathrm{II}}$), 73.1 (*C*H$_2$Ph), 72.9 (*C*H$_2$Ph), 71.2 (C-2$^{\mathrm{IV}}$), 70.5 (C-2$^{\mathrm{I}}$), 69.8 (C-6$^{\mathrm{III}}$), 69.3 (C-5$^{\mathrm{III}}$), 68.8 (C-6$^{\mathrm{IV}}$), 67.8 (C-6$^{\mathrm{II}}$), 63.0 (C-6$^{\mathrm{I}}$), 55.6 (C-2$^{\mathrm{III}}$), 24.2 (S*C*H$_2$CH$_3$), 23.1 (NHCO*C*H$_3$), 14.9 (SCH$_2$*C*H$_3$); ES-HRMS calcd for $C_{98}H_{103}NO_{23}S$ $[Na]^+$ 1716.6539 found 1716.6548.

Ethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)]-(1 → 4)-(6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (1). To an ice-cold solution of **18**[36] (0.635 g, 1.33 mmol) in CH_2Cl_2 (6 mL) was added bromine (75.0 µL, 1.46 mmol) and the solution stirred for 10 min. Cyclohexene (50 µL) was then added and the mixture concentrated and dried *in-vacuo*. The residue was dissolved in CH_2Cl_2 (4 mL) and added to a mixture of **17** (450 mg, 0.265 mmol) and tetraethylammonium bromide (0.084 g, 0.398 mmol) in CH_2Cl_2 (6 mL) and DMF (2.8 mL) which had been stirred with molecular sieves 4 Å at room temperature for 40 min. The resulting reaction mixture was stirred under a N_2 – atmosphere for 3 d and excluded from light. Afterwards the mixture was filtered through a pad of Celite, the solution concentrated, and the mixture purified by two successive flash chromatography columns (column 1: pentane–EtOAc 5 : 1 to 2 : 1, column 2: toluene–acetonitrile 7 : 1 to 4 : 1) to obtain **1** (0.600 g, 89%) as a colourless solid. R_f 0.72 (toluene–EtOAc 2 : 1), $[\alpha]_D^{20}$ −36.9° (*c* 0.73, $CHCl_3$); ^{13}C NMR (125 MHz, CD_2Cl_2) *δ* 169.63 (NH*C*OCH$_3$), 166.3, 166.1 and 165.7 (*C*OPh), 140.3, 140.0, 139.8, 139.7, 139.5, 139.3, 139.0, 138.9, 138.8, 138.6, 133.9, 133.7, 133.3, 130.8, 130.5, 130.3, 130.2, 130.1, 129.9, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4 and 127.0 (Ar), 104.0 (C-1$^{\mathrm{II}}$), 102.7 (C-1$^{\mathrm{III}}$), 102.0 (C-1$^{\mathrm{IV}}$), 99.2 (C-1$^{\mathrm{VI}}$), 98.2 (C-1$^{\mathrm{V}}$), 84.3 (C-3$^{\mathrm{IV}}$), 84.1 (C-1$^{\mathrm{I}}$), 81.1 (C-2$^{\mathrm{II}}$), 80.8 (C-3$^{\mathrm{V}}$), 80.5 (C-3$^{\mathrm{II}}$), 79.5 (C-3$^{\mathrm{VI}}$), 79.1 (C-4$^{\mathrm{VI}}$), 78.8 (C-4$^{\mathrm{V}}$), 78.1 (C-5$^{\mathrm{I}}$), 76.6 (2C, C-2$^{\mathrm{V}}$, C-4$^{\mathrm{II}}$), 76.5 (2C, C-2$^{\mathrm{VI}}$, C-4$^{\mathrm{I}}$), 76.2 (*C*H$_2$Ph), 76.0 (C-5$^{\mathrm{III}}$), 75.8 (C-3$^{\mathrm{III}}$), 75.5 and 75.3 (*C*H$_2$Ph), 75.2 (2C, *C*H$_2$Ph), 74.8 (2C, C-3$^{\mathrm{I}}$, C-2$^{\mathrm{IV}}$), 74.3 (C-4$^{\mathrm{IV}}$), 74.1 (*C*H$_2$Ph), 73.8 (C-5$^{\mathrm{II}}$), 73.6 (*C*H$_2$Ph), 73.5 (2C, C-5$^{\mathrm{IV}}$, *C*H$_2$Ph) 73.3 (C-4$^{\mathrm{III}}$), 73.2 (*C*H$_2$Ph), 72.2 (2C, *C*H$_2$Ph), 71.9 (*C*H$_2$Ph), 71.3 (C-2$^{\mathrm{I}}$), 69.2 (C-6$^{\mathrm{IV}}$), 68.2 (2C, C-6$^{\mathrm{III}}$, C-6$^{\mathrm{II}}$), 67.3 (C-5$^{\mathrm{V}}$), 66.9 (C-5$^{\mathrm{VI}}$), 63.5 (C-6$^{\mathrm{I}}$), 56.5 (C-2$^{\mathrm{III}}$), 24.9 (*C*H$_2$CH$_3$), 23.8 (NHCO*C*H$_3$), 16.5 (2C, C-6$^{\mathrm{V}}$, C-6$^{\mathrm{VI}}$), 15.4 (CH$_2$*C*H$_3$); ES-HRMS calcd for $C_{152}H_{159}NO_{31}S$ $[Na]^+$ 2549.0515 found 2549.0415.

2-Azidoethyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)]-(1 → 4)-(6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranosyl)-(1 → 6)-3-*O*-benzoyl-2-deoxy-2-acetamido-α-D-galactopyranoside (22). A mixture of donor **1** (90 mg, 0.036 mmol), acceptor **21** (21 mg, 0.053 mmol) and molecular sieves 4 Å in CH_2Cl_2 (3 mL) was stirred for 20 min. The mixture was cooled to −40 °C, NIS (16 mg, 0.071 mmol) and AgOTf (cat) added and the mixture stirred for 2 h while gradually warming to 0 °C. Upon completion of the reaction, Et_3N (20 µL) was added and the mixture diluted with CH_2Cl_2 (20 mL) and filtered through a pad of Celite. The mixture was washed with 10% aq Na_2SO_3 and the organic layer dried over $MgSO_4$, filtered and concentrated. Purification by flash chromatography (toluene–EtOAc) gave **22** (0.086 g, 84%) as a colourless solid. R_f 0.35 (toluene–EtOAc 1 : 1); $[\alpha]_D^{20}$ −21.1° (*c* 2.33, $CHCl_3$); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 170.1 and 169.1 (NH*C*OCH$_3$), 166.4, 166.0, 165.7 and 165.3 (*C*OPh), 139.5, 139.3, 139.2, 138.9, 138.6, 138.4, 138.2, 138.0, 137.7, 133.4, 133.3, 133.2, 132.6, 130.1, 130.0, 129.8, 129.6, 129.4, 128.6, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.3, 127.1, 127.0, 126.9, 126.5 and 126.2 (Ar), 103.0 (C-1$^{\mathrm{II}}$), 101.8 (C-1$^{\mathrm{III}}$), 101.6 (C-1$^{\mathrm{IV}}$), 101.2 (C-1$^{\mathrm{I}}$), 98.4 (C-1$^{\mathrm{VI}}$), 97.8 (2C, C-1$^{\mathrm{V}}$, C-1$^{\mathrm{VII}}$), 83.8 (C-3$^{\mathrm{IV}}$), 80.6 (C-2$^{\mathrm{II}}$), 80.3 (C-3$^{\mathrm{V}}$), 79.7 (C-3$^{\mathrm{II}}$), 79.1 (C-3$^{\mathrm{VI}}$), 78.1 (C-4$^{\mathrm{V}}$), 78.1 (C-4$^{\mathrm{VI}}$), 75.8 (C-4$^{\mathrm{II}}$), 75.7 (C-2$^{\mathrm{V}}$), 75.4 (3C, C-2$^{\mathrm{VI}}$, C-5$^{\mathrm{III}}$, *C*H$_2$Ph), 75.1 (C-3$^{\mathrm{III}}$), 75.0 (C-4$^{\mathrm{I}}$), 74.9 and 74.8 (*C*H$_2$Ph), 74.7 (2C, *C*H$_2$Ph), 74.5 (*C*H$_2$Ph), 74.0 (C-2$^{\mathrm{IV}}$), 73.8 (C-5$^{\mathrm{I}}$), 73.6 (*C*H$_2$Ph), 73.5 (C-5$^{\mathrm{II}}$), 73.2 (*C*H$_2$Ph), 73.1 (2C, C-4$^{\mathrm{IV}}$, C-4$^{\mathrm{III}}$), 72.9 (2C, *C*H$_2$Ph, C-5$^{\mathrm{IV}}$), 72.6 (2C, C-3$^{\mathrm{I}}$, *C*H$_2$Ph), 71.7 (4C, C-2$^{\mathrm{I}}$, C-3$^{\mathrm{VII}}$, 2 x *C*H$_2$Ph) 71.2 (*C*H$_2$Ph), 69.4 (C-5$^{\mathrm{VII}}$), 68.9 (C-6$^{\mathrm{VII}}$), 68.5 (C-6$^{\mathrm{IV}}$), 67.9 (C-6$^{\mathrm{II}}$), 67.3 (C-6$^{\mathrm{III}}$), 67.1 (C-4$^{\mathrm{VII}}$), 67.0 (*C*H$_2$CH$_2$N$_3$), 66.8 (C-5$^{\mathrm{V}}$), 66.6 (C-5$^{\mathrm{VI}}$), 62.2 (C-6$^{\mathrm{I}}$), 56.4 (C-2$^{\mathrm{III}}$), 50.2 (*C*H$_2$*C*H$_2$N$_3$), 47.3 (C-2$^{\mathrm{VII}}$), 23.4 and 23.2 (NHCO*C*H$_3$), 16.3 and 16.2 (C-6$^{\mathrm{V}}$ and C-6$^{\mathrm{VI}}$); ES-HRMS calcd for $C_{167}H_{175}N_5O_{38}$ $[Na]^+$ 2881.1813 found 2881.1875.

2-Aminoethyl (α-L-fucopyranosyl)-(1 → 2)-(β-D-galactopyranosyl)-(1 → 3)-[(α-L-fucopyranosyl)]-(1 → 4)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-(β-D-

glucopyranosyl)-(1 → 6)-2-deoxy-2-acetamido-α-D-galactopyranoside (23). Sodium methoxide was added to a solution of **22** (35 mg, 0.012 mmol) in dry MeOH (5 mL) until a pH of 13 was reached, the solution was stirred overnight. Upon completion of the reaction the mixture was neutralized using Dowex 50 W+ ion exchange resin, filtered, concentrated and dried *in vacuo*. The residue was dissolved in a mixture of THF (2 mL), EtOH (2 mL) and H_2O (55 μL). HCl (aq, 1 N, 20 μL) and Pd/C (35 mg) was added and the suspension was stirred under H_2 atmosphere (5 bar) for 18 h. Additional H_2O (3 mL), HCl (aq, 1 N, 10 μL) and Pd/C (35 mg) were added and the mixture was stirred under a H_2 atmosphere (5 bar) for another 18 h. Upon completion of the reaction the mixture was neutralized with saturated aq $NaHCO_3$, filtered through frits (20 μm, 10 μm, 5 μm), and the solution concentrated. The crude material was purified on a P2 Biogel column to obtain **23** (14 mg, 92%) after freeze drying as a colourless solid. R_f 0.23 (EtOAc–MeOH–H_2O 2 : 2 : 2 +0.5% AcOH); $[\alpha]_D^{20}$ −12.8° (*c* 0.67, H_2O); ^{13}C NMR (150 MHz, D_2O) *δ* 174.6 and 174.1 (NH*C*OCH$_3$), 103.2 (C-1^{III}), 102.9 (C-1^{II}), 102.4 (C-1^{V}), 100.6 (C-1^{IV}), 99.5 (C-1^{VI}), 97.7 (C-1^{I}), 97.6 (C-1^{VII}), 81.5, 78.1, 76.4, 75.1, 74.8, 74.7 (2C), 74.4, 74.2, 73.6, 72.7, 71.9 (2C), 71.7, 70.2, 70.1, 69.4, 69.2, 69.0, 68.7, 68.5, 68.4, 68.2, 67.7, 67.4, 67.0, 66.2, 64.5, 61.5, 60.9, 59.9, 59.4, 55.7, 49.5, 39.2 (CH_2*C*H_2N_3), 22.1, 21.9 (NHCO*C*H$_3$), 15.3, 15.2 (C-6^{VI}, C-6^{VII}); ES-HRMS calcd for $C_{48}H_{83}N_3O_{34}$ $[Na]^+$ 1268.4756 found 1268.4805.

***N*-[(*tert*-Butoxy)carbonyl] *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)]-(1 → 4)-(6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-L-serine benzyl ester (24).** Dichloromethane (6 mL) was added to a mixture of **1** (150 mg, 0.059 mmol), Boc-Ser-OBn (27 mg, 0.089 mmol) and 4 Å molecular sieves. The suspension was cooled to −40 °C before adding NIS (27 mg, 0.119 mg) and AgOTf (catalytic). The mixture was stirred for 2 h while gradually warming to 0 °C. The reaction was quenched with Et_3N (20 μL) and diluted with dichloromethane (20 mL) before filtering through a Celite pad. The mixture was washed with 1-% aqueous $Na_2S_2O_3$ and the organic layer dried over $MgSO_4$, filtered and concentrated. Purification by silica gel *via* Biotage (column 1 gradient elution toluene/ethyl acetate, column 2 gradient toluene/acetonitrile) gave **24** (118 mg, 72%) as a colourless solid. ^1H NMR (500 MHz, MeO*D*) *δ* 7.91–7.86 (m, 3H, ArH), 7.79–7.77 (dd, *J* = 8.3, 1.2 Hz, 2H, ArH), 7.50–7.43 (m, 2H, ArH), 7.36–7.00 (ArH), 6.91–6.89 (d, *J* = 7.0 Hz, ArH), 5.56–5.53 (m, 2H), 5.41–5.40 (d, *J* = 3.9 Hz, 1H), 5.25–5.22 (m, 1H), 5.20 (t, 3H), 5.14–5.13 (d, 1 H), 4.97–4.92 (m, 2H), 4.86–4.86 (d, *J* = 3.5 Hz, 1H) 4.84–4.77 (m, 3H), 4.71–4.58 (m 7H), 4.54–4.38 (m, 9H), 4.35–4.24 (m, 8H), 4.21–4.19 (m, 2H), 4.16–4.14 (m, 1H), 4.09–3.99 (m, 4H), 3.90–3.85 (m, 3H), 3.81–3.76 (m, 3H), 3.74–3.64 (m, 7H), 3.57–3.54 (dd, *J* = 9.9, 3.0 Hz, 2H), 3.50–3.42 (m, 4H), 3.27–3.22 (m, 2H), 3.08 (bs, 1H), 2.85–2.80 (m, 2H), 1.55 (s, 3H, $COCH_3$), 1.21 (s, 9H, ^{t}Bu), 1.08–1.07 (d, *J* = 6.5 Hz, 6H, $CH_3^{IV,V}$); ^{13}C NMR (126 MHz, MeO*D*) *δ* 165.3 (*C*OOPh), 164.6 (NH*C*OCH$_3$), 161.3, 161.0, 160.7 (*C*OPh), 150.7 (HN*C*OO^{t}Bu), 135.3, 135.1, 135.0, 134.8, 134.6, 134.5, 134.5, 134.2, 133.9, 133.9, 133.8, 133.6, 131.1, 128.8, 128.6, 128.3 (17 qC), 125.6, 125.4, 125.3, 125.2, 125.1, 124.9, 124.2, 124.1, 124.0, 124.0, 124.0, 123.9, 123.9, 123.9, 123.8, 123.8, 123.8, 123.8, 123.7, 123.7, 123.6, 123.6, 123.5, 123.5, 123.5, 123.4, 123.3, 123.2, 123.1, 123.1, 123.0, 122.9, 122.9, 122.8, 122.8, 122.7, 122.7, 122.6, 122.6, 122.5, 122.4, 122.0, 122.0 (ArC), 98.9, 97.6, 97.0, 96.5, 94.1, 93.1 (C1^{I-VI}), 79.2, 76.1, 75.8, 75.1, 74.5, 74.1, 73.8, 71.6, 71.5, 71.4, 71.2, 71.2, 71.0, 70.7, 70.5, 70.3, 70.2, 69.8, 69.2, 69.1, 69.1, 68.8, 68.6, 68.4, 68.4, 68.4, 68.3, 68.1, 67.3, 67.2, 66.9, 65.1, 64.2, 63.2, 63.1, 62.5, 62.3, 61.9, 58.2, 49.5, 23.4 (^{t}Bu), 18.8 (NCO*C*H$_3$), 11.5 (C6^{V,VI}); ES-HRMS calcd for $C_{165}H_{174}N_2O_{36}Na_2$ 1402.5821 found 1402.5828.

***N*-[((3-Methyl-3*H*-diazirine-3-yl)propanoxy)carbonyl] *O*-(α-L-fucopyranosyl)-(1 → 2)-(β-D-galactopyranosyl)-(1 → 3)-[(α-L-fucopyranosyl)]-(1 → 4)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-*O*-(β-D-glucopyranosyl)-L-serine D-biotin hydrazide (25).** To a solution of compound **24** (115 mg, 0.042 mmol) in dry THF, Pd/C (20 mg) was added and the mixture was stirred for 1 h. Pd/C was filtered off and the solvent was removed. The residue was taken up in a mixture of THF (4 mL), EtOH (4 mL), H_2O (110 μL) and 1N aqueous HCl (40 μL). Pd/C was added, and the mixture was stirred under a H_2 atmosphere (5 bar) for 4 h. Additional H_2O (4 mL) was added, and the mixture was stirred under a H_2 atmosphere overnight. Upon completion of the reaction, the mixture was neutralised with saturated $NaHCO_3$, filtered through frits (20 μm, 10 μm and 5 μm) and reduced to dryness. The residue was dissolved in dry methanol (5 mL) and sodium methoxide (cat.) was added until a pH of 12–13 was reached. The reaction was stirred for 4 h then neutralised with Dowex W50$^+$ ion exchange resin. The resin was filtered off and the liquors were concentrated. Crude material was passed through a short C18 column to afford 40 mg (88%) of *O*-(α-L-fucopyranosyl)-(1 → 2)-(β-D-galactopyranosyl)-(1 → 3)-[(α-L-fucopyranosyl)]-(1 → 4)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-*O*-(β-D-glucopyranosyl)-L-serine ES-HRMS: calcd for $C_{41}H_{69}N_2O_{31}$ $[M - H]^-$ 1085.3884, found 1085.3845. This crude compound was dissolved in PBS buffer (2.00 mL, 100 mM sodium phosphate, pH 7.4) and the *N*-succinimidyl-diazirine (SDA) (10.7 mg), dissolved in DMF (100 μL) was added. The mixture was stirred overnight then concentrated. NMR analysis showed incorporation of the diazirine linker. ^1H NMR (500 MHz, D_2O) *δ* 5.16–5.15 (d, *J* = 3.7 Hz, 1H), 5.04–5.03 (d, *J* = 3.7 Hz 1H), 4.89–4.85(m, 1H), 4.67–4.66 (d, *J* = 7.5 Hz, 1H), 4.62–4.60 (d, *J* = 8.6 Hz, 1H), 4.49–4.47(d, *J* = 7.9 Hz, 1H), 4.44–4.41(m, 2H), 4.37–4.33 (dd, *J* = 12.5, 5.9 Hz, 1H), 4.28–4.25 (dd, *J* = 10.6, 5.1 Hz, 1H), 4.16–4.12 (t, *J* = 9.8 Hz, 1H), 3.99–3.85 (m, 7H), 3.83–3.68 (m, 13H), 3.65–3.47 (m, 8H), 3.35(t, *J* = 8.4 Hz, 1H), 2.73 (s, 1H), 2.27 (t, *J* = 7.5 Hz, 2H, CH_2), 2.07 (s, 3H, $COCH_3$), 1.73–1.69 (m, *J* = 14.8, 7.4 Hz, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 6H, $CH_3^{VI,V}$), 1.04 (CH_3) The crude was dissolved in water, biotin hydrazate (13.0 mg, 0.051 mmol), EDCl (9.70 mg, 0.051 mmol) and HOBt·H_2O (2.60 mg, 0.017 mmol) were added. The reaction was stirred overnight and then concentrated. The residue was purified by C18 column chromatography (H_2O/MeOH 90 : 10 to 50 : 50) followed by a Biogel P2 size exclusion column to give **25** (6.5 mg, 0.0045 mmol, 11% over four steps). ^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, J = 4.1 Hz, 1H), 5.04 (d, J = 3.9 Hz, 1H), 4.88 (d, J = 6.8 Hz, 1H), 4.75 (d, J = 5.1 Hz,1H), 4.65–4.59 (m, 2H), 4.49–4.40 (m, 2H), 4.35 (q, J = 6.6 Hz, 1H), 4.25 (dd, J = 10.7, 5.3 Hz, 1H), 4.15 (m, 3H), 4.00–3.50 (m, 28H), 3.36 (m, 2H), 3.02 (dd, J = 13.1, 5.0 Hz, 1H, biotin h), 2.80 (d, J = 13.0 Hz, 1H, biotin h), 2.35 (t, J = 7.3 Hz, 2H, biotin a), 2.29 (t, J = 7.4 Hz, 2H, diazirine i), 2.07 (s, 3H, $COOCH_3$), 1.78–1.67 (m, 4H, biotin d′, b and diazirine j), 1.62 (dd, J = 14.1, 7.6 Hz, 1H, biotin d″), 1.48 (q, J = 7.6 Hz, 2H, biotin c), 1.28 (dd, J = 9.2, 6.6 Hz, 6H, $CH_3^{V,VI}$), 1.04 (s, 3H, diazirine CH_3). ^{13}C NMR (151 MHz, D_2O) δ 175.5, 174.1, 165.3 ($C{=}O$), 103.17, 102.93, 102.08, 100.57, 99.49, 97.71 (C-1^{I-VI}), 81.48, 78.12, 76.42, 75.11, 74.72, 74.40, 74.06, 73.56, 72.62, 71.91, 71.71, 70.07, 69.37, 69.04, 68.67, 68.55 (CH_2 Serine), 68.20, 67.73, 66.96, 66.17, 61.90, 61.52, 60.88, 59.9, 59.4 ($C6^{I-IV}$), 60.19, 55.68, 55.15, 52.33, 39.63 (CH_2 biotin h), 33.09 (CH_2 biotin a), 29.81 (CH_2 diazirine i), 29.42 (CH_2 biotin b), 27.59 (CH_2 biotin c), 27.45 (CH_2 biotin d), 24.59 (CH_2 diazirine j), 22.10 ($COOCH_3$), 18.49 (CH_3 diazirine k), 15.29 ($CH_3^{V,VI}$). MALDI-TOF MS: $C_{56}H_{92}N_8NaO_33S$ $[M + Na]^+$ calcd 1459.5385, found 1459.49.

Conflicts of interest

There are no conflicts to declare.

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