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Synthesis of a B-Antigen Hexasaccharide, a B-Lewis b Heptasaccharide and Glycoconjugates Thereof to Investigate Binding Properties of *Helicobacter pylori*

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Abstract: Infecting the stomach of almost 50% of people, *Helicobacter pylori* is a causative agent of gastritis, peptic ulcers and stomach cancers. Interactions between bacterial membrane-bound lectin, Blood group Antigen Binding Adhesin (BabA), and human blood group antigens are key in the initiation of infection. Herein, the synthesis of a B-antigen hexasaccharide (B6) and a B-Lewis b heptasaccharide (BLeb7) and Bovine Serum Albumin glycoconjugates thereof is reported to assess the binding properties and preferences of BabA from different strains. From a previously reported trisaccharide acceptor a versatile key Lacto-*N*-tetraose tetrasaccharide intermediate was synthesized, which allowed us to explore various routes to the final targets, either via initial introduction of fucosyl residues followed by introduction of the B-determinant or vice versa. The first approach proved unsuccessful, whereas the second afforded the target structures in good yields. Protein conjugation using isothiocyanate methodology allowed us to reach high glycan loadings (up to 23 per protein) to mimic multivalent displays encountered in Nature. Protein glycoconjugate inhibition binding studies were performed with *H. pylori* strains displaying high or low affinity for Lewis b hexasaccharide structures showing that the binding to the high affinity strain was reduced due to the presence of the B-determinant in the Bleb7-conjugates and further reduced by the absence of the Lewis fucose residue in the B6-conjugate.

Introduction

First reported by Marshall and Warren in 1982 and previously referred to as *Campylobacter pylori*, *Helicobacter pylori* is a helical-shaped, Gram-negative bacterium that is infectious in the stomach of almost 50% of the population.[1,2] Most cases are asymptomatic but approximately 10% result in severe gastroduodenal diseases such as chronic gastritis, peptic ulceration, adenocarcinoma and stomach cancer.[3–5] Oral-oral and oral-fecal contact are accepted to be the most common modes of transmission.[6]

Lewis antigens are a set of human oligosaccharides that are closely related to the ABO blood group system (Figure 1).[7] They are mostly displayed on the surface of epithelial cells but have also been found on a range of other cell types, including sensory neurons. Biosynthesised through sequential addition of monosaccharide residues by glycosyl transferases, Lewis antigens are presented at the non-reducing end of glycans belonging to glycolipids of the lacto, ganglio and globo series along with *O*-glycans and *N*-glycans.[8–13]

H. pylori strains are classed as either generalists or specialists depending on their blood group antigen binding preferences. Generalists can bind to structures belonging to blood types A, B, AB and O while specialists can only bind to the smaller, non-extended glycans expressed by those who are blood type O.[14]

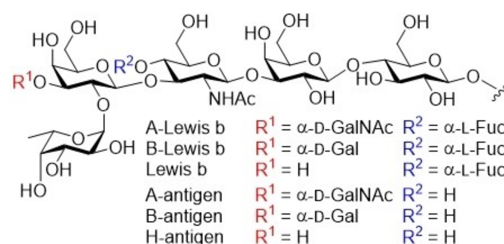

Figure 1. Blood group antigen structure of the lacto series.

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This phenomenon arises due to a single difference in the amino acid sequences of a bacterial membrane-bound lectin known as Blood group Antigen Binding Adhesin (BabA).[15,16] The binding of BabA to Lewis b blood group antigens of the lacto series on the surface of epithelial cells in the stomach facilitates adherence of *H. pylori* to the host and increases the virulence of the strain by allowing the transfer of effector proteins.[17,18] With the B-Lewis b heptasaccharide and B hexasaccharide being unavailable from commercial sources, due to the low abundance of blood group B in most populations, the study of these protein-glycan interactions heavily depends on synthetic structures.

Our group has previously published various pathways to synthesise Lewis b pentasaccharides and hexasaccharides (including a structure with a ^{13}C-labelled fucose residue) and protein conjugates thereof that have been used to investigate interactions with BabA.[14,16,19–21] The B-Lewis b heptasaccharide has been synthesised and used in these interaction studies,[14,16] but the synthesis has not been published. This manuscript summarises our experiences in the synthesis of B-Lewis b heptasaccharide **1**, B-antigen hexasaccharide **2**, and Bovine Serum Albumin (BSA) glycoconjugates thereof (**3** and **4**, Figure 2) and preliminary inhibition experiments.

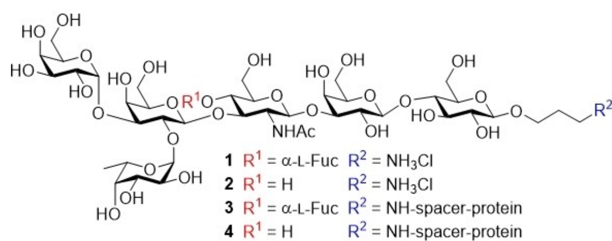


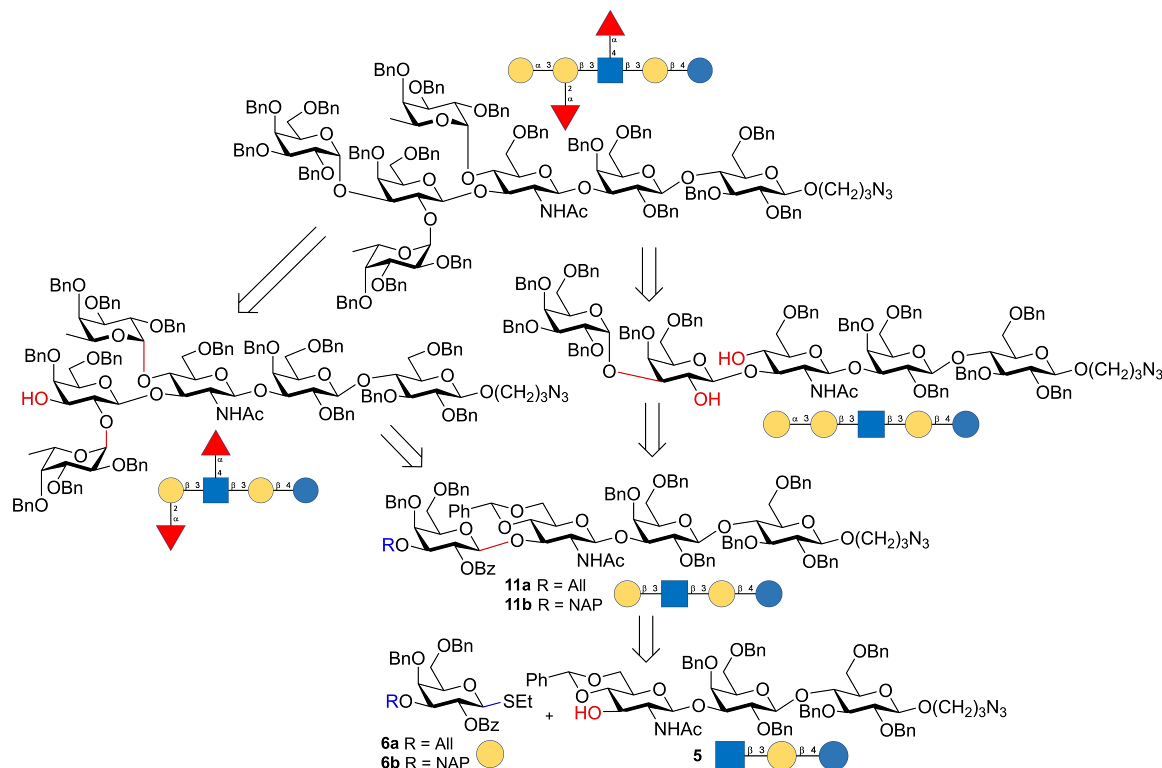
Figure 2. B-Lewis b heptasaccharide and B-antigen hexasaccharide targets.

Results and Discussion

Retrosynthesis of targets **1** and **2** gives two possible disconnections, either at the α-L-fucose glycosidic linkages or at the B-determinant (α-D-Gal) linkage (Scheme 1), giving a pentasaccharide diol acceptor or a hexasaccharide acceptor intermediate respectively (the latter being attractive as a common precursor for both the B- and A-Lewis b heptasaccharides). The next disconnection will bring these paths together to a versatile 2‴-, 3‴-, 4″-orthogonally protected tetrasaccharide, which disconnects to a known trisaccharide **5** (used in earlier Lewis b syntheses) and a galactosyl donor **6**.[19–21]

The retrosynthetic pathway from the B-antigen hexasaccharide target **2** was envisioned to include the same key tetrasaccharide intermediate **11** outlined in Scheme 1. In contrast to the B-Lewis b pathway, preservation of the benzylidene acetal would be key to protect the 4″-OH, allowing access to the monofucosylated target.

In contrast to previous Lewis b syntheses, galactosyl donor **6** requires an orthogonal protecting group in the 3-positions to allow introduction of the B- (or A)-determinant. Several protecting groups were initially tested, but only two were



Scheme 1. Retrosynthesis of B-Lewis b heptasaccharide via a key tetrasaccharide intermediate **11**.

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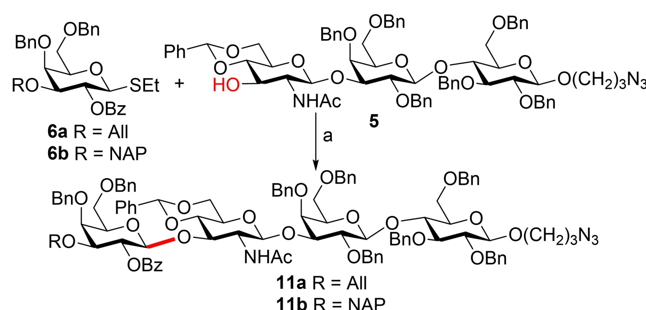
investigated in the synthesis of the tetrasaccharide intermediate: the allyl group (Gal donor **6a**) and the methylnaphtyl group (NAP, Gal donor **6b**). The NAP group was attractive because of the capacity to be removed either chemoselectively, using, for example, CAN or DDQ, or together with the permanent benzyl groups via catalytic hydrogenolysis.

Initially, the synthesis of Gal donor **6a** was attempted using the 2,3-butadione acetal as intermediate, but problems were encountered reproducing the literature procedures,[22,23] which is why another approach starting from 4,6-*O*-benzylidene acetal **7** was used (Scheme S1). Hydroxyls activation of **7** with Bu_2SnO in refluxing MeOH and subsequent regioselective alkylation with allyl bromide and CsF in refluxing THF furnished 3-*O*-All compound **8** in a 70% yield. Benzoylation through reaction with benzoyl chloride and Et_3N in CH_2Cl_2 afforded 88% of compound **9**. Reductive benzylidene acetal ring opening was then performed with $PhBCl_2$ and Et_3SiH in CH_2Cl_2 at $-78\,^{\circ}C$ (→ **10**, 79%) and subsequent benzylation under dry conditions allowed access to galactosyl donor **6a** (87% yield). Donor **6b** was synthesised in a similar manner.[24,25]

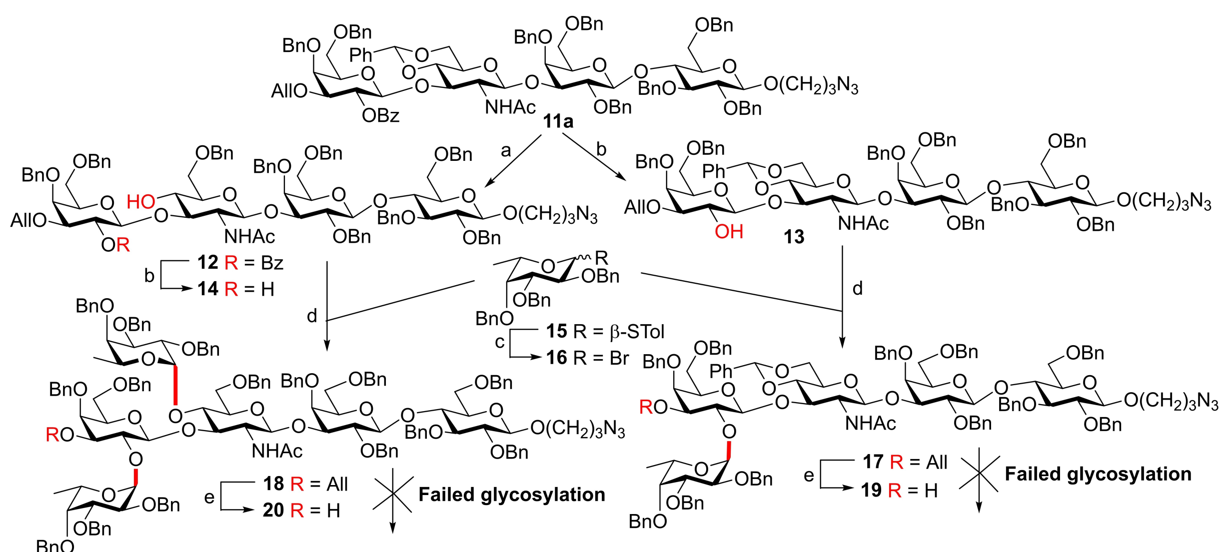
Galactosyl donors **6a** and **6b** underwent NIS/AgOTf promoted glycosylations with trisaccharide acceptor **5** to furnish key tetrasaccharide intermediates **11a** and **11b** in 65–85% and 72% yields, respectively (Scheme 2). Attempts to chemoselectively remove the NAP group from **11b**, to access acceptor **21**, were made (see Table 1 in the SI for details) but with no success, which is why the use of this intermediate was abandoned. We, as well as others, have reported problems with the orthogonal removal of the NAP protecting group on substrates containing many benzyl groups.[26,27] We then explored the possibility of manipulating intermediate **11a** to proceed towards targets **1** and **2** either by introducing the fucosyl residues first or the galactosyl residue first.



Scheme 2. Synthesis of intermediate **11** a) NIS, AgOTf (21 mol%), AW-300 4 Å molecular sieves, CH_2Cl_2, rt, 20 min. **11a**: 65–85%, **11b**: 72%.

Pathway A. Initial introduction of the fucosyl residues

Reductive benzylidene acetal ring opening with $NaBH_3CN$ and 1 M HCl/Et_2O in THF to reveal the 4''-OH, delivered compound **12** in a 61% yield (Scheme 3). This transformation was unsuccessful when tried with $BF_3\cdot Et_2O$ and Et_3SiH in CH_2Cl_2 or $Cu(OTf)_2$ (cat.) and Me_2EtSiH in MeCN due to hydrolysis of the acetal rather than opening, even in the presence of molecular sieves.[28,29] Saponification of the benzoyl ester in **11a** and **12** with 10 M aq. NaOH afforded 57% of mono-ol acceptor **13** and 77% of diol acceptor **14**, respectively. These conditions were employed since the use of NaOMe in MeOH for this transformation required elevated temperatures (60-70 °C) and led to



Scheme 3. Unsuccessful routes towards targets **1** and **2** a) $NaBH_3CN$, 1 M HCl/Et_2O, 4 Å molecular sieves, THF, rt, 1.5 h, 61%; b) 10 M aq. NaOH, THF/EtOH/H_2O (5:2:1, v/v/v), 35 °C, 23 h, **13**: 57%, **14**: 77%; c) Br_2, CH_2Cl_2, 0 °C, 15 min; d) Bu_4NBr, 4 Å molecular sieves, CH_2Cl_2/DMF (9:1, v/v), rt, 3 days, **17**: 64%, **18**: 76%; e) H_2, $Ir(COD)(PPh_2Me)_2PF_6$ (20 mol%), THF, rt, 2 h, then $NaHCO_3$, H_2O, I_2, rt, 21 h, **19**: 85%, **20**: 69%.

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prolonged reaction times (3–4 days). Fucosylation through Lemieux's halide-assisted glycosylation[30] of **13** and **14** produced pentasaccharide **17** and hexasaccharide **18** in a 64% and a 76% yield respectively, with only the 1,2-*cis* linkages being observed. Due to the incompatibility of allyl groups with Br_2, donor **15** was first converted to the bromosugar **16** through reaction with Br_2 in CH_2Cl_2 before being added to acceptors. The allyl group in **17** and **18** was removed via Ir(COD)$(PPh_2Me)_2PF_6$ catalysis followed by sequential addition of $NaHCO_3$, H_2O and I_2, affording 3‴-OH acceptors **19** (85%) and **20** (69%), respectively (Scheme 3).

Addition of galactosyl building blocks **22** or **22a** (B-determinant) to hexasaccharide acceptor **18** and pentasaccharide acceptor **20** was attempted using different glycosylation conditions including halide-assisted glycosylation conditions. In a halide-assisted glycosylation carried out with an in situ formation of the galactosyl bromide in the presence of the acceptor, no consumption of the acceptor was observed, and addition of AgOTf to further activate the bromosugar, did not improve the outcome. This led to abandonment of the pathways including late-stage introduction of the B-determinant.

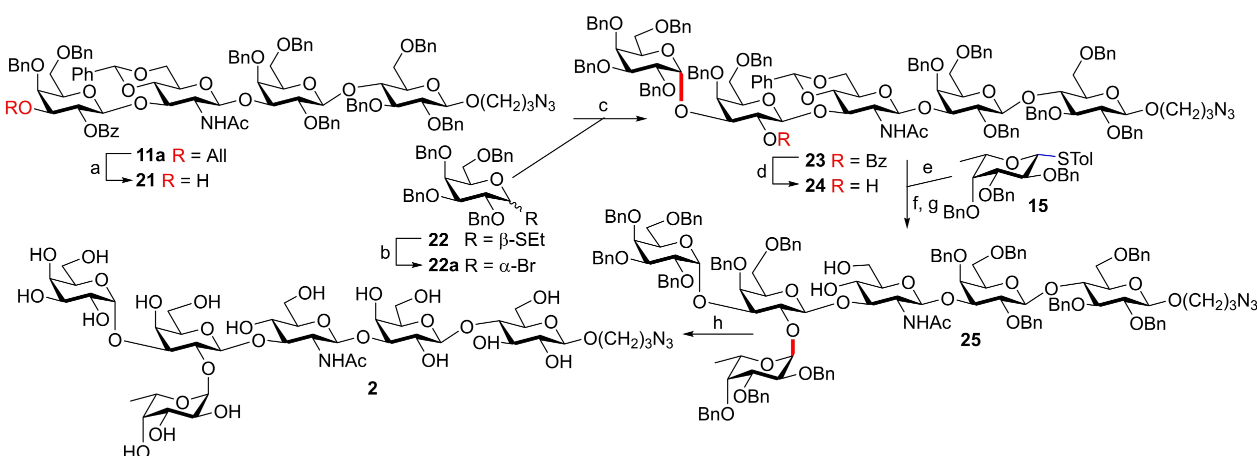
Earlier syntheses of the B-antigen Type 1 tetrasaccharide using a final galactosylation step to introduce the B determinant all utilized a 4,6-*O*-benzylidene group in the acceptor galactosyl residue, in contrast to our 4,6-di-O-benzyl protection, which might be an explanation to the different results.[31] In parallel with this approach, we also pursued the pathway from the same intermediate **11a** but changing the order of introduction of the fucosyl and galactosyl residues.

Pathway B. Initial introduction of the B-determinant

The allyl group of tetrasaccharide intermediate **11a** was removed via Ir(COD)$(PPh_2Me)_2PF_6$ catalysis followed by sequential addition of $NaHCO_3$, H_2O and I_2, affording acceptor **21** in a 73% yield (Scheme 4). Conversion of galactosyl thioglycoside **22** to bromosugar **22a** and subsequent reaction with acceptor **21** and Bu_4NBr resulted in formation of pentasaccharide **23** in a 48% yield. Bromine activation of donor **22** in situ led to a complex mixture due to removal of the acid labile benzylidene acetal of **21**. The 2‴-OBz group was removed with 10 M aq. NaOH in THF/EtOH/H_2O (5:2:1, v/v/v), delivering 75% of compound **24**. Halide-assisted fucosylation of this was sluggish, but afforded the B-antigen hexasaccharide, which due to purification issues, was isolated as compound **25** following removal of the benzylidene acetal. To access the B-Lewis b diol acceptor **26**, reductive ring opening of the benzylidene acetal in **24** was attempted, which proved troublesome. Numerous conditions were tested, among which 1 M HCl/Et_2O and $NaBH_3CN$ in THF delivered the only positive results, albeit with poor reproducibility (see Table 2 in the Supporting Information).

Because of these problems encountered in the conversion of **24** to **26**, we investigated if acceptor **26** could be accessed through a different route starting from tetrasaccharide intermediate **12** in which the benzylidene acetal had already been opened (Scheme 5). The 4″-OH of tetrasaccharide **12** was temporarily protected with an acetyl group (Ac_2O/DMAP/pyridine) with subsequent removal of the allyl group, this time using $PdCl_2$ in CH_2Cl_2/MeOH, delivering acceptor **27** in an 85% yield over 2 steps.

Halide-assisted glycosylation with galactosyl building block **21** (in situ activation), followed by hydrolysis of the acyl groups



Scheme 4. Synthesis of B hexasaccharide **2** a) H_2, Ir(COD)$(PPh_2Me)_2PF_6$ (20 mol%), THF, rt, 2 h, then $NaHCO_3$, H_2O, I_2, rt, 20 h, 73%; b) Br_2, CH_2Cl_2, 0 °C, 15 min; c) Bu_4NBr, 4 Å molecular sieves, CH_2Cl_2/DMF (9:1, v/v), rt, 10 days, 48%; d) 10 M aq. NaOH, THF/EtOH/H_2O (5:2:1, v/v/v), 35 °C, 48 h, 75%; e) Br_2, CH_2Cl_2, 0 °C, 10 min; f) Bu_4NBr, 4 Å molecular sieves, CH_2Cl_2/DMF (9:1, v/v), 21 days; g) *p*-TsOH·H_2O, CH_2Cl_2/MeOH, rt, 48 h, 26% over 2 steps; h) H_2 (1 atm), 5% Pd/C (10 mol%), 20% $Pd(OH)_2$/C (62 mol%), 0.1 M aq. HCl, EtOAc/EtOH/ H_2O, rt, 5 days, 55%.

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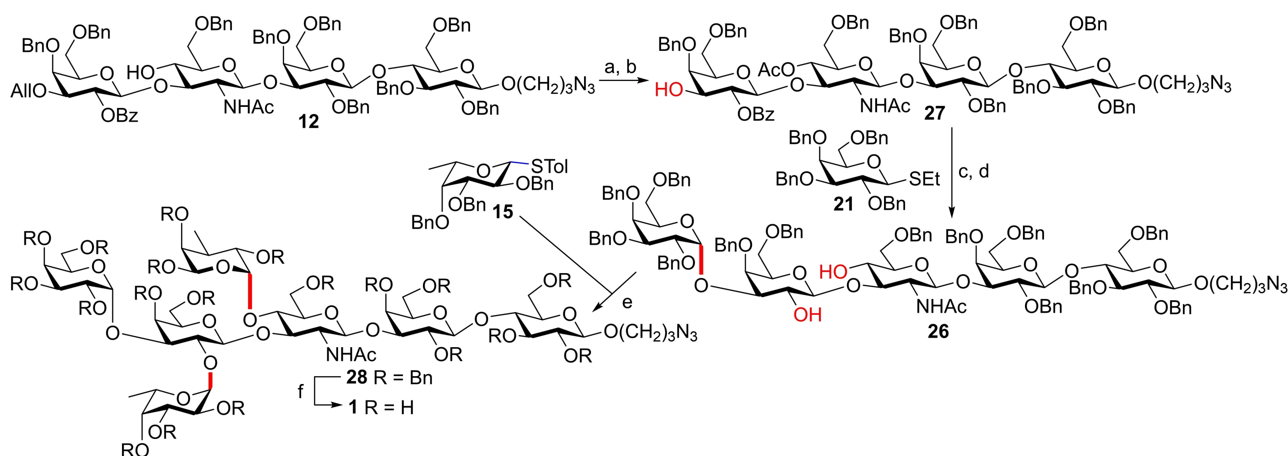

Scheme 5. Synthesis of B-Lewis b heptasaccharide **1** a) Ac_2O/Py (1:2, v/v), DMAP (56 mol%), rt, 3 h; b) $PdCl_2$ (50 mol%), CH_2Cl_2/MeOH (1:1, v/v), rt, 6 h, 85% over 2 steps; c) Br_2, Bu_4NBr, 4 Å molecular sieves, CH_2Cl_2/DMF (9:1, v/v), rt, 5 days; d) 10 M aq. NaOH, THF/EtOH/H_2O (5:2:1, v/v/v), 35 °C, 21 h, 56% over 2 steps; e) Br_2, Bu_4NBr, 4 Å molecular sieves, CH_2Cl_2/DMF (9:1, v/v), rt, 3 days, 83%; f) H_2 (1 atm), 5% Pd/C, 0.1 M aq. HCl, THF/^{t}Bu-OH/H_2O (6:1:3, v/v/v), rt, 42 h, quant.

with 10 M aq. NaOH furnished diol acceptor **26** in a 56% yield over 2 steps, representing a much-improved route to acceptor **26** from intermediate **11 a**. Difucosylation of acceptor **26** via halide-assisted glycosylation, with in situ activation of donor **15**, smoothly delivered 83% of heptasaccharide **28** (Scheme 5).

Hydrogenolysis of compounds **25** and **28** with catalytic amounts of Pd/C (10 mol%) and $Pd(OH)_2$/C (62 mol%) and 1 equivalent of 0.1 M aq. HCl in EtOAc/EtOH/H_2O delivered targets B hexasaccharide **2** and B-Lewis b heptasaccharide **1** in almost quantitative yield and 55% yield, respectively, after size-exclusion chromatography and lyophilisation (Schemes 4 and 5). These variant yields show the difficulty in ascertaining reproducible results in these hydrogenolysis reactions, partly because of different quality of commercial palladium catalysts.[32]

Conjugations

We have made several protein conjugates of the Lewis b hexasaccharide varying in both loading and conjugation methodology. Binding interactions with *H. pylori* have shown that multivalency is important for a strong binding.[14] Only one conjugate had been made with the B Lewis b heptasaccharide, using squarate ester methodology, which resulted in a loading of approximately 5 glycans per protein, too low to be suitable for Scatchard affinity analysis and for detection of strains with low binding affinities.[14] Proving to be optimal for binding to Lewis b glycoconjugates, we now targeted a loading of ~20 glycans per protein, similar to loadings of commercial Lewis b and A Lewis b conjugates.

Initially, N-hydroxysuccinimide (NHS) ester methodology was tried (Scheme 6), which had worked well in previous conjugations with Lewis b structures.[20] To limit dimerisation, heptasaccharide **1** underwent a reaction with a large excess of heterobifunctional compound **30** (10 equiv.) with Et_3N in DMSO. The reaction was monitored by MALDI-TOF mass spectrometry (super-DHB matrix, Supporting Information S45–S47) which showed that heptasaccharide **1** was consumed after 1 h, with limited amounts of dimerisation evident. Cold water was added to the reaction mixture, and the unwanted by-products and remaining compound **30** were filtered off. The filtrate was lyophilised to remove water and DMSO and the resulting solid **31** (60 equiv.) underwent conjugation with BSA (1 equiv.) in pH 10 aq. borate buffer. Analysis of the reaction mixture by MALDI-TOF mass spectrometry (sinapinic acid matrix) revealed that a loading of only 3.5 glycans per protein (compound **32**, Bleb7-L4) had been achieved after 24 h (6% incorporation) and allowing the reaction to proceed for longer periods of time did not improve the loading, as earlier found. We hypothesised that the NHS ester of **31** was rapidly hydrolysing in the alkaline buffer before conjugation could take place, however, performing the conjugation step in pH 7.5 aq. phosphate buffer saline (PBS) resulted in the same level of incorporation.

Isothiocyanate methodology was then investigated (Scheme 6).[33] Using a biphasic procedure, similar to Trattnig et al.,[34] heptasaccharide **1** (20 equiv.) was converted to the corresponding isothiocyanate **33** in a mixture of 24 μM thiophosgene/$CHCl_3$ (60 equiv.) and 0.1 M aq. $NaHCO_3$. Monitoring of the reaction by MALDI-TOF mass spectrometry (super-DHB matrix) after 17 h revealed partial conversion of **1** to isothiocyanate **33**. Additional equivalents of thiophosgene and/or extended reaction times did not improve the outcome. Nevertheless, the organic phase was removed, and the aqueous layer was added to a solution of BSA (1 equiv.) in 0.3 M aq. NaCl/0.1 M aq. $NaHCO_3$. The reaction was monitored daily by MALDI-TOF mass spectrometry (trans-ferulic acid matrix) and appeared to progress for up to 5 days before no further conjugation was taking place. At this stage, a loading of 7 glycans per protein was observed (35% glycan incorporation). Ultracentrifugation with a 30 kD molecular weight filter and subsequent freeze-drying gave glycoconjugate **34**. To increase

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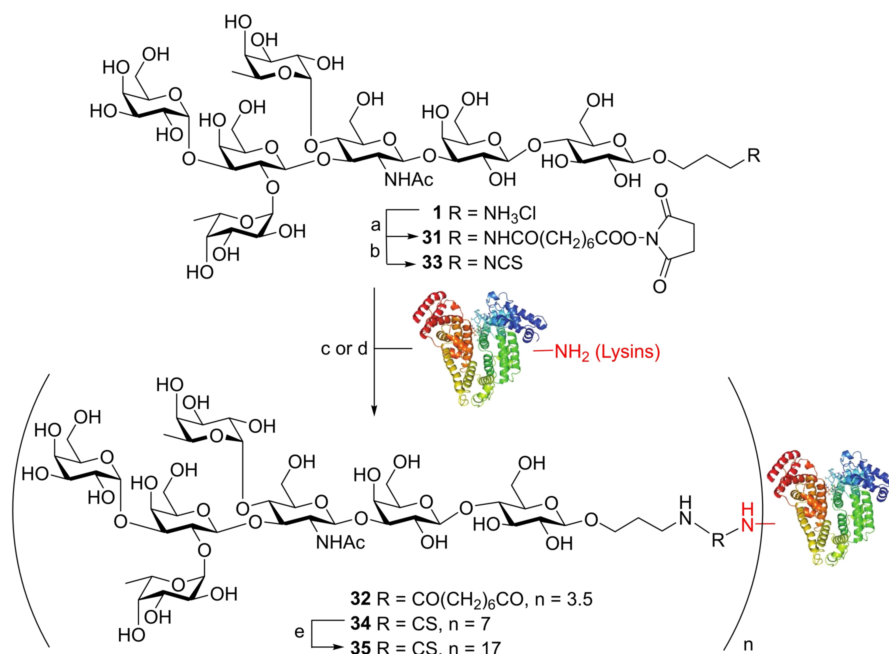

Scheme 6. Synthesis of glycoconjugates **32**, **34**, and **35** a) suberic acid bis(N-hydroxysuccinimide ester (**30**, 10 equiv.), Et_3N, DMSO, rt, 1 h; b) 24 μM thiophosgene (3 equiv.)/$CHCl_3$, 0.1 M aq. $NaHCO_3$, rt, 17 h; c) **31** (60 equiv.), borate buffer (pH 10), rt, 3 days, or PBS buffer (pH 7.5), rt, 3 days; d) **33** (20 equiv.), 0.3 M NaCl/0.1 M aq. $NaHCO_3$, rt, 5 days; e) **33** (40 equiv.), 0.3 M NaCl/0.1 M aq. $NaHCO_3$, rt, 5 days.

the loading of **34**, 40 equiv. of heptasaccharide **1** were converted into isothiocyanate **33** and subsequently reacted with glycoconjugate **34** as described above. After 5 days, MALDI-TOF mass spectrometry (trans-ferulic acid matrix) revealed an average further incorporation of 10 glycans (28%) per protein (compound **35**, Bleb7-L17). Glycoconjugate **35** was isolated via ultracentrifugation and freeze-dried. B-antigen hexasaccharide **2** was conjugated to BSA in a similar two-step procedure using 33 equivalents of the glycan **2** in each step, resulting in glycoconjugate **36** (B6-L23) with a loading of 23 glycans (35% conjugation) per protein.

Inhibition experiments

With the access to BLeb7 conjugates with different loadings, L4, L13, and L17, and a B6 conjugate with high loading, L23, their binding to *H. pylori* was investigated in preliminary inhibition experiments (Figure 3 and Supporting Information page S48-S49). Two different strains were investigated, strain 17875, a high affinity strain for Lewis b structures, and strain J166, a low affinity strain for Lewis b structures. As expected, there was a strong correlation between binding strength and loading of the conjugates, with the multivalency effect decreasing at higher loadings. Thus, the Bleb7-L4 conjugate only showed weak binding (IC_{50} ~ 500) to 17875 and even weaker binding to J166 (IC_{50} ~ 6200), at this loading indicating J166 to be a low affinity strain also for BLeb7 structures. However, the Bleb7-L13 and -L17 conjugates showed similar binding both to 17875 and J166 (IC_{50} ~ 22-30) and no difference in binding between the different loadings. The B6-L23 conjugate showed weaker binding to the 17875 strain (IC_{50} ~ 60) but comparable binding to Bleb7 to the J166 strain (IC_{50} ~ 25). Thus, the absence of the 4-linked Lewis fucose residue resulted in a weaker binding to the BabA of strain 17875. This has earlier been observed also for the corresponding A6 (α-GalNAc) structure, for which ITC measurements showed a 3.5 and 2-fold lower monovalent affinity compared to BLeb7 and Leb5 structures, respectively.[16]

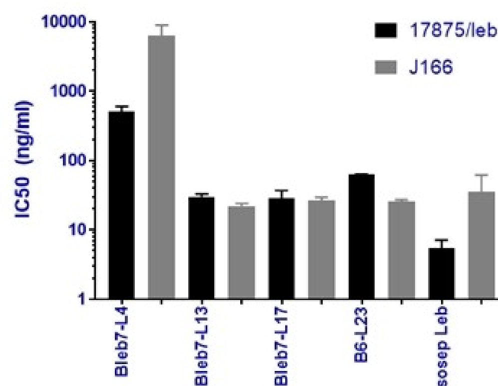

Figure 3. IC_{50} (ng/ml) of glycoconjugates versus *H. pylori* strains 17875/leb and J166. IsoSep Leb is a commercial conjugate with an average loading of 22.

However, the absence of the Lewis fucose is having minor effect on the binding to the J166 BabA. B6 conjugates with different loadings and a Bleb7 conjugate with higher loading (~ 23) are now being prepared to further investigate their binding to various *H. pylori* strains and to search for B-specific strains.

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Conclusions

In this article, we have outlined the first reported syntheses of a lactose series B-Lewis b heptasaccharide, a B-antigen hexasaccharide, and BSA-glycoconjugates of both synthetic structures. Proceeding through a versatile tetrasaccharide intermediate, we found that introducing the fucosyl residues first proved to be unsuccessful due to poor reactivity of the 3‴-OH acceptors. Proceeding through [1 + 1 + 5] and [1 + 5] glycosylation was the key to reaching the B-Lewis b heptasaccharide and the B hexasaccharide targets, respectively. For the B-Lewis b synthesis reductive ring opening at the tetrasaccharide level proved to be more reliable than at the pentasaccharide stage. In all pathways the unusual instability of the 4″,6″-*O*-benzylidene acetal caused problems, both in reductive opening of the acetal and in glycosidation reactions, for example, attempting halide-assisted glycosylation with in situ activation of the thioglycoside donors with Br_2 caused hydrolysis of the acetal. Conjugation to BSA using NHS ester methodology resulted in poor glycan incorporations (up to 3.5 per protein) while exploration of isothiocyanates led to a step-wise method allowing us to achieve high loadings (up to 23 per protein). Inhibition binding studies with our synthetic B6 and BLeb7 glycoconjugates and a Leb high affinity *H. pylori* strain, 17875, and a Leb low affinity strain, J166, showed the same affinity difference between the two strains for the Bleb7 conjugates at low loading (L4), but equal affinity to the two strains at higher loading (L13, 17, and 23) due to a lower affinity for the 17875 strain as compared to Leb conjugates with similar loadings. Furthermore, absence of the Lewis fucose in the B6 conjugate resulted in a lower affinity to the 17875 strain but had no effect on the binding to the J166 strain.

Experimental Section

Please see the Supporting Information for the experimental procedures towards compounds **8–10**, **6a**, **11b**, **13**, **14** and **17–20**, NMR spectra of all compounds, and for inhibition experiment methods.

General Methods: All reactions were monitored by thin-layer chromatography (TLC) on Merck DC-Alufolien plates precoated with silica gel 60 F_{254}. Visualisation was performed with UV-light (254 nm) fluorescence quenching, and/or by staining with an 8% H_2SO_4 dip (stock solution: 8 mL conc. H_2SO_4, 92 mL EtOH). All chemicals for the synthesis were purchased from commercial suppliers (Acros, Carbosynth Ltd, Fisher Scientific Ltd., Glycom A/S, Merck, Sigma-Aldrich, VWR) and were used without purification. Dry CH_2Cl_2 and THF were obtained from a PureSolv-EN™ solvent purification system (Innovative Technology Inc.). All other anhydrous solvents were used as purchased from Sigma-Aldrich in AcroSeal® bottles. Silica gel flash chromatography was carried out using Davisil® LC60A (40–63 μm) silica gel or with automated flash chromatography systems, Buchi Reveleris® X2 (UV 200–500 nm and ELSD detection, Reveleris® silica cartiges 40 μm, BÜCHI Labortechnik AG®) and Biotage® SP4 HPFC (UV 200–500 nm, Biotage® SNAP KP-Sil 50 μm irregular silica, Biotage® AB). ^1H NMR and ^{13}C NMR spectra were recorded on Varian Inova spectrometers at 25 °C. Assignments were aided by homonuclear ^1H–^1H (COSY, TOCSY) and ^1H–^{13}C heteronuclear (HSQC, HMBC) two-dimensional correlation spectroscopies. ^{13}C chemical shifts were reported to one decimal point unless an additional digit was required to distinguish overlapping peaks. Software for data processing: MestReNova, version 11.0.0–17609 (MestReLab Research S.L.). High-resolution mass spectrometry (HRMS) data were recorded on a Waters Micromass LCT LC-TOF instrument using electrospray ionisation (ESI) in positive mode. MALDI-TOF mass spectrometry data were recorded on a Scientific Analysis Instruments MALDI-TOF mass spectrometer in reflectron mode for oligosaccharide structures, and linear mode for glycoconjugates using laser power in the range of 60–280. Samples were prepared by pre-mixing 1 μL of a solution containing the analyte with 20 μL of a matrix solution (10 mg/mL, MeCN/H_2O, 1:1, v/v + 1% TFA), pipetting 1 μL of the mixture onto the sample plate and drying under gentle heat from a heat gun. Optical rotations were recorded in a Perkin-Elmer polarimeter (Model 343) at the sodium D-line (589 nm) at 20 °C using a 1 dm cell. Samples were prepared at the concentration (g/100 mL) in the solvent indicated. Deprotected sugars were lyophilised using a freeze-dryer Alpha 1–2 LDplus (Christ Ltd): pressure: 0.055 mbar; ice-condenser temperature: −55 °C. Carbohydrate structures are named according to IUPAC recommendations.[35] Individual monosaccharide residues belonging to oligosaccharides are labelled as: Fuc (fucose), Gal (galactose), Glc (glucose) and GlcNAc (2-acetamido-2-deoxy-glucopyranoside).

3-Azidopropyl 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11a): Donor **6a** (0.58 g, 1.1 mmol) and acceptor **5** (0.86 g, 0.68 mmol) were placed under N_2 and stirred at room temperature in dry CH_2Cl_2 (14 mL) with AW-300 4 Å molecular sieves (1.53 g) for 2 h. NIS (0.31 g, 1.4 mmol) and AgOTf (35 mg, 0.14 mmol) were added sequentially, and the reaction was stirred at room temperature for 20 minutes. The mixture was then neutralised with Et_3N and filtered through Celite®. The filtrate was concentrated under reduced pressure and purified via flash chromatography on silica gel (toluene→toluene/EtOAc, 4:1), yielding **11a** as a pale-yellow foam (0.77 g, 65%); R_f = 0.4 (toluene/EtOAc, 3:1); [α] + 5.4 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.08–7.95 (m, 2H, Ar–H), 7.55 (m, 1H, Ar–H), 7.49–7.38 (m, 4H, Ar–H), 7.37–7.05 (m, 43H, Ar–H), 5.63 (m, 1H, $CH_2{=}\underline{CH}_{(OAll)}$), 5.51 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2$_{Gal'''}$), 5.48 (s, 1H, $CH_{(acetal)}$), 5.29–5.27 (m, 2H, H-1$_{GlcNAc}$, NH), 5.11 (m, 1H, $C\underline{H_2}{=}CH_{(A)OAll}$), 5.01 (m, 1H, $C\underline{H_2}{=}CH_{(B)OAll}$), 4.97 (d, *J* = 10.6 Hz, 1H, OCH$\underline{H}$Ph), 4.91–4.86 (m, 2H, 2×OCH$\underline{H}$Ph), 4.79 (d, *J* = 11.0 Hz, 1H, OCH$\underline{H}$Ph), 4.72 (d, *J* = 10.9 Hz, 1H, OCH$\underline{H}$Ph), 4.68–4.43 (m, 9H, H-1$_{Gal'''}$, H-3$_{GlcNAc}$, 7×OCH$\underline{H}$Ph), 4.34–4.24 (m, 5H, H-1$_{Gal'}$, H-1$_{Glc}$, H-6$_{(A)GlcNAc}$, 2×OCH$\underline{H}$Ph), 4.21–4.18 (m, 2H, 2×OCH$\underline{H}$Ph), 4.01 (m, 1H, $OCH_{2(A)OAll}$), 3.95–3.81 (m, 6H, H-4$_{Gal'''}$, H-4$_{Gal'}$, H-4$_{Glc}$, $OCH_{2(B)OAll}$, $OCH_{2(A)Linker}$), 3.72–3.67 (m, 2H, H-4$_{GlcNAc}$, H-6$_{(B)GlcNAc}$), 3.66–3.51 (m, 7H, H-5$_{GlcNAc}$, H-5, H-6$_{(A+B)Glc}$, H-6$_{(A)}$, $OCH_{2(B)Linker}$), 3.48–3.44 (m, 4H, H-3$_{Gal'}$, H-3$_{Glc}$, H-6$_{(B)}$), 3.40–3.31 (m, 7H, H-2$_{Gal'}$, H-2$_{Glc}$, H-3$_{Gal'''}$, H-5, H-6$_{(A)}$, $CH_2N_{3(Linker)}$), 3.28 (dd, *J* = 8.5, 5.2 Hz, 1H, H-6$_{(B)}$), 3.15 (m, 1H, H-5$_{Glc}$), 2.97 (m, 1H, H-2$_{GlcNAc}$), 1.92–1.79 (m, 2H, $-CH_2-_{(Linker)}$), 0.81 (s, 3H, $CH_{3(NHAc)}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7 ($C{=}O_{(NHAc)}$), 165.3 ($C{=}O_{(OBz)}$), 139.5, 139.2, 138.9, 138.7, 138.6, 138.38, 138.30, 137.9, 137.7 (Ar–$C_{(quat)}$), 134.4 ($CH_2{=}\underline{C}H_{(OAll)}$), 133.2 (Ar–CH), 130.2 (Ar–$C_{(quat)}$), 129.8, 129.2, 128.59, 128.52, 128.48, 128.45, 128.44, 128.42, 128.40, 128.39, 128.28, 128.27, 128.25, 128.09, 128.03, 128.02, 127.97, 127.87, 127.75, 127.73, 127.72, 127.70, 127.69, 127.63, 127.42, 127.40, 127.2, 126.4 (Ar–CH), 117.3 ($\underline{C}H_2{=}CH_{(OAll)}$), 103.6 (C-1$_{Gal'}$), 102.6 (C-1$_{Glc}$), 101.4 ($CH_{(acetal)}$), 101.0 (C-1$_{Gal'''}$), 100.7 (C-1$_{GlcNAc}$), 83.0 (C-3$_{Glc}$), 82.5 (C-3$_{Gal'}$), 81.8 (C-2$_{Glc}$), 81.3 (C-4$_{GlcNAc}$), 80.2 (C-3$_{Gal'''}$), 79.5 (C-5), 77.1 (C-3$_{GlcNAc}$), 76.5 (C-4$_{Gal'}$), 76.3 (C-4$_{Glc}$), 75.5 (Ph–$CH_2$), 75.17 (Ph–$CH_2$), 75.09 (C-5$_{Glc}$), 74.96 (Ph–$CH_2$), 74.8 (Ph–$CH_2$), 74.6 (Ph–$CH_2$), 73.6 (Ph–$CH_2$), 73.4 (Ph–$CH_2$), 73.2 (Ph–$CH_2$), 73.05 (C-2$_{Gal'}$), 72.98 (C-5), 72.8 (C-2$_{Gal'''}$), 72.5 (C-4$_{Gal'''}$), 71.2 ($OCH_{2(OAll)}$), 69.0 (C-6$_{GlcNAc}$), 68.37 (C-6), 68.29 (C-6), 68.1 (C-6$_{Glc}$), 66.5

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(OCH$_{2(Linker)}$), 65.6 (C-5$_{GlcNAc}$), 59.2 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_{3(Linker)}$), 29.4 (–CH$_2$–$_{(Linker)}$), 22.4 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{102}H_{110}N_4O_{22}$ [*M*+Na]$^+$ 1765.7509, found 1765.7482.

3-Azidopropyl 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12): Compound **11 a** (0.75 g, 0.43 mmol) was placed under N_2 and stirred in dry THF (22 mL) with 4 Å molecular sieves (1.00 g) at room temperature for 1 h. NaBH$_3$CN (0.54 g, 8.6 mmol) was added and stirring was continued for 1 h. 1 M HCl/Et$_2$O was then added dropwise until pH 1 was reached. The reaction was stirred for a further 1.5 h, then diluted with CH$_2$Cl$_2$ (200 mL) and filtered through Celite®. The organic phase was then washed with sat. aq. NaHCO$_3$ (200 mL), water (200 mL) and brine (200 mL). The combined aqueous phase was extracted with CH$_2$Cl$_2$ (200 mL) and the collective organic layer was dried over MgSO$_4$, filtered and concentrated in vacuo. Flash chromatography on silica gel (toluene/EtOAc, 4:1→1:1) yielded **12** as a white foam (0.46 g, 61 %). R$_f$=0.4 (toluene/EtOAc, 3:2); [α] −50 (*c* 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.03–7.97 (m, 2H, Ar–H), 7.54 (m, 1H, Ar–H), 7.47–7.36 (m, 2H, Ar–H), 7.36–6.99 (m, 45H, Ar–H), 5.68 (m, 1H, CH$_2$=C$\underline{H}_{(OAll)}$), 5.56 (dd, *J*=10.1, 8.0 Hz, 1H, H-2$_{Gal'''}$), 5.16 (m, 1H, $\underline{CH_{2(A)}}$=CH$_{(OAll)}$), 5.14–5.03 (m, 2H, H-1$_{GlcNAc}$, $\underline{CH_{2(B)}}$=CH$_{(OAll)}$), 5.02–4.87 (m, 4H, N$\underline{H}$, 3×OCH$\underline{H}$Ph), 4.79 (d, *J*=11.2 Hz, 1H, OCH$\underline{H}$Ph), 4.76–4.62 (m, 2H, 2×OCH$\underline{H}$Ph), 4.61–4.53 (m, 4H, H-1$_{Gal'''}$, 3×OCH$\underline{H}$Ph), 4.53–4.38 (m, 7H, H-3$_{GlcNAc}$, 6×OCH$\underline{H}$Ph), 4.36–4.23 (m, 4H, H-1$_{Glc}$, H-2$_{Gal'}$, 2×OCH$\underline{H}$Ph), 4.17 (m, 1H, OCH$\underline{H}$Ph), 4.07 (m, 1H, OCH$_{2(A)OAll}$), 3.99–3.81 (m, 6H, H-4$_{Gal'''}$, H-4$_{Gal'}$, H-4$_{Glc}$, H-6$_{(A)GlcNAc}$, OCH$_{2(B)OAll}$, OC$\underline{H}_{2(A)Linker}$), 3.72 (m, 1H, H-5), 3.68–3.39 (m, 12H, H-2$_{Gal'}$, H-3$_{Gal'''}$, H-3$_{Gal'}$, H-3$_{Glc}$, H-5$_{GlcNAc}$, H-6$_{(B)GlcNAc}$, H-6$_{(A+B)Glc}$, H-6$_{(A+B)}$, H-6$_{(A)}$, $\underline{OCH_{2(B)Linker}}$), 3.38–3.29 (m, 5H, H-2$_{Glc}$, H-5, H-6$_{(B)}$, C$\underline{H}_2N_{3(Linker)}$), 3.17 (m, 1H, H-5$_{Glc}$), 2.77 (m, 1H, H-2$_{GlcNAc}$), 1.90–1.79 (m, 2H, –C$\underline{H}_2$–$_{(Linker)}$), 0.87 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.7 (C=O$_{(NHAc)}$), 165.2 (C=O$_{(OBz)}$), 139.8, 139.2, 138.9, 138.8, 138.6, 138.4, 138.3, 138.2, 137.6 (Ar–C$_{(quat)}$), 134.2 CH$_2$=$\underline{C}$H$_{(OAll)}$), 133.4, 129.9, 128.7, 128.6, 128.46, 128.45, 128.44, 128.40, 128.37, 128.36, 128.32, 128.21, 128.12, 128.10, 128.05, 128.04, 127.9, 127.79, 127.76, 127.73, 127.70, 127.57, 127.56, 127.52, 127.33, 127.27, 127.16, (Ar–CH), 117.9 ($\underline{C}$H$_2$=CH$_{(OAll)}$), 103.6 (C-1$_{Glc}$), 102.6 (C-1$_{Gal'}$), 101.8 (C-1$_{Gal'''}$), 99.9 (C-1$_{GlcNAc}$), 83.2 (C-3$_{GlcNAc}$), 83.0 (C-3$_{Glc}$), 81.9 (C-3$_{Gal'}$), 81.7 (C-2$_{Glc}$), 80.0 (C-3$_{Gal'''}$), 79.5 (C-2$_{Gal'}$), 76.8 (C-4$_{Gal'}$), 76.3 (C-4$_{Glc}$), 75.4 (Ph–CH$_2$), 75.2 (Ph–CH$_2$), 75.12 (C-5$_{Glc}$), 75.08 (C-5$_{GlcNAc}$), 74.9 (Ph–CH$_2$), 74.8 (Ph–CH$_2$), 74.6 (Ph–CH$_2$), 73.9 (C-5), 73.8 (Ph–CH$_2$), 73.66 (Ph–CH$_2$), 73.33 (Ph–CH$_2$), 73.4 (C-5), 73.3 (Ph–CH$_2$), 72.5 (C-4$_{Gal'''}$), 72.3 (C-2$_{Gal'''}$), 71.7 (OCH$_{2(OAll)}$), 70.3 (C-6$_{GlcNAc}$), 70.1 (C-4$_{GlcNAc}$), 68.8 (C-6), 68.5 (C-6), 68.0 (C-6$_{Glc}$), 66.5 (OCH$_{2(Linker)}$), 58.7 (C-2$_{GlcNAc}$), 48.5 (–CH$_2$–$_{(Linker)}$), 29.4 (CH$_2$N$_{3(Linker)}$), 22.8 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{102}H_{112}N_4O_{22}$ [*M*+Na]$^+$ 1767.7666, found 1767.7649.

3-Azidopropyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (21): Ir(COD)(PMePh$_2$)$_2$PF$_6$ (80 mg, 95 μmol) was placed under N_2 dry THF (5 mL) was added. The flask was purged with H_2 until the red colour of the solution turned to pale-yellow (20 minutes). The H_2 atmosphere was then replaced with N_2. A solution of compound **11 a** (813 mg, 0.466 mmol) in dry THF (18 mL) was added to the iridium complex and the reaction was stirred at room temperature for 2 h. NaHCO$_3$ (1.57 g, 18.7 mmol), water (7 mL) and I$_2$ (242 mg, 0.953 mmol) were then added sequentially, and the resulting suspension was stirred at room temperature for 17 h. The mixture was concentrated in vacuo, taken up in EtOAc (250 mL) and washed with water (250 mL) and brine (250 mL). The combined aqueous phase was then extracted with EtOAc (2×200 mL) and the collective organic layer was dried over MgSO$_4$, filtered and concentrated. Flash chromatography on silica gel (toluene→toluene/EtOAc, 3:2) yielded **21** as a pale-yellow foam (576 mg, 73 %). R$_f$=0.4 (toluene/EtOAc, 7:3); [α]+7.2 (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.12–7.90 (m, 2H, Ar–H), 7.59–7.42 (m, 3H, Ar–H), 7.44–7.02 (m, 45H, Ar–H), 5.50 (s, 1H, CH$_{(acetal)}$), 5.23 (d, *J*=8.1 Hz, 1H, H-1$_{GlcNAc}$), 5.22–5.19 (m, 2H, H-2$_{Gal'''}$, NH), 4.97 (d, *J*=10.7 Hz, 1H, OCH$\underline{H}$Ph), 4.88 (d, *J*=11.7 Hz, 1H, OCH$\underline{H}$Ph), 4.79 (d, *J*=11.0 Hz, 1H, OCH$\underline{H}$Ph), 4.75–4.56 (m, 7H, H-1$_{Gal'''}$, H-3$_{GlcNAc}$, 5×OCH$\underline{H}$Ph), 4.56–4.45 (m, 3H, 3×OCH$\underline{H}$Ph), 4.36–4.17 (m, 8H, H-1$_{Gal'}$, H-2$_{Glc}$, H-6$_{(A)GlcNAc}$, 5×OCH$\underline{H}$Ph), 3.95–3.86 (m, 2H, H-4$_{Glc}$, OCH$_{2(A)Linker}$), 3.84 (d, *J*=3.0 Hz, 1H, H-4$_{Gal'}$), 3.81 (d, *J*=3.6 Hz, 1H, H-4$_{Gal'''}$), 3.76–3.67 (m, 2H, H-4$_{GlcNAc}$, H-6$_{(B)GlcNAc}$), 3.66–3.52 (m, 7H, H-2$_{Gal'}$, H-3$_{Gal'''}$, H-5$_{GlcNAc}$, H-6$_{(A+B)Glc}$, H-6$_{(A)}$, OCH$_{2(B)Linker}$), 3.50–3.39 (m, 4H, H-3$_{Gal'}$, H-3$_{Glc}$, H-5, H-6$_{(A)}$), 3.39–3.29 (m, 6H, H-2$_{Glc}$, H-5, H-6$_{(B)}$, H-6$_{(B)}$, CH$_2$N$_{3(Linker)}$), 3.16 (m, 1H, H-5$_{Glc}$), 3.03 (m, 1H, H-2$_{GlcNAc}$), 2.21 (d, *J*=10.2 Hz, 1H, OH), 1.91–1.79 (m, 2H, –CH$_2$–$_{(Linker)}$), 0.85 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.6 (C=O$_{(NHAc)}$), 166.4 (C=O$_{(OBz)}$), 139.5, 139.1, 138.9, 138.7, 138.4, 138.3, 138.1, 137.68, 137.59 (Ar–C$_{(quat)}$), 133.4, 129.9, 129.8, 129.3, 129.2, 128.68, 128.60, 128.56, 128.46, 128.44, 128.41, 128.34, 128.28, 128.25, 128.18, 128.09, 128.02, 128.01, 127.9, 127.77, 127.73, 127.71, 127.70, 127.64, 127.32, 127.25, 126.3 (Ar–CH), 103.6 (C-1$_{Glc}$), 102.6 (C-1$_{Gal'}$), 101.5 (CH$_{(acetal)}$), 100.8 (C-1$_{GlcNAc}$), 100.6 (C-1$_{Gal'''}$), 83.0 (C-3$_{Glc}$), 82.3 (C-3$_{Gal'}$), 81.8 (C-2$_{Glc}$), 81.3 (C-4$_{GlcNAc}$), 79.6 (C-2$_{Gal'}$), 77.1 (C-3$_{GlcNAc}$), 76.45 (C-4$_{Gal'}$), 76.38 (C-4$_{Gal'''}$), 76.29 (C-4$_{Glc}$), 75.54 (Ph–CH$_2$), 75.46 (Ph–CH$_2$), 75.2 (Ph–CH$_2$), 75.10 (C-5$_{Glc}$), 74.91 (Ph–CH$_2$), 74.84 (Ph–CH$_2$), 74.6 (C-2$_{Gal'''}$), 73.6 (Ph–CH$_2$), 73.4 (Ph–CH$_2$), 73.2 (Ph–CH$_2$), 73.1 (C-5), 72.94 (C-3$_{Gal'''}$), 72.90 (C-5), 69.0 (C-6$_{GlcNAc}$), 68.26 (C-6), 68.12 (C-6), 68.07 (C-6$_{Glc}$), 66.5 (OCH$_{2(Linker)}$), 65.6 (C-5$_{GlcNAc}$), 58.9 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_{3(Linker)}$), 29.4 (–CH$_2$–$_{(Linker)}$), 22.5 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{99}H_{106}N_4O_{22}$ [*M*+Na]$^+$ 1725.7191, found 1725.7230.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (23): Acceptor **21** (250 mg, 0.147 mmol) and Bu$_4$NBr (428 mg, 1.33 mmol) were placed under N_2 together and dissolved in dry CH$_2$Cl$_2$/DMF (2.5 mL, 4:1, v/v). 4 Å molecular sieves (770 mg) were added and the resulting suspension was stirred at room temperature for 3 h. In a separate flask, donor **22** (516 mg, 0.882 mmol) was placed under N_2 and dissolved in dry CH$_2$Cl$_2$ (17 mL). The solution was cooled to 0 °C and Br$_2$ (68 μL, 1.3 mmol) was added. The reaction was stirred at 0 °C for 10 minutes, then quenched with cyclohexene until the solution became colourless and concentrated under reduced pressure. The resulting residue was added to the flask containing the acceptor in dry CH$_2$Cl$_2$ (2 mL) and the reaction was stirred at room temperature for 10 days. The mixture was then diluted with CH$_2$Cl$_2$, filtered through Celite® and concentrated in vacuo. Flash chromatography on silica gel (toluene→toluene/EtOAc, 7:3) yielded **23** as a white foam (158 mg, 48 %). R$_f$=0.6 (toluene/EtOAc, 7:3); [α]+32 (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.93–7.89 (m, 2H, Ar–H), 7.49–7.38 (m, 3H, Ar–H), 7.37–7.05 (m, 65H, Ar–H), 5.57 (dd, *J*=10.2, 8.0 Hz, 1H, H-2$_{Gal'''}$), 5.46 (s, 1H, CH$_{(acetal)}$), 5.27 (d, *J*=8.0 Hz, 1H, H-1$_{GlcNAc}$), 5.23 (d, *J*=6.9 Hz, 1H, NH), 4.98–4.96 (m, 2H, 2×OCH$\underline{H}$Ph), 4.91 (d, *J*=3.5 Hz, 1H, H-1$_{α\text{-}Gal}$), 4.87 (d, *J*=11.7 Hz, 1H, OCH$\underline{H}$Ph), 4.80–4.71 (m, 4H, 4×OCH$\underline{H}$Ph), 4.66–4.63 (m, 2H, H-1$_{Gal'''}$, OCH$\underline{H}$Ph), 4.61–4.44 (m, 7H, H-3$_{GlcNAc}$, 6×OCH$\underline{H}$Ph), 4.43–4.23 (m, 10H, H-1$_{Gal'}$, H-1$_{Glc}$, H-6$_{(A)GlcNAc}$, 7×OCH$\underline{H}$Ph), 4.23–4.16 (m, 2H, 2×OCH$\underline{H}$Ph), 4.14 (d, *J*=11.7 Hz, 1H, OCH$\underline{H}$Ph), 3.99–3.81 (m, 6H, H-2$_{α\text{-}Gal}$, H-4$_{Gal'''}$, H-4$_{Gal'}$, H-4$_{Glc}$, H-5, OCH$_{2(A)Linker}$), 3.74–3.51 (m, 10H, H-2$_{Gal'}$, H-3$_{α\text{-}Gal}$, H-3$_{Gal'''}$, H-4$_{α\text{-}Gal}$, H-4$_{GlcNAc}$, H-5$_{GlcNAc}$, H-6$_{(B)GlcNAc}$, H-6$_{(A+B)Glc}$, OCH$_{2(B)Linker}$), 3.49–3.43 (m, 4H, H-3$_{Gal'}$, H-3$_{Glc}$, 2×H-6$_{(A)}$), 3.39–3.23 (m, 7H, H-2$_{Glc}$, 2×H-5, 2×H-6$_{(B)}$, CH$_2$N$_{3(Linker)}$), 3.19 (t, *J*=8.4 Hz, 1H, H-6$_{(A)}$), 3.15 (m, 1H, H-5$_{Glc}$), 3.04–2.93 (m, 2H, H-2$_{GlcNAc}$, H-6$_{(B)}$), 1.89–1.79 (m, 2H, –CH$_2$–$_{(Linker)}$), 0.77 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (151 MHz, CDCl$_3$) δ 170.6 (C=O$_{(NHAc)}$), 165.2 (C=O$_{(OBz)}$), 139.5, 139.2, 138.97, 138.91, 138.76,

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138.72, 138.68, 138.45, 138.38, 138.30, 138.27, 138.0, 137.7 (Ar–C$_{(quat)}$), 133.2, 130.0, 129.9, 129.18, 129.15, 128.57, 128.51, 128.46, 128.43, 128.40, 128.38, 128.34, 128.31, 128.27, 128.26, 128.23, 128.22, 128.18, 128.12, 128.08, 128.02, 127.94, 127.88, 127.84, 127.82, 127.79, 127.73, 127.70, 127.67, 127.62, 127.54, 127.48, 127.46, 127.43, 127.39, 127.32, 127.23, 126.4, 125.4 (Ar–CH), 103.6 (C-1$_{Glc}$), 102.6 (C-1$_{Gal'}$), 101.4 (CH$_{(acetal)}$), 100.9 (C-1$_{Gal'''}$), 100.7 (C-1$_{GlcNAc}$), 98.5 (C-1$_{\alpha\text{-Gal}}$), 83.0 (C-3$_{Glc}$), 82.4 (C-3$_{Gal'}$), 81.8 (C-2$_{Glc}$), 81.2 (C-4$_{GlcNAc}$), 79.7 (C-3$_{Gal'''}$), 79.5 (C-2$_{Gal'}$), 79.0 (C-3$_{\alpha\text{-Gal}}$), 77.0 (C-3$_{GlcNAc}$), 76.53 (C-2$_{\alpha\text{-Gal}}$), 76.45 (C-4$_{Gal'}$), 76.3 (C-4$_{Glc}$), 75.4 (Ph–CH$_2$), 75.17 (Ph–CH$_2$), 75.09 (C-5$_{Glc}$), 74.94 (Ph–CH$_2$), 74.91 (Ph–CH$_2$), 74.80 (Ph–CH$_2$), 74.77 (C-4$_{\alpha\text{-Gal}}$), 74.4 (Ph–CH$_2$), 73.43 (C-4$_{Gal'''}$), 73.41 (Ph–CH$_2$), 73.36 (Ph–CH$_2$), 73.30 (Ph–CH$_2$), 73.2 (Ph–CH$_2$), 73.05 (C-5), 73.01 (C-5), 72.7 (Ph–CH$_2$), 72.3 (C-2$_{Gal'''}$), 69.7 (C-5), 69.0 (C-6$_{GlcNAc}$), 68.5 (C-6), 68.3 (C-6), 68.1 (C-6$_{Glc}$), 68.0 (C-6), 66.5 (OCH$_{2(Linker)}$), 65.6 (C-5$_{GlcNAc}$), 59.2 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_{3(Linker)}$), 29.4 (–CH$_2$–$_{(Linker)}$), 22.4 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{133}H_{140}N_4O_{27}$ $[M+Na]^+$ 2247.9603, found 2247.9632.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (24): 10 M aq. NaOH (266 μL, 2.66 mmol) was added to a stirring solution of compound **23** (148 mg, 66.5 μmol) in THF/EtOH/H_2O (3.5 mL, 5:2:1, v/v). The reaction was stirred at 35 °C for 16 h, then neutralised with Amberlite® IR120 (H^+ form) resin, filtered and concentrated in vacuo. Flash chromatography on silica gel (toluene→toluene/EtOAc, 3:2) yielded **24** as a white foam (95 mg, 67%). R$_f$ = 0.5 (toluene/EtOAc, 7:3); [α] + 7.2 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50–7.43 (m, 2H, Ar–H), 7.39–7.34 (m, 2H, Ar–H), 7.34–7.05 (m, 61H, Ar–H), 5.53–5.52 (m, 2H, NH, CH$_{(acetal)}$), 5.06 (d, *J* = 8.3 Hz, 1H, H-1$_{GlcNAc}$), 5.01–4.90 (m, 5H, H-1$_{\alpha\text{-Gal}}$, 4× OCH<u>H</u>Ph), 4.84 (d, *J* = 11.7 Hz, 1H, OCH<u>H</u>Ph), 4.81 (d, *J* = 11.1 Hz, 1H, OCH<u>H</u>Ph), 4.77–4.60 (m, 7H, 7×OCH<u>H</u>Ph), 4.57–4.53 (m, 2H, 2× OCH<u>H</u>Ph), 4.50 (d, *J* = 12.1 Hz, 1H, OCH<u>H</u>Ph), 4.44 (d, *J* = 11.8 Hz, 1H, OCH<u>H</u>Ph), 4.40–4.30 (m, 5H, H-1$_{Gal'}$, H-6$_{(A)GlcNAc}$, 3×OCH<u>H</u>Ph), 4.29 (d, *J* = 7.8 Hz, 1H, H-1$_{Glc}$), 4.25 (d, *J* = 11.7 Hz, 1H, OCH<u>H</u>Ph), 4.22–4.05 (m, 7H, H-1$_{Gal'''}$, H-2$_{\alpha\text{-Gal}}$, H-3$_{GlcNAc}$, H-5, 3×OCH<u>H</u>Ph), 3.97–3.77 (m, 7H, H-2$_{Gal'''}$, H-3$_{\alpha\text{-Gal}}$, H-4$_{\alpha\text{-Gal}}$, H-4$_{Gal'''}$, H-4$_{Gal'}$, H-4$_{Glc}$, OCH$_{2(A)Linker)}$), 3.74 (t, *J* = 10.2 Hz, 1H, H-6$_{(B)GlcNAc}$), 3.70–3.64 (m, 2H, H-2$_{Gal'}$, H-6$_{(A)Glc}$), 3.63–3.53 (m, 4H, H-3$_{Gal'}$, H-4$_{GlcNAc}$, H-6$_{(B)Glc}$, OCH$_{2(B)Linker)}$), 3.51–3.43 (m, 6H, H-2$_{GlcNAc}$, H-3$_{Glc}$, H-5$_{GlcNAc}$, 3×H-6$_{(A)}$), 3.42–3.27 (m, 9H, H-2$_{Glc}$, H-3$_{Gal'''}$, 2× H-5, 3×H-6$_{(B)}$, CH$_2$N$_{3(Linker)}$), 3.20 (m, 1H, H-5$_{Glc}$), 1.91–1.79 (m, 2H, –CH$_2$–$_{(Linker)}$), 1.45 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3 (C=O$_{(NHAc)}$), 139.5, 139.3, 139.18, 139.12, 138.72, 138.68, 138.62, 138.56, 138.4, 138.3, 138.07, 138.03, 137.3 (Ar–C$_{(quat)}$), 129.2, 128.48, 128.47, 128.44, 128.39, 128.36, 128.34, 128.27, 128.22, 128.19, 128.15, 128.10, 128.09, 128.03, 127.96, 127.85, 127.83, 127.78, 127.72, 127.69, 127.65, 127.60, 127.51, 127.44, 127.36, 127.32, 127.2, 127.0, 126.3 (Ar–CH), 104.1 (C-1$_{Gal'''}$), 103.6 (C-1$_{Glc}$), 102.67 (C-1$_{GlcNAc}$), 102.62 (C-1$_{Gal'}$), 101.3 (CH$_{(acetal)}$), 98.9 (C-1$_{\alpha\text{-Gal}}$), 84.1 (C-3$_{Gal'''}$), 83.0 (C-3$_{Glc}$), 82.3 (C-3$_{Gal'}$), 81.8 (C-2$_{Glc}$), 80.5 (C-4$_{GlcNAc}$), 79.7 (C-2$_{Gal'}$), 78.8 (C-3$_{\alpha\text{-Gal}}$), 77.3 (C-3$_{GlcNAc}$), 76.9 (C-2$_{\alpha\text{-Gal}}$, under $CDCl_3$ peak), 76.5 (C-4$_{Glc}$), 76.4 (C-4$_{Gal'}$), 75.5 (Ph–CH$_2$), 75.18 (Ph–CH$_2$), 75.13 (C-5$_{Glc}$), 75.08 (C-4$_{\alpha\text{-Gal}}$), 74.87 (Ph–CH$_2$), 74.78 (2×Ph–CH$_2$), 74.71 (Ph–CH$_2$), 74.19 (Ph–CH$_2$), 74.13 (C-4$_{Gal'''}$), 73.48 (C-5), 73.41 (Ph–CH$_2$), 73.38 (Ph–CH$_2$), 73.33 (Ph–CH$_2$), 73.2 (C-5, Ph–CH$_2$), 73.0 (Ph–CH$_2$), 70.5 (C-2$_{Gal'''}$), 69.9 (C-5), 69.3 (C-6), 68.9 (C-6$_{GlcNAc}$), 68.4 (C-6), 68.3 (C-6), 68.2 (C-6$_{Glc}$), 66.5 (OCH$_{2(Linker)}$), 66.2 (C-5$_{GlcNAc}$), 57.5 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_{3(Linker)}$), 29.4 (–CH$_2$–$_{(Linker)}$), 23.3 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{126}H_{136}N_4O_{26}$ $[M+Na]^+$ 2143.9341, found 2143.9388.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (26): Compound **24** (115 mg, 73 μmol) was placed under N_2 and dissolved in dry THF (5 mL). 3 Å molecular sieves were added and the solution was stirred at room temperature for 30 min. $NaBH_3CN$ (30 mg, 0.48 mmol) was added and stirring was continued for a further 30 minutes. 1 M HCl/Et_2O was added dropwise until evolution of gas was observed to cease and the reaction was allowed to proceed for an additional 6 h. Et_3N (0.5 mL) was then added to quench the reaction and the mixture was filtered through Celite®. The filtrate was concentrated and purified by flash chromatography on silica gel (toluene →toluene/EtOAc 3:1) to deliver compound **26** as a white foam (78 mg, 52%). [α] + 19 (*c* 1.0, CH_2Cl_2); R$_f$ = 0.4 (toluene/EtOAc, 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.03 (m, 65H, Ar–H), 5.02–4.99 (m, 2H, 2×OCH<u>H</u>Ph), 4.96–4.83 (m, 6H, H-1$_{\alpha\text{-Gal}}$, H-1$_{GlcNAc}$, 4×OCH<u>H</u>Ph), 4.80 (d, *J* = 11.1 Hz, 1H, OCH<u>H</u>Ph), 4.75–4.71 (m, 2H, 2×OCH<u>H</u>Ph), 4.69–4.59 (m, 4H, 4× OCH<u>H</u>Ph), 4.58–4.48 (m, 4H, 4×OCH<u>H</u>Ph), 4.47–4.22 (m, 10H, H-1$_{Gal'}$, H-1$_{Glc}$, 8×OCH<u>H</u>Ph), 4.18 (d, *J* = 12.1 Hz, 1H, OCH<u>H</u>Ph), 4.10 (dd, *J* = 10.5, 3.8 Hz, 1H, H-2$_{\alpha\text{-Gal}}$), 4.06–3.98 (m, 2H, H-1$_{Gal'''}$), 3.96–3.83 (m, 5H, H-3$_{\alpha\text{-Gal}}$, H-4$_{Glc}$, OCH$_{2(A)Linker)}$), 3.82–3.71 (m, 2H, H-3$_{GlcNAc}$), 3.72–3.61 (m, 4H, H-6$_{(A)Glc}$), 3.60–3.31 (m, 17H, H-2$_{GlcNAc}$, H-2$_{Glc}$, H-3$_{Glc}$, H-6$_{(B)Glc}$, OCH$_{2(B)Linker}$, CH$_2$N$_{3(Linker)}$), 3.28 (m, 1H), 3.19 (m, 1H, H-5$_{Glc}$), 1.90–1.80 (m, 2H, –CH$_2$–$_{(Linker)}$), 1.34 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5 (C=O$_{(NHAc)}$), 139.62, 139.60, 139.23, 139.21, 138.75, 138.69, 138.59, 138.58, 138.56, 138.47, 138.3, 138.1, 137.8 (Ar–C$_{(quat)}$), 128.53, 128.50, 128.45, 128.38, 128.33, 128.29, 128.25, 128.18, 128.15, 128.09, 128.05, 127.94, 127.88, 127.84, 127.80, 127.69, 127.64, 127.60, 127.56, 127.2, 126.6 (Ar–CH), 104.8 (C-1$_{Gal'''}$), 103.6 (C-1$_{Glc}$), 102.6 (C-1$_{Gal'}$), 101.6 (C-1$_{GlcNAc}$), 99.3 (C-1$_{\alpha\text{-Gal}}$), 85.9 (C-3$_{GlcNAc}$), 84.48, 83.1 (C-3$_{Glc}$), 81.8 (C-2$_{Glc}$), 79.76, 78.83, 77.36, 76.85 (C-2$_{\alpha\text{-Gal}}$, under $CDCl_3$ peak), 76.61, 76.5 (C-4$_{Glc}$), 75.50, 75.36, 75.18, 75.11 (C-5$_{Glc}$), 75.01, 74.86, 74.68, 74.49, 74.26, 73.89, 73.86, 73.69, 73.47, 73.41, 73.34, 72.96, 70.42, 70.12, 69.81, 69.49, 68.90, 68.58, 68.0 (C-6$_{Glc}$), 66.5 (OCH$_{2(Linker)}$), 56.7 (C-2$_{GlcNAc}$), 48.5 (CH$_2$N$_{3(Linker)}$), 29.8 (–CH$_2$–$_{(Linker)}$), 23.2 (CH$_{3(NHAc)}$). HRMS (ESI) *m/z* calculated for $C_{126}H_{138}N_4O_{26}$ $[M+Na]^+$ 2145.9497, found 2145.9423.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (25): Acceptor **24** (86 mg, 41 μmol) and Bu_4NBr (120 mg, 0.372 mmol) were placed under N_2 together and dissolved in dry CH_2Cl_2/DMF (1.0 mL, 4:1, v/v). 4 Å molecular sieves (220 mg) were added and the suspension was stirred at room temperature for 3 h. In a separate flask, donor **15** (132 mg, 0.244 mmol) was placed under N_2 and dissolved in dry CH_2Cl_2 (5 mL). The solution was cooled to 0 °C and Br_2 (19 μL, 0.37 mmol) was added. After stirring at 0 °C for 15 minutes, the Br_2 was quenched by adding cyclohexene until the solution turned colourless and the solution was concentrated. The resulting syrup was re-dissolved in dry CH_2Cl_2 (1.0 mL) and added to the flask containing the acceptor. After stirring at room temperature for 7 days, an additional portion of donor **15** (220 mg, 0.407 mmol) was converted to the corresponding α-bromosugar in a similar manner to that outlined above and added to the reaction mixture as a solution in dry CH_2Cl_2 (1.0 mL). Further Bu_4NBr (168 mg, 0.521 mmol) was then added, and the reaction was stirred at room temperature for an additional 14 days. The mixture was then then filtered through Celite® and concentrated in vacuo. Flash chromatography on silica gel (toluene/EtOAc, 17:3) yielded a mixture of a hexasaccharide product and fucose-related impurities as a white foam (55 mg). This mixture (55 mg) was placed under N_2 and dissolved in dry CH_2Cl_2/MeOH (2 mL, 1:1, v/v). *p*-TsOH·H_2O (4 mg, 20 μmol) was added, and the solution was stirred at room temperature for 48 h. Et_3N was then added to quench the reaction and the mixture was then reduced to dryness. Flash chromatography on silica gel (toluene→toluene/EtOAc, 1:1) yielded **25** as a white foam

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(26 mg, 26% over 2 steps). R_f = 0.5 (toluene/EtOAc, 3:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44–7.33 (m, 6H, Ar–H), 7.33–7.03 (m, 69H, Ar–H), 5.26 (d, *J* = 3.5 Hz, 1H, H-1), 5.21 (d, *J* = 4.0 Hz, 1H, H-1), 5.09 (d, *J* = 11.6 Hz, 1H, OCHHPh), 4.97 (d, *J* = 10.6 Hz, 1H, OCHHPh), 4.90–4.84 (m, 2H, 2×OCHHPh), 4.80 (d, *J* = 11.1 Hz, 1H, OCHHPh), 4.77–4.66 (m, 4H, 4×OCHHPh), 4.66–4.50 (m, 11H, H-1$_{GlcNAc}$, 10x OCHHPh), 4.48 (d, *J* = 12.1 Hz, 1H, OCHHPh), 4.45–4.35 (m, 5H, H-1$_{Gal'''}$, H-5$_{Fuc}$, 3×OCHHPh), 4.35–4.29 (m, 6H, H-1$_{Gal'}$, 5×OCHHPh), 4.28–4.26 (m, 2H, H-1$_{Glc}$, OCHHPh), 4.25–4.17 (m, 2H, OCHHPh), 4.09 (dd, *J* = 10.1, 3.3 Hz, 1H), 4.05 (d, *J* = 2.6 Hz, 1H), 4.00–3.81 (m, 8H, H-2$_{GlcNAc}$, H-4$_{Gal'}$, H-4$_{Glc}$, OCH$_{2(A)Linker}$), 3.80–3.79 (m, 2H), 3.76–3.69 (m, 2H), 3.67–3.55 (m, 6H, H-2$_{Gal'}$, H-3$_{Gal'}$, H-6$_{(A)Glc}$, OCH$_{2(B)Linker}$), 3.55–3.47 (m, 4H, H-6$_{(B)Glc}$), 3.44 (m, 1H, H-3$_{Glc}$), 3.39–3.31 (m, 5H, H-2$_{Glc}$, CH$_2$N$_{3(Linker)}$), 3.29–3.25 (m, 2H), 3.16–3.12 (m, 2H, H-5$_{Glc}$), 1.89–1.79 (m, 2H, –CH$_2$–$_{(Linker)}$), 1.55 (s, 3H, CH$_{3(NHAc)}$), 0.91 (d, *J* = 6.4 Hz, 3H, H-6$_{Fuc}$); ^{13}C NMR data are reported using combined information from ^{13}C NMR (126 MHz, $CDCl_3$) and ^{1}H-^{13}C HSQC spectra: δ 170.0 (C=O$_{(NHAc)}$), 139.5, 139.03, 138.95, 138.76, 138.69, 138.63, 138.60, 138.34, 138.29, 138.1, 137.9, 137.4 (Ar–C$_{(quat)}$), 128.53, 128.50, 128.47, 128.36, 128.33, 128.26, 128.23, 128.22, 128.18, 128.14, 128.03, 128.01, 128.00, 127.95, 127.91, 127.81, 127.70, 127.68, 127.64, 127.59, 127.53, 127.49, 127.41, 127.28, 127.15, 127.11, 127.05, 126.88, 126.85 (Ar–CH), 103.5 (C-1$_{Glc}$), 103.2 (C-1$_{GlcNAc}$), 102.8 (C-1$_{Gal'''}$), 102.4 (C-1$_{Gal'}$), 97.7 (C-1), 95.3 (C-1), 83.0 (C-3$_{Glc}$), 81.6 (C-2$_{Glc}$), 80.7 (C-3$_{Gal'}$), 80.1 (C-2$_{Gal'}$), 79.1, 79.0, 78.1, 77.0, 76.53, 76.44, 76.37, 76.1 (C-4$_{Glc}$), 75.7 (C-4$_{Gal'}$), 75.4 (Ph–CH$_2$), 75.2, 75.1 (Ph–CH$_2$), 75.02 (C-5$_{Glc}$), 74.69 (Ph–CH$_2$), 74.61 (Ph–CH$_2$), 74.43, 74.34 (Ph–CH$_2$), 74.1 (Ph–CH$_2$), 73.8 (Ph–CH$_2$), 73.69 (Ph–CH$_2$), 73.64, 73.62 (Ph–CH$_2$), 73.36, 73.32 (Ph–CH$_2$), 73.1 (Ph–CH$_2$), 72.3 (Ph–CH$_2$), 71.9 (Ph–CH$_2$), 71.3, 70.5, 70.1 (C-6), 69.54 (C-6), 69.49 (C-5$_{Fuc}$), 68.43 (C-6), 68.0 (C-6$_{Glc}$), 67.2, 66.4 (OCH$_{2(Linker)}$), 63.0 (C-6), 54.2 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_{3(Linker)}$), 29.3 (–CH$_2$–$_{(Linker)}$), 23.0 (CH$_{3(NHAc)}$), 16.8 (C-6$_{Fuc}$). HRMS (ESI) *m/z* calculated for $C_{146}H_{161}N_4O_{30}$ $[M+Na]^+$ 2473,1093, found. 2473.1023.

3-Azidopropyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (27): Compound **12** (0.44 g, 0.25 mmol) was placed under N_2 and dissolved in Ac_2O/Py (12 mL, 1:2, v/v). DMAP (17 mg, 0.14 mmol) was added, and the reaction was stirred at room temperature for 3 h. The solution was then concentrated and purified by flash chromatography on silica gel (toluene/EtOAc, 4:1→13:7), yielding a white foam (441 mg). The white foam isolated above (410 mg, assumed as 0.229 mmol) was placed under N_2 and dissolved in dry CH_2Cl_2/MeOH (22 mL, 1:1, v/v). $PdCl_2$ (20 mg, 0.11 mmol) was added, and the reaction was stirred at room temperature for 6 h. The mixture was then filtered through Celite® and concentrated under reduced pressure. Compound **27** was obtained after flash chromatography on silica gel (toluene/EtOAc, 4:1→11:9) as a white foam (348 mg, 85% over 2 steps). R_f = 0.3 (toluene/EtOAc, 7:3); [α] −21 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02–7.98 (m, 2H, Ar–H), 7.52 (m, 1H, Ar–H), 7.48–6.91 (m, 47H, Ar–H), 5.15 (d, *J* = 7.2 Hz, 1H, NH), 5.12–5.05 (m, 2H, H-2$_{Gal'''}$, H-1$_{GlcNAc}$), 4.99 (d, *J* = 10.6 Hz, 1H, OCHHPh), 4.90–4.86 (m, 2H, H-4$_{GlcNAc}$, OCHHPh), 4.82–4.58 (m, 6H, 6×OCHHPh), 4.57–4.49 (m, 7H, H-1$_{Gal'''}$, H-3$_{GlcNAc}$, 5×OCHHPh), 4.44–4.42 (m, 2H, 2×OCHHPh), 4.35–4.24 (m, 4H, H-1$_{Gal'}$, H-1$_{Glc}$, 2×OCHHPh), 4.16 (d, *J* = 11.8 Hz, 1H, OCHHPh), 3.96–3.84 (m, 4H, H-4$_{Gal'''}$, H-4$_{Gal'}$, H-4$_{Glc}$, OCH$_{2(A)Linker}$), 3.74–3.40 (m, 16H, H-2$_{Gal'}$, H-3$_{Gal'''}$, H-3$_{Gal'}$, H-3$_{Glc}$, H-5$_{GlcNAc}$, H-5, H-6$_{(A+B)GlcNAc}$, H-6$_{(A+B)Glc}$, H-6$_{(A+B)}$, H-6$_{(A+B)}$, H-6$_{(A)}$, OCH$_{2(B)Linker}$), 3.39–3.30 (m, 5H, H-2$_{Glc}$, H-5, H-6$_{(B)}$, CH$_2$N$_{3(Linker)}$), 3.15 (m, 1H, H-5$_{Glc}$), 2.95 (m, 1H, H-2$_{GlcNAc}$), 2.34 (d, *J* = 10.1 Hz, 1H, OH), 1.90–1.78 (m, 5H, CH$_{3(OAc)}$, –CH$_2$–$_{(Linker)}$), 1.36 (s, 3H, CH$_{3(NHAc)}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5 (C=O$_{(NHAc)}$), 169.8 (C=O$_{(OAc)}$), 166.4 (C=O$_{(OBz)}$), 139.5, 139.2, 139.0, 138.7, 138.34, 138.26, 138.16, 138.0, 137.7, 133.4 (Ar–C$_{(quat)}$), 130.0, 129.8, 128.73, 128.66, 128.64, 128.54, 128.47, 128.44, 128.39, 128.35, 128.33, 128.30, 128.28, 128.25, 128.18, 128.14, 128.12, 128.10, 128.08, 128.04, 128.02, 128.00, 127.96, 127.91, 127.87, 127.81, 127.77, 127.72, 127.62, 127.52, 127.48, 127.3, 127.2, 127.0, 126.8 (Ar–CH), 103.6 (C-1$_{Glc}$), 102.5 (C-1$_{Gal'}$), 100.4 (C-1$_{Gal'''}$), 100.3 (C-1$_{GlcNAc}$), 83.0 (C-3$_{Glc}$), 81.9 (C-3$_{Gal'}$), 81.7 (C-2$_{Glc}$), 79.5 (C-2$_{Gal'}$), 76.7 (C-4$_{Gal'}$), 76.5 (C-4$_{Gal'''}$), 76.3 (C-4$_{Glc}$), 76.1 (C-3$_{GlcNAc}$), 75.49 (Ph–CH$_2$), 75.45 (Ph–CH$_2$), 75.2 (Ph–CH$_2$), 75.1 (C-5$_{Glc}$), 74.8 (Ph–CH$_2$), 74.74 (C-2$_{Gal'''}$), 74.6 (Ph–CH$_2$), 73.7 (Ph–CH$_2$), 73.6 (Ph–CH$_2$), 73.4 (Ph–CH$_2$), 73.30 (C-5), 73.25 (C-5), 73.15 (C-2$_{Gal'''}$), 73.0 (C-5$_{GlcNAc}$), 70.5 (C-4$_{GlcNAc}$), 70.1 (C-6$_{GlcNAc}$), 68.4 (C-6), 67.98 (C-6), 67.90 (C-6$_{Glc}$), 66.5 (OCH$_2$), 58.9 (C-2$_{GlcNAc}$), 48.4 (CH$_2$N$_3$), 29.4 (–CH$_2$–), 23.2 (CH$_{3(NHAc)}$), 21.0 (CH$_{3(OAc)}$). HRMS (ESI) *m/z* calculated for $C_{101}H_{110}N_4O_{23}$ $[M+Na]^+$ 1769.7459, found 1769.7454.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (26) Alternative route: Donor **22** (281 mg, 479 μmol), acceptor **27** (280 mg, 160 μmol) and Bu_4NBr (208 mg, 639 μmol) were placed under N_2 together and dissolved in dry CH_2Cl_2/DMF (1.6 mL, 9:1, v/v). 4 Å molecular sieves (347 mg) were added and the mixture was stirred at room temperature for 1 h. Br_2 (41 μL, 0.80 mmol) was then added and the reaction was stirred at room temperature. Additional donor **22** (187 mg, 0.320 mmol) and Br_2 (25 μL, 0.49 mmol) were added after 24 h and stirring was continued at room temperature. After an additional 24 h, an extra portion of Bu_4NBr (103 mg, 0.320 mmol) was added and the reaction was stirred for a further 3 days. Et_3N and cyclohexene were added to quench the reaction and the mixture was filtered through Celite®. The filtrate was concentrated in vacuo and purified via flash chromatography on silica gel (toluene/EtOAc, 9:1→3:1), yielding a mixture of the pentasaccharide and hydrolysed donor as a white foam (272 mg). The mixture (272 mg, assumed as 120 μmol) was dissolved in THF/EtOH/H_2O (5.5 mL, 5:2:1, v/v) and 10 M aq. NaOH (0.55 mL, 5.5 mmol) was added. The reaction was stirred at 35 °C for 20 h, then neutralised with Amberlite® IR120 (H^+ form) resin, filtered and concentrated. Purification by flash chromatography on silica gel (toluene/EtOAc, 3:1→2:3) yielded **26** as a white foam (189 mg, 56% over 2 steps). NMR data were identical to those reported above for compound **26**.

3-Azidopropyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)]-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (28): Donor **15** (240 mg, 444 μmol), acceptor **26** (73.0 μmol) and Bu_4NBr (190 mg, 589 μmol) were placed under N_2 together and dissolved in dry CH_2Cl_2/DMF (1.46 mL, 9:1, v/v). 4 Å molecular sieves (420 mg) were added and the mixture was stirred at room temperature for 2 h. Br_2 (37 μL, 0.72 mmol) was then added and the reaction was stirred at room temperature for 4 days. Cyclohexene and Et_3N were then added to quench the reaction. The mixture was filtered through Celite®, concentrated and purified by flash chromatography on silica gel (toluene→toluene/EtOAc, 4:1), yielding **28** as a white foam (180 mg, 83%). R_f = 0.7 (toluene/EtOAc, 7:3); [α] −11 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40–6.99 (m, 95H, Ar–H), 5.63 (d, *J* = 4.2 Hz, 1H, H-1$_{Fuc}$), 5.32 (d, *J* = 3.2 Hz, 1H, H-1$_{Fuc}$), 5.04 (d, *J* = 11.4 Hz, 1H, OCHHPh), 5.01–4.87 (m, 4H, H-1$_{α\text{-}Gal}$, 3×OCHHPh), 4.84–4.69 (m, 8H, 8×OCHHPh), 4.68–4.30 (m, 20H, H-5$_{Fuc}$, 19×OCHHPh), 4.30–4.15 (m, 8H, 2×H-1, 6×OCHHPh), 4.08 (m, 1H), 4.02–3.76 (m, 11H, H-6$_{(A)}$, OCH$_{2(A)Linker}$, OCHHPh), 3.73–3.49 (m, 12H, H-6$_{(B)}$, 3×H-6$_{(A+B)}$, H-6$_{(A)}$, OCH$_{2(B)Linker}$), 3.49–3.31 (m, 8H, H-6$_{(B)}$, CH$_2$N$_{3(Linker)}$), 3.20–3.19 (m, 2H, H-5$_{Glc}$), 3.08 (br s, 1H), 1.92–1.79 (m, 2H, –CH$_2$–$_{(Linker)}$), 1.57 (s, 3H, CH$_{3(NHAc)}$), 1.15 (d, *J* = 6.5 Hz, 3H, H-6$_{Fuc}$), 1.09 (d, *J* = 6.3 Hz, 3H, H-

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6_{Fuc}); ^{13}C NMR data are reported using combined information from ^{13}C NMR (151 MHz, $CDCl_3$) and ^{1}H-^{13}C HSQC spectra: δ 169.1 (C=$O_{(NHAc)}$), 139.5, 139.34, 139.28, 138.94, 138.88, 138.76, 138.54, 138.44, 138.37, 138.21, 138.14, 138.0, 137.9 (Ar–$C_{(quat)}$), 128.55, 128.50, 128.44, 128.37, 128.27, 128.21, 128.14, 128.04, 127.97, 127.88, 127.79, 127.64, 127.55, 127.47, 127.44, 127.33, 127.29, 127.22, 127.1, 126.6 (Ar–CH), 103.4 (C-1), 102.43 (C-1), 102.42 (C-1), 102.0 (C-1), 98.0 (C-1_{Fuc}), 97.8 (C-$1_{\alpha\text{-Gal}}$), 97.0 (C-1_{Fuc}), 82.90, 81.57, 80.18, 79.89, 78.20, 78.14, 77.49, 76.29, 76.10, 75.67, 75.43, 75.39, 75.24, 74.99, 74.87, 74.83, 74.74, 74.59, 74.47, 74.40, 73.99, 73.47, 73.39, 73.30, 73.22, 73.19, 72.99, 71.80, 71.63, 71.46, 69.92, 68.7 (2× C-6), 68.3 (C-6), 68.0 (C-6), 67.6 (C-6), 66.67 (C-5_{Fuc}), 66.59 (C-5_{Fuc}), 66.4 ($OCH_{2(Linker)}$), 48.3 ($CH_2N_{3(Linker)}$), 29.2 (–CH_2–$_{(Linker)}$), 23.1 ($CH_{3(NHAc)}$), 16.40 (C-6_{Fuc}), 16.33 (C-6_{Fuc}). HRMS (ESI) *m/z* calculated for $C_{180}H_{194}N_4O_{34}$ $[M+Na]^+$ 2978.3472, found 2978.3425.

3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside hydrochloride (1): Compound **28** (90 mg, 30 μmol) was dissolved in EtOH (2.3 mL). 0.1 M aq. HCl (304 μL, 30.4 μmol) and H_2O (304 μL) were then added, followed by dropwise addition of EtOAc until a homogenous solution was formed. 5% Pd/C (20 mg, 9.4 μmol) and 20% $Pd(OH)_2$/C (20 mg, 0.56 mmol) were added and the reaction was stirred at room temperature under H_2 at atmospheric pressure. After 24 h, additional H_2O (2 mL) was added and stirring was continued for a further 6 days. The mixture was then diluted with H_2O, filtered through Celite®, concentrated in vacuo and lyophilised. Purified by size-exclusion chromatography on Biogel® P-2 gel (H_2O/^{t}Bu-OH, 99:1) and subsequent lyophilisation yielded **1** as a white solid (21 mg, 55%). ^{1}H NMR (500 MHz, D_2O) δ 5.25 (d, *J*=3.8 Hz, 1H, H-1), 5.18 (d, *J*=4.2 Hz, 1H, H-1), 5.05 (d, *J*=3.9 Hz, 1H, H-1), 4.85 (m, 1H, H-5_{Fuc}), 4.74 (d, *J*=7.6 Hz, 1H, H-1), 4.61 (d, *J*=8.4 Hz, 1H, H-1), 4.52 (d, *J*=8.0 Hz, 1H, H-1), 4.44–4.38 (m, 2H, H-1, H-5_{Fuc}), 4.33–4.22 (m, 2H), 4.22–4.11 (m, 2H), 4.07 (m, 1H), 4.03–3.46 (m, 32H), 3.33 (m, 1H), 3.17 (t, *J*=7.0 Hz, 2H, $CH_2N_{(Linker)}$), 2.08 (s, 3H, $CH_{3(NHAc)}$), 2.06–1.97 (m, 2H, –CH_2–$_{(Linker)}$), 1.31–1.28 (m, 6H, 2×H-6_{Fuc}); ^{13}C NMR (D_2O, taken from ^{1}H–^{13}C HSQC) δ 103.3 (C-1), 102.9 (C-1), 102.0 (C-1), 100.6 (C-1), 99.2 (C-1), 97.6 (C-1), 93.2 (C-1), 81.5, 78.2, 76.7, 75.1, 74.74, 74.73, 74.6, 74.27, 74.23, 74.0, 72.6, 72.0, 71.9, 71.1, 70.0, 69.6, 69.2, 69.0, 68.5, 68.0, 67.9, 67.7, 67.0 (C-5_{Fuc}), 66.4, 63.30, 61.26, 61.15, 61.11, 60.0, 59.4, 55.7 (C-2_{GlcNAc}), 37.5 ($CH_2N_{(Linker)}$), 26.6 (–CH_2–$_{(Linker)}$), 22.3 ($CH_{3(NHAc)}$), 15.3 (2×C-6_{Fuc}). HRMS (ESI) *m/z* calculated for $C_{47}H_{82}N_2O_{34}$ $[M+Na]^+$ 1241.4647, found 1241.4623.

3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside hydrochloride (2): Compound **25** (22 mg, 9.0 μmol) was dissolved in THF/^{t}Bu-OH/H_2O (2 mL, 6:1:3, v/v/v) and 0.1 M aq. HCl (90 μL, 9.0 μL) was added. 5% Pd/C (53 mg, 24 μmol) was then added, and the resulting suspension was stirred under H_2 (1 atm) at room temperature for 42 h. The mixture was then diluted with water, filtered through Celite® and concentrated in vacuo. Purification on a GE Healthcare® PD-10 desalting column (H_2O/^{t}Bu-OH, 99:1) and subsequent lyophilisation yielded **2** as a white solid (10 mg, quant.); ^{1}H NMR (500 MHz, D_2O) δ 5.26–5.24 (m, 2H, 2×H-1), 4.73 (d, *J*=7.6 Hz, 1H, H-1), 4.64 (d, *J*=8.4 Hz, 1H, H-1), 4.52 (d, *J*=7.8 Hz, 1H, H-1), 4.43 (d, *J*=7.9 Hz, 1H, H-1), 4.36 (q, *J*=6.6 Hz, 1H, H-5_{Fuc}), 4.30–4.23 (m, 2H), 4.16 (d, *J*=3.2 Hz, 1H), 4.09–3.69 (m, 24H), 3.67–3.44 (m, 7H), 3.34 (t, *J*=8.4 Hz, 1H), 3.12 (t, *J*=6.0 Hz, 2H, $CH_2N_{(Linker)}$), 2.08 (s, 3H, $CH_{3(NHAc)}$), 2.00 (br s, 2H, –CH_2–$_{(Linker)}$), 1.25 (d, *J*=6.6 Hz, 3H, H-6_{Fuc}); ^{13}C NMR (D_2O, taken from HSQC) δ 103.2 (C-1), 102.9 (C-1), 102.0 (C-1), 100.0 (C-1), 99.2 (C-1), 93.0 (C-1), 81.54, 78.17, 77.20, 76.04, 75.12, 74.79, 74.69, 74.25, 73.81, 72.70, 71.83, 71.25, 70.13, 69.72, 69.34, 69.26, 68.54, 68.51, 68.07, 67.8 ($OCH_{2(Linker)}$), 67.7, 66.6 (C-5_{Fuc}), 63.35, 61.17 (C-6), 61.07 (C-6), 60.34 (C-6), 60.32 (C-6), 60.0 (C-6), 54.8 (C-2_{GlcNAc}), 37.5 ($CH_2N_{(Linker)}$), 27.2 (–CH_2–$_{(Linker)}$), 22.2 ($CH_{3(NHAc)}$), 15.2 (C-6_{Fuc}). HRMS (ESI) *m/z* calculated for $C_{41}H_{73}N_2O_{30}$ $[M]^+$ 1073.42498, found 1073.4250.

B-Lewis b-BSA Glycoconjugate, Loading of 17 Heptasaccharides per Protein (35): Heptasaccharide **1** (3.8 mg, 3.0 μmol) was dissolved in 0.1 M aq. $NaHCO_3$ (76 μL) and 0.24 M thiophosgene/$CHCl_3$ (76 μL, 18 μmol) was added. The biphasic mixture was shaken vigorously at room temperature for 17 h, analysed using MALDI-TOF mass spectrometry (super-DHB matrix), and the organic layer was then removed. The aqueous phase was washed with $CHCl_3$ (2× 76 μL) and then added to a solution of bovine serum albumin (10 mg, 0.15 μmol) in 0.1 M $NaHCO_3$/0.3 M aq. NaCl (76 μL). The solution was shaken at room temperature for 5 days and monitored daily by MALDI-TOF mass spectrometry (*trans*-ferulic acid matrix). The mixture was then purified via ultracentrifugation in a Pall Nanostep® 30 kD centrifuge filter and lyophilisation of the retained material afforded compound **34** as a white solid (10 mg, average loading=7 glycans per protein, 35% incorporation).

An additional portion of **1** (7.6 mg, 6.1 μmol) was converted to corresponding isothiocyanate **33** in a similar manner to above and then added to a solution of glycoconjugate **34** in 0.1 M $NaHCO_3$/0.3 M aq. NaCl (76 μL). The reaction was shaken at room temperature for 5 days and monitored daily using MALDI-TOF mass spectrometry (*trans*-ferulic acid matrix). The mixture was then purified as before through ultracentrifugation and lyophilisation, yielding **35** as a white solid (10 mg, loading=17 glycans per protein, 28% overall incorporation).

B-Antigen-BSA Glycoconjugate, Loading of 23 Hexasaccharides per Protein (36): Hexasaccharide **2** (0.5 mg, 0.5 μmol) was dissolved in 0.1 M aq. $NaHCO_3$ (16 μL) and 0.34 M thiophosgene/$CHCl_3$ (16 μL, 3 μmol) was added. The biphasic mixture was shaken vigorously at room temperature for 22 h, analysed using MALDI-TOF mass spectrometry (super-DHB matrix), and the organic layer was then removed. The aqueous phase was washed with $CHCl_3$ (3×20 μL) and then added to a solution of bovine serum albumin (1.0 mg, 15 nmol) in 0.1 M $NaHCO_3$/0.3 M aq. NaCl (8 μL). The solution was shaken at room temperature for 5 days and monitored daily by MALDI-TOF mass spectrometry (*trans*-ferulic acid matrix). The mixture was then purified via ultracentrifugation in a Pall Nanostep® 30 kD centrifuge filter and lyophilisation of the retained material afforded a white solid (0.8 mg, average loading=11 glycans per protein, 33% incorporation).An additional portion of **2** (0.5 mg, 0.5 μmol) was converted to the corresponding isothiocyanate in a similar manner to above and then added to a solution of the isolated glycoconjugate in 0.1 M $NaHCO_3$/0.3 M aq. NaCl (8 μL). The reaction was shaken at room temperature for 5 days and monitored daily using MALDI-TOF mass spectrometry (*trans*-ferulic acid matrix). The mixture was then purified as before through ultracentrifugation and lyophilisation, yielding **36** as a white solid (0.8 mg, loading=23 glycans per protein, 35% overall incorporation).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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