SUPPORTING INFORMATION

Surface Plasomon Resonance imaging of glycoarrays identifies novel carbohydrate-based ligands for potential ricin sensor development

Margherita Fais,[a,b‡] Rositsa Karamanska,[a,b‡] Sarah Allman,[c] Shirley A. Fairhurst,[a] Paolo Innocenti,[c] Antony J. Fairbanks,[c,d] Timothy J. Donohoe,[c] Benjamin G. Davis,[c] David A. Russell,[b] Robert A. Field[a,b]*

[a] *Department of Biological Chemistry, Norwich Research Park, John Innes Centre, Norwich Research Park,, Norwich, NR4 7UH, UK. E-mail: rob.field@bbsrc.ac.uk*
[b] *School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.*
[c] *Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK.*
[d] *Present address: Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand*

1.1 General methods

All the reagents were used as purchased without further purification. 6-Biotinamido hexanoic acid hydrazide (commercial name EZ-Link Biotin-LC-hydrazide) and Biotin-LC-LC-sulfoNHS ester were purchased from Pierce. RCA_{120} was supplied by Sigma in stock solution of 6.12 mg of protein/mL and diluted to required concentrations. The library of 40 reducing glycans was purchased by the Consortium for Functional Glycomics (http://www.functionalglycomics.org/static/index.shtml).[1] Phosphate-buffered saline pH 7.4 (PBS) contained 10 mM phosphate and 150 mM NaCl and was used freshly prepared. Reactions were carried out in oven-dried glassware under nitrogen atmosphere.

When necessary, solvents were dried by passing them through a column of activated basic alumina according to Grubbs.[*] Analytical TLC was carried out on Merck (aluminium sheets) silica gel plates using short wave (254 nm) UV light, $KMnO_4$ and vanillin to visualise components. Silica gel (silica gel 60, 230-400 mesh, BDH) was used for flash column chromatography.

[*] A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, *Organometallics*, **1996**, *15*, 1518.

Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a water-jacketed 1 cm^3 cell with a path length of 1 dm and are quoted in units of $°\cdot cm^2 \cdot g^{-1}$. Concentrations (*c*) are given in g/100 cm^3, solvent and temperature are quoted. ^{1}H-NMR and ^{13}C-NMR were recorded on Bruker spectrometers (200, 400 and 500 MHz). For STD NMR studies, see text associated with Fig. 6 in the main manuscript. Infra-red spectra were recorded on a Fourier transform Perkin-Elmer 150 IR spectrometer. Low resolution mass spectra were recorded on a VG Micromass Platform 1 or a Waters Micromass LCT Premier XE using atmospheric pressure electrospray ionisation (ESI) for reducing disaccharides, whilst high resolution mass spectra were recorded on a Bruker Daltonics MicroTOF mass spectrometer using electrospray ionisation (ESI).

A full list of compounds from the Consortium for Functional Glycomics[1] collection that were used in this study can be found in Table 1.

Sample solutions of biotinylated sugars were filtered before HPLC through Whatman polytetrafluoroethylene syringe filters (0.45 μm pores) or through Millipore polyvinylidene difluoride membrane filters (0.22 μm pores) for making stock solutions of purified samples. Biotinylated sugars were purified using a Dionex Ultimate 3000 Series HPLC equipped with UV detector and Chromeleon software equipped with a Phenomenex Jupiter C18 column (4.6 × 250 mm, 5 μm, V_0 2.6 ml). Low resolution mass spectra for biotinylated disaccharides were recorded using electrospray ionisation mass spectrometry (ESI-MS) on a ThermoScientific spectrometer equipped with DecaXPplus ion trap, capillary temperature 350 ºC, spray voltage 5.2kV positive mode/5.0kV negative, m/z detection range 0–2000 amu.

1.2 The SPR imaging experiment using the Biacore Flexchip

1.2.1 Immobilisation by pin-printing of 40 different biotinylated glycans

Biotinylated sugars were prepared in phosphate-buffered saline (PBS; 10 mM phosphate buffer pH 7.4, containing 137 mM NaCl and 2.7 mM KCl) at concentrations of 100, 500 and 1000 µg/ml in 386-well V-bottom plates (Genetix). Samples were spotted onto NeutrAvidin® affinity chips (Figure S1; neutravidin-coated gold chips, Biacore) using a Genomic Solutions Omnigrid contact microarrayer. Split-pin (CMP-5) printing with a pin dwell time of 25 ms gave a delivery volume of *ca.* 1.3 nl, which gave a typical spot size of 150-160 µm diameter. Printed arrays were incubated in a humid atmosphere for 1 hour (40%) and stored dry at 4 ^{o}C prior to use.

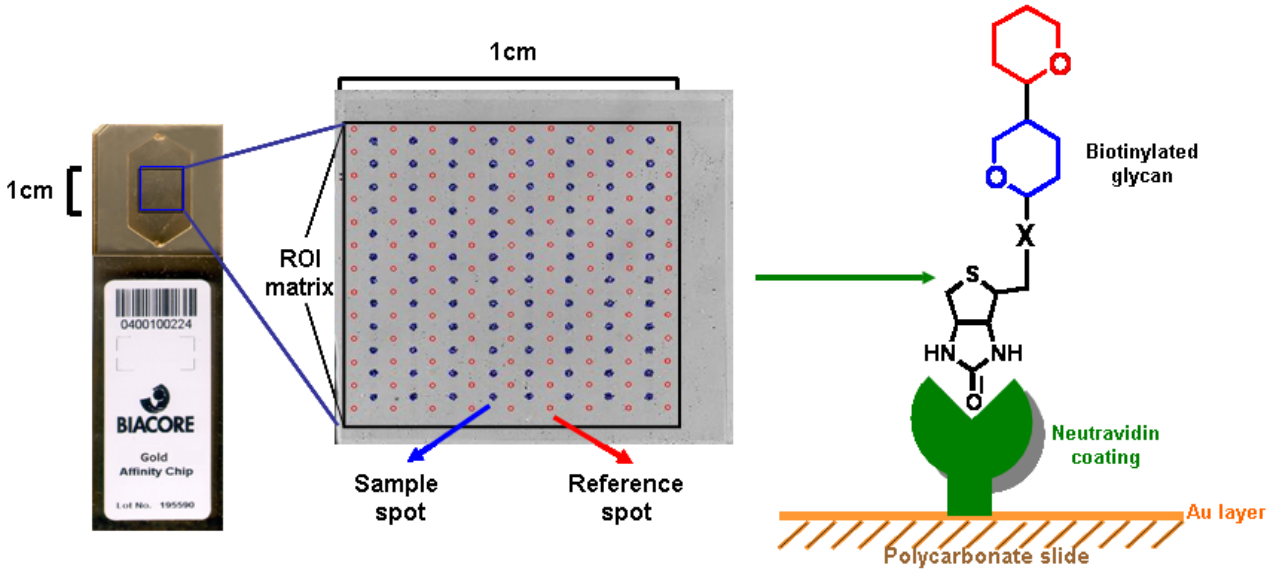


Figure S1. Carbohydrate microarray preparation for SPR imaging with Biacore Flexchip: a region of 1 cm^2 can be spotted with 400 dots of biotinylated glycans on a neutravidin-coated Au chip (a polycarbonate slide) with a split-pin contact printer. The array, a region of interest (ROI) matrix (12 rows × 8 columns), is thus created bearing active spots (blue rings) and reference spots (red rings). The array appears as a mask on the actual spot array as shown by the Biacore Flexchip Control Software.

1.2.2 Binding assay with the Biacore FlexChip

Regions of interest (ROI) on the printed chip were assigned while the chip was dry, as described by the manufacturer. Each ROI has associated reference spots that surround the ROI and were used to correct for bulk refractive index changes and instrumental drift. Experiments were performed at a flow rate of 500 µl/min at 25 ^{o}C. The chip was blocked with Flexchip blocking buffer prior to use. The array was subsequently washed for 10 min with PBS running buffer prior to the injection of lectin (in PBS) across the chip for 10 min to observe glycan-lectin association. A further 10 min

wash with PBS was used to observe the dissociation phase of the glycan-lectin interaction. Samples of RCA_{120} (Sigma) were diluted to obtain 833 nM and 2 µM concentrations in 1.6 ml buffer to be used in separate experiments. Sensorgrams for each target were recorded while running a specific method. All the sensorgrams were recorded simultaneously (Figure S2).

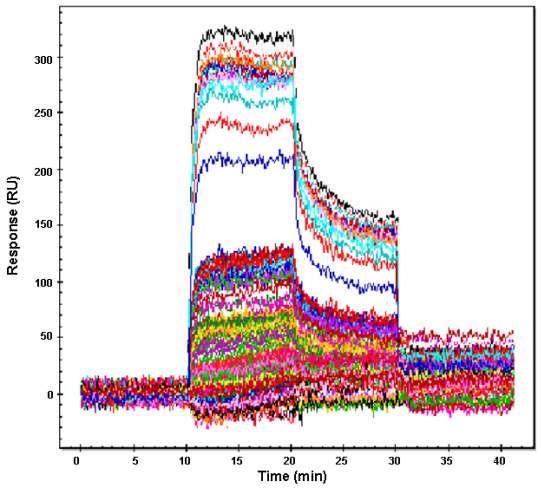


Figure S2. Simultaneous record of sensorgrams for the array of studied compounds. Plots report SPR response units (RU) versus time (min).

Data analysis was performed using the package provided with the Flexchip instrument. Relative ranking is represented as the average value of duplicates from a 'binding late' analysis (as defined by the manufacturer), which equates to equilibrium binding. Four sensorgrams obtained for quadruplicates of each sample at a specific concentration were designated as a 'group'. SPR responses at four specific time intervals in each sensorgram were chosen. The so-called 'report points' are designated as 'binding early', 'binding late', 'stability early' and 'stability late', as illustrated in (Figure S3), and were set automatically for all obtained sensorgrams.

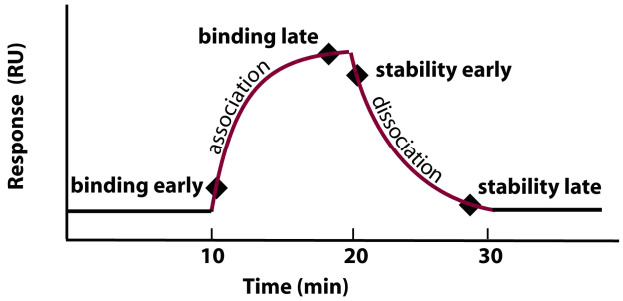


Figure S3. Report points: black spots indicate 'report points' set at specific time intervals. 'Binding early' represents the response at the early phase of complex formation, 'binding late' is the response recorded at the equilibrium, 'stability early' is the early phase of dissociation of the complex and 'stability late' refers to complete dissociation.

1.3 The SPR imaging experiment using the Horiba-Genoptics SPRi-plex

1.3.1 Immobilisation by pin-printing of 40 different biotinylated glycans

The same library of 40 biotinylated glycans (Table 1) was printed using a Xtend microarray pin-printer (0.35 mm tip diameter) onto an SPRi COe chip (extravidin-coated, Genoptics) at 500 µg/ml conc. in triplicates (dilutions in 10 mM PBS buffer at pH 7.4, 150 mM NaCl from stock solutions). Spotting was performed at room temperature with a relative humidity of 70 %. Each compounds act as negative control for the rest of the set.

1.3.2 Binding assay with SPRi-Plex

The sensor chip was used straightaway for SPRi assay, running on top of the sensor surface the same buffer as for the immobilisation (10 mM PBS buffer at pH 7.4, 150 mM NaCl). The flow rate was set to 25 µl/min at room temperature throughout the experiment, with the injection loop being a 50 µl volume (contact time 2 min). Prior to injection of the analyte, a calibration injection with PBS buffer at 12.5 mM concentration (1.25x of the running buffer concentration) was performed, as recommended by the manufacturer, to check for proper functioning of the optical system. SNA was diluted from a stock solution in immobilisation buffer (10 mM PBS buffer at pH 7.4, 150 mM NaCl) to 100 µg/ml (667 nM, data are reported in Figure 4 of the main paper) and injected (Figure S4). The surface was regenerated between each analyte injection with 20 µl of 100 mM HCl. When required, regeneration was also accompanied by the injection of 20 µl of 10 mM NaOH solution.

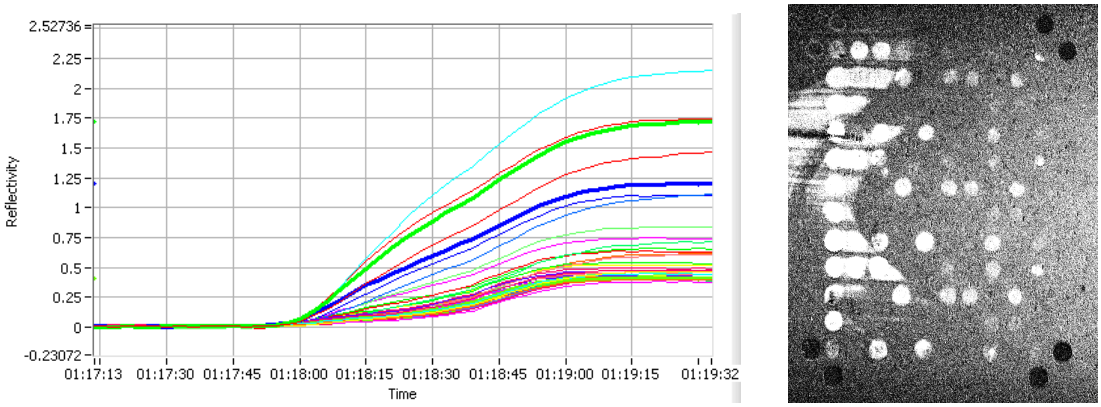


Figure S4. Responses in reflectivity changes over time and real-time image of the corresponding spots (highlighted in white) of the array for the library of triplicates of biotinylated glycans, as assessed with the by Genoptics SPRi-plex.

Assuming a conversion between reflectivity variation (%) and surface coverage (pg/mm^2) of *ca.* 1 %, equal to 200 pg/mm^2, the SPRi-analysis software afforded results that can be summarised into a bar chart representing equilibrium binding for SNA versus the glycan library (Figure 4, main text).

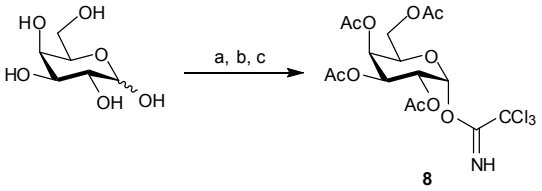
1.4 Development of novel carbohydrate-based ligands for RCA_{120}: Regioselective glycosylation

1.4.1 Syntheses of donor 8-12

Five trichloroacetimidate donors[5] with various structural modifications were synthesized initially in order to access the galactosyl, fucosyl, arabinosyl, 6-deoxy-6-fluoro and 6-deoxy-6-azido target compounds **8-12**.

1.4.1.1 Synthesis of 2,3,4,6-tetra-*O*-acetyl-D-galactosyl trichloroacetimidate 8

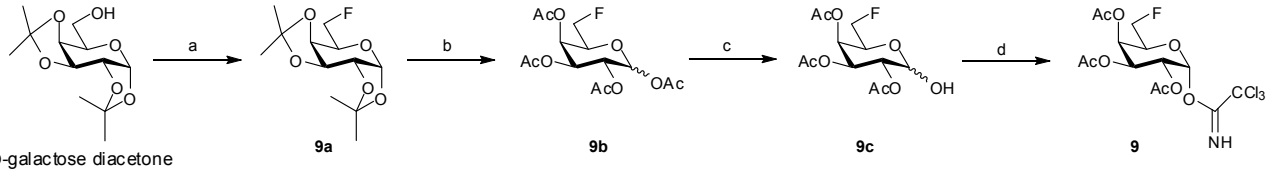
Per-*O*-acetyl galactosyl tricholoracetimidate[6] **8** could be prepared directly from galactose in three steps (Scheme S5). Anomeric deprotection of galactose penta-acetate using benzylamine in THF gave hemiacetal in reasonable yield.[7,8] Trichloroacetimidate formation could be affected by the procedure outlined by Schmidt and co-workers[5,9] and gave the desired trichloroacetimidate compound **8** as a white crystalline solid in 97 % yield.



Scheme S5. Synthesis of the 2,3,4,6-tetra-*O*-acetyl-D-galactosyl trichloroacetimidate **8**. *Reagents and conditions:* a) Ac_2O/Py, 0 ºC to r.t., overnight, quantitative; b) $BnNH_2$, THF, r.t., 16 h, 38 %; c) Cl_3CCN, DBU, DCM, r.t., 3 h, 97 %.

1.4.1.2 Synthesis of the 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-D-galactosyl trichloroacetimidate 9

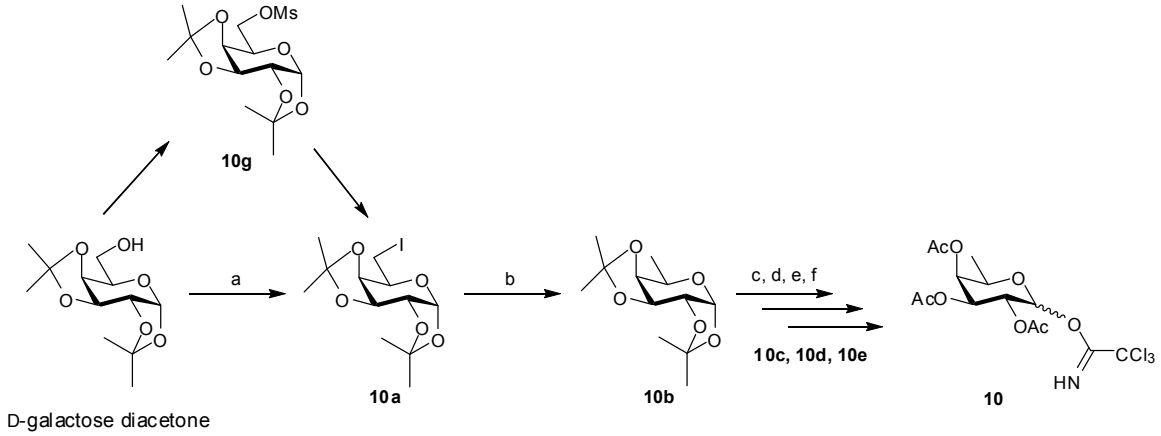
The 6-deoxy-6-fluoro trichloroacetimidate donor **9** could be prepared as described in Scheme S6. Diisopropylidene galactose was fluorinated in high yield (81 %) by refluxing in DCM with diethylaminosulfur trifluoride (DAST) in accordance with the procedure outlined by Burkart *et al*[10] to give fluorinated compound **9a**. The protected 6-deoxy-6-fluoro sugar was then deprotected by acid hydrolysis with trifluoroacetic acid and the deprotected product acetylated to aid purification to give compound **9b**. The per-*O*-acetylated compound was then anomerically deprotected using hydrazine acetate[11] in reasonable yield (49 %) to give hemi-acetal **9c**. The corresponding trichloroacetimidate compound **9** was synthesized by reaction of the anomerically deprotected sugar with trichloroacetonitrile in DCM in the presence of a catalytic amount of DBU.[11]



Scheme S6. Synthesis of the 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-D-galactose trichloroacetimidate **9**. *Reagents and conditions:* a) DAST, DCM, 81 %; b) i. TFA; ii. Ac_2O, I_2, 81 %; c) hydrazine acetate, DMF, 42 %; d) Cl_3CCN, DBU, DCM, 97 %.

1.4.1.3 Synthesis of the 2,3,4-tri-*O*-acetyl-D-fucosyl trichloroacetimidate 10

2,3,4-Tri-*O*-acetyl D-fucose **10** was prepared from diacetone galactose following literature procedure.[10] Although commercially available, D-fucose may be prepared economically in large quantities from diisopropylidene galactose. Iodination of diisopropylidene galactose was carried out at postion 6 by direct reaction with triphenyl phosphine and iodine[12] to give compound **10a**. Hydrogenolysis of the iodide gave the protected 6-deoxygenated sugar[13] **10b**. The protected sugar was treated with trifluoroacetic acid in order to remove the diisopropylidene protecting groups to give deprotected D-fucose **10c** and acetylated (acetic anhydride/pyridine) to give compound[14] **10d**. The resultant per-*O*-acetylated D-fucose **10d** was then anomerically deprotected using hydrazine acetate in reasonable yield (51 % over two steps) to give hemiacetal **10e**. The 2,3,4-tri-*O*-acetyl-D-fucose trichloroacetimidate **10** was then synthesized by the reaction of the partially deprotected sugar with trichloroacetonitrile and catalytic DBU[15] (Scheme S7).

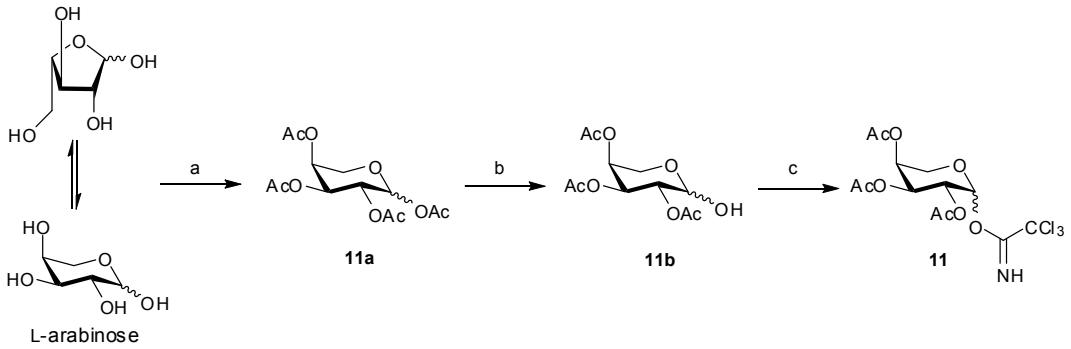


Scheme S7. Synthesis of the 2,3,4 tri-O-acetyl-D-fucose trichloroacetimidate **10**. *Reagents and conditions:* a) PPh_3, I_2, THF, 57 %; b) H_2, Pd/C, 79 %; c) i. TFA, **10c**, 100 %; ii. Ac_2O/Py, **10d**, quantitative; iii. hydrazine acetate, **10e**, 51%; iv. Cl_3CCN, DBU, DCM, 64 %.

The iodide may also be prepared from the diisopropylidene protected mesylate **10g** by nucleophilic displacement, and then reduced in accordance with the procedure outlined above. Attempts to reduce the mesylate directly were unsuccessful.

1.4.1.4 Synthesis of 2,3,4-tri-*O*-acetyl-L-arabinosyl trichloroacetimidate 11

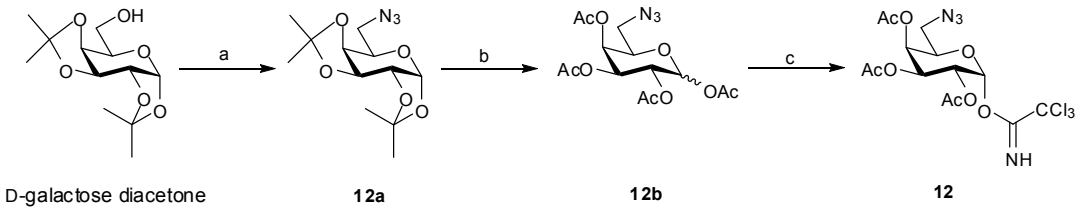
The acetylated arabinosyl trichloroacetimidate donor **11** was synthesized from arabinose as described below (Scheme S8). L-arabinose was peracetylated using the iodine-mediated method described by Mukhopadhyay and co-workers[16] to give the per-*O*-acetylated pyranose **11a**, which was then anomerically deprotected using benzylamine to give protected hemi-acetal[17] **11b**. Reaction of the anomerically deprotected sugar with trichloroacetonitrile and catalytic DBU gave the desired trichloroacetimidate **11** in 95 % yield as a pale yellow oil which crystallised on standing to give pale yellow needles.[18]



Scheme S8. Synthesis of the 2,3,4-tri-*O*-acetyl-L-arabinosyl trichloroacetimidate **11**. *Reagents and conditions:* a) Ac_2O, I_2, quantitative; b) $BnNH_2$, THF, r.t., 16 h, 46 %; c) Cl_3CCN, DBU, DCM, r.t., 3 h, 95 %.

1.4.1.5 Synthesis of the 2,3,4-tri-*O*-acetyl-6-deoxy-6-azido-D-galactosyl trichloroacetimidate 12

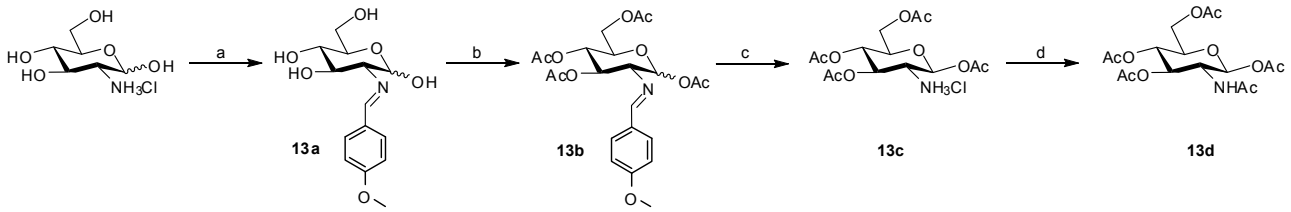
The synthesis of 6-deoxy-6-azidogalactosamine donors was carried out according to known procedures. Diisopropylidene galactose was converted into the corresponding mesylate and reacted with sodium azide to produce bis acetonide **12a** (Scheme S9). After deprotection by acid hydrolysis, the sugar was converted into the peracetylated galactosyl derivative **12b**. Selective deprotection of the anomeric position on azido derivative **12b** was carried out using hydrazine acetate. Treatment with DBU in dichoromethane/trichloroacetonitrile afforded the stable trichloroacetimidate **12**.



Scheme S9. Synthesis of the 2,3,4-tri-*O*-acetyl-6-deoxy-6-azido-D-galactosyl trichloroacetimidate **12**. *Reagents and conditions:* a) i. MsCl, Et_3N; ii. NaN_3, 79 %; b) i. TFA; ii. Ac_2O/Py, **12b**, 81 %; c) i. hydrazine acetate; ii. Cl_3CCN, DBU, DCM, 50 %.

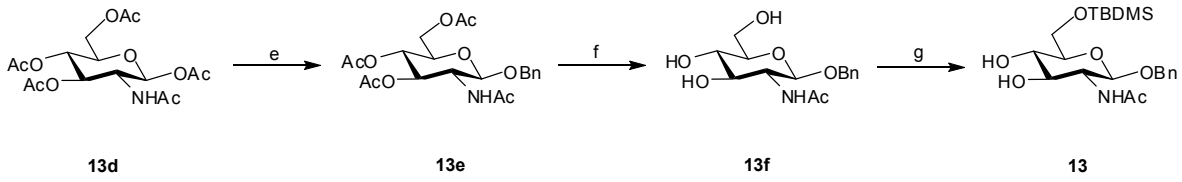
1.4.2 Synthesis of the acceptor 13

D-Glucosamine hydrochloride may be converted to the anisaldehyde adduct **13a** in 79 % yield by stirring the salt with aqueous sodium hydroxide followed by the addition of *p*-anisaldehyde.[19] This adduct may be acetylated using pyridine and acetic anhydride to give the beta acetate **13b** exclusively in 88 % yield. Cleavage of the anisaldehyde moiety and subsequent acetylation of the amine salt **13c** provides per-*O*-acetyl-β-GlcNAc[20] **13d** (Scheme S10).



Scheme S10. Synthesis of the per-*O*-acetyl-β-GlcNAc **13d**. *Reagents and conditions:* a) *p*-anisaldehyde, NaOH, H_2O, 6h, 79 %; b) Ac_2O/Py, 88 %; c) HCl, acetone, 2.5h, 69 %; d) TEA, Ac_2O, DCM, 3.5h, quantitative.

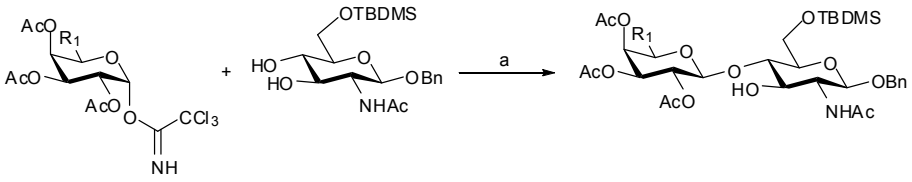
Glycosylation of the per-*O*-acetyl-β-GlcNAc **13d** with benzyl alcohol and ytterbium triflate gave the benzyl glycoside **13e** which could then be deprotected under Zemplén conditions to give compound **13f**, and a silyl protecting group was introduced selectively on the primary hydroxyl to give the suitably protected glycosyl acceptor **13** (Scheme S11).



Scheme S11. Synthesis of the 6-*tert*-butyldimethylsialyl-benzyl-2-acetamido-2-deoxy-β-D-glucopyranoside acceptor **13**. *Reagents and conditions:* e) BnOH, $Yb(OTf)_3$, DCM, reflux, 36h, 63 %; f) NaOMe, MeOH, 15 min, quantitative; g) TBDMSCl/Py, 12 h, quantitative.

1.4.3 Synthesis of β-1,4-linked disaccharide compounds 14-18

Synthesis of the β-1,4-linked disaccharide compounds bearing modifications at position 5 of Gal residue were carried out as described in Scheme S12.



8 $R_1 = CH_2OAc$
9 $R_1 = CH_2F$
10 $R_1 = CH_3$
11 $R_1 = H$
12 $R_1 = CH_2N_3$

13

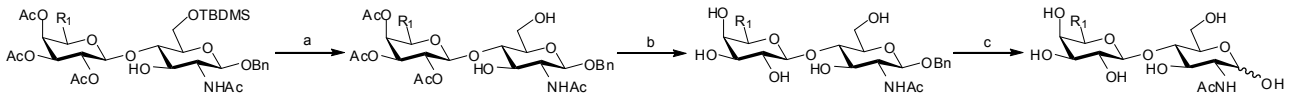
14 $R_1 = CH_2OAc$, 30%
15 $R_1 = CH_2F$, 50%
16 $R_1 = CH_3$, 27%
17 $R_1 = H$, 44%
18 $R_1 = CH_2N_3$, 53%

Scheme S12. Synthesis of β-1,4-linked disaccharide compounds **14-18**. *Reagents and conditions:* a) $BF_3{\cdot}OEt_2$, DCM, -40 ºC.

The desired products can be isolated from the reaction in yields ranging between 27 and 53 %. No trace of the 1,3-linked compound or the α-anomer was observed under these conditions, although extended reaction times may sometimes result in recovery of desilylated products. Unreacted glycosyl acceptor could be recovered from the reaction and recycled; although it is noted that no desilylated acceptor is isolated on the time-scale of the reaction.

1.4.4 Deprotection of β1,4-linked compounds 14-18 for target compounds 1-7

Galβ1,4GlcNAc disaccharide and the modified compounds could be effectively deprotected in high yield. The silyl protecting group at the 6-position of GlcNAc was removed by Lewis acid mediated hydrolysis using boron trifluoride diethyl etherate in acetonitrile; capitalising on solvent dependent increase in rate of hydrolysis of the silyl ether. Deacetylation under Zemplen conditions (sodium methoxide in anhydrous methanol) proceeded cleanly to give the desired anomerically protected disaccharides (Scheme S13). The removal of the anomeric benzyl protecting group was carried out using Pearlmann's catalyst in methanol or methanol and tetrahydrofuran mixtures, to give the reducing disaccharide in high yield. Methanol – tetrahydrofuran mixtures were initially used for this deprotection due to the known relative rates of deprotection in tetrahydrofuran versus methanol. Nevertheless, the reaction proceeds as cleanly and expediently in methanol with the systems tested.



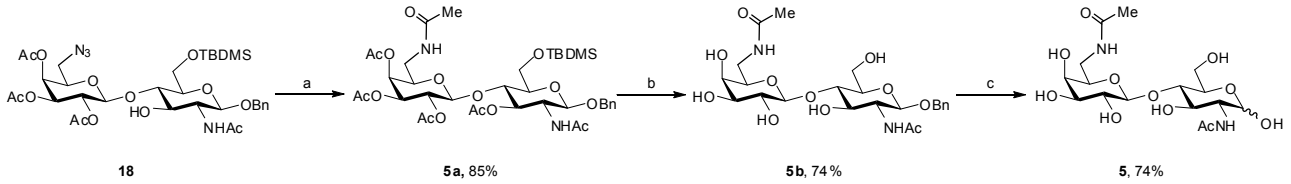
14 $R_1 = CH_2OAc$
15 $R_1 = CH_2F$
16 $R_1 = CH_3$
17 $R_1 = H$

14b R = CH_2OH, 94%
15b R = CH_2F, 83%
16b R = CH_3, 100%
17b R = H, 75%

1 R = CH_2OH, 90%
2 R = CH_2F, 88%
3 R = CH_3, 100%
4 R = H, 90%

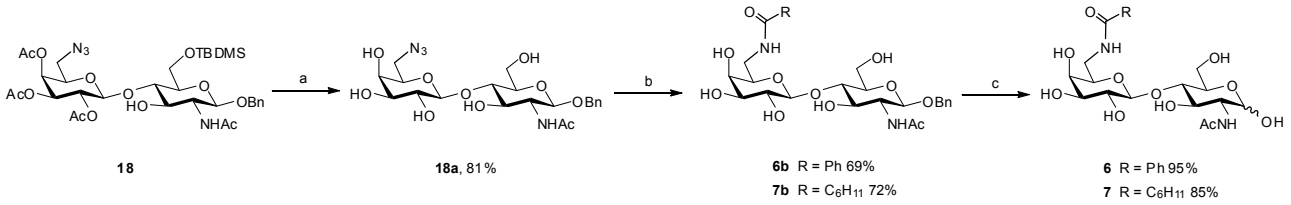
Scheme S13. Deprotection of β1,4-linked LacNAc analogues **14-17**. *Reagents and conditions:* a) $BF_3{\cdot}OEt_2$, CH_3CN, r.t.; b) NaOMe, MeOH, r.t.; c) $Pd(OH)_2$, H_2, MeOH, r.t.

Staudinger reduction followed by peracetylation of compound **18** gave bis acetamide **5a**, which could be fully deprotected leading to the reducing sugar **5** (Scheme S14):



Scheme S14. Reduction and deprotection of β1,4-linked LacNAc analogue **18** for target compound **5**. *Reagents and conditions:* a) i. PPh_3, H_2O; ii. Ac_2O, Py; b) i. $BF_3{\cdot}OEt_2$; ii. MeONa, MeOH, r.t.; c) H_2, $Pd(OH)_2/C$, MeOH, r.t.

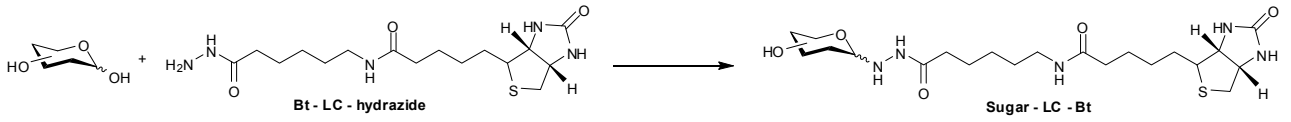
Alternatively, similar amides **6** and **7** could be produced by deprotection of azide **18** followed by Staudinger reduction and formation of amides under Schotten-Baumann conditions (Scheme S15):



Scheme S15. Deprotection and reduction of β1,4-linked LacNAc analogue **18** for target compounds **6** and **7**. *Reagents and conditions:* a) i. $BF_3{\cdot}OEt_2$; ii. MeONa, MeOH, r.t.; b) i. PPh_3, H_2O; ii. RCOCl, aq. $NaHCO_3$; c) H_2, $Pd(OH)_2/C$, MeOH, r.t.

1.4.5 Biotinylation of the reducing disaccharides 1-7

Disaccharides (**1-7**) were dissolved in a 25% aqueous acetic acid and mixed with 6-biotinamido hexanoic acid hydrazide[21,22] (Bt-LC-hydrazide) dissolved in MeOH to afford the target compounds **1Bt-7Bt** (Scheme S16).



Scheme S16. Synthesis of biotinylated disaccharides (sugar-LC-Bt) from biotinyl hydrazide (Bt-LC-hydrazide).

Reactions were typically carried out on a 1 mg scale with respect to the disaccharides, which were used in 2-fold molar excess with respect to the biotinyl hydrazide reagent. The methanolic and acetic acid solutions were mixed to afford a mixture MeOH/H_2O/AcOH of ratio 92:6:2 in volume of 300 µl, reacting for 1 day at 40 ºC. The crude mixtures were purified by a reversed phase C_{18} HPLC column (Phenomenex Jupiter C_{18} column 4.6 × 250 mm, 5 µm, V_0 2.6 ml, flow rate 5 ml/min),

isolated and analysed by ESI-MS. Retention times for the library of compounds were found in a range between 15 and 20 min.

1.5 Biotinylated compounds versus PAA-glycans from CFG array

Data abstracted from the CFG web portal: http://www.functionalglycomics.org/static/index.shtml. The PAA-glycan array presented polyacrylamide-based glycoconjugates (PAA-glycans)[23] immobilised onto NHS-activated glass slides by the formation of an amide bond, and the glycans have been spotted at two different concentrations (10 and 100 μM), according to a protocol used for glycan array fabrication in binding assays with other proteins.[24] The microarrays have been printed by robotic pin deposition (delivery volume *ca.* 0.6 nl), wherein each sample has been spotted in a replicate of six per each slide. FITC-labelled RCA_{120} for the assay has been diluted to obtain a 0.2 μg/ml (*ca.* 1.7 nM) concentration in binding buffer. Buffer used for dilutions and binding was TSM (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$). The lectin has been applied (50 μl) to the printed surfaces and coverslipped. The slides have been incubated in a humid and dark atmosphere for 1 h and rinsed with buffer prior to analysis. The binding image has been read out by a Perkin Elmer Microscan array scanner (Figure S17).

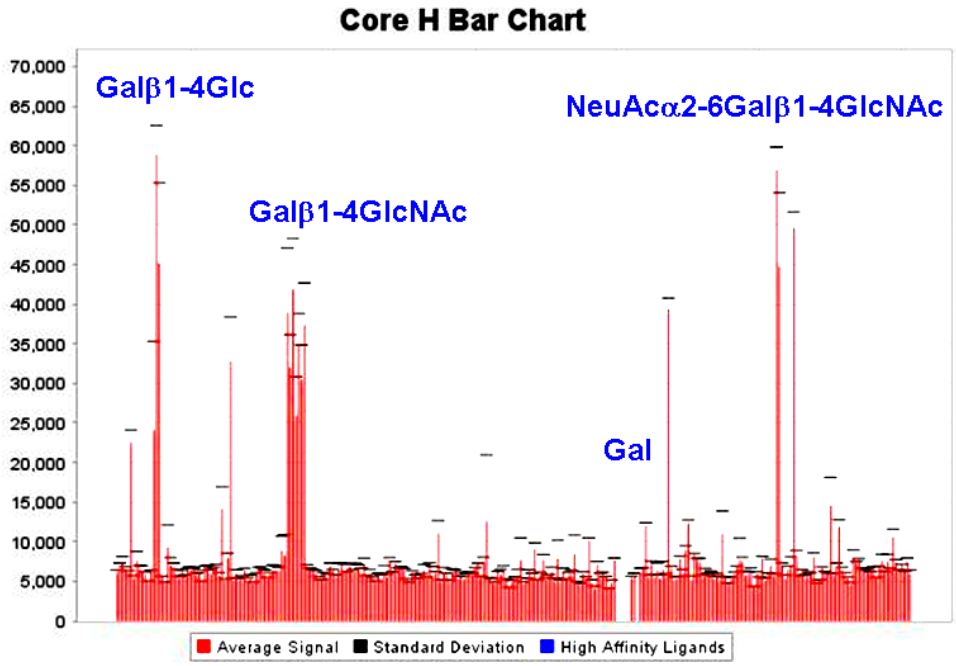


Figure S17. FITC-RCA_{120} binding to glycan array from CFG.

1.6 STD-NMR for binding of RCA_{120} to Lactose (Lac) and α-2,6'-sialyl-lactose (6'SL)

All STD NMR experiments were performed on a Bruker Avance II 600 MHz spectrometer equipped with a TCI cryoprobe. All spectra were processed on Bruker Topspin 2.1 software. A Bruker pulsed sequence for STD-NMR (stddiffesgp.3) was used with a shaped pulse train for saturation alternating between on and off resonance and a spoil pulse to destroy unwanted magnetization. The sequence includes solvent suppression using excitation sculpting with gradients and spinlock to suppress protein signals. On resonance was set at 360 Hz, whilst off resonance -50,000 Hz. The number of scans varied with sample, and was typically 256 scans.

NMR samples were prepared in 500 μL of 99.9 % D_2O buffer containing 10 mM phosphate buffer, 150 mM NaCl at pH 7.4 (not corrected for D_2O). Solute exchange was achieved by ultrafiltration of the RCA_{120} sample with Amicon® Ultra (Millipore) centrifugal filters (membrane cut-off value: 30 kDa). Galβ1,4Glc and Neu5Acα2,6Galβ1,4Glc were purchased by Carbosynth Limited. Addition of the saccharides to the protein NMR sample was from concentrated stock solutions in 99.9 % buffered D_2O. In competition experiments, the competing carbohydrate ligand was in 4-fold excess with respect to the primary carbohydrate ligand and therefore in a 1:132 ratio with the protein. As far as carbohydrate epitope mapping analysis was concerned, the STD integrals of the individual protons of the two disaccharides (Galβ1,4Glc and Neu5Acα2,6Galβ1,4Glc) were referenced to the strongest STD signal in each spectrum (H2'-Gal in both the saccharides), which was assigned a value of 100 %.

Using the H2-Glc signal as a reference for the intensity of the STD signals, we observed that the 1D NMR spectrum for the 4:1 mixture of Lac/6'SL (Figure S18-A) reflects the excess of Lac in peak intensities, which is also observable in the corresponding STD spectrum (Figure S18-B). Furthermore, the latter is similar to the STD NMR for Lac only (Figure S18-D), indicating that Lac is the preferred ligand in solution. Further experiments with 6'SL in excess of Lac (4:1) (Figure S19) show STD NMR signals for both Lac and 6'SL (Figure S19-B; compare to Figure S19-D for Lac only and to Figure S19-C for 6'SL only). This again indicates that Lac is the preferred ligand for RCA_{120}. When the Lac/6'SL ratio is 1:1 (Figure S20-B), both ligands are still recognised, as observed from the STD NMR signals (Figure S20-B), with Lac slightly preferred over 6'SL. Competing binding is evident in Figure S21, in which the STD NMR spectrum for the 1:4 molar ratio Lac/6'SL (Figure S21-C) is compared to the 1D NMR spectrum for a 1:1 mixture of ligands (Figure S21-A).

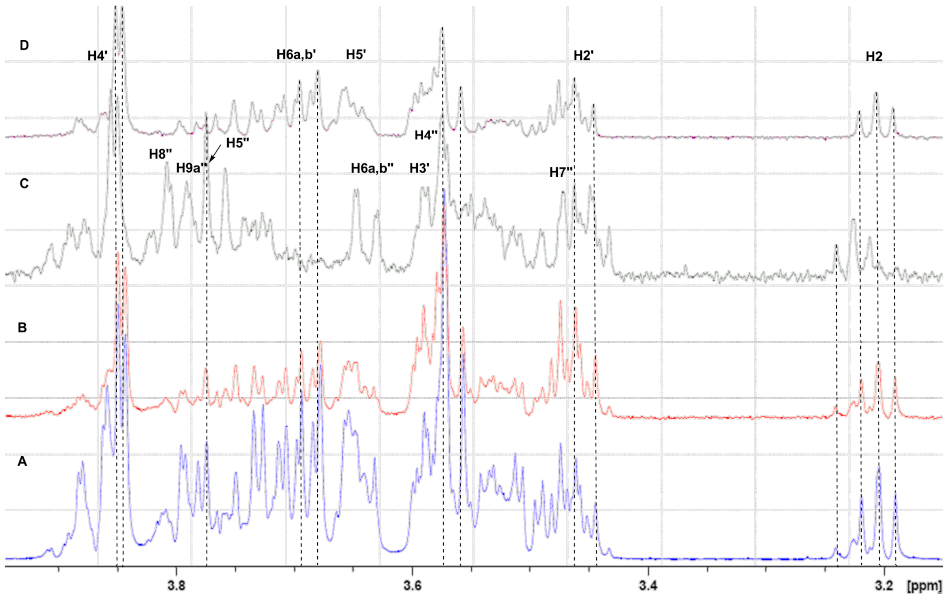


Fig. S18. The interaction of Neu5Acα2-6Galβ1-4Glc (1 mM) in the presence of Galβ1-4Glc (4 mM) with RCA_{120} (30 μM) by STD-NMR. (A) The reference 1D NMR spectrum, showing the 4:1 ratio in Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc mixture noticeable from the intensity of H2-Glc signal. The latter is also up-field shifted in the case of Galβ1-4Glc. (B) The corresponding STD NMR spectrum. (C) The STD NMR spectrum of RCA_{120} with only Neu5Acα2-6Galβ1-4Glc (1 mM) and (D) only Galβ1-4Glc (1 mM).

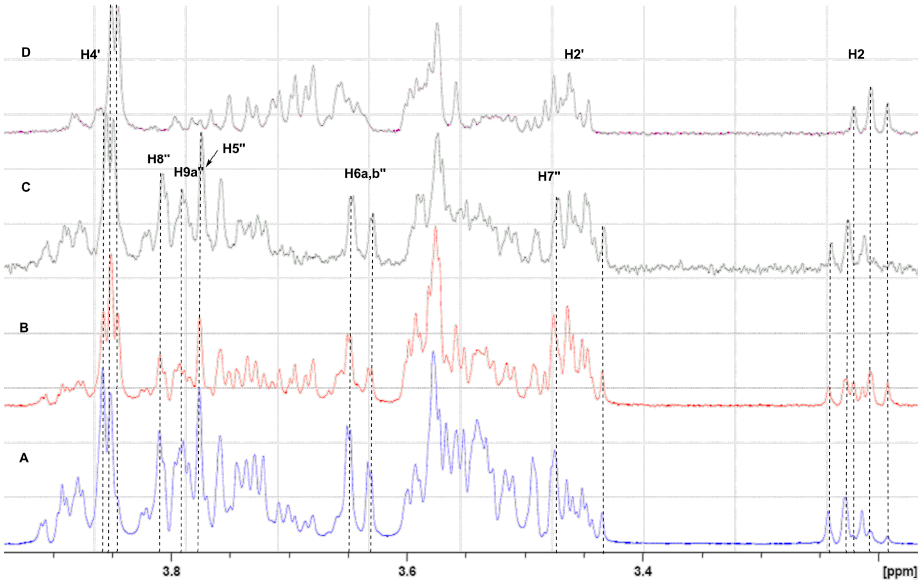


Fig. S19. The interaction of Galβ1-4Glc (1 mM) in the presence of Neu5Acα2-6Galβ1-4Glc (4 mM) with RCA_{120} (30 μM) by STD-NMR. (A) The reference 1D NMR spectrum, showing the 1:4 ratio in Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc mixture noticeable from the intensity of H2-Glc signal. The latter is also down-field shifted in the case of Neu5Acα2-6Galβ1-4Glc. (B) The corresponding STD NMR spectrum. (C) The STD NMR spectrum of RCA_{120} with only Neu5Acα2-6Galβ1-4Glc (1 mM) and (D) only Galβ1-4Glc (1 mM).

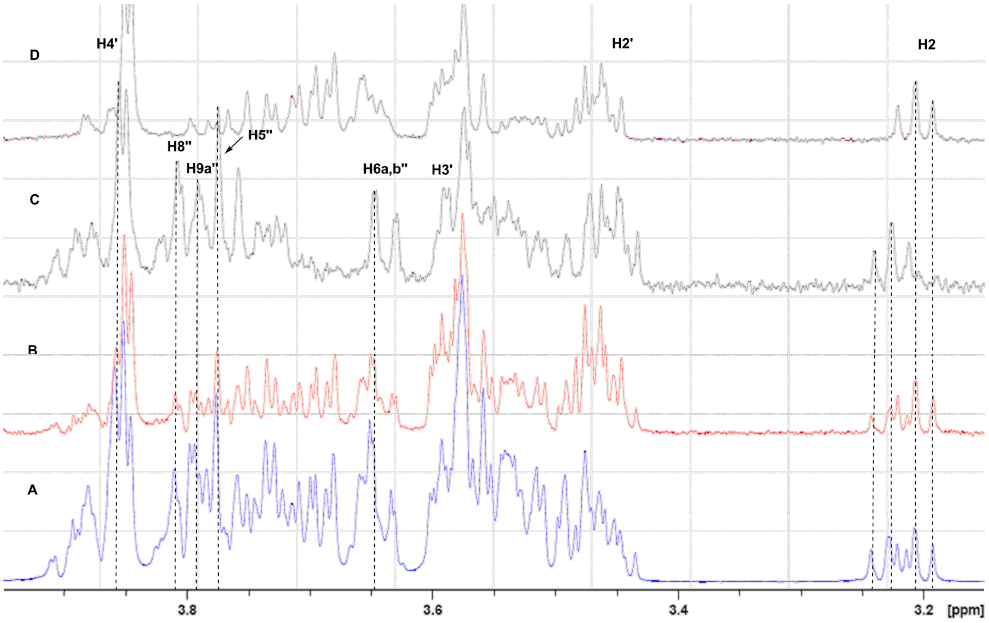


Fig. S20. The interaction of Galβ1-4Glc (1 mM) and Neu5Acα2-6Galβ1-4Glc (1 mM) with RCA_{120} (30 μM) by STD-NMR. (A) The reference 1D NMR spectrum, showing the 1:1 ratio in Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc mixture noticeable from the intensity of H2-Glc signal. (B) The corresponding STD NMR spectrum. (C) The STD NMR spectrum of RCA_{120} with only Neu5Acα2-6Galβ1-4Glc (1 mM) and (D) only Galβ1-4Glc (1 mM).

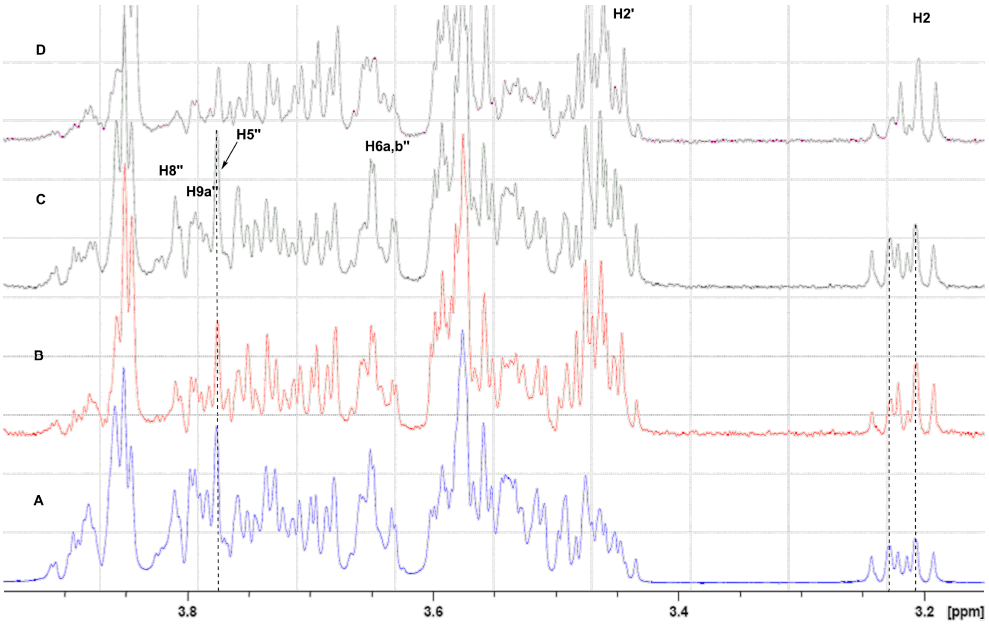
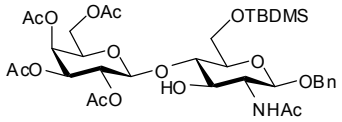


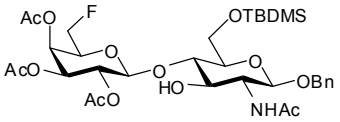
Fig. S21. The interaction of Galβ1-4Glc and Neu5Acα2-6Galβ1-4Glc with RCA_{120} (30 μM) by STD-NMR. (A) The reference 1D NMR spectrum for a 1:1 molar ratio of ligands. (B) The corresponding STD NMR spectrum. (C) The STD NMR spectrum of RCA_{120} for a Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc 1:4 molar ratio, showing a nearly 1:1 bound ligand fraction ratio. (D) The STD NMR spectrum of RCA_{120} with Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc 4:1 molar ratio, showing the same 4:1 bound ligand fraction ratio. Similarity between the reference 1D NMR spectrum (A) for 1:1 molar ratio and the STD NMR spectrum (C) (Galβ1-4Glc/Neu5Acα2-6Galβ1-4Glc 1:4 molar ratio) suggests a 1:1 competition for the binding of RCA_{120} despite the excess of sialylated sugar in solution.

1.7 Benzyl 2,3,4,6-tetra-*O*-acetyl-D-galactosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 14



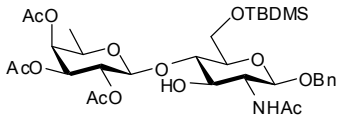
D-Galactose trichloroacetimidate **8** (500 mg, 1.00 mmol) and protected acceptor **13** (409 mg, 1.00 mmol) were combined and taken up in toluene. The mixture was evaporated from toluene to drive off any residual water and this process thrice repeated. The residue was dissolved in anhydrous dichloromethane (15 ml) and molecular sieves (3Å, 1 weight equivalent). The reaction mixture was cooled to – 41 ºC and allowed to stir for 20 min. Boron trifluoride diethyl etherate (284 mg, 0.22 ml, 2.0 mmol) was added and the solution left to stir for 6 h. Sodium hydrogen carbonate (10 ml, saturated solution) was injected in the reaction mixture and left to stir for 10 min. The organic layer was extracted with brine (10 ml, saturated solution), dried over anhydrous magnesium sulphate, filtered to remove the solid and concentrated. The residue was purified by flash chromatography in ethyl acetate to give the desired product **14** as a white solid (260 mg, 30 % yield). R_f 0.35 (EtOAc); $[\alpha]^{22}_D$ -13.4 (c = 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.10 (s, 3 H, -OTB*DM*S), 0.11 (s, 3 H, -OTB*DM*S), 0.92 (s, 9 H, -O*TB*DMS), 1.98, 1.98, 2.05, 2.08, 2.15 (s, 3 H, -OAc, -NHAc), 3.37 (ddd, *J*=9.1, 3.5, 1.8 Hz, 1 H, H-5 GlcNAc), 3.51 - 3.58 (m, 1 H, H-2 GlcNAc), 3.61 (t, *J*=8.6 Hz, 1 H, H-4 GlcNAc), 3.70 - 3.77 (m, *J*=11.6, 3.8 Hz, 1 H, H-6 GlcNAc), 3.86 (dd, *J*=11.6, 2.0 Hz, 1 H, H-6' GlcNAc), 3.92 (t, *J*=9.2 Hz, 1 H, H-3 GlcNAc), 3.97 (app t, *J*=6.9 Hz, 1 H, H-5 Gal), 4.11 - 4.15 (m, 2 H, H-6,H-6' Gal), 4.56 (d, *J*=11.9 Hz, 1 H, -C*HH*Ph), 4.62 (d, *J*=8.1 Hz, 1 H, H-1 Gal), 4.71 (d, *J*=8.1 Hz, 1 H, H-1 GlcNAc), 4.84 (d, *J*=11.9 Hz, 1 H, -C*HH*Ph), 4.98 (dd, *J*=10.4, 3.5 Hz, 1 H, H-3 Gal), 5.22 (dd, *J*=10.6, 8.1 Hz, 1 H, H-2 Gal), 5.38 (d, *J*=3.5 Hz, 1 H, H-4 Gal), 5.59 (d, *J*=7.8 Hz, 1 H, -*NH*Ac), 7.25 - 7.36 (m, 5 H, -OBn); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm; - 5.2 (CH_3, -OTB*DM*S), - 4.9 (CH_3, -OTB*DM*S), 18.3 (C, -OTBDMS), 20.5, 20.6, 20.7, 23.6 (CH_3, -OAc, -NHAc), 25.9 (CH_3, -O*TB*DMS), 56.4 (CH, C-2 GlcNAc), 61.4 (CH_2, C-6 Gal), 61.7 (CH_2, C-6 GlcNAc), 66.8 (CH, C-4 Gal), 68.8 (C-2 Gal), 70.3 (CH_2, -*C*HHPh), 70.9 (CH, C-3 Gal), 71.2 (CH, C-5 Gal), 71.7 (CH, C-3 GlcNAc), 74.7 (CH, C-5 GlcNAc), 80.8 (CH, C-4 GlcNAc), 98.9 (CH, C-1 GlcNAc), 101.43 (CH, C-1 Gal), 127.9, 128.0, 128.4 (CH, -Ar), 137.3 (C, -Ar), 169.3, 170.0, 170.1, 170.4 (C, -OAc, -NHAc); IR (thin film) cm^{-1}: 3494, 3267, 2932, 2887, 1752, 1654; HRMS (ESI^+): calculated for $C_{35}H_{53}NO_{15}SiNa$ $[M + Na]^+$: 778.3082, found: 778.3077 $[M + Na]^+$.

1.8 Benzyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-D-galactosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 15



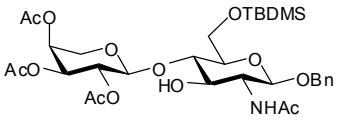
2,3,4-Tri-*O*-acetyl-6-deoxy-6-fluoro-D-galactose trichloroacetimidate **9** (200 mg, 0.44 mmol) and benzyl 2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **13** (170 mg, 0.40 mmol) were dissolved in toluene (4 ml). The mixture was evaporated to dryness and the procedure thrice repeated in order to drive off any excess water. Molecular sieves (1 weight equivalent) were added and the residue dissolved in anhydrous dichloromethane (10 ml). The mixture was cooled to – 41 ºC and boron trifluoride diethyl etherate (113.5 mg, 101.4 µl, 0.80 mmol) was added. The reaction was left to stir for 6 h. before being quenched by the addition of saturated sodium hydrogen carbonate solution (5 ml). The organic layer was separated and the aqueous phase re-extracted with dichlormethane (3 x 20 ml). The combined organic phases were dried over anhydrous sodium sulphate and reduced *in vacuo*. The residue was purified by flash chromatography (1:1 ethyl acetate: hexane) to give the desired product **15** as a white foam (156 mg, 50 %); R_f 0.51 (ethyl acetate), $[\alpha]^{21}_D$ - 28.7 (c = 10 mg/ml, $CHCl_3$), ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 0.10 (s, 6 H, -OTB*DM*S), 0.92 (s, 9 H, -O*TB*DMS), 1.95 (s, 3 H, -OAc), 1.99 (s, 3 H, -OAc), 2.07 (s, 3 H, -OAc), 2.16 (s, 3 H, -OAc), 3.18 (dd, *J*=18.0, 7.9 Hz, 1 H, H-2 GlcNAc), 3.50 (app t, *J*=9.1 Hz, 1 H, H-4 GlcNAc), 3.84 (dd, *J*=11.4, 5.4 Hz, 1 H, H-6 GlcNAc), 4.01 (dd, *J*=11.4, 2.2 Hz, 1 H, H-6' GlcNAc), 4.02 - 4.07 (m, 1 H, H-5 GlcNAc), 4.32 - 4.42 (m, 2 H, H-6 Gal, H-3 GlcNAc), 4.46 - 4.57 (m, 2 H, H-6' Gal, H-5 GlcNAc), 4.61 (d, *J*=7.9 Hz, 1 H, H-1 Gal), 4.65 (d, *J*=8.2 Hz, 1 H, -C*H*HPh), 4.85 (d, *J*=11.7 Hz, 1 H, H-1 GlcNAc), 4.86 (d, *J*=8.2 Hz, 1 H, -CH*H*Ph), 5.01 (dd, *J*=10.4, 3.5 Hz, 1 H, H-3 Gal), 5.24 (dd, *J*=10.6, 8.0 Hz, 1 H, H-2 Gal), 5.41 (app d, *J*=2.8 Hz, 1 H, H-4 Gal), 5.55 (d, *J*=7.6 Hz, 1 H, -N*H*Ac), 7.29 - 7.35 (m, 5 H, Ar), ^{13}C-NMR (126 MHz, $CDCl_3$) δ ppm -5.2 (CH_3, -OTB*DM*S), -5.2 (CH_3, -OTB*DM*S), 18.4 (C, -O*TB*DMS), 20.5 (CH_3, -OAc), 20.6 (CH_3, -OAc), 20.8 (CH_3, -OAc), 23.8 (CH_3, NH*Ac*), 25.9 (3 CH_3, -O*TB*DMS), 57.5 (CH, C-2 GlcNAc), 62.9 (CH_2, C-6 GlcNAc), 66.8 (CH, C-4 Gal), 68.9 (CH, C-2 Gal), 69.5 (CH, C-4 GlcNAc), 70.7 (CH, C-3 Gal), 70.8 (CH_2, -*C*HHPh), 71.9 (CH, C-5 Gal), 76.2 (CH, C-5 GlcNAc), 80.6 (d, *J*=172.6 Hz, CH_2, C-6 Gal), 83.2 (CH, C-3 GlcNAc), 98.1 (CH, C-1 GlcNAc), 101.1 (CH, C-1 Gal), 128.0 (CH, Ar), 128.2 (2 x CH, Ar), 128.5 (2 x CH, Ar), 169.1 (C, -OAc), 170.0 (C, -OAc), 170.1 (C, -OAc), 170.5 (C, -NH*Ac*), ^{19}F-NMR ($CDCl_3$-*d*, 376.5 MHz) δ_F -230.9; IR (thin film) cm^{-1}: 2927, 1752, 1656; HRMS (ESI^+): calculated for $C_{33}H_{50}NO_{13}FSiNa$ $[M + Na]^+$: 738.2933, found 738.2928 $[M + Na]^+$.

1.9 Benzyl 2,3,4-tri-*O*-acetyl-D-fucosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 16



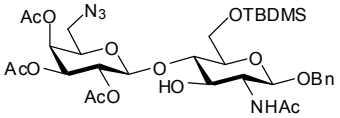
2,3,4-Tri-*O*-acetyl D-fucose trichloroacetimidate **10** (360 mg, 0.83 mmol) and benzyl 2-acetamido-6-O-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **13** (320.5 mg, 0.75 mmol) were taken up in toluene (5 ml). The solvent was removed *in vacuo* and the process thrice repeated in order to drive off any water. The residue was dissolved in anhydrous dichloromethane and 4 Å molecular sieves (1 weight equivalent) were added. The solution was cooled to -41 ºC and boron trifluoride diethyl etherate (213 mg, 190.1 µl, 1.50 mmol) was added. The solution was left to stir for 6.5 h. The reaction was quenched by the injection of saturated sodium bicarbonate solution (10 ml). The organic phase was separated and the aqueous re-extracted with dichloromethane (4 x 20 ml). The combined organic phases were dried over sodium sulphate, filtered and reduced *in vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate) to give the desired product **16** as a white foam (157 mg, 27 %); R_f (ethyl acetate) 0.34, $[\alpha]^{22}_D$ + 23.4 (c = 10 mg/ml, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.10 (s, 3 H, -OTB*DM*S), 0.11 (s, 3 H, -OTB*DM*S), 0.94 (s, 9 H, -O*TB*DMS), 1.19 (d, *J*=6.3 Hz, 3 H, H-6 Fuc), 1.98 (s, 3 H, -OAc), 1.98 (s, 3 H, -OAc), 2.06 (s, 3 H, -OAc), 2.17 (s, 3 H, -N*H*Ac), 3.47 (dd, *J*=8.7, 4.3 Hz, 1 H, H-2 GlcNAc), 3.56 (app t, *J*=8.8 Hz, 1 H, H-3 Fuc), 3.62 - 3.69 (m, 1 H, H-3 GlcNAc), 3.77 (dd, *J*=6.4, 0.9 Hz, 1 H, H-5 Fuc), 3.82 (dd, *J*=11.5, 6.2 Hz, 1 H, H-2 Fuc), 4.14 (dd, *J*=11.5, 1.9 Hz, 1 H, H-4 Fuc), 4.35 (d, *J*=7.8 Hz, 1 H, H-1 GlcNAc), 4.61 (d, *J*=11.9 Hz, 1 H, -C*H*HPh), 4.82 (d, *J*=7.8 Hz, 1 H, H-1 Fuc), 4.88 (d, *J*=11.6 Hz, 1 H, -CH*H*Ph), 5.02 (dd, 1 H, *J*=10.4, 3.3 Hz, H-6 GlcNAc), 5.08 - 5.15 (m, 2 H, H-5 GlcNAc, H-4 GlcNAc), 5.22 (dd, *J*=3.4, 0.9 Hz, 1 H, H-6' GlcNAc), 5.56 (d, *J*=4.0 Hz, 1 H, -N*H*Ac), 7.32 - 7.40 (m, 5 H, -Ar), ^{13}C-NMR (126 MHz, $CDCl_3$) δ ppm -5.2 (CH_3, -OTB*DM*S), -5.1 (CH_3, -OTB*DM*S), 16.0 (CH_3, C-6 Fuc), 18.3 (C, -OTBDMS), 20.6 (CH_3, -OAc), 20.7 (CH_3, -OAc), 21.0 (CH_3, -OAc), 23.5 (CH_3, -NH*Ac*), 26.0 (3 x CH_3, -O*TB*DMS), 58.5 (CH, C-4 Fuc), 62.9 (CH_2, -*C*HHPh), 68.9 (CH, C-4 GlcNAc), 69.3 (CH, C-5 GlcNAc), 70.2 (CH_2, C-6 GlcNAc), 70.2 (CH, C-5 Fuc), 71.3 (CH, C-2 GlcNAc), 75.4 (CH, C-5 GlcNAc), 76.1 (CH, C-3 GlcNAc), 78.6 (CH, C-5 Fuc), 98.0 (CH, C-1 GlcNAc), 102.1 (CH, C-1 Fuc), 128.4 (2 CH, -Ar), 128.5 (2 CH, -Ar), 128.7 (3 CH, -Ar), 170.1 (C, -OAc), 170.3 (C, -OAc), 170.7 (C, -OAc), 172.4 (C, -NHAc); IR (thin film) cm^{-1}: 3328, 2931, 1751, 1664; HRMS (ESI^+): calculated for $C_{33}H_{52}NO_{13}SiNa$ $[M + Na]^+$: 698.3208, found: 698.3202 $[M + Na]^+$.

1.10 Benzyl 2,3,4-tri-*O*-acetyl-L-arabinopyranosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 17



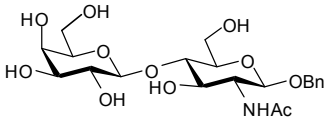
2,3,4-Tri-*O*-acetyl-L-arabinopyranose-α/β-trichloroacetimidate **11** (488 mg, 1.16 mmol) and benzyl 2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **13** (438 mg, 1.10 mmol) were dissolved in toluene (20 ml). The solution was reduced *in vacuo* and this process thrice repeated to drive off the excess water. Molecular sieves (1 weight equivalent) were added and the residue taken up in anhydrous dichloromethane (15 ml). The solution was cooled to – 41 ºC (dry ice/acetonitrile) and boron trifluoride diethyl etherate (284 mg, 0.25 ml, 2.00 mmol) was added. The solution was left to stir for 4 h. The reaction was quenched by the injection of saturated sodium hydrogen carbonate solution (15 ml) and left to stir for 10 min. The aqueous phase was extracted with dichloromethane (3 x 20 ml), dried over anhydrous magnesium sulphate, filtered and reduced *in vacuo*. The resultant crude glassy solid was purified by flash chromatography (EtOAc) to give the resultant product **17** as a white foam (348 mg, 44 %); R_f (EtOAc) 0.43, $[\alpha]^{22}_D$ 0.0 (c = 10 mg/ml, $CHCl_3$), ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 0.11 (s, 6 H, -OTB*DM*S), 0.92 (s, 9 H, -O*TB*DMS), 1.99 (s, 3 H, -OAc), 2.01 (s, 3 H, -OAc), 2.08 (s, 3 H, -OAc), 2.15 (s, 3 H, -NHAc), 3.36 (dd, *J*=9.4, 1.5 Hz, 1 H, H-4 GlcNAc), 3.58 (dd, *J*=18.0, 8.1 Hz, 1 H, H-2 GlcNAc), 3.53 - 3.69 (m, 2 H, H-6 GlcNAc, H-5 GlcNAc), 3.77 (dd, *J*=11.4, 3.5 Hz, 1 H, H-5 Ara), 3.84 - 3.93 (m, 2 H, H-5' Ara, H-3 GlcNAc), 4.05 (dd, *J*=13.4, 2.3 Hz, 1 H, H-6' GlcNAc), 4.57 (d, *J*=11.6 Hz, 1 H, -CH*H*Ph), 4.54 (d, *J*=7.6 Hz, 1 H, H-1 Ara), 4.70 (d, *J*=8.1 Hz, 1 H, H-1 GlcNAc), 4.85 (d, *J*=11.9 Hz, 1 H, -C*H*HPh), 4.99 (dd, *J*=10.1, 3.5 Hz, 1 H, H-3 Ara), 5.22 (dd, *J*=10.1, 7.6 Hz, 1 H, H-2 Ara), 5.27 (app s, 1 H, H-4 Ara), 5.57 (d, *J*=7.8 Hz, 1 H, -N*H*Ac), 7.33 (s, 5 H, -Ar), ^{13}C-NMR (100 MHz, $CDCl_3$), δ ppm -5.3 (CH_3, -OTB*DM*S), -5.0 (CH_3, -OTB*DM*S), 20.6 (CH_3, -OAc), 20.7 (CH_3, -OAc), 20.9 (CH_3, -OAc), 23.6 (CH_3, -NHAc), 25.9 (CH_3, -O*TB*DMS), 56.6 (CH, C-2 GlcNAc), 61.6 (CH_2, C-5 Ara), 64.2 (CH_2, C-6 GlcNAc), 67.6 (CH, C-4 Ara), 67.6 (CH, C-2 Ara), 69.1 (CH_2, -*C*HHPh), 70.2 (CH, C-3 Ara), 71.7 (CH, C-3 GlcNAc), 74.9 (CH, C-4 GlcNAc), 79.9 (CH, C-5 GlcNAc), 99.0 (CH, C-1 GlcNAc), 101.5 (CH, C-1 Ara), 127.9, 128.0, 128.1, 128.4 (4 x CH, -Ar), 137.3 (C, -Ar), 169.3, 170.1, 170.3, 170.6 (4 x C, 3 x -OAc, -NHAc), IR (thin film) cm^{-1}: 3020, 1748, 1216; *m/z* (ESI^+) 742.34 (M + MeCN, 100 %); HRMS (ESI^+): calculated for $C_{32}H_{50}NO_{13}Si$ $[M+H]^+$ 684.3051, found: 684.3046 $[M+H]^+$.

1.11 Benzyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-azido-D-galactosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 18



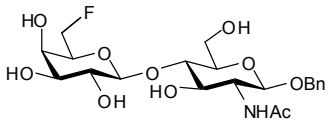
Trichloroacetimidate **12** (198 mg, 0.42 mmol, 1.1 eq.) and glucosamine derivative **13** (161 mg, 0.38 mmol, 1.0 eq.) were dried (azeotropic removal of H_2O with toluene × 2) and dissolved in dry DCM (2 mL). The resulting solution was cooled at - 40 °C and treated with $BF_3 \cdot Et_2O$ (100 µl, 0.79 mmol, 2.1 eq.). After stirring for 2h, the reaction was quenched with sat. aq. $NaHCO_3$ at -40 °C, the mixture allowed to reach rt and extracted with DCM. The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified *via* flash chromatography (hexane:EtOAc, 50:50 → 20:80) to give azide **37** (149 mg, 53%) as a white solid. $[\alpha]_D^{22}$ –7.8 (*c* = 0.95 mg/ml, $CHCl_3$); ν_{max} (thin film) 3491, 2932, 2108, 1754, 1660, 1370, 1221, 1062; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 0.10 (s, 3H, -OTB*DM*S), 0.12 (s, 3H, -OTB*DM*S), 0.93 (s, 9H, -O*TB*DMS), 1.99 (s, 6H, 2 × -OAc), 2.08 (s, 3H, -OAc), 2.18 (s, 3H, -OAc), 3.33 (dd, $J_{5,6}$ 5.2 Hz, $J_{6,6'}$ 12.6 Hz, 1H, H-6 Gal), 3.39-3.46 (m, 2H, H-2_a, H-5 GlcNAc), 3.53 (dd, $J_{5,6'}$ 7.6 Hz, $J_{6,6'}$ 12.6 Hz, H-6' Gal), 3.68 (1H, at, *J* 8.5 Hz, H-4 GlcNAc), 3.76 (1H, dd, $J_{5,6}$ 3.9 Hz, $J_{6,6'}$ 11.4 Hz, 1H, H-6 GlcNAc), 3.80 (dd, $J_{5,6}$ 5.2 Hz, $J_{5,6'}$ 7.6 Hz, 1H, H-5 Gal), 3.88 (dd, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 11.4 Hz, 1H, H-6' GlcNAc), 3.96 (br d, *J* 2.2 Hz, 1H, OH), 4.06-4.10 (m, 1H, H-3 GlcNAc), 4.56 (d, *J* 11.7 Hz, 1H, PhC*H*H), 4.68 (d, $J_{1,2}$ 8.2 Hz, 1H, H-1 Gal), 4.82 (d, $J_{1,2}$ 7.9 Hz, 1H, H-1 GlcNAc), 4.85 (d, *J* 11.7 Hz, 1H, PhCH*H*), 4.99 (dd, $J_{3,4}$ 3.5 Hz, $J_{2,3}$ 10.4 Hz, 1H, H-3 Gal), 5.21 (dd, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 10.4 Hz, 1H, H-2 Gal), 5.38 (dd, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 0.6 Hz, 1H, H-4 Gal), 5.61 (br d, *J* 7.3 Hz, 1H, NH), 7.29-7.36 (m, 5H, Ph); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm -5.2, -5.0 (2 × -OTB*DM*S), 18.3 (CH_3, -OAc), 20.5, 20.6, 20.7, 23.6 (4 × CH_3, -OAc), 25.9 (CH_3, -O*TB*DMS), 50.3 (CH_2, C-6 GlcNAc), 57.0 (CH, C-2 GlcNAc), 61.7 (CH_2, C-6 GlcNAc), 67.5 (CH, C-4 Gal), 68.8 (CH, C-2 Gal), 70.5 (CH_2, -*C*HHPh), 70.8 (CH, C-3 Gal), 71.4 (CH, C-3 GlcNAc), 72.2 (CH_2, C-5 Gal), 74.8 (CH, C-5 GlcNAc), 80.2 (CH, C-4 GlcNAc), 98.7 (CH, C-1 GlcNAc), 101.1 (CH, C-1 Gal), 128.0, 128.1, 128.5 (3 × CH, -Ar), 137.3 (C, -Ar), 169.3, 170.0, 170.1, 170.7 (4 x C, 3 x -OAc); *m/z* (ESI^+) 761 $[M + Na]^+$, 739 $[M + H]^+$; HRMS (ESI^+) calculated for $C_{33}H_{50}N_4O_{13}SiNa$ $[M + Na]^+$: 761.3036, found 761.3029 $[M + Na]^+$.

1.12 Benzyl D-galactosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 14b



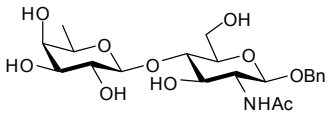
Benzyl tetra-*O*-acetyl-D-galactosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **14** (260 mg, 0.34 mmol) was dissolved in anhydrous acetonitrile (5 ml). Boron trifluoride diethyletherate (54 mg, 47.8 µl, 0.39 mmol) was added and the solution left to stir for 4 h at rt. Sodium hydrogen carbonate (10 ml) was added and the solution concentrated under reduced pressure. The solution was then diluted with dichloromethane (20 ml) and the aqueous layer re-extracted with dichloromethane (3 x 20 ml). The combined organic phases were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to give a clear oil (218 mg, 0.34 mmol, 100 %). The oil (84 mg, 0.13 mmol) was dissolved in anhydrous methanol (6 ml). Sodium methoxide (10 mg, 0.19 mmol) was added and the solution left to stir for 30 min. The reaction mixture was neutralised with pre-washed acidic Dowex 50W and filtered through a cotton wool plug to remove the resin. The solution was then reduced *in vacuo* to give a white foam **14b** (57 mg, 94 % yield); (57 mg, 94 % yield); m.p. 100 – 102 °C (ether/hexane) ; lit m.p. 102-103 °C[25]; $[\alpha]^{21}_{D}$ – 19.3 (c = 10 mg/ml, DMSO) lit. $[\alpha]^{21}_{D}$ - 23.2 (c = 16 mg/ml, DMSO)[26]; ^{1}H-NMR (500 MHz, D_2O) δ ppm 1.84 (s, 3 H, -NHAc), 3.44 (dd, *J*=10.1, 7.9 Hz, 1 H, H-2 GlcNAc), 3.49 (ddd, *J*=9.8, 5.0, 2.2 Hz, 1 H, H-5 GlcNAc), 3.55 (dd, *J*=10.4, 1.9 Hz, 1 H, H-4 GlcNAc), 3.58 (dd, *J*=10.1, 3.5 Hz, 1 H, H-3 $GlcNA_c$), 3.60 - 3.71 (m, 5 H, H-2 Gal, H-3 Gal, H-5 Gal, H-6 GlcNAc, H-6' GlcNAc), 3.76 (dd, *J*=12.3, 5.0 Hz, 1 H, H-6 Gal), 3.83 (app d, *J*=3.5 Hz, 1 H, H-4 Gal), 3.93 (dd, *J*=12.1, 2.0 Hz, 1 H, H-6' Gal), 4.38 (d, *J*=7.9 Hz, 1 H, H-1 GlcNAc), 4.46 (d, *J*=8.5 Hz, 1 H, H-1 Gal), 4.59 (d, *J*=12.3 Hz, 1 H, -CHHPh), 4.80 (d, *J*=12.0 Hz, 1 H, -CHHPh), 7.26 - 7.32 (m, 2 H, -OBn), 7.32 - 7.42 (m, 3 H, -OBn), ^{13}C-NMR (125 MHz, D_2O) δ ppm 22.1 (CH_3, -NHAc), 55.0 (CH, C-2 GlcNAc), 60.1 (CH_2, C-6 Gal), 61.0 (CH_2, C-6 GlcNAc), 68.5 (CH, C-4 Gal), 70.9 (C-2 Gal), 71.4 (CH_2, -CHHPh), 72.3 (CH, C-3 Gal), 72.5 (CH, C-5 Gal), 74.8 (C-3 GlcNAc), 75.3 (C-5 GlcNAc), 78.4 (CH, C-4 GlcNAc), 99.8 (CH, C-1 GlcNAc), 102.8 (CH, C-1 Gal), 128.4, 128.6, 128.7 (CH, -Ar), 136.7 (C, -Ar), 174.4 (C, -NHAc); IR (thin film) cm^{-1}: no significant peaks; HRMS (ESI^+): calculated for $C_{21}H_{31}NO_{11}Na$ $[M + Na]^+$: 496.1795, found: 496.1789 $[M + Na]^+$.

1.13 Benzyl 6-deoxy-6-fluoro-D-galactosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 15b



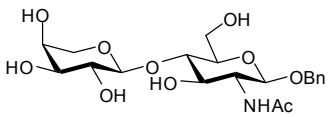
Benzyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-D-galactose-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **15** (100 mg, 0.14mmol) was dissolved in anhydrous acetonitrile (5 ml). Boron trifluoride diethyl etherate (21.6 mg, 19.5 µl, 0.15 mmol) was added and the solution left to stir for 4 h. The reaction was quenched by the addition of sodium hydrogen carbonate solution (2 ml, saturated solution) and the mixture evaporated to remove the acetonitrile. The residue was dissolved in dichloromethane and washed with water (5 ml). The aqueous phase was re-extracted with dichloromethane (2 x 2 ml). The combined organic phases were dried over sodium sulphate, filtered and reduced *in vacuo*. The residue was then taken up in anhydrous methanol (1.5 ml) and sodium methoxide (9.4 mg, 0.17 mmol) was added the solution was left to stir at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50Wx8 acidic ion exchange resin, filtered and reduced *in vacuo*. The desired product **15b** was obtained as an amorphous white solid (55 mg, 83 %); R_f 0.43 (0.8:2:3 water:isopropanol:ethyl acetate), $[\alpha]^{22}_D$ (MeOH) - 10.7 (c = 10 mg/ml); ^{1}H-NMR (500 MHz, CD_3OD) δ ppm 1.97 (s, 3 H, -NHAc), 3.45 (dd, *J*=9.3, 7.1 Hz, 1 H, H-5 GlcNAc), 3.45 (dd, *J*=9.3, 1.7 Hz, 1 H, H-2 Gal), 3.52 - 3.61 (m, 3 H, H-4 GlcNAc, H-4 Gal, H-3 Gal), 3.64 (dd, *J*=17.0, 9.8 Hz, 1 H, H-3 GlcNAc), 3.81 (dd, *J*=10.1, 8.5 Hz, 1 H, H-2 GlcNAc), 3.84 - 3.93 (m, 2 H, H-6 GlcNAc, H-6' GlcNAc), 3.96 - 4.02 (m, 1 H, H-5 Gal), 4.44 (d, *J*=7.3 Hz, 1 H, H-1 Gal), 4.50 (d, *J*=8.2 Hz, 1 H, H-1 GlcNAc), 4.62 (d, *J*=12.3 Hz, 1 H, -C*H*HPh), 4.62 - 4.66 (m, 2 H, H-6 Gal), 4.91 (app s, 1 H, -CH*H*Ph, partially obscured by HDO peak), ^{13}C-NMR (126 MHz, CD_3OD), δ ppm 23.0 (CH_3, -NH*Ac*), 55.6 (CH, C-2 GlcNAc), 62.1 (CH_2, C-6 GlcNAc), 65.3 (CH, C-2 GlcNAc), 69.7 (CH, C-4 GlcNAc), 69.8 (CH_2, -CHHPh), 72.3 (CH, C-3 GlcNAc), 74.0 (CH, C-4 Gal), 74.5 (CH, C-2 Gal), 75.1 (CH, C-5 GlcNAc), 75.3 (d, *J*=311.9 Hz, CH, C-5 Gal), 76.5 (CH, C-3 Gal), 83.7 (CH_2, d, *J*=167.8 Hz, C-6 Gal), 101.8 (CH, C-1 Gal), 105.3 (CH, C-6 GlcNAc), 128.7, 128.8, 129.0, 129.2, 129.4 (5 x CH, -Ar), 139.1 (C, -Ar), 173.6 (C, -NHAc), ^{19}F-NMR ($CDCl_3$-*d*, 376.5 MHz) δ ppm -232.1, IR (thin film) cm^{-1}: 3370, 2094, 1643, HRMS (ESI$^+$): calculated for $C_{21}H_{30}NO_{10}FNa$ $[M + Na]^+$: 498.1751, found 498.1746 $[M + Na]^+$.

1.14 Benzyl D-fucosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 16b



Benzyl 2,3,4-tri-*O*-acetyl-D-fucosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **16** (149 mg, 0.21 mmol) was dissolved in anhydrous acetonitrile (5 ml). Boron trifluoride diethyl etherate (33 mg, 30 µl, 0.23 mmol) was added and the reaction left to stir at room temperature for 5 h. Sodium hydrogen carbonate solution (5 ml) was added and the acetonitrile removed under reduced pressure. The solution was diluted with dichloromethane (20 ml) and the aqueous phase re-extracted with dichloromethane (3 x 20 ml). The combined organic phases were dried over anhydrous sodium sulphate, filtered and reduced under vacuum to give the desialylated product as a clear oil. The residue was taken up in methanol (5 ml). Sodium methoxide (16.1 mg, 0.30 mmol) was added and the solution left to stir for 35 min. The reaction was neutralized by the addition of solid carbon dioxide, reduced *in vacuo* then lyophilised to give the desired product **16b** as a white powder (97 mg, quant.); R_f 0.64 (1:2:2 water:isopropanol:ethyl acetate), m.p. 192 – 195 ºC, ^{1}H-NMR (500 MHz, CD_3OD) δ ppm 1.30 (d, *J*=6.62 Hz, 3 H, H-6 Fuc), 1.97 (s, 3 H, -NHAc), 3.43 (dd, *J*=9.46, 1.89 Hz, 1 H, H-3 Fuc), 3.49 - 3.54 (m, 2 H, H-2 Fuc, H-5 GlcNAc), 3.55 - 3.62 (m, 1 H, H-4 Fuc), 3.62 - 3.65 (m, 2 H, H-3 GlcNAc, H-4 GlcNAc), 3.74 - 3.85 (m, 2 H, H-2 GlcNAc, H-5 Fuc), 3.89 (dd, *J*=12.30, 4.41 Hz, 1 H, H-6 GlcNAc), 3.97 (dd, *J*=11.98, 2.62 Hz, 1H, H-6' GlcNAc), 4.36 (d, *J*=5.99 Hz, 1 H, H-1 Fuc), 4.49 (d, *J*=8.20 Hz, 1 H, H-1 GlcNAc), 4.62 (d, *J*=12.30 Hz, 1 H, -C*H*HPh), 4.90 - 4.92 (m, 1 H, -C*H*HPh, partially obscured by HDO peak), ^{13}C-NMR (125 MHz, CD_3OD) δ ppm 16.6 (CH_3, C-6 Fuc), 23.0 (CH_3, -NH*Ac*), 56.6 (CH, C-5 Fuc), 62.0 (CH_2, C-6 GlcNAc, C-6' GlcNAc), 71.6 (CH_2, -*C*H_2Ph), 72.2 (CH, C-5 GlcNAc), 72.5 (CH, C-3 GlcNAc), 72.9 (CH, C-4 GlcNAc), 74.1 (CH, C-2 GlcNAc), 75.0 (CH, C-2 Fuc), 76.5 (CH, C-3 Fuc), 81.7 (CH, C-4 Fuc), 101.8 (CH, C-1 GlcNAc), 105.2 (CH, C-1 Fuc), 128.1 (CH, Ar), 128.7 (2 x CH, Ar), 129.4 (2 x CH, Ar), 173.60 (C, -NH*Ac*), IR (thin film): 3424, 1642; HRMS (ESI$^+$): calculated for $C_{21}H_{31}NO_{10}Na$ $[M + Na]^+$: 480.1846, found: 480.1854 $[M + Na]^+$.

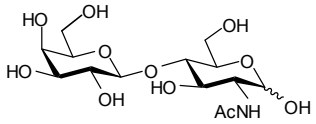
1.15 Benzyl L-arabinopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 17b



Benzyl 2,3,4-tri-*O*-acetyl-L-arabinopyranosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **17** (100 mg, 0.14 mmol) was dissolved in anhydrous acetonitrile (5

ml). Boron trifluoride diethyl etherate (20.7 mg, 18.4 ml, 0.16 μmol) was added and the solution left to stir for 4 h. The reaction was quenched with saturated sodium hydrogen carbonate (10 ml) and the acetonitrile removed *in vacuo*. The resultant aqueous mixture was extracted with dichloromethane (4 x 20 ml). The organic layers were combined, dried over magnesium sulphate, filtered and reduced *in vacuo*. The crude residue was taken up in anhydrous methanol (4 ml) and sodium methoxide (10 mg, 0.18 mmol) was added. The reaction mixture was left to stir at room temperature for 1 h. The mixture was the neutralized with acidified and washed Dowex 50WX8 ion exchange resin, filtered to remove the resin and concentrated *in vacuo* to give the desired product **17b** as a white powder (47 mg, 75 % over two steps); R_f (0.8:2:3 water:isopropanol:ethyl acetate) 0.40, ^{1}H-NMR (500 MHz, D_2O) δ ppm 1.84 (s, 3 H, -NHAc), 3.49 (dd, *J*=9.1, 4.7 Hz, 1 H, H-2 GlcNAc), 3.46 (dd, *J*=9.8, 7.9 Hz, 1 H, H-3 GlcNAc), 3.51 - 3.57 (m, 2 H, H-5 Ara, H-5' Ara), 3.57 - 3.61 (m, 3 H, H-4 GlcNAc, H-4 Ara, H-3 Ara), 3.65 (dd, *J*=10.1, 8.5 Hz, 1 H, H-2 Ara), 3.76 (dd, *J*=12.3, 5.0 Hz, 1 H, H-6 GlcNAc), 3.82 - 3.88 (m, 1 H, H-5 GlcNAc), 3.92 (dd, *J*=12.5, 2.1 Hz, 1 H, H-6' GlcNAc), 4.30 (d, *J*=7.9 Hz, 1 H GlcNAc), 4.46 (d, *J*=8.2 Hz, 1 H Ara), 4.59 (d, *J*=12.0 Hz, 1 H, -C*H*HPh), 4.80 (d, *J*=12.0 Hz, 1 H, -CH*H*Ph), 7.26 - 7.31 (m, 2 H, -Ar), 7.32 - 7.40 (m, 3 H, -Ar), ^{13}C-NMR (126 MHz, D_2O) δ ppm 22.1 (CH_3, -NHAc), 55.1 (CH, C-4 GlcNAc), 60.0 (CH_2, C-6 GlcNAc), 66.3 (CH_2, -*C*HHPh), 68.3 (CH, C-4 Ara), 70.9 (CH_2, C-5 Ara), 71.5 (CH, C-5 GlcNAc), 72.1 (C-2 Ara), 72.2 (CH, C-3 Ara), 74.9 (CH, C-3 GlcNAc), 78.4 (CH, C-2 GlcNAc), 99.8, (CH, C-1 Ara), 103.3 (CH, C-1 GlcNAc), 128.4, 128.6, 128.7 (5 x C, -Ar), 136.7 (C, -Ar), 174.5 (C, -NH*Ac*), IR (thin film) cm^{-1}: 3287, 1656, 1595, 1256, 1164, 1082, 1001, 941, 836, 781; HRMS (ESI$^+$) calculated for $C_{20}H_{29}NO_{10}Na$ $[M + Na]^+$: 466.1689, found: 466.1684 $[M + Na]^+$.

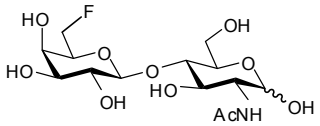
1.16 D-Galactosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranose 1



Benzyl tetra-*O*-acetyl-D-galactose-(β1,4)-2-acetamido-2-deoxy-D-glucopyranoside (100 mg, 0.11 mmol) was dissolved methanol (1 ml). Sodium methoxide (11.8 mg, 0.22 mmol) was added and the solution left to stir for 10 min. The reaction was neutralised by the addition of prewashed and acidified Dowex50Wx8 acidic ion exchange resin. The resin was removed by filtration and the eluent concentrated to give a white powder. The residue was taken up in methanol and Pearlman's catalyst (10 mg) was added. The solution was degassed and placed under a positive pressure of hydrogen. The degassing procedure was repeated three times and reaction mixture left to stir under a positive pressure of hydrogen for 5.5 h. The mixture was then filtered through Celite to remove

the catalyst, reduced *in vacuo* and purified by flash chromatography (0.8:2:3 water isopropanol:ethyl acetate) to give the desired product **1** as a white powder (38.4 mg, 90 %). R_f (0.8:2:3 water:isopropanol:ethyl acetate) 0.19; ^{1}H-NMR (400 MHz, D_2O) δ ppm 1.95 (s, 3 H, -NHAc), 3.32 - 3.91 (m, 12 H, H-2 Gal, H-2 GlcNAc, H-3 Gal, H-3 GlcNAc, H-4 Gal, H-4 GlcNAc, H-5 Gal, H-5 GlcNAc, H-6 Gal, H-6 GlcNAc, H-6' Gal, H-6' GlcNAc), 4.38 (d, *J*=7.8 Hz, 1 H, H-1 Gal), 4.63 (d, *J*=7.1 Hz, 1 H, H-1β GlcNAc), 5.11 (br. s., 1 H, H-1α GlcNAc); ^{13}C-NMR (100 MHz, D_2O) δ ppm 22.3 (CH_3, -NHAc), 54.1 (CH, C-2 GlcNAc), 60.3, 61.4 (CH_2, C-6 GlcNAc, C-6 Gal), 68.9, 69.6, 71.3, 72.9, 75.2, 75.7, 79.1 (CH, C-2 Gal, C-3 Gal, C-3 GlcNAc, C-4 Gal, C-4 GlcNAc, C-5 Gal, C-5 GlcNAc), 90.9 (CH, C-1α GlcNAc), 95.9 (CH, C-1β GlcNAc), 103.3 (CH, C-1 Gal), 174.8 (C, -NHAc); IR (thin film) no significant peaks; HRMS (ESI$^+$) calculated for: $C_{14}H_{25}NO_{11}Na$ $[M + Na]^+$: 406.1325, found: 406.1320 $[M + Na]^+$.

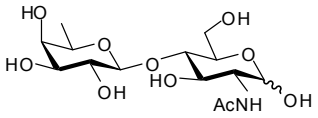
1.17 6-Deoxy-6-fluoro-D-galactosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranose 2



Benzyl 6-deoxy-6-fluoro-D-galactosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranoside **15b** (24 mg, 0.05 mmol) was dissolved in tetrahydrofuran (3 ml). Methanol (200 µl) was added to solubilise the compound. Pearlman's catalyst (5 mg) was added. The sample vessel was evacuated and filled with hydrogen. The degassing cycle was thrice repeated and the sample left to stir under a positive pressure of hydrogen for 4 h. The sample was then filtered through Celite to remove the catalyst and the solvents removed *in vacuo* to give the desired product **2** as a white powder (17.1 mg, 88 %, 2:3 α/β); R_f (0.8:2:3 water:isopropanol:ethyl acetate) 0.35, 0.30; ^{1}H-NMR (500 MHz, CD_3OD) δ ppm 2.00 (s, 3 H, -NHAcβ GlcNAc), 2.01 (s, 3 H, NHAcα GlcNAc), 3.43 - 3.49 (m, 1 H, H-2 Gal), 3.52 - 3.63 (m, 3 H, H-3 Gal, H-4 Gal, H-4β GlcNAc), 3.64 - 3.72 (m, 1 H, H-6 GlcNAc), 3.81 - 3.98 (m, 5 H, H-5 Gal, H-2α GlcNAc, H-2β GlcNAc, H-3 GlcNAc, H-5 GlcNAc), 4.43 (d, *J*=7.3 Hz, 1 H, H-1β GlcNAc), 4.54 - 4.58 (m, 1 H, H-1 Gal), 4.59 - 4.63 (m, 1 H, H-1 Gal), 4.63 - 4.69 (m, 1 H, H-6' Gal), 5.13 (d, *J*=2.8 Hz, 1 H, H-1α GlcNAc); ^{13}C-NMR (125 MHz, CD_3OD) δ ppm 22.6 (CH_3, -NHAcβ), 22.9 (CH_3, -NHAcα), 58.2 (CH, C-3 GlcNAc), 62.2 (CH_2, C-6 GlcNAc), 69.8 (d, *J*=6.7 Hz, C-5 Gal), 70.7 (CH, C-5 GlcNAc), 71.4 (CH, C-4 GlcNAc), 72.3 (CH, C-4 Gal), 74.0 (CH, C-2β GlcNAc), 75.1 (CH, C-2α GlcNAc), 76.5 (CH, C-2 Gal), 82.6 (CH_2 d, *J*=119.2 Hz, C-6 Gal), 84.4 (CH, C-3 Gal), 92.3 (CH, C-1α GlcNAc), 97.2 (CH, C-1 Gal), 105.4 (CH, C-1β GlcNAc), 173.6 (C, -NHAc); ^{19}F-NMR ($CDCl_3$-*d*, 376.5 MHz) δ ppm -232.6; IR (thin film) cm^{-1}: no significant peaks; *m/z* (ESI$^+$) 444.26 (M +59; MeCN, NH_4^+, 100 %); HRMS (ESI$^+$):

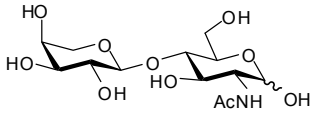
$C_{14}H_{24}FNO_{10}Na$ $[M + Na]^+$: 408.1282, found: 408.1276 $[M + Na]^+$.

1.18 D-Fucosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranose 3



Benzyl D-fucosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranoside **16b** (15 mg, 0.03 mmol) was dissolved in 1:1 tetrahydrofuran:methanol (4 ml). Pearlman's catalyst (2 mg) was added and the solution degassed and placed under a positive pressure of hydrogen. The degassing procedure was repeated three times and the solution left to stir under a hydrogen atmosphere for 8 h. The reaction mixture was then filtered through Celite to remove the catalyst and concentrated *in vacuo*. The residue was then taken up in water and lypohilised to give the desired product **3** as a white solid (11 mg, quant.); R_f 0.45, 0.48 (1:2:2 water:isopropanol:ethyl acetate) ^{1}H-NMR (500 MHz, D_2O) δ ppm 1.17 (d, *J*=6.6 Hz, 3 H, H-6 Fuc), 1.96 (s, 3 H, -NHAc), 3.38 - 3.46 (m, 1 H, H-2 Fuc), 3.56 - 3.64 (m, 3 H, H-2 GlcNAc, H-5 GlcNAc, H-3 Fuc), 3.68 (br. s., 1 H, H-3 GlcNAc), 3.73 - 3.77 (m, 2 H, H-4 GlcNAc, H-5 Fuc), 3.77 - 3.83 (m, 3 H, H-6 GlcNAc, H-4 Fuc), 3.84 - 3.91 (m, 1 H, H-6' GlcNAc), 4.34 (d, *J*=7.9 Hz, 1 H, H-1 Fuc), 4.64 (d, *J*=7.9 Hz, 1 H, H-1β GlcNAc), 5.12 (d, *J*=2.2 Hz, 1 H, H-1α GlcNAc), ^{13}C-NMR (125 MHz, D_2O) δ ppm 15.3 (CH_3, C-6 Fuc), 21.8 (CH_3, -NHAc), 53.7 (CH, C-4 Fuc), 55.9 (CH, C-2 GlcNAc), 59.9 (CH_2, C-6 GlcNAc), 69.2 (CH, C-4 GlcNAc), 70.1 (CH, C-2 Fuc), 71.1 (CH, C-5 Fuc), 72.5 (CH, C-3 GlcNAc), 74.7 (CH, C-3 Fuc), 79.4 (CH, C-5 GlcNAc), 90.5 (CH, C-1α GlcNAc), 94.8 (CH, C-1β GlcNAc), 103.0 (CH, C-1 Fuc), 174.7 (C, -NHAc); IR (thin film) cm^{-1}: no significant peaks; HRMS (ESI$^+$): calculated for $C_{14}H_{25}NO_{10}Na$ $[M + Na]^+$: 390.1376, found: 390.1371 $[M + Na]^+$.

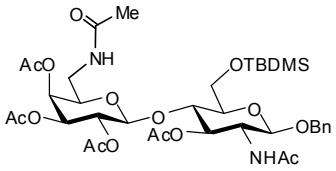
1.19 L-Arabinosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranose 4



Benzyl L-arabinosyl-(β1,4)-2-acetamido-2-deoxy-D-glucopyranoside **17b** (20 mg, 0.045 mmol) was dissolved in tetrahydrofuran (3 ml) and methanol (200 µl). Pearlman's catalyst (5 mg) was added. The solution was degassed and placed under a positive pressure of hydrogen. The degassing procedure was repeated three times and reaction mixture left to stir under a positive pressure of hydrogen for 5.5 h. The mixture was then filtered through a 0.2 µm syringe filter to remove the catalyst and reduced *in vacuo* to give the desired product **4** as a white powder (14.3 mg, 90 %, 1:2 α/β); R_f (0.8:2:3 water:isopropanol:ethyl acetate) 0.23, 0.25; $[\alpha]^{22}_D$ + 17.1 (c = 7 mg/ml, H_2O); ^{1}H-

NMR (500 MHz, D_2O) δ ppm 1.95 (s, 3 H, -NHAcβ), 2.00 (s, 3 H, -NHAcα), 3.43 - 3.56 (m, 1 H, H-2 Ara), 3.53 - 3.68 (m, 5 H, H-2β GlcNAc, H-6 GlcNAc, H-6 GlcNAc, H-3 Ara, H-5 Ara), 3.71 - 3.93 (m, 5 H, H-2 GlcNAc, H-3 GlcNAc, H-4 GlcNAc, H-4 Ara, H-5' Ara), 4.30 (d, *J*=7.9 Hz, 1 H, H-1 Ara), 4.34 (d, *J*=3.5 Hz, 1 H, H-3 Ara), 4.37 (d, *J*=7.9 Hz, 1 H, -NHAc), 4.64 (d, *J*=7.6 Hz, 1 H, H-1β GlcNAc), 5.12 (d, *J*=2.5 Hz, 1 H, H-1α GlcNAc), ^{13}C-NMR (MeOD-d_4, 125 MHz) δ ppm 22.0 (CH_3, -NHAcβ), 22.1 (CH_3, -NHAcα), 53.8 (CH, C-2 GlcNAc), 59.9 (CH_2, C-6 GlcNAc), 66.4 (CH_2, C-5 Ara), 68.4, 69.1, 70.9, 71.1, 74.8, 78.4, 78.7 (CH, C-2 Ara, C-3 Ara, C-3α GlcNAc, C-3β GlcNAc, C-4 Ara, C-4 GlcNAc, C-5 GlcNAc), 90.5 (CH, C-1α GlcNAc), 94.8 (CH, C-1 Ara), 103.5 (CH, C-1β GlcNAc), 174.5 (C, NHAcβ), 174.7 (C, NHAcα); *m/z* 412.20 (M + 59, 100%), HRMS (ESI$^+$): calculated for $C_{13}H_{23}NO_{10}Na$ $[M + Na]^+$: 376.1220, found: 376.1214 $[M + Na]^+$.

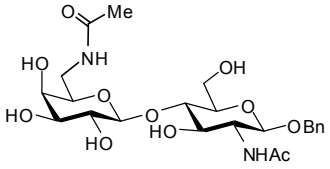
1.20 Benzyl 6-acetamido-2,3,4-tri-*O*-acetyl-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside 5a



A solution of azide **18** (401 mg, 0.54 mmol, 1.0 eq.) and PPh_3 (217 mg, 0.83 mmol, 1.5 eq.) was dissolved in THF (4 ml) and H_2O (500 µl, 28 mmol, 51 eq.) and stirred overnight. The solvents were removed under reduced pressure, the resulting solid was treated with Ac_2O (2.5 ml) and pyridine (2.5 ml) and the mixture stirred overnight. The solvents were removed under reduced pressure (toluene azeotrope) and the residue was purified *via* flash chromatography (EtOAc → EtOAc:MeOH 96:4) to give acetamide **5a** (364 mg, 85%) as a white solid, $[\alpha]_D^{22}$ –21.1 (*c* = 0.90 mg/ml, $CHCl_3$); ν_{max} (thin film) 3312, 2933, 1753, 1663, 1550, 1370, 1221, 1060; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 0.07 (6H, s, ^{t}BuSi(C*H*$_3$)$_2$), 0.90 (9H, s, (C*H*$_3$)$_3$CSiMe$_2$), 1.99 (3H, s, CH_3C(O)), 2.01 (6H, s, 2 × CH_3C(O)), 2.05 (3H, s, CH_3C(O)), 2.07 (3H, s, CH_3C(O)), 2.16 (3H, s, CH_3C(O)), 3.16 (1H, m, H-6 Gal), 3.49 (1H, aq, *J* 5.4 Hz, H-5 GlcNAc), 3.56-3.61 (1H, m, H-6' Gal), 3.67 (1H, dd, $J_{5,6}$ 4.7 Hz, $J_{5,6'}$ 8.2 Hz, H-5 Gal), 3.95 (1H, dd, $J_{5,6}$ 5.4 Hz, $J_{6,6'}$ 10.9 Hz, H-6 GlcNAc), 4.00 (1H, t, *J* 6.4 Hz, H-4 GlcNAc), 4.18-4.22 (1H, m, H-2 GlcNAc), 4.51 (1H, d, $J_{1,2}$ 6.0 Hz, H-1 GlcNAc), 4.55-4.59 (2H, m, H-1 Gal, PhC*H*H), 4.84 (1H, d, *J* 12.3 Hz, PhCH*H*), 4.98 (1H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.5 Hz, H-3 Gal), 5.02 (1H, t, *J* 7.1 Hz, H-3 GlcNAc), 5.10 (1H, dd, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.4 Hz, H-2 Gal), 5.34 (1H, d, $J_{4,5}$ 3.5 Hz, H-4 Gal), 5.79 (1H, br d, *J* 9.1, NH), 6.32-6.34 (1H, br m, NH), 7.29-7.36 (5H, m, Ph); ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm -5.3, -5.2 (2 × ^{t}BuSi(*C*H$_3$)$_2$), 18.2 ((CH$_3$)$_3$*C*), 20.6, 20.7, 20.8, 21.0, 23.1, 23.3 (6 × *C*H$_3$C(O)), 25.8 ((*C*H$_3$)$_3$C), 39.0 (C-6 Gal),

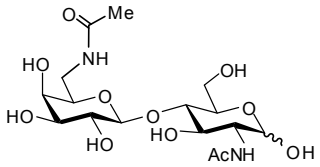
51.6 (C-2 GlcNAc), 61.7 (C-6 GlcNAc), 68.1 (C-4 Gal), 69.3 (C-2 Gal), 69.9 ($PhCH_2$), 70.6 (C-3 Gal), 71.9 (C-3 GlcNAc), 72.1 (C-5 Gal), 72.8 (C-4 GlcNAc), 75.9 (C-5 GlcNAc), 99.0 (C-1 GlcNAc), 100.4 (C-1 Gal), 127.6, 127.9, 128.4 (3 × Ph), 137.2 (ipso Ph), 169.7, 169.9, 170.1, 170.3, 170.5, 170.7 (6 × $CH_3C(O)$); *m/z* (ESI^+) 819 ($M+Na^+$), 814 ($M+NH_4^+$), 797 ($M+H^+$); HRMS (ESI^+) calculated for $C_{37}H_{56}N_2O_{15}Si$ $[M + Na]^+$: 819.3342, found 819.3372 $[M + Na]^+$.

1.21 Benzyl 6-acetamido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 5b



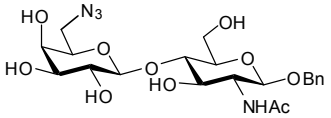
Acetamide **5a** (324 mg, 0.41 mmol, 1.0 eq.) was dissolved in dry CH_3CN (2.5 ml), treated with $BF_3{\cdot}Et_2O$ (62 µL, 0.49 mmol, 1.2 eq.) and stirred at rt for 5h. The reaction was quenched with sat. aqueous $NaHCO_3$ (2 ml) and the volatiles removed under reduced pressure. After addition of H_2O, the residue was extracted with DCM; the organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (4 ml), treated with NaOMe (~ 10 mg, 0.2 mmol, 0.5 eq.) and stirred at rt overnight. The reaction was quenched with Amberlyst 15 (acidic form) until neutrality, filtered and dried under reduced pressure to give disaccharide **5b** (155 mg, 74%) as a white solid. $[\alpha]_D^{22}$ –2.3 (*c* = 0.65 mg/ml, MeOH); ν_{max} (KBr) 3387, 1654, 1566, 1377, 1060; ^{1}H-NMR (500 MHz, CD_3OD) δ ppm 1.97 (3H, s, $CH_3C(O)$), 1.98 (3H, s, $CH_3C(O)$), 3.39 (1H, dd, $J_{5,6}$ 8.8 Hz, $J_{6,6'}$ 13.9 Hz, H-6_b), 3.43 (1H, m, H-5 GlcNAc), 3.49 (1H, dd, $J_{5,6'}$ 4.1 Hz, $J_{6,6'}$ 13.9 Hz, H-6' Gal), 3.51 (1H, dd, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.2 Hz, H-3 Gal), 3.54 (1H, dd, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.8 Hz, H-2 Gal), 3.61-3.66 (3H, m, H-3 GlcNAc, H-4 GlcNAc, H-5 Gal), 3.77 (1H, d, $J_{3,4}$ 3.2 Hz, H-4 Gal), 3.82-3.86 (1H, m, H-2 GlcNAc), 3.90 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.0 Hz, H-6 GlcNAc), 3.97 (1H, dd, $J_{5,6'}$ 2.5 Hz, $J_{6,6'}$ 12.0 Hz, H-6' GlcNAc), 4.37 (1H, d, $J_{1,2}$ 7.6 Hz, H-1 Gal), 4.49 (1H, d, $J_{1,2}$ 8.5 Hz, H-1 GlcNAc), 4.63 (1H, d, *J* 12.3 Hz, PhC*H*H), 4.90 (1H, d, *J* 12.3 Hz, PhC*H*H), 7.27-7.36 (5H, m, Ph); ^{13}C-NMR (125 MHz, CD_3OD) δ ppm 22.6, 22.9 (2 × $CH_3C(O)$), 41.4 (C-6 Gal), 56.6 (C-2 GlcNAc), 61.9 (C-6 GlcNAc), 70.7 (C-4 Gal), 71.7 ($PhCH_2$), 72.4 (C-2 Gal), 74.1 (C-3_b), 74.7 (C-3 GlcNAc), 74.8 (C-5 Gal), 76.7 (C-5 GlcNAc), 81.1 (C-4 GlcNAc), 101.8 (C-1 GlcNAc), 105.1 (C-1 Gal), 128.7, 128.8, 129.4 (3 × Ph), 139.1 (ipso Ph), 173.5, 174.1 (2 × $CH_3C(O)$); *m/z* (ESI^+) 537 $[M+Na]^+$; HRMS (ESI^+) calculated for $C_{23}H_{34}N_2O_{11}Na$ $[M + Na]^+$ 537.2055, found 537.2066).

1.22 6-Acetamido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranose 5



A suspension of acetamide **5b** (55 mg, 0.11 mmol) and $Pd(OH)_2/C$ (20% w/w, 11 mg, 0.016 mmol, 0.15 eq.) in MeOH (1.0 ml) was stirred under an atmosphere of H_2 for 5h. After the addition of a fresh batch of $Pd(OH)_2/C$ (20% w/w, 15 mg, 0.021 mmol, 0.19 eq.), the mixture was stirred for a further 2h, filtered through Celite and concentrated under reduced pressure. The residue was purified *via* flash chromatography (n-BuOH:acetone:H_2O, 5:4:1) to give the free reducing sugar **5** (35 mg, 74 %) as a white solid. ν_{max} (KBr) 3145, 1643, 1561, 1405, 1059: ^{1}H-NMR (500 MHz, D_2O, 1.5:1 mixture of anomers) δ ppm 1.91 (3H, s, $CH_3C(O)$), 1.96 (3H, s, $CH_3C(O)$), 3.36 (2H, ad, $J_{6,6'}$ 6.6 Hz, H-6 Gal, H-6' Gal), 3.43-3.48 (1H, m, H-2 Gal), 3.50-3.53 (1H, m, H-$5_{a\ minor}$), 3.56-3.70 (3H + $1H_{major}$ + $1H_{minor}$, m, H-2 $GlcNAc_{minor}$, H-3 Gal, H-4 GlcNAc, H-5 $GlcNAc_{major}$, H-5 Gal), 3.72-3.91 (4H + $1H_{major}$, m, H-2 $GlcNAc_{major}$, H-3 GlcNAc, H-4 Gal, H-6 GlcNAc, H-6' GlcNAc), 4.35-4.37 (1H, m, H-1 Gal), 4.65 ($1H_{minor}$, d, $J_{1,2\ minor}$ 8.2 Hz, H-1 $GlcNAc_{minor}$), 5.12 ($1H_{major}$, d, $J_{1,2\ major}$ 3.2 Hz, H-1 $GlcNAc_{major}$); ^{13}C-NMR (125 MHz, D_2O) δ ppm 21.8, 22.1 (2 × $CH_3C(O)$), 39.9 (C-6 Gal), 53.7 (C-2 $GlcNAc_{major}$), 56.2 (C-2 $GlcNAc_{minor}$), 59.9 (C-6 $GlcNAc_{major}$), 60.0 (C-6 $GlcNAc_{minor}$), 68.9 (C-4 Gal), 69.1 (C-3 $GlcNAc_{minor}$), 70.2 (C-3 $GlcNAc_{major}$), 70.8 (C-2 Gal,), 72.4 (C-3 Gal, C-5 $GlcNAc_{major}$), 73.0 (C-5 Gal), 74.8 (C-5 $GlcNAc_{minor}$), 78.5 (C-4 $GlcNAc_{minor}$), 78.8 (C-4 $GlcNAc_{major}$), 90.6 (C-1 $GlcNAc_{major}$), 94.8 (C-1 $GlcNAc_{minor}$), 102.8 (C-1 Gal_{major}), 102.9 (C-1 Gal_{minor}), 174.4, 174.6 (2 × C(O)); *m/z* (ESI^+) 447 $[M+Na]^+$; HRMS (ESI^+) calculated for $C_{16}H_{28}N_2O_{11}Na$ $[M + Na]^+$: 447.1585, found 447.1585 $[M + Na]^+$.

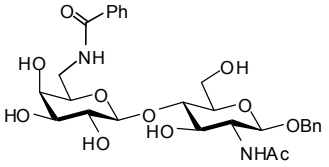
1.23 Benzyl 6-azido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 18a



Benzyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-D-galactosyl-(β1,4)-2-acetamido-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-glucopyranoside **18** (2.34 g, 3.2 mmol, 1.0 eq.) was dissolved in dry CH_3CN (20 ml), treated with $BF_3 \cdot Et_2O$ (480 µl, 3.8 mmol, 1.2 eq.) and stirred at rt for 3h. The reaction was quenched with sat. aqueous $NaHCO_3$ (20 ml) and the volatiles removed under reduced pressure. After addition of H_2O, the residue was extracted with DCM, the organic layers were

combined, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in MeOH (30 ml), treated with NaOMe (23 mg, 0.4 mmol, 0.1 eq.) and stirred at rt for 3h. The reaction was quenched with Amberlyst 15 (acidic form) until neutrality, filtered and dried under reduced pressure. The residue was purified *via* flash chromatography (EtOAc → EtOAc:MeOH, 80:20) to give disaccharide **18a** (1.29 g, 81%) as a white solid. $[\alpha]_D^{22}$ –27.0 (*c* = 0.60 mg/ml, MeOH); ν_{max} (KBr) 3407, 2927, 2108, 1655, 1557, 1374, 1309, 1063; ^{1}H-NMR (500 MHz, CD_3OD) δ ppm 1.97 (3H, s, $CH_3C(O)$), 3.42-3.45 (1H, m, H-5 GlcNAc), 3.51-3.58 (4H, m, H-2 Gal, H-5 Gal, H-6 Gal, H-6' Gal), 3.60-3.69 (2H, m, H-3 GlcNAc, H-4 GlcNAc), 3.72-3.74 (1H, m, H-3 Gal), 3.79-3.82 (2H, m, H-2 GlcNAc, H-4 Gal), 3.90 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.3 Hz, H-6 GlcNAc), 3.98 (1H, dd, $J_{5,6'}$ 2.5 Hz, $J_{6,6'}$ 12.3 Hz, H-6' GlcNAc), 4.43 (1H, d, $J_{1,2}$ 7.3 Hz, H-1 Gal), 4.51 (1H, d, $J_{1,2}$ 8.5 Hz, H-1 GlcNAc), 4.62 (1H, d, *J* 12.3 Hz, PhC*H*H), 4.90 (1H, d, *J* 12.3 Hz, PhCH*H*), 7.27-7.36 (5H, m, Ph); ^{13}C-NMR (125 MHz, CD_3OD) δ ppm 23.0 ($CH_3C(O)$), 52.3 (C-6 Gal), 56.7 (C-2 GlcNAc), 62.1 (C-6 GlcNAc), 70.4 (C-4 Gal), 71.7 ($PhCH_2$), 72.3 (C-2 Gal), 74.0 (C-3 GlcNAc), 74.6 (C-5 Gal), 74.8 (C-3 Gal), 76.6 (C-5 GlcNAc), 81.4 (C-4 GlcNAc), 101.8 ((C-1 GlcNAc), 105.2 (C-1 Gal), 128.7, 128.8, 129.4 (3 × Ph), 139.2 (ipso Ph), 173.6 (C(O)); *m/z* (ESI^+) 521 $[M+Na]^+$, 499 $[M+H]^+$; HRMS (ESI^+) calculated for $C_{21}H_{30}N_4O_{10}Na$ $[M + Na]^+$: 521.1854, found 521.1858 $[M + Na]^+$.

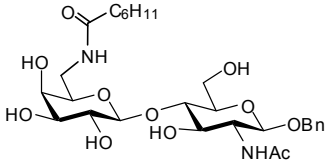
1.24 Benzyl 6-benzanamido-6-deoxy-D-galactopyranosyl-(β1,4)- 2-acetamido-2-deoxy-β-D-glucopyranoside 6b



A solution of azide **18a** (95 mg, 0.19 mmol, 1.0 eq.) and PPh_3 (76 mg, 0.29 mmol, 1.5 eq.) was dissolved in THF (1.4 ml) and H_2O (170 µl, 9 mmol, 49 eq.) and stirred overnight. The solvents were removed under reduced pressure, the resulting solid suspended in sat. aqueous $NaHCO_3$ (0.8 ml) and treated with a solution of benzoyl chloride (25 µl, 0.22 mmol, 1.2 eq.) in DCM (0.8 ml). The mixture was stirred for 1 h, the solvents were removed under reduced pressure and the residue purified *via* flash chromatography (DCM → DCM:MeOH, 80:20) to give acetamide **6b** (76 mg, 69%) as a white solid. $[\alpha]_D^{20}$ +19.6 (*c* = 0.25 mg/ml, MeOH); ν_{max} (KBr) 3426, 2873, 1646, 1552, 1372, 1064; ^{1}H-NMR (500 MHz, D_2O) δ ppm 1.74 (3H, s, $CH_3C(O)$), 3.41-3.61 (8H, m, H-2 GlcNAc, H-2 Gal, H-3 GlcNAc, H-3 Gal, H-4 GlcNAc, H-5 GlcNAc, H-6 Gal, H-6' Gal), 3.70 (1H, dd, $J_{5,6}$ 4.9 Hz, $J_{6,6'}$ 12.1 Hz, H-6 GlcNAc), 3.81 (1H, dd, $J_{5,6}$ 4.3 Hz, $J_{5,6'}$ 9.0 Hz, H-5 Gal), 3.85-3.88 (2H, m, H-4 Gal, H-6' GlcNAc), 5.35 (1H, d, $J_{1,2}$ 5.1 Hz, H-1 Gal), 5.37 (1H, d, $J_{1,2}$ 5.5 Hz, H-

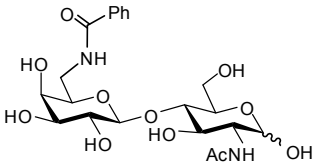
1 GlcNAc), 4.51 (1H, d, *J* 12.3 Hz, PhC*H*H), 4.74 (1H, d, *J* 12.3 Hz, PhCH*H*), 7.22-7.52 (7H, m, Ph), 7.64-7.66 (2H, m, Ph), 7.68-7.80 (1H, m, Ph); ^{13}C-NMR (125 MHz, D_2O) δ ppm 22.1 (CH_3C(O)), 40.5 (C-6 Gal), 54.9 (C-2 GlcNAc), 60.1 (C-6 GlcNAc), 69.0 (C-4 Gal), 70.8 (C-2 Gal), 71.5 ($PhCH_2$), 72.0, 72.5, 73.1 (C-3 GlcNAc, C-3 Gal, C-5 Gal), 74.7 (C-5 GlcNAc), 78.8 (C-4 GlcNAc), 99.9 (C-1 GlcNAc), 102.8 (C-1 Gal), 127.1, 128.5, 128.8, 129.2, 132.2, 132.7 (6 × Ph), 133.4, 136.7 (2 × ipso Ph), 171.4, 174.2 (2 × C(O)); *m/z* (ESI$^+$) 1175 (2 × M+Na$^+$), 599 (M+Na$^+$); HRMS (ESI$^+$) calculated for $C_{28}H_{36}N_2O_{11}Na$ [M + Na]$^+$: 599.2211, found 599.2210 [M + Na]$^+$.

1.25 Benzyl 6-cyclohexanamido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside 7b



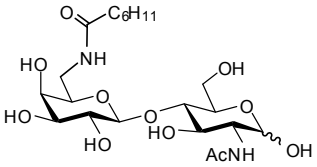
A solution of azide **18a** (110 mg, 0.22 mmol, 1.0 eq.) and PPh_3 (85 mg, 0.32 mmol, 1.5 eq.) was dissolved in THF (1.5 ml) and H_2O (200 µl, 11 mmol, 50 eq.) and stirred overnight. The solvents were removed under reduced pressure, the resulting solid suspended in sat. aqueous $NaHCO_3$ (1.0 ml) and treated with a solution of cyclohexanecarbonyl chloride (38 µl, 0.25 mmol, 1.1 eq.) in DCM (1.0 ml). The mixture was stirred for 30 min., the solvents were removed under reduced pressure and the residue purified *via* flash chromatography (^{n}BuOH:acetone:H_2O, 5:4:1) to give acetamide **7b** (92 mg, 72%) as a white solid. $[\alpha]_D^{22}$ +3.3 (*c* = 0.15 mg/ml, MeOH); ν_{max} (KBr) 3291, 2930, 1654, 1557, 1056; ^{1}H-NMR (500 MHz, D_2O) δ ppm 1.09-1.30 (4H, m, Cy), 1.55-1.78 (6H, m, Cy), 1.84 (3H, s, CH_3C(O)), 2.12-2.20 (1H, m, CHC(O)), 3.30-3.37 (2H, m, H-6 Gal, H-6' Gal), 3.44 (1H, dd $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.8 Hz, H-2 Gal), 3.46-3.50 (1H, m, H-5 GlcNAc), 3.50-3.60 (3H, m, H-3 GlcNAc, H-3 Gal, H-4 GlcNAc), 3.65-3.69 (2H, m, H-2 GlcNAc, H-5 Gal), 3.74 (1H, dd, $J_{5,6}$ 5.0 Hz, $J_{6,6'}$ 12.3 Hz, H-6 GlcNAc), 3.80 (1H, d, *J* 3.2 Hz, H-4 Gal), 3.91 (1H, dd, $J_{5,6'}$ 1.9 Hz, $J_{6,6'}$ 12.3 Hz, H-6' GlcNAc), 4.34 (1H, d, $J_{1,2}$ 7.6 Hz, H-1 Gal), 4.44 (1H, d, $J_{1,2}$ 8.5 Hz, H-1 GlcNAc), 4.59 (1H, d, *J* 12.3 Hz, PhC*H*H), 4.80 (1H, d, *J* 12.3 Hz, PhCH*H*), 7.28-7.39 (5H, m, Ph); ^{13}C-NMR (125 MHz, D_2O) δ ppm 22.1 (CH_3C(O)), 25.2 (Cy CH_2), 28.9 (Cy CH_2), 29.3 (Cy CH_2), 39.8 (C-6 Gal), 45.0 (*C*HC(O)), 54.9 (C-2 GlcNAc), 60.0 (C-6 GlcNAc), 68.9 (C-4 Gal), 70.8 (C-2 Gal), 71.4 ($PhCH_2$), 72.1, 72.5 (C-3 GlcNAc, C-3 Gal), 73.0 (C-5 Gal), 74.7 (C-5 GlcNAc), 78.5 (C-4 GlcNAc), 99.9 (C-1 GlcNAc), 102.9 (C-1 Gal), 128.4, 128.6, 128.7 (3 × Ph), 136.7 (ipso Ph), 174.2, 180.7 (2 × C(O)); *m/z* (ESI$^+$) 1187 (2 × M+Na$^+$), 605 (M+Na$^+$); HRMS (ESI$^+$) calculated for $C_{28}H_{42}N_2O_{11}Na$ [M + Na]$^+$: 605.2681, found 605.2667 [M + Na]$^+$.

1.26 6-Benzanamido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranose 6



A suspension of acetamide **6b** (15 mg, 0.026 mmol) and $Pd(OH)_2/C$ (20% w/w, 5 mg, 0.007 mmol, 0.27 eq.) in MeOH (0.6 ml) was stirred under an atmosphere of H_2 for overnight. After the addition of a fresh batch of $Pd(OH)_2/C$ (20% w/w, 4 mg, 0.006 mmol, 0.23 eq.), the mixture was stirred for a further 2 days, filtered through Celite and concentrated under reduced pressure. The residue was purified *via* flash chromatography (n-BuOH:acetone:H_2O, 5:4:1) to give the free reducing sugar **6** (12 mg, 95%) as a white solid. ν_{max} (KBr) 3406, 2937, 1635, 1542, 1458, 1312, 1068; ^{1}H-NMR (500 MHz, D_2O, 2:1 mixture of anomers) δ ppm 1.89 ($3H_{minor}$, s, $CH_3C(O)_{minor}$), 1.90 ($3H_{major}$, $CH_3C(O)_{major}$), 3.46-3.66 (6H + $1H_{minor}$, m, H-2 $GlcNAc_{minor}$, H-2 Gal, H-3 Gal, H-4 GlcNAc, H-5 GlcNAc, H-6 Gal, H-6' Gal), 3.68-3.73 ($1H_{major}$ + $1H_{minor}$, m, H-2 $GlcNAc_{major}$, H-6 $GlcNAc_{minor}$), 3.76-3.89 (4H + $1H_{major}$, m, H-3 GlcNAc, H-4 Gal, H-5 Gal, H-6 $GlcNAc_{major}$, H-6' GlcNAc), 4.37-4.39 (1H, m, H-1 Gal), 4.57-4.58 ($1H_{minor}$, d, $J_{1,2}$ 7.6 Hz, H-1 $GlcNAc_{minor}$), 5.08 ($1H_{major}$, d, $J_{1,2}$ 3.5 Hz, H-1 $GlcNAc_{major}$), 7.39-7.56 (2H, m, Ph), 7.52-7.56 (1H, m, Ph), 7.68-7.70 (2H, m, Ph); ^{13}C-NMR (125 MHz, D_2O) δ ppm 21.8 ($CH_{3\ major}$), 22.1 ($CH_{3\ minor}$), 40.5 (C-6 Gal), 53.8 (C-2 $GlcNAc_{major}$), 56.0 (C-2 $GlcNAc_{minor}$), 59.8 (C-6 $GlcNAc_{major}$), 60.0 (C-6 $GlcNAc_{minor}$), 68.8 (C-3 $GlcNAc_{minor}$), 69.0 (C-4 Gal), 70.1 (C-3 $GlcNAc_{major}$), 70.8 (C-2 Gal), 72.2 (C-5 $GlcNAc_{major}$), 72.5 (C-3 Gal), 73.0 (C-5 Gal), 74.7 (C-5 $GlcNAc_{minor}$), 78.8 (C-4 $GlcNAc_{minor}$), 79.1 (C-4 $GlcNAc_{major}$), 90.5 (C-1 $GlcNAc_{major}$), 95.9 (C-1 $GlcNAc_{minor}$), 103.0 (C-1 Gal), 127.1, 128.7, 132.2 (3 × Ph), 133.4 (ipso Ph), 171.4, 174.1, 174.5 (3 × C(O)); *m/z* (ESI^+) 995 (2 × $M+Na^+$), 509 ($M+Na^+$); HRMS (ESI^+) calculated for $C_{21}H_{30}N_2O_{11}Na$ $[M + Na]^+$: 509.1742, found 509.1742 $[M + Na]^+$.

1.27 6-Cyclohexanamido-6-deoxy-D-galactopyranosyl-(β1,4)-2-acetamido-2-deoxy-β-D-glucopyranose 7



A suspension of acetamide **7b** (35 mg, 0.06 mmol) and $Pd(OH)_2/C$ (20% w/w, 29 mg, 0.04 mmol, 0.7 eq.) in MeOH (1.5 ml) was stirred under an atmosphere of H_2 for 1 day. The mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified *via* flash chromatography (n-BuOH:acetone:H_2O, 5:4:1) to give the free reducing sugar **7** (25 mg, 85%) as a

white solid. ν_{max} (KBr) 3309, 2930, 1649, 1554, 1376, 1058; ^{1}H-NMR (500 MHz, D_2O, 2:1 mixture of anomers) δ ppm 1.09-12.9 (4H, m, Cy), 1.56-1.72 (6H, m, Cy), 1.96 (3H, s, $CH_3C(O)$), 2.14 (1H, dt, *J* 2.8 Hz, *J* 11.7 Hz, CH Cy), 3.30-3.39 (2H, m, H-6 Gal, H-6$^{'}$ Gal), 3.42-3.52 (1H + $1H_{minor}$, m, H-2 Gal, H-5 $GlcNAc_{minor}$), 3.56-3.88 (8H + $1H_{major}$, m, H-2 GlcNAc, H-3 GlcNAc, H-3 Gal, H-4 GlcNAc, H-4 Gal, H-5 $GlcNAc_{major}$, H-5 Gal, H-6 GlcNAc, H-6$^{'}$ GlcNAc,), 4.33 (1H, d, $J_{1,2}$ 7.9 Hz, H-1 Gal), 4.62 ($1H_{minor}$, d, $J_{1,2}$ 7.6 Hz, H-1 Gal_{minor}), 5.11 ($1H_{major}$, d, $J_{1,2}$ 2.5 Hz, H-1 Gal_{major}); ^{13}C-NMR (125 MHz, D_2O) δ ppm 21.8 (CH_3), 25.16, 25.20, 25.28, 29.0, 29.22 (5 × CH_2 Cy), 39.8 (C-6_b), 45.0 (CH Cy), 53.9 (C-2 $GlcNAc_{major}$), 56.1 (C-2 $GlcNAc_{minor}$), 59.8 (C-6 $GlcNAc_{major}$), 60.0 (C-6 $GlcNAc_{minor}$), 68.8 (C-3 $GlcNAc_{minor}$), 68.9 (C-4 Gal), 70.2 (C-3 $GlcNAc_{major}$), 70.8 (C-2 Gal), 72.2 (C-5 $GlcNAc_{major}$), 72.5 (C-3 Gal), 73.0 (C-5 Gal), 74.7 (C-5 $GlcNAc_{minor}$), 78.6 (C-4 $GlcNAc_{minor}$), 78.9 (C-4 $GlcNAc_{major}$), 90.5 (C-1 $GlcNAc_{major}$), 94.9 C-1 $GlcNAc_{minor}$), 102.9 (C-1 Gal), 174.5, 180.6 (2 × C(O)); *m/z* (ESI^+) 515 ($M+Na^+$), 493 ($M+H^+$); HRMS (ESI^+) calculated for $C_{21}H_{36}N_2O_{11}Na$ $[M + Na]^+$: 515.2211, found 515.2218 $[M + Na]^+$.

References

1. http://www.functionalglycomics.org/static/index.shtml.
2. Eklind, K.; Gustafsson, R.; Tiden, A. K.; Norberg, T.; Aberg, P. M. *J. Carbohydr. Chem.* **1996,** *15*, 1161.
3. Dahmen, J.; Frejd, T.; Magnusson, G.; Noori, G. *Carbohydr. Res.* **1982,** *111*, C1.
4. Dahmen, J.; Frejd, T.; Gronberg, G.; Lave, T.; Magnusson, G.; Noori, G. *Carbohydr. Res.* **1983,** *116*, 303.
5. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994,** *50*, 21.
6. Cheng, H.; Cao, X. H.; Xian, M.; Fang, L. Y.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D. X.; Wang, P. G. *J. Med. Chem.* **2005,** *48*, 645.
7. Cai, T. B.; Lu, D. N.; Tang, X. P.; Zhang, Y. L.; Landerholm, M.; Wang, P. G. *J. Org. Chem.* **2005,** *70*, 3518.
8. Misra, A. K.; Agnihotri, G. *Carbohydr. Res.* **2004,** *339*, 885.
9. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991,** *32*, 3353.
10. Burkart, M. D.; Vincent, S. P.; Duffels, A.; Murray, B. W.; Ley, S. V.; Wong, C. H. *Bioorg. Med. Chem.* **2000,** *8*, 1937.
11. Elchert, B.; Li, J.; Wang, J. H.; Hui, Y.; Rai, R.; Ptak, R.; Ward, P.; Takemoto, J. Y.; Bensaci, M.; Chang, C. W. T. *J. Org. Chem.* **2004,** *69*, 1513.
12. Garegg, P. J.; Samuelsson, B. *J. Chem. Soc. , Perkin Trans. 1* **1980,** 2866.
13. Ariatti, M.; Zemlicka, J. *J. Org. Chem.* **1981,** *46*, 5204.
14. Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1993,** *249*, 163.
15. Chang, C. W. T.; Hui, Y.; Elchert, B.; Wang, J. H.; Li, J.; Rai, R. *Org. Lett.* **2002,** *4*, 4603.
16. Mukhopadhyay, B.; Kartha, K. P. R.; Russell, D. A.; Field, R. A. *J. Org. Chem.* **2004,** *69*, 7758.
17. Saito, S.; Sumita, S.; Ichinose, K.; Kanda, Y. *Chem. Pharm. Bull.* **1993,** *41*, 90.
18. Murakami, T.; Matsuda, H.; Inadzuki, M.; Hirano, K.; Yoshikawa, M. *Chem. Pharm. Bull.* **1999,** *47*, 1717.
19. Medgyes, A.; Farkas, E.; Liptak, A.; Pozsgay, V. *Tetrahedron* **1997,** *53*, 4159.
20. Kim, T. Y.; Davidson, E. A. *J. Org. Chem.* **1963,** *28*, 2475.
21. Shinohara, Y.; Sota, H.; Kim, F.; Shimizu, M.; Gotoh, M.; Tosu, M.; Hasegawa, Y. *J. Biochem.* **1995,** *117*, 1076.
22. Leteux, C.; Childs, R. A.; Chai, W. G.; Stoll, M. S.; Kogelberg, H.; Feizi, T. *Glycobiology* **1998,** *8*, 227.
23. Bovin, N. V. *Glycoconj. J.* **1998,** *15*, 431.
24. Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C. *Proc. Natl. Acad. Sci. USA* **2004,** *101*, 17033.
25. Aspinall, G. O.; Przybylski, E.; Ritchie, R. G. S.; Chung, O. W. *Carbohydr. Res.* **1978**, *66*, 225.
26. Baggett, N.; Marsden, B. J. *Carbohydr. Res.* **1982**, *110*, 11.