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Mimics of the Sialyl Lewis X Tetrasaccharide. Replacement of the *N*-Acetylglucosamine Sugar with Simple C_2-Symmetric 1,2-Diols

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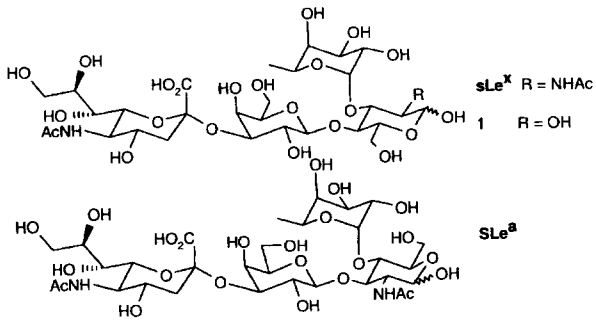
Abstract—Analogues of sialyl Lewis X have been synthesized that feature replacement of the *N*-acetylglucosamine residue with C_2-symmetric diols. The diols used contain different levels of torsional constraint and various functional groups. The cyclohexyl derived compound **27** was equipotent to sLex in vitro (IC_{50} 0.5 mM). Copyright © 1996 Elsevier Science Ltd

Introduction

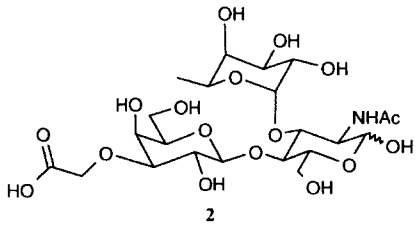
Cell adhesion processes of the immune system are currently of particular interest in that they have implications in the therapeutic areas of infection, inflammation and cancer. The selectins are three cell adhesion molecules that play important roles as mediators of inflammatory reactions in man.[1] L-, *P*- and *E*-selectin are unified structurally by the inclusion of lectin, EGF-like and complement binding-like domains,[2] and functionally by their ability to mediate cell binding through interactions between their lectin domains and cell surface carbohydrate ligands.[3] L-Selectin is found on leukocytes and is involved with the trafficking of lymphocytes to peripheral lymphoid tissues and with neutrophil-mediated inflammatory responses. Although the exact structure of the endothelial ligand(s) for L-selectin is unknown, researchers have identified endothelial ligands for L-selectin as sialylated, fucosylated and sulfated.[4] *P*-Selectin is implicated in acute inflammatory responses and is stored within the endothelial cells before being released onto the surface within minutes of exposure to inflammatory mediators.[1] *E*-Selectin is associated with chronic inflammation and requires de novo synthesis before being expressed on the endothelium 4–6 h after activation. Each of the selectins have been implicated in the recruitment of neutrophils to a site of tissue injury, although their relative importance is still unclear. The neutrophil adhesion to the endothelium is a multistep process, the initial stage of which involves the rolling of the neutrophil along the cell surface.[5] A constitutive glycoprotein on the neutrophil possesses a terminal tetrasaccharide unit sialyl Lewis X (sLe^x), this has been shown to bind to *E*- and *P*-selectin and to mediate the rolling phenomenon. Activation of neutrophils leads to firm attachment to the endothelium through an integrin dependent protein–protein interaction, followed by extravasation of the neutrophil through the blood vessel wall to the site of tissue injury. In vivo experiments have suggested that if the sLe^x/selectin interaction is prevented, by either antibodies to selectins or by the sLe^x tetrasaccharide, inflammation is substantially reduced.[6]

Reports have appeared in the literature that explore the key structural features required for recognition of oligosaccharides by *E*-selectin.[7–14] It was noted that *E*-selectin bound sLe^a,[7] the positional isomer of sLe^x, as well as or slightly better than sLe^x. Sle^x and sLe^a are related, to a first approximation, by rotational symmetry within the *N*-acetylglucosamine residue through 180°. In studies on various oligosaccharides, it was shown that both the fucose and the sialic acid residues of sLe^x were required for full recognition and that the sialyl α(2,6)-Le^x was not recognized at all.[8] It has been suggested that the 4- and the 6-hydroxyl groups of galactose are also important, although this has not been confirmed experimentally.[9] A detailed investigation showed that modification of the sialic acid moiety, such as trimming of the polyol chain or replacement of the *N*-acetyl group had no effect on *E*-selectin recognition.[8–10] In fact, the key structural feature of the sialic acid residue is the carboxylic acid moiety and this is borne out by the observation that the 3′-carboxymethyl substituted sLe^x analogue **2** has similar binding activity to sLe^x.[11] It has been shown that the *N*-acetylglucosamine residue of sLe^x can be replaced by glucose to give **1** with no apparent loss of activity.[12] In addition, activity is retained even if the glucose unit of this derivative is ring-opened by reduction.[9] These results suggest that the *N*-acetylglucosamine residue may primarily act as a two-carbon linker between the fucose and the galactose. However, Nelson demonstrated that in some modifications to the *N*-acetylglucosamine residue of sLe^a and sLe^x, signifi-

cant additional binding of sLe^x analogues to *E*-selectin could be achieved.[13] The most potent inhibitor reported was an analogue of sLe^a where *N*-acetylglucosamine is replaced with glucosamine, which was reported to be 36-fold more potent than the reducing tetrasaccharide sLe^x.[13]

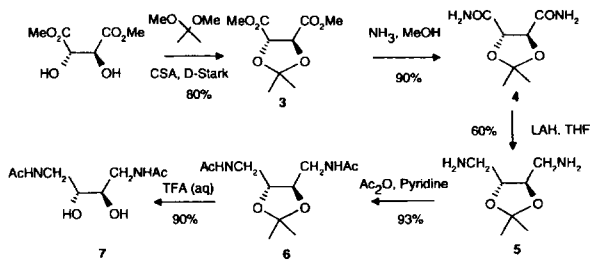


In this article, we report the results from our continuing investigation of sLe^x/*E*-selectin-mediated cell adhesion. The aim of our research is to rationally design and identify antagonists of the sLe^x/*E*-selectin interaction that have greater potency compared to sLe^x, reduced molecular weight and improved pharmacokinetic properties.[14] In addition to our studies, other researchers have reported the synthesis of sLe^x analogues.[15] Herein, we report a number of analogues of sLe^x which incorporate a carboxymethyl group as replacement for sialic acid and C_2-symmetric 1,2-diols as replacements for the *N*-acetylglucosamine residue. These analogues possess replacements for *N*-acetylglucosamine with different levels of torsional constraint and contain a variety of functional groups to probe for additional binding interactions to the *E*-selectin protein. In addition, the C_2-symmetric nature of our *N*-acetylglucosamine replacements investigate the intriguing symmetry element of sLe^x and sLe^a.



Results and Discussion

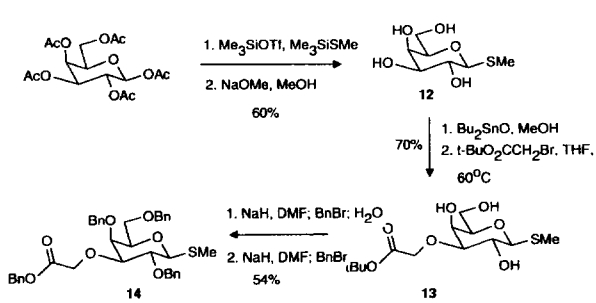
After extensive investigation, two synthetic routes were developed to provide access to analogues containing a variety of functional groups. Analogues containing nonaromatic 1,2-diol replacements for *N*-acetylglucosamine were generally synthesized according to Scheme 3. Aromatic 1,2-diol replacements required an alternative strategy which features a base-mediated phase-transfer glycosidation reaction and is illustrated in Scheme 4. The required symmetric 1,2-diols **8–11** and



Scheme 1.

32 were commercially available. However, the amide-containing diol **7** was synthesized from dimethyl tartrate according to Scheme 1. The 1,2-diol unit of dimethyl tartrate was protected as the isopropylidine acetal to give **3**. Subsequent treatment of **3** with methanolic ammonia furnished the amide **4**, which was reduced to the diamine **5**[16] using lithium aluminium hydride in 60% yield. N-Acetylation using acetic anhydride/pyridine gave the diamide **6** in 93% yield. Finally, removal of the isopropylidine group using aqueous trifluoroacetic acid gave the required diol **7**[17] in 90% yield.

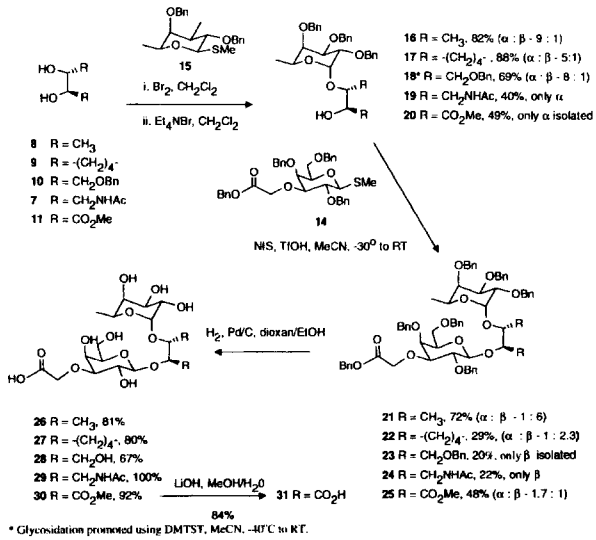
The fucose donor **15** (Scheme 3) was prepared from L-fucose on a large scale and in high yield using the method of Hasegawa.[12] A number of galactose donors were investigated which contain directing groups at the 2-position. Low yielding glycosidation, potentially due to concomitant orthoester formation,[18] led to the development of the galactose donor **14** which features both the carboxymethyl replacement for sialic acid and benzyl protecting groups. We anticipated that the use of acetonitrile as a participating solvent in our galactosidation reaction would provide the required β-selectivity.[19] The required galactose donor **14** was synthesized according to Scheme 2. Conversion of β-D-galactose pentaacetate to the thiomethyl glycoside **12** was achieved using trimethylsilyl triflate and thiomethyltrimethyl silane followed by Zemplen deacetylation in 60% yield. The protected carboxymethyl replacement for sialic acid was introduced using tin acetal chemistry to give **13** in 70% yield. Unfortunately, benzylbromoacetate gave low yields of alkylated product. Treatment of **13** with sodium hydride and benzyl bromide followed by hydrolytic work up alkylated the free hydroxyl groups but also, suprisingly, the acid which was converted into the required donor **14** in 54% yield for two steps.



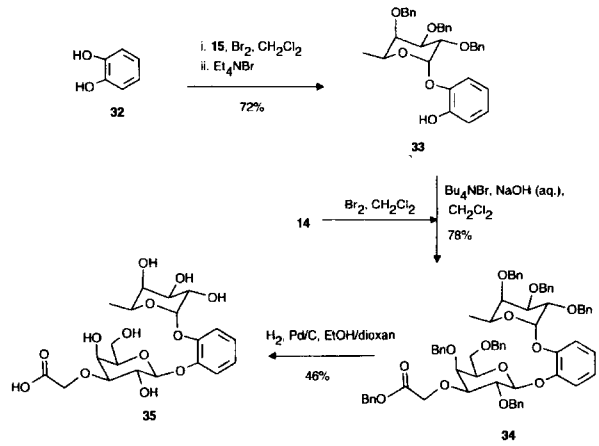
Scheme 2.

The nonaromatic diols **7–11** were coupled to the fucose donor **15** using a halide-mediated glycosidation protocol (Scheme 3).[20] The thioglycoside was converted to its bromide in situ using molecular bromine. In some cases complete α-selectivity was observed in the coupling reaction. The fucose-diol adducts **16–20** were coupled to the galactose donor **14** using N-iodosuccinimide/triflic acid (NIS/TfOH) in acetonitrile solvent under conditions of kinetic control.[19] In most cases good β-selectivity was observed, although the adduct **20** inexplicably furnished predominantly the α-product. The anomeric mixtures from both coupling reactions were separated either using flash column chromatography or HPLC. Removal of the benzyl protecting groups was effected by hydrogenation to furnish the sLe^x analogues **26–30** in high yield and **30** was converted to the acid **31** using lithium hydroxide in 84% yield.

Catechol **32** (Scheme 4) was coupled to the fucosyl donor **15** using the halide-mediated glycosidation procedure to give the fucose diol adduct **33**, only the predicted α-adduct was obtained. The reduced nucleophilicity of the adduct **33** precluded use of Koenigs Knorr-type glycosidation reactions. Phenolic glycosides have been formed using base-mediated phase-transfer methodology.[21] In these methods glycosyl bromides are treated with the phenol under phase-transfer conditions using sodium hydroxide as base. The galactose donor **14** was converted to the bromide in situ using molecular bromine. Subsequent reaction with the fucose diol adduct **33**, under phase-transfer conditions, furnished only the required β-galactoside **34** in 78% yield. We postulate that the anion formed from reaction of **33** with sodium hydroxide reacts rapidly with the α-bromide to give solely the observed β-product. Finally, removal of protecting groups using the same methods as previously described for the non-aromatic linkers furnished the required analogue **35**.



Scheme 3.



Scheme 4.

In vitro activity was determined in a scintillation proximity assay (SPA). The assay is based upon human *E*-selectin-*ZZ*, which is immobilized on SPA beads via an IgG linkage. Radiolabelled HL-60 cell membranes are then prepared and incubated with the *E*-selectin coated beads. The amount of bound ligand is determined by scintillation counting and the results are shown in Table 1. The cyclohexyl derivative **27** retains activity compared with sLe^x in the assay, despite significant structural simplification and reduced molecular weight. However, the less constrained butane diol analogue **26** and the alternatively constrained catechol analogue **35** showed very low levels of inhibition. It is apparent that the GlcNAc residue in sLe^x is only acting as a linker, but that the level of conformational constraint and the dihedral angle between the two glycosidic bonds contained in that linker is important. The derivatives **28–31** contain C_2-symmetric acyclic linkers with a variety of functional groups in order to probe for additional binding interactions with the protein. Our analogues **28–31** exhibited only very low levels of inhibition in the assay. Perhaps a functionalized system with a higher level of torsional constraint is required for higher levels of inhibition.

The sLe^x mimic **27** is structurally much simpler than sLe^x, has reduced carbohydrate character and a vastly reduced molecular weight. We are currently using these results to help us design further novel sLe^x

Table 1. Inhibition of *E*-selectin/sLe^x adhesion

Compound	M_r	IC_{50} (mM)
sLe^x	820	0.3
26	456	2.1
27	482	0.5
28	488	1.6
29	570	> 10.0
30	544	4.0
31	516	5.0
35	467	> 10.0

mimics and the results of these studies will be published shortly.

Experimental

Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were determined on an Optical Activity AA100 polarimeter. Analytical TLC was performed on Silica Gel 60-F_{254} glass-backed plates (E. Merck, Darmstadt). Silica column chromatography was performed using Silica Gel (9384, Merck Kieselgel) with flash elution. All reagents were used as supplied. ^{1}H NMR spectra were recorded at 250 (Bruker AM250) or 400 MHz (Bruker AM400) in deuterated solvents. COSY, HETCOR, DEPT135 and TOCSY experiments were performed on a representative number of compounds in each series. Mass spectra were recorded using VG Autospec (FAB +ve), Hewlett Packard HP Engine (TSP), VG Platform (ES) or VG Autospec QM (HRMS) spectrometers. IR spectra were obtained using a Nicolet 5SXC FTIR spectrometer.

Method for biological assay

E- and *P*-selectin-dependent adhesion of sLex^{+ve}-HL60 cell membranes was determined in a scintillation proximity assay (SPA). This method avoids washing steps that can lead to losses of weakly bound ligands. To prepare radiolabelled membranes, HL60 cells (5×10^5 cells/mL) were incubated for 16 h at 37 °C in 100 mL methionine-free RPMI medium containing 10% fetal calf serum, 2 mM glutamine and 400 μCi L-[methyl-^{3}H]methionine. The cell pellet was washed extensively and the cells were then homogenized on ice in the presence of protease inhibitors using a hand-held glass homogenizer. The resulting membranes were isolated by differential centrifugation and stored at −70 °C until used. *E*-selectin-*ZZ* and *P*-selectin-*ZZ* chimeras were obtained and expressed in insect cells as described previously[22] [The *ZZ* domain from protein A allows immobilization of the selectin by binding to IgG on the beads]. Adhesion assays were conducted in Microbeta 96-well plates. Each well contained 50 μg anti-rabbit IgG-coated SPA beads (Amersham International), 1 μg rabbit IgG (Sigma), 1.25 μg E- or *P*-selectin-*ZZ*, HL60 cell membranes (derived from 5×10^6 cells) plus the compounds under test at 0.5–10 mM in 100 μL of 'adhesion' buffer (HBSS, 25 mM HEPES, pH 7.2, 0.4 mM $CaCl_2$, 0.4 mM $MgCl_2$ and 0.5% bovine serum albumin). Following a 30 min incubation at 21 °C with shaking, the plates were read in a Wallac Microbeta scintillation counter to determine bound cell membranes. Nonspecific binding of HL60 cell membranes to beads in the absense of *E*- or *P*-selectin was negligible (<10% of total binding). Specific binding was inhibited completely in the presence of 10 mM-EDTA or antibodies to the respective selectin. Binding was also inhibited if the membranes were treated with neuraminidase prior to the incubations. Experiments were carried out in triplicate.

***N*-[5*R*-(Acetylamino-methyl)-2,2-dimethyl)-[1,3]dioxolan-4*R*-ylmethyl]-acetamide (6).** To a solution of the diamine **5**[16] (500 mg, 3.12 mmol) in pyridine (10 mL) at room temperature was added acetic anhydride (3 mL, 32 mmol). The solution was stirred overnight and then concentrated in vacuo to give an oil. Purification by flash column chromatography (SiO_2, 7% methanol in ethyl acetate) furnished **6** (720 mg, 93%) as a pale orange oil: $[\alpha]_D^{25}$ +4.0° (*c* 0.25, methanol); ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.00 (br, 2H, NH), 3.70 (m, 2H), 3.25 (m, 4H), 1.82 (s, 6H, COMe), 1.30 (s, 6H, CMe_2); FABHRMS *m/e* 245.1505 ($[M+H]^+$, $C_{11}H_{21}N_2O_4$ requires 245.1501).

***N* - (4 - Acetylamino - 2*R*,3*R* - dihydroxy - butyl) - acetamide (7).**[17] The diamide **6** (605 mg, 2.47 mmol) was treated with a solution of 30% aqueous TFA (15 mL) at room temperature. After 1 h the mixture was concentrated in vacuo and treated with diethyl ether (50 mL). The resulting suspension was filtered and washed with ether (25 mL) to give the title compound **7** (451 mg, 90%) as a white powder: mp 180 °C (dec.); $\alpha]_D^{25}$ −16.0° (*c* 0.5, methanol); ^{1}H NMR (250 MHz, DMSO-d_6): δ 7.86 (2H, brm, NH), 3.52 (brs, 2H), 3.40 (t, 2H, $J = 5.6$ Hz), 3.10–3.30 (m, 2H), 2.92–3.10 (m, 2H), 1.80 (s, 6H, COMe); FABHRMS *m/e* 205.1187 ($[M+H]^+$, $C_8H_{17}N_2O_4$ requires 205.1188).

(1-Deoxy-1-methylsulfanyl-β-D-galactopyranos-3-yl)-acetic acid *tert*-butyl ester (13). A suspension of **12**[12] (2.00 g, 9.51 mmol) and dibutyltin oxide (3.51 g, 14.10 mmol) in methanol (300 mL) was heated to 60 °C for 2 h. The clear solution was concentrated in vacuo to give a white foam. The residue was dissolved in anhydrous tetrahydrofuran (280 mL) and treated with tetrabutylammonium bromide (1.51 g, 4.68 mmol) and *tert*-butyl-bromoacetate (9 mL, 55 mmol). The pale yellow solution was heated to 60 °C overnight with the exclusion of light. The solvent was removed in vacuo to give an orange oil. Purification by flash column chromatography (SiO_2, ethyl acetate) furnished **13** (2.15 g, 70%) as a white solid: mp 139–142 °C; $[\alpha]_D^{25}$ +8° (*c* 0.5, methanol); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.03 (d, 1H, $J = 6.0$ Hz, OH), 4.56 (m, 1H), 4.42 (d, 1H, $J = 4.0$ Hz), 4.12 and 4.22 (AB, 2H, $J = 16.0$ Hz), 4.11 (d, 1H, $J = 10.0$ Hz, H1), 3.92 (m, 1H), 3.52 (dt, 1H, $J = 5.0$ and 10.0 Hz), 3.45 (m, 2H), 3.33 (t, 1H, $J = 6.0$ Hz), 3.22 (dd, 1H, $J = 3.0$ and 10.0 Hz), 2.07 (s, 3H), 1.42 (s, 9H); *m/e* (thermospray) 325 ($[M+H]^+$); Found: C, 47.76; H, 7.50; S, 9.79. $C_{13}H_{24}O_7S$ requires: C, 48.13; 7.46; S, 9.88.

(2,4,6-Tri-*O*-benzyl-1-deoxy-1-methylsulfanyl-β-D-galactopyranos-3-yl)-acetic acid benzyl ester (14). Tetraol **13** (6.9 g, 21.0 mmol) was dissolved in anhydrous DMF (70 mL) and cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 6.72 g, 168 mmol) was added and the mixture allowed to attain room temperature. After stirring at this temperature

for 2 h, benzyl bromide (21.55 g, 126 mmol) was added and the mixture was stirred overnight. The mixture was cooled to 0 °C and a solution of acetic acid (9.53 mL, 168 mmol) in dichloromethane (380 mL) added slowly. The mixture was diluted with dichloromethane (300 mL) and water (300 mL). The organic phase was washed with water (2 × 500 mL) and then concentrated in vacuo. The residue was applied to a column of silica gel and the excess alkylating agent removed by eluting ethyl acetate/cyclohexane (1:8), the acid was then obtained by eluting chloroform/methanol (4:1). The acid was subsequently redissolved in anhydrous DMF (100 mL) and treated with sodium hydride (60% dispersion in mineral oil, 891 mg, 22.0 mmol) and benzyl bromide (4.13 g, 24 mmol) as before. The reaction mixture was cooled to 0 °C and acetic acid (2.11 mL, 37 mmol) added. The mixture was diluted with ethyl acetate (500 mL) and washed with water (2 × 500 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (SiO_2, ethyl acetate:cyclohexane, 1:8) furnished **14** (6.46 g, 54%) as a colorless oil: $[\alpha]_D^{25}$ −17.3Û° (*c* 1.27, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.20–7.50 (20H, m), 5.15 and 5.20 (AB, 2H, J = 11.3 Hz), 4.96 and 4.63 (AB, 2H, J = 10.0 Hz), 4.90 and 4.75 (AB, 2H, J = 10.0 Hz), 4.05–4.50 (m, 6H), 3.82 (t, 1H, J = 8.8 Hz), 3.42–3.62 (m, 4H), 2.21 (s, 3H, SMe); *m/e* (thermospray) 646 ($[M+NH_4]^+$); Found: C, 70.66; H, 6.44; S, 4.92. $C_{37}H_{40}O_7S$ requires: C, 70.67; H, 6.41; S, 5.10.

General procedure for the fucosylation of diols 8–11 and 32

A solution of the fucose donor **15** in dichloromethane (ca. 30 mL/mmol) was cooled to 0 °C. Bromine (1.1 equiv) was added and the mixture stirred for 5 min, then cyclohexene was added to quench the excess bromine. Powdered 4 Å molecular sieves (ca. 3 g/mmol of substrate) and tetraethylammonium bromide (2–5 equiv) were added followed by the diol (2–5 equiv). The mixture was generally stirred for 2 h at 0 °C before warming to room temperature and stirring overnight. The reaction mixture was diluted with chloroform, filtered to remove the molecular sieves, and sequentially washed with water and brine. The mixture was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (SiO_2, chloroform/diethyl ether mixtures) or HPLC furnished the required α-fucose diol adducts.

(2*R*,3*R*)3-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranos-1-yl)-butan-2-ol (16). Treatment of the fucose donor **15** (1.6 g, 3.44 mmol) in dichloromethane (100 mL) with bromine (0.195 mL, 3.78 mmol), molecular sieves (10 g), tetraethylammonium bromide (3.62 g, 17.20 mmol) and (2*R*,3*R*)-(−)-2,3-butanediol (1.57 mL, 17.20 mmol), according to the general procedure outlined above, furnished the required product as a mixture of anomers (2.35 g, 82%, α:β 9:1). Purification by flash column chromatography (SiO_2, chloroform/ether gradient) furnished a sample of the desired α-anomer **16** (0.96 g, 55%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22–7.45 (m, 15H, aromatic H), 4.97 (d, 1H, J = 3.7 Hz, H_1), 4.60–5.10 (m, 7H), 4.08 (dd, 1H, J = 3.7 and 10.3 Hz, H_2), 4.00 (q, 1H, J = 6.6 Hz, H_5), 3.92 (dd, 1H, J = 2.5 and 10.3 Hz, H_3), 3.60–3.70 (m, 2H), 3.48 (m, 1H), 1.10–1.20 (m, 9H); IR ($CHBr_3$): ν_{max} 2976, 2932, 1453, 1045 cm^{-1}; FABHRMS *m/e* 524.3003 ($[M+NH_4]^+$, $C_{31}H_{42}NO_6$ requires 524.3012).

(1*R*,2*R*)-2-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranos-1-yl)-cyclohexanol (17). Treatment of the fucose donor **15** (4.0 g, 8.60 mmol) in dichloromethane (240 mL) with bromine (0.488 mL, 9.46 mmol), molecular sieves (24.0 g), tetraethylammonium bromide (3.61 g, 17.20 mmol) and (1*R*,2*R*)-(−)-1,2-cyclohexanediol (2 g, 17.20 mmol) according to the general procedure outlined above, furnished the required product as a mixture of anomers (4.03 g, 88%, α::β 5:1). A sample of the desired α-anomer **17** (1.33 g of the anomeric mixture gave 0.87 g of **17**) was obtained by HPLC (85% aqueous acetonitrile) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.23 (m, 15H, aromatic H), 4.98 and 4.60 (2 ds, 2H, J = 11.6 Hz), 4.98 (d, 1H, J = 3.9 Hz, H_1), 4.74 and 4.86 (ds, 2H), 4.67 and 4.81 (ds, 2H,), 4.07 (dd, 1H, J = 3.9 and 10.1 Hz, H_2), 4.07 (qd, 1H, J = 1.3 and 6.3 Hz, H_5), 3.94 (dd, 1H, J = 2.8 and 10.1 Hz, H_3), 3.69 (dd, 1H, J = 1.3 and 2.8 Hz, H_4), 3.60 (d, 1H, J = 1.8 Hz, OH), 3.40–3.44 (m, 1H), 3.20–3.24 (m, 1H), 1.98–2.05 (m, 2H), 1.66–1.74 (m, 2H), 1.17–1.35 (m, 4H), 1.14 (d, 3H, J = 1.8 Hz, H_6); HRMS *m/e* (electrospray$^+$) 533.2900 ($[M+H]^+$, $C_{33}H_{41}O_6$ requires 533.2903).

(2*R*,3*R*)-1,4-Bis-benzyloxy-3-(2,3,4-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-butan-2-ol (18). Treatment of the fucose donor **15** (1.54 g, 3.31 mmol) in dichloromethane (100 mL) with bromine (0.187 mL, 3.64 mmol), molecular sieves (10 g), tetraethylammonium bromide (1.39 g, 6.62 mmol) and (+)-1,4-di-O-benzyl-D-threitol (2.0 g, 6.61 mmol) according to the general procedure outlined above furnished the product as a mixture of anomers (1.65 g, 69%, α:β 8:1). Further purification by flash column chromatography (SiO_2, chloroform/ether gradient) furnished a sample of the desired α-anomer **18** (0.65 g, 29%) as a clear oil: ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.20–7.45 (m, 25H), 5.17 (d, 1H, J = 3.75 Hz, H_1), 4.96 (d, 1H, J = 11.3 Hz), 4.82 (d, 1H, J = 10.0 Hz), 4.60–4.77 (m, 4H), 4.37–4.55 (m, 4H), 3.84–4.10 (m, 5H), 3.45–3.75 (m, 5H), 3.07 (d, 1H, OH), 1.08 (d, 3H, J = 6.25 Hz); FABHRMS *m/e* 741.3407 ($[M+Na]^+$, $C_{45}H_{50}NaO_8$ requires 741.3403).

***N*-[4-Acetylamino-3*R*-hydroxy-2*R*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-butyl]-acetamide (19).** Treatment of the fucose donor **15** (158 mg, 0.34 mmol) in dichloromethane (5 mL) with bromine (0.02 mL, 0.37 mmol), molecular sieves (780 mg), tetraethylammonium bromide (350 mg, 1.66 mmol) and **7** (155 mg, 0.75 mmol), according to the general procedure outlined above, except that the diol **7** was added as a solution in DMF (5 mL) and the mixture was heated at 40 °C for 2 h. The procedure furnished the required

product **19** (80 mg, 40%) as a colorless oil and a single anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27–7.43 (m, 15H), 6.66 (dd, 1H, J = 3.5 and 6.5 Hz, NH), 6.11 (t, 1H, J = 5.5 Hz, NH), 4.97 and 4.94 (AB, 2H, J = 11.4 Hz), 4.89 (d, 1H, J = 3.7 Hz), 4.78 (d, 2H, J = 11.7 Hz), 4.70 and 4.64 (AB, 2H, J = 11.4 Hz), 4.13 (dd, 1H, J = 3.7 and 10.1 Hz, H_2), 4.07 (qd, 1H, J = 1.2 and 6.5 Hz, H_5), 3.98 (dd, 1H, J = 2.6 and 10.1 Hz, H_3), 3.74 (dd, 1H, J = 1.2 and 2.7 Hz, H_4), 3.62 (m, 1H), 3.50 (m, 3H), 3.10–3.20 (m, 2H), 1.97 and 1.57 (2s, 6H, COMes), 1.17 (d, 3H, J = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.56, 170.51, 138.30, 138.23, 138.81, 128.63–127.42 (aromatic signals), 99.51, 82.35, 79.49, 76.64, 76.43, 76.38, 75.01, 74.91, 74.77, 72.59, 71.62, 68.03, 41.55, 39.74, 23.11, 22.50, 16.71; FABHRMS *m/e* 621.3174 ($[M+H]^+$, $C_{35}H_{45}N_2O_8$ requires 621.3176).

(2*S*,3*S*)-2-Hydroxy-3-(3,4,5-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-succinic acid dimethyl ester (20). Treatment of the fucose donor **15** (4.0 g, 8.61 mmol) in dichloromethane (200 mL) with bromine (0.488 mL, 9.47 mmol), molecular sieves (26 g), tetraethylammonium bromide (3.62 g, 17.20 mmol) and dimethyl-D-tartrate (3.07 g, 17.20 mmol), according to the general procedure outlined above, furnished the required product **20** (2.49 g, 49%) as a single anomer: $[\alpha]_D^{25}$ −74° (*c* 1.18, chloroform); ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.20–7.45 (m, 15H), 5.23 (d, 1H, J = 3 Hz, H_1), 4.85–5.00 (m, 3H), 4.60–4.75 (m, 5H), 4.10 (dd, 1H, J = 3.0 and 11.0 Hz), 3.81 (dd, 1H, J = 3.0 and 11.0 Hz), 3.80 (s, 3H), 3.71 (s, 3H), 3.50–3.61 (2H, m), 3.46 (d, 1H, J = 10 Hz), 1.09 (d, 3H, J = 6.3 Hz); HRMS *m/e* (CI, NH_3) 612.2805 ($[M+NH_4]^+$, $C_{33}H_{42}NO_{10}$ requires 612.2809).

2-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranos-1-yl)-phenol (33). Treatment of the fucose donor **15** (600 g, 1.3 mmol) in dichloromethane (35 mL) with bromine (0.074 mL, 1.42 mmol), molecular sieves (3.9 g), tetraethylammonium bromide (0.545 g, 2.58 mmol) and catechol (286 g, 2.58 mmol) according to the general procedure outlined above furnished the required product **33** (490 mg, 72%) as a colorless oil and a single anomer: $[\alpha]_D^{25}$ −86° (*c* 1.0, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.47 (m, 15H), 7.05 (dd, 1H, J = 1.6 and 7.7 Hz), 7.02 (m, 1H), 6.96 (dd, 1H, J = 1.7 and 8.1 Hz), 6.78 (m, 1H), 5.02 and 4.70 (ds, 2H, J = 11.6 Hz), 4.94 and 4.70 (ds, 2H, J = 11.5 Hz), 4.85 and 4.83 (ds, 2H, J = 11.0 Hz), 5.00 (m, 1H), 4.27 (qd, 1H, J = 1.3 and 6.3 Hz, H_5), 4.17 (m, 2H), 3.78 (m, 1H, H_4), 1.24 (d, 3H, J = 6.5 Hz, H_6); FABHRMS *m/e* 525.2278 ($[M-H]^-$, $C_{33}H_{33}O_6$ requires 525.2277).

General procedure for the galactosylation of adducts 16–20

A solution of the acceptor and the donor **14** (1.2 equiv) were stirred with 4 Å molecular sieves in anhydrous acetonitrile (20 mL/mmol acceptor). After 20 min the mixture was cooled to −30 °C and NIS (1.2 equiv) followed by triflic acid (0.12 equiv) was added (for the preparation of **23**, DMTST was used as promotor). Stirring was continued at −30 °C for 1 h, then the mixture was allowed to warm to room temperature and left at this temperature overnight. The mixture was diluted with ethyl acetate, filtered to remove the molecular sieves and washed sequentially with saturated aqueous sodium hydrogen carbonate, water and brine. The mixture was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (SiO_2, diethyl ether/cyclohexane/methanol mixtures) furnished the required β-glycosides.

{2,4,6-Tri-*O*-benzyl-1-*O*-[1*R*-methyl-2*R*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-propyl]-β-D-galactopyranos-3-yl}-acetic acid benzyl ester (21). The acceptor **16** (50.6 mg, 0.10 mmol) and the galactose donor **14** (75.4 mg, 0.12 mmol) in acetonitrile (2 mL) were treated with molecular sieves (440 mg), NIS (27 mg, 0.12 mmol) and triflic acid (0.001 mL, 0.012 mmol), according to the method outlined above, to give a mixture of glycosides (79 mg, 72%, α:β 1:6). Purification by flash column chromatography (SiO_2, diethyl ether:cyclohexane:methanol 10:30:0.5) furnished the required β-anomer **21** (61 mg, 57%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15–7.40 (m, 35H), 5.12 and 5.17 (AB, 2H, J = 12 Hz), 4.22–4.96 (m, 17H), 4.08 (d, 1H, J = 2.0 Hz), 3.75–4.05 (m, 4H), 3.70 (dd, 1H, J = 10.0 and 11.0 Hz), 3.40–3.59 (m, 5H), 1.15 (t, 6H, J = 7.0 Hz), 1.05 (d, 3H, J = 6.0 Hz); *m/e* (electrospray +, % relative intensity) 1087.5 ($[M+H]^+$, 100%), 1088.5 (76%), 1089.5 (25%); Predict 1087.5 ($[M+H]^+$, 100%), 1088.5 (76%), 1089.5 (32%).

{2,4,6-Tri-*O*-benzyl-1-*O*-[2*R*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranos-1-l)-cyclohex-1*R*-yl]-β-D-galactopyranos-3-yl}-acetic acid benzyl ester (22). The acceptor **17** (813 mg, 1.53 mmol) and the galactose donor **14** (1.15 g, 1.84 mmol) in acetonitrile (30 mL) were treated with molecular sieves (7 g), NIS (412 mg, 1.84 mmol) and triflic acid (0.016 mL, 0.18 mmol) according to the method outlined above to give a mixture of glycosides (493 mg, 29%, α:β 1:2.3). Purification by flash column chromatography (SiO_2, diethyl ether:cyclohexane:methanol 10:50:0.25) furnished the required β-anomer **22** (340 mg, 20%) as a clear oil: $[\alpha]_D^{25}$ −52.3° (*c* 0.88, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15–7.40 (m, 35H), 5.11 and 5.16 (AB, 2H, J = 17.0 Hz), 4.97 and 4.94 (AB, 2H, J = 7.0 Hz), 4.86 (d, 1H, J = 2.5 Hz, H_1-fuc), 4.20–4.74 (series m, 13H), 4.10 (d, 1H, J = 1.5 Hz), 3.91–3.94 (tight m, 2H), 3.52–3.72 (m, 5H), 3.47 (dd, 1H, J = 5.0 and 7.0 Hz), 3.43 (dd, 1H, J = 3.0 and 10.0 Hz), 3.39 (s, 1H), 1.90–2.07 (2H, m), 1.14–1.72 (m, 8H), 1.07 (d, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.61, 139.34, 139.26, 139.00, 138.71, 138.10, 135.44, 128.56–127.12 (aromatic signals), 101.16, 94.73, 83.61, 80.48–72.69 (14 signals), 69.75, 68.66, 66.50, 66.03, 29.93, 29.23, 23.33, 16.59; *m/e* (electrospray +, % relative intensity) 1135.7 ($[M+Na]^+$, 100%), predict 1135.5 ($[M+Na]^+$, 100%).

{2,4,6 - Tri - *O*- benzyl - 1 - *O* - [3 - benzyloxy - 1*R* - (benzyloxy-methyl)-2*R*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-propyl]-β-D-galactopyranos-3-yl}-acetic acid benzyl ester (23). The acceptor **18** (600 mg, 0.82 mmol) and the galactose donor **14** (430 mg, 0.684 mmol) in acetonitrile (12 mL) were treated with molecular sieves (2.4 g) and DMTST (3.42 mmol) according to the procedure outlined above. Purification by flash column chromatography (SiO_2, diethyl ether:cyclohexane:methanol 10:30:0.5) furnished the required β-anomer **23** (174 mg, 20%) as a clear oil: $[\alpha]_D^{25}$ −47.7° (*c* 0.88, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14–7.42 (m, 42H), 5.10–5.20 (m, 2H), 4.97 (AB, 2H, *J* = 8.0 Hz), 4.70–3.30 (m, 31H), 1.02 (d, 3H, *J* = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.63, 139.26, 139.11, 139.05, 138.95, 138.48, 138.24, 138.22, 135.51, 128.57–127.14 (aromatic signals) 102.71, 97.00, 83.53, 80.10–66.43 (25 signals), 16.60; *m/e* (electrospray +, % relative intensity) 1321.4 ($[M+Na]^+$, 100%), 1322.4 (88%), 1323.4 (40%); predict 1321.6 ($[M+Na]^+$, 100%), 1322.6 (90%), 1323.6 (45%).

{1-*O*-[3-Acetylamino-*R*-(acetylamino-methyl)-2*R*-(2,3,4-tri-*O*-benzyl-α-fucopyrano-1-yl)propyl]-2,4,6-tri-*O*-benzyl-β-D-galactopyranos-3-yl}-acetic acid benzyl ester (24). The acceptor **19** (60 mg, 0.097 mmol) and the galactose donor **14** (121 mg, 0.194 mmol) in acetonitrile (3 mL) were treated with molecular sieves (360 mg), NIS (44 mg, 0.194 mmol) and triflic acid (0.002 mL, 0.02 mmol) according to the method outlined above. Purification by flash column chromatography (SiO_2, diethyl ether/methanol-gradient elution) furnished the required β-anomer **24** (22 mg, 22%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–7.50 (m, 35H), 5.18 (1H, d, *J* = 4.0 Hz, H_1-fuc), 3.10–5.00 (series m, 32H), 1.54 and 1.49 (2s, 6H, 2AcMes), 1.09 (d, 3H, *J* = 6H, Me-fuc); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.36, 170.31, 169.93, 138.57, 138.15, 137.65, 135.28, 127.35–128.63 (aromatic signals), 104.03, 99.62, 83.98, 66.69–79.11 (18 signals), 39.33, 38.89, 22.71, 22.51, 16.66; *m/e* (CI, NH_3) 1202 ($[M+H]^+$).

(2*S*,3*S*)2-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranos-1-yl)-3-[2,4,6-tri-*O*-benzyl-3-*O*-(benzyloxycarbonyl-methyl)-β-D-galactopyranos-1-yl]-succinic acid dimethyl ester (25). The acceptor **20** (1.29 g, 2.17 mmol) and the galactose donor **14** (1.65 g, 2.6 mmol) in acetonitrile (80 mL) were treated with molecular sieves (11 g), NIS (590 mg, 2.6 mmol) and triflic acid (0.023 mL, 0.26 mmol) according to the method outlined above to give a mixture of glycosides (1.22 g, 48%, α:β 1.7:1). Purification of a portion of this mixture by HPLC furnished a sample of the required product **25** (63.2 mg) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17–7.41 (m, 35H), 5.17 and 5.12 (AB, 2H, *J* = 12.2 Hz), 4.91 and 4.56 (AB, 2H, *J* = 11.7 Hz), 4.88 (d, 1H, 11.8 Hz), 4.78 and 4.67 (AB, 2H, *J* = 11.7 Hz), 4.72 (d, 1H, J = 11.8 Hz), 4.65 and 4.57 (AB, 2H, *J* = 12.4 Hz), 4.51 (d, 2H, *J* = 11.8 Hz), 5.07 (d, 1H, *J* = 3.7, H_1-fuc), 4.97 (d, 1H, 3.7 Hz, H_1-gal), 4.65 (d, 1H, *J* = 6.2 Hz), 4.47 (d, 1H, *J* = 6.2 Hz), 4.42 and 4.39 (AB, 2H, *J* = 12.1 Hz), 4.36 (d, 1H, *J* = 16.5 Hz), 4.30 (d, 1H, *J* = 16.5 Hz), 4.18 (ddd, 1H, *J* = 1.4, 5.9, 7.0 Hz, H_5-gal), 4.14 (dd, 1H, *J* = 1.4 and 2.8 Hz, H_4-gal), 4.07 (qd, 1H, *J* = 0.9 and 6.3 Hz, H_5-fuc), 4.04 (dd, 1H, *J* = 3.7 and 10.2 Hz, H_2-fuc), 3.99 (dd, 1H, *J* = 3.7 and 10.3 Hz, H_2-gal), 3.89 (dd, 1H, *J* = 2.8 and 10.2 Hz, H_3-fuc), 3.87 (dd, 1H, *J* = 2.8 and 10.3 Hz, H_3-gal), 3.59 (s, 3H, OMe), 3.52 (s, 3H, OMe), 3.43 (dd, 1H, *J* = 7.0 and 9.4 Hz), 3.38 (dd, 1H, *J* = 0.9 and 2.8 Hz, H_4-fuc), 3.32 (dd, 1H, *J* = 5.9 and 9.4 Hz), 1.04 (d, 3H, *J* = 6.3 Hz); *m/e* (electrospray +, % relative intensity) 1197.6 ($[M+Na]^+$, 100%), 1198.6 (82%), 1199.6 (36%); predict 1197.5 ($[M+Na]^+$, 100%), 1198.5 (80%), 1199.5 (32%).

{2,4,6-Tri-*O*-benzyl-1-*O*-[2-(3,4,5-tri-*O*-benzyl-α-L-fucopyranos-1-yl)-phenyl]-β-D-galactopyranos-3-yl}-acetic acid benzyl ester (34). The donor **14** (0.86 g, 1.37 mmol) dissolved in dichloromethane (3.5 mL) was cooled to 0 °C and treated with bromine (0.077 mL, 1.51 mmol). The mixture was stirred for 5 min and then the excess bromine was quenched by the addition of cyclohexene. Tetrabutylammonium bromide (442 mg, 1.37 mmol) was added followed by the acceptor **33** (360mg, 0.69 mmol) and aqueous sodium hydroxide solution (1.0 M, 1.37 mL, 1.37 mmol). The mixture was generally stirred for 2 h at 0 °C before warming to room temperature and stirring overnight. The reaction mixture was diluted with chloroform and sequentially washed with water and brine. The mixture was dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (SiO_2, diethyl ether:cyclohexane:methanol 10:25:1) furnished the required β-galactoside **34** (591 mg, 78%) as a clear oil: $[\alpha]_D^{25}$ −93.2° (*c* 1.55, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.80–7.40 (m, 39H), 5.49 (d, 1H, *J* = 3.7 Hz, H_1-fuc), 5.20 (d, 1H, *J* = 10.7 Hz), 5.13 and 5.16 (AB, 2H, *J* = 12.0 Hz), 5.02 (d, 1H, *J* = 11.2 Hz), 4.97 (d, 1H, *J* = 7.8 Hz, H_1-gal), 4.89 (d, 1H, *J* = 11.2 Hz), 4.68 (s, 2H), 4.61–4.70 (m, 1H), 4.47–4.55 (m, 4H), 4.30–4.41 (m, 4H), 4.06–4.15 (m, 3H), 3.56–3.85 (m, 5H), 3.51 (dd, 1H, *J* = 2.4 and 9.6 Hz), 3.31 (d, 1H, *J* = 2.4 Hz), 1.03 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.80, 147.83, 147.41, 139.07, 138.88, 138.68, 138.65, 138.61, 138.12, 135.40, 128.62–127.13 (aromatic signals), 122.59, 117.34, 116.43, 102.34, 97.51, 83.02, 79.63, 79.09, 77.55, 76.55, 76.15, 75.35, 74.86, 74.31, 73.63, 72.93, 69.79, 67.11, 66.53, 26.95, 16.57; *m/e* (electrospray +, % relative intensity) 1129.3 ($[M+Na]^+$, 100%), 1130.3 (78%), 1131.3 (36%); predict 1129.5 ($[M+Na]^+$, 100%), 1130.5 (78%), 1131.5 (32%).

General procedure for the hydrogenation of compounds 21–25 and 34

A solution of the perbenzylated compound in ethanol/dioxan (5 mL/mmol) was strirred over Pd/C (10%, 1:1 by weight) under an atmosphere of hydrogen. The reaction was continued until TLC indicated the formation of one product. The catalyst was removed by filtration through a pad of Celite and the deprotected

compounds purified by flash column chromatography (SiO_2, propan-2-ol:ethyl acetate:water 3:5:2).

[1-*O*-(2*R*-α-L-Fucopyranos-1-yl-1*R*-methyl-propyl)-β-D-galactopyranos-3-yl]-acetic acid (26). Treatment of compound **21** (44 mg, 0.04 mmol) as a solution in ethanol (15 mL) with Pd/C (10%, 50 mg) according to the above procedure furnished **26** (15 mg, 81%) as a clear oil: $[\alpha]_D^{25}$ +56.7° (*c* 0.3, water); ^{1}H NMR (400 MHz, D_2O): δ 4.97 (d, 1H, J = 4.0 Hz, H_1-fuc), 4.48 (d, 1H, 7.0 Hz, H_1-gal), 4.33 (dd, 1H, J = 13.0 and 7.0 Hz), 4.06 (s, 2H), 3.95–4.03 (m, 1H), 3.89 (dd, 1H, J = 3.0 and 10.0 Hz), 3.50–3.84 (m, 7H), 3.45 (dd, 1H, J = 3.0 and 10.0 Hz), 1.14–1.24 (m, 9H); FABHRMS *m/e* 455.1763 ([M-H]$^-$, $C_{18}H_{31}O_{13}$ requires 455.1764). Found: C, 40.95. H, 6.95; $C_{18}H_{32}O_{13}$·4 H_2O requires: C, 40.91; H, 7.63.

[1-*O*-(2*R*-α-L-Fucopyranos-1-yl-cyclohex-1*R*-yl)-β-D-galactopyranos-3-yl]-acetic acid (27). Treatment of compound **22** (340 mg, 0.305 mmol) as a solution in ethanol:dioxan (2:1, 15 mL) with Pd/C (10%, 340 mg), according to the above procedure, furnished **27** (117 mg, 80%) as a white solid; mp 253–257 °C; $[\alpha]_D^{25}$ −82.0° (*c* 0.5, water); ^{1}H NMR (400 MHz, D_2O): δ 4.98 (d, 1H, J = 4.0 Hz, H_1-fuc), 4.62 (qd, 1H, J = 1.0 and 6.6 Hz, H_5-fuc), 4.51 (d, 1H, J = 7.8 Hz, H_1-gal), 4.07 (dd, 1H, J = 1.0 and 3.0 Hz, H_4-fuc), 4.06 (s, 2H), 3.91 (dd, 1H, J = 3.4 and 10.4 Hz), 3.77 (m, 1H), 3.74 (m, 1H), 2.02–2.16 (m, 2H), 1.65–1.75 (m, 2H), 1.29–1.34 (m, 4H) and 1.19 (d, 3H, J = 6.6 Hz); ^{13}C NMR (100 MHz, D_2O): δ 178.47, 99.79, 95.50, 82.30, 78.21, 77.13, 74.57, 72.08, 72.01, 69.95, 69.65, 68.51, 68.47, 68.01, 66.50, 66.47, 65.42, 69.39, 61.49, 29.63, 29.12, 23.12, 15.31; FABHRMS *m/e* 481.1919 ([M-H]$^-$, $C_{20}H_{33}O_{13}$ requires 481.1921).

[1-*O*-(2*R*-α-L-Fucopyranos-1-yl-3-hydroxy-1*R*-hydroxymethyl-propyl)-β-D-galactopyranos-3-yl]-acetic acid (28). Treatment of compound **23** (199 mg, 0.153 mmol) as a solution in ethanol:dioxan (2:1, 6 mL) with Pd/C (10%, 200 mg) according to the above procedure furnished **28** (50.4 mg, 67%) as a white solid: mp 244–246 °C; $[\alpha]_D^{25}$ −65.6° (*c* 0.32, water); ^{1}H NMR (400 MHz, D_2O): δ 5.06 (d, 4.2 Hz, 1H, H_1-fuc), 4.58 (d, 1H, H_1-gal), 4.28 (dd, 1H, J = 7 and 14 Hz), 4.04–4.12 (m, 4H), 3.84–3.96 (m, 3H), 3.50–3.84 (m, 7H), 3.65 (dd, 1H, J = 8 and 10 Hz), 3.64–3.68 (m, 1H), 3.49 (dd, 1H, J = 3 and 10 Hz), 1.22 (d, 3H, J = 5 Hz); ^{13}C NMR (100 MHz, D_2O): δ 178.51, 102.92, 98.31, 82.31, 79.51, 77.52, 74.95, 72.04, 70.13, 69.70, 68.67, 68.38, 67.27, 65.53, 61.34, 61.02, 59.91, 15.51; FABHRMS *m/e* 487.1658 ([M−H]$^-$, $C_{18}H_{31}O_{15}$ requires 487.1663).

{1-*O*-[3-Acetylamino-1*R*-(acetylamino-methyl)-2*R*-α-L-fucopyranos-1-yl]-β-D-galactpyranos 3-yl}-acetic acid (29). Treatment of compound **24** (35 mg, 0.029 mmol) as a solution in ethanol (3 mL) with Pd/C (10%, 50 mg), according to the above procedure, furnished **29** (17 mg, quantitative) as a yellow solid: ^{1}H NMR (400 MHz, D_2O): δ 5.05 (d, 1H, J = 4.4 Hz, H_1-fuc), 4.50 (d, 1H, J = 7.8 Hz, H_1-gal), 3.35–4.20 (ms, 27H), 2.04 (s, 3H), 2.01 (s, 3H), 1.21 (d, 3H, J = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.41, 174.43, 174.31, 103.54, 98.55, 82.01, 78.03, 76.51, 75.70, 74.91, 71.83, 69.84, 69.48, 68.48, 68.07, 67.35, 65.41, 61.30, 40.15, 38.40, 23.34, 15.41; *m/e* (electrospray) 571 ([M+H]$^+$).

(2*S*,3*S*)-2-(3-*O*-Carboxymethyl-β-D-galactopyranos-1-yl)-3-α-L-fucopyranos-1-yl-succinic acid dimethyl ester (30). Treatment of compound **25** (260 mg, 0.22 mmol) as a solution in ethanol:dioxan (2:1, 10 mL) with Pd/C (10%, 260 mg), according to the above procedure, furnished **30** (110 mg, 92%) as a white solid: mp 180 °C (dec); $[\alpha]_D^{25}$ −85.4° (*c* 0.5, water); ^{1}H NMR (400 MHz, D_2O): δ 5.19 (d, 1H, J = 2.4 Hz), 5.02 (d, 1H, J = 3.6 Hz, H_1-fuc), 4.93 (d, 1H, J = 2.4 Hz), 4.49 (d, 1H, J = 7.7 Hz, H_1-gal), 4.11 (dd, 1H, J = 1.1 and 2.9 Hz), 3.84 (s, 3H), 3.83 (s, 3H), 3.70–3.80 (m, 6H), 3.65 (dd, 1H, J = 7.7 and 9.7 Hz), 3.59 (ddd, 1H, J = 1.1, 5.1, 6.8 Hz), 3.50 (dd, 1H, J = 3.2 and 9.7 Hz), 1.02 (d, 3H, J = 6.5 Hz); ^{13}C NMR (100 MHz, D_2O): δ 176.48, 171.31, 171.06, 102.22, 98.59, 82.52, 76.69, 75.69, 75.57, 72.22, 70.36, 69.95, 68.50, 68.26, 68.04, 66.06, 61.66, 53.84, 15.90; FABHRMS *m/e* 567.1544 ([M+Na]$^+$, $C_{20}H_{32}NaO_{17}$ requires 567.1537). Found: C, 40.38; H, 5.94. $C_{20}H_{31}O_{17}$·1.6H_2O·Na requires: C, 40.35; H, 5.79.

[1-*O*-(2-α-L-Fucopyranos-1-yl-phenyl)-β-D-galactopyranos-3-yl]-acetic acid (35). Treatment of compound **34** (535 mg, 0.48 mmol) as a solution in ethanol:dioxan (2:1, 12 mL) with Pd/C (10%, 540 mg), according to the above procedure, furnished **35** (106 mg, 46%) as a white solid: $[\alpha]_D^{25}$ −98.0° (*c* 0.25, water); ^{1}H NMR (400 MHz, D_2O): δ 7.10–7.30 (m, 4H), 5.63 (d, 1H, J = 3.8 Hz, H_1-fuc), 5.07 (d, 1H, J = 8.0 Hz, H_1-gal), 4.65–4.80 (m, 1H), 4.38 (qd, 1H, J = 1.0 and 6.6 Hz, H_5-fuc), 4.05–4.10 (m, 3H), 3.92–3.99 (m, 2H), 3.89 (d, 1H, J = 3.2 Hz), 3.72–3.83 (m, 3H), 3.59 (dd, 1H, J = 3.2 and 9.8 Hz), 1.17 (d, 3H, J = 6.6 Hz); ^{13}C NMR (100 MHz, D_2O): δ 178.38, 147.47, 145.75, 124.36, 124.11, 119.66, 118.09, 101.50, 98.70, 82.04, 75.24, 71.89, 69.67, 69.51, 68.62, 68.13, 67.96, 65.34, 60.84, 15.27; FABHRMS *m/e* 475.1450 ([M−H]$^-$, $C_{20}H_{27}O_{13}$ requires 475.1451). Found: C, 45.08; H, 5.66. $C_{20}H_{27}O_{13}$·Na·1.9 H_2O requires: C, 45.10; H, 5.83.

(2*S*,*S*)-2-(3-*O*-Carboxymethyl-β-D-galactopyranos-1-yl)-3-α-L-fucopyranos-1-yl-succinic acid (31). The diester **30** (89 mg, 0.16 mmol) in methanol:water 5:1, 2.2 mL) was treated with an aqueous solution of lithium hydroxide (5.0 M, 0.65 mL). The mixture was stirred at room temperature until TLC indicated the complete conversion of the starting material. The alkaline solution was neutralized with Dowex 50W acidic ion-exchange resin, filtered and freeze dried to give the diacid **31** (70 mg, 84%) as a white solid: mp 165 °C (dec); $[\alpha]_D^{25}$ −68.0° (*c* 0.2, water); ^{1}H NMR (400 MHz, D_2O): δ 5.10 (d, 1H, J = 2.5 Hz), 5.03 (d, 1H, J = 3.5 Hz, H_1-fuc), 4.86 (d, 1H, J = 2.5 Hz), 4.51 (d, 1H, J = 7.8 Hz, H_1-gal), 4.32 and 4.37 (AB, 2H,

J = 16.6 and 20.5 Hz), 4.14 (d, 1H, J = 3.2 Hz, H_4-gal), 3.93 (q, 1H, J = 6.5 Hz, H_5-fuc), 3.86 (dd, 1H, J = 3.1 and 10.5 Hz, H_3-fuc), 3.66–3.82 (m, 5H), 3.61 (dd, 1H, J = 4.3 and 6.8 Hz), 3.55 (dd, 1H, J = 3.2 and 9.7 Hz), 1.18 (d, 3H, J = 6.5 Hz); HRMS *m/e* 539.1229 ($[M+Na]^+$, $C_{18}H_{28}NaO_{17}$ requires: 539.1224). Found: C, 36.76; H, 5.64. $C_{18}H_{27}O_{17}\cdot 1Na\cdot 0.8\ H_2O$ requires: C, 36.72; H, 5.58.

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