Carbohydrate−Carbohydrate Recognition Between Lewis X Blood Group Antigens, Mediated by Calcium Ions

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Dedicated to Hans Paulsen on the occasion of his 80th birthday

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Bivalent Lewis X (Le^X) oligosaccharides were synthesised in order to study the conformational details of carbohydrate clusters by NMR spectroscopy. To this end, two Le^X trisaccharide moieties (**1**) were covalently linked through the 6-hydroxy group or through the anomeric oxygen of GlcNAc to yield dimers **2** and **3**, respectively. The two Le^X halves of the model saccharide **2** exhibited cooperativity in calcium binding. Three different lactosides served as control compounds for NMR titration with calcium chloride.

Introduction

Lewis X (Le^X) is the terminal trisaccharide moiety of numerous cell surface glycolipids and glycoproteins involved in selectin-mediated cell-cell adhesion and recognition processes.[1,2] In addition to protein-carbohydrate binding, there are also intercellular adhesive forces based on direct carbohydrate-carbohydrate interactions: on homophilic Le^X-Le^X binding, for instance.[3−7] Molecular details of such homophilic recognition between neutral carbohydrate moieties are incompletely understood because of the chemical complexity of the cell membrane. Oligovalency and cooperativity are often discussed as major principles in the amplification of receptor binding. Ligand-receptor complexes with weak affinity constants between singular entities (K_a values in the mM^{-1} range) multiply to polyvalent adhesive forces of high affinity.[8−10] The cation-dependent clustering of membrane-anchored neutral Lewis glycolipids has been described,[11] but the experimental characterization of the synergistic multiplication of weak carbohydrate-carbohydrate affinities to provide considerable intercellular forces remains an analytical challenge.[12−14] In isotropic solution, no self-assembly of Le^X glycoconjugates or significant metal complexation was detectable.[15,16] The very weak calcium affinity of the neutral Le^X oligosacchararides required their tethering to a lipid bilayer in order to make the weak carbohydrate-carbohydrate binding observable by NMR spectroscopy.[17] From this preliminary study, the Le^X trisaccharide was identified as the minimal structural unit capable of stabilizing homophilic Le^X-Le^X recognition. However, transferred NOE measurements cannot characterise the contact surface within a pair of structurally identical sugar subunits. Therefore, the preorientation of Le^X epitopes on a cell surface was mimicked by use of a flexible spacer between two Le^X trisaccharides. A covalent linker should keep the oligosaccharides in close contact without the necessity to employ extraordinary high sugar concentrations. This strategy should allow the direct conformational analysis of a Le^X cluster with bound calcium.[18]

For this purpose, Le^X monomer **1** (Scheme 1) was tethered either through the 6-hydroxy group of GlcNAc (→ **2**) or, in a second bivalent model, through the anomeric oxygen of GlcNAc (→ **3**). The two linkages were chosen because they were distant from the expected calcium binding site, which involves the hydroxy groups of the terminal Gal and Fuc residues. Six rotatable bonds in **2** and **3** permitted several relative orientations of the Le^X moieties. In the presence of calcium ions, a single mode of self-assembly was observed by NMR spectroscopy for **2**, and conformational details of a Le^X cluster could be quantified for the first time.[18] The cooperative calcium ion complexation of a simple Le^X cluster such as **2** or **3** should contrast with the ion-binding behaviour of other carbohydrate clusters. Because of this, and for reasons of comparison, methyl lactoside **4**, the corresponding 6a-*O*-tethered dimer **5**, and a

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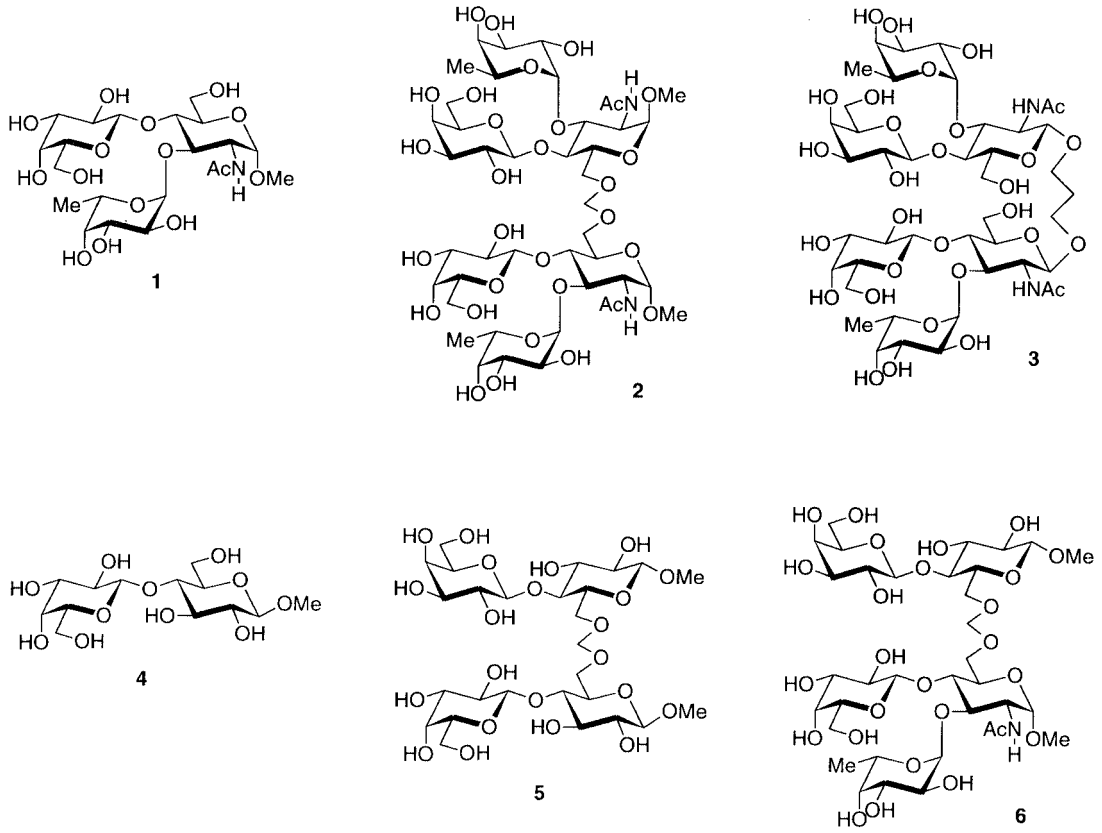


Scheme 1. Target molecules **1**–**6**

6a-*O*-tethered dimer **6**, consisting of a lactoside and a Le^X moiety, were also investigated. The synthesis of these compounds and results of their calcium ion-mediated binding are reported and discussed.

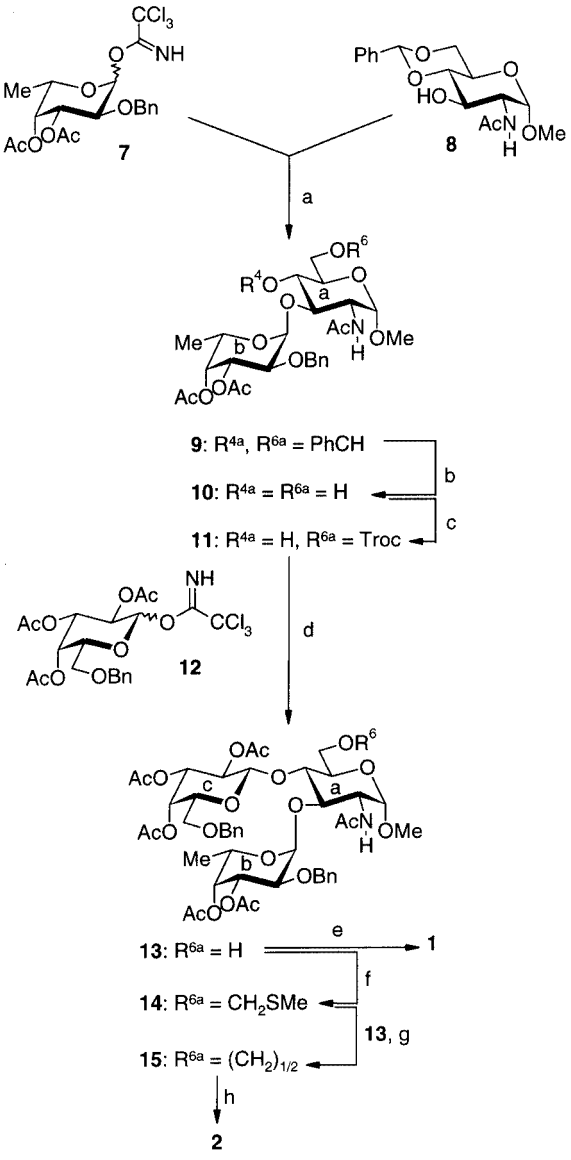
Results and Discussion

Synthesis of Compounds 1–6

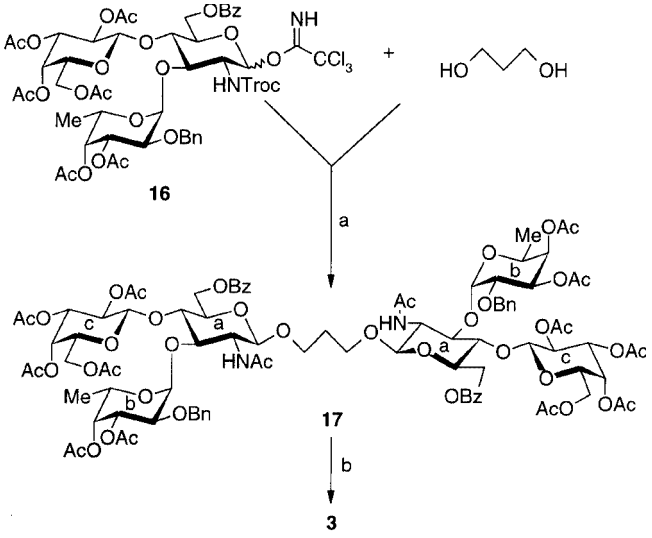
The synthesis of Le^X trisaccharide **1** was based on known building blocks (Scheme 2). Fucosylation of 3-*O*-unprotected *N*-acetylglucosamine derivative **8**[19] as acceptor with the known fucosyl donor **7**,[20] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst, at −10 °C and by the inverse procedure (IP),[21] afforded the desired α(1−3)-linked disaccharide **9** in high yield. When the reaction was instead carried out at room temperature, considerable amounts of the β anomer were also obtained, thus supporting the configurational assignment (^{1}H NMR: **9**, $J_{1b,2b}$ = 3.4 Hz; β anomer, $J_{1b,2b}$ = 7.6 Hz). Treatment of **9** with ethyl mercaptan in the presence of *p*-toluenesulfonic acid (*p*-TsOH) as catalyst resulted in 4a,6a-*O*-debenzylidenation (→ **10**). Regioselective 6a-*O*-protection of **10** could be carried out with different reagents,[22] but the best results in the subsequent glycosylation were obtained with the trichloroethoxycarbonyl (Troc) group, introduced as Troc chloride in pyridine (→ **11**). Galactosylation of **11** with the known donor **12**[23] in the presence of TMSOTf as catalyst afforded the expected Le^X trisaccharide, which was immediately deprotected at the 6a-*O* position with activated zinc in acetic acid, thus furnishing **13**, with the desired β-linked galactosyl residue (^{1}H NMR: $J_{1c,2c}$ = 7.8 Hz), in acceptable yield. Hydrogenolytic *O*-debenzylation with palladium on carbon as catalyst and subsequent *O*-deacetylation by treatment with methylamine in ethanol afforded Le^X trisaccharide **1**.

For the tethering of two molecules of **1** with a methylene group, various methods were tested.[22] Finally, a methylthiomethyl group was introduced at the 6a-oxygen of **13**. To this end, treatment of **13** either with DMSO/acetic anhydride/acetic acid[24] or with benzoyl peroxide/dimethyl sulfide/lutidine in acetonitrile[25,26] was carried out, providing **14** in 83% and 89% yields, respectively. Treatment of **14** with **13** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) as promoters[27,28] gave the desired tethered dimer **15** in 58% yield. Hydrogenolytic *O*-debenzylation and subsequent *O*-deacetylation under Zemplén conditions[29] (NaOMe in MeOH) furnished the desired target molecule **2** in 69% yield.

Linkage of two Le^X moieties through the anomeric centre with six rotatable bonds could readily be accomplished with 1,3-propanediol as acceptor (Scheme 3). To this end, known trichloroacetimidate **16**,[30] as Le^X donor, was treated with 1,3-propanediol in the presence of TMSOTf as catalyst, thus affording the β-linked dimer **17** (^{1}H NMR: $J_{1a,2a}$ = 8.3 Hz) after removal of the Troc protecting group with ac-



Scheme 2. Synthesis of Le^X derivative **1** and its dimer **2**: a) TMSOTf, −10 °C, CH_2Cl_2, IP (84 %). b) EtSH, *p*-TsOH (90 %). c) Troc-Cl, pyridine (93 %). d) TMSOTf, 0 °C → room temp., CH_2Cl_2; Zn powder, THF, HOAc (54 %). e) Pd/C, H_2; $MeNH_2$, EtOH (82 %). f) DMSO, HOAc, Ac_2O (83 %) or Bz_2O_2, Me_2S, lutidine, 0 °C → room temp. (89 %). g) NIS, TfOH (58 %). h) Pd/C, H_2; NaOMe, MeOH (69 %)



Scheme 3. Synthesis of dimer **3**: a) TMSOTf, 4-Å mol. sieves, room temp., CH_2Cl_2; Zn powder, Ac_2O, HOAc (63 %). b) Pd/C, H_2; NaOMe, MeOH (79 %)

tivated zinc in acetic anhydride/acetic acid. Hydrogenolytic *O*-debenzylation and subsequent *O*-deacetylation as described above afforded the target molecule **3** in good overall yield.

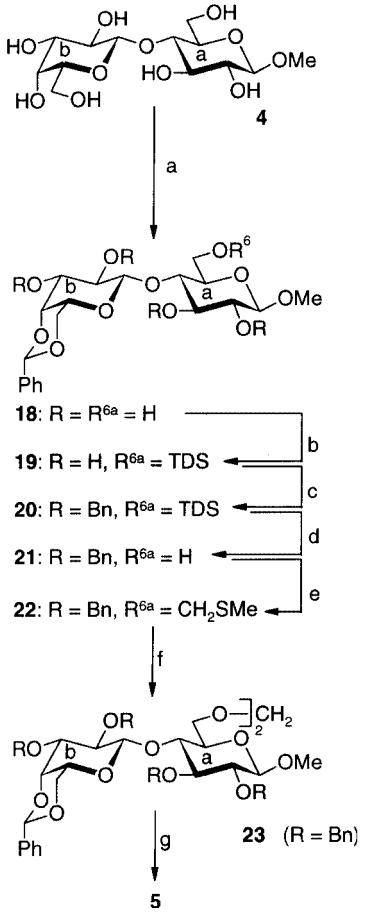
For the comparison studies, methyl β-lactoside (**4**, Scheme 4) was prepared as previously described.[31] The corresponding 6a-*O*-methylene-bridged dimer was also obtained from **4**. To achieve this, 4b,6b-*O*-benzylidenation of **4** was performed as previously described (→ **18**).[31] Regioselective 6a-*O*-silylation with thexyldimethylsilyl (TDS) chloride in the presence of imidazole (→ **19**) and subsequent *O*-perbenzylation by treatment with benzyl bromide in the presence of sodium hydride and tetrabutylammonium iodide (TBAI) afforded fully protected lactoside **20**. 6a-*O*-Desilylation with TBAF in THF gave 6a-*O*-unprotected derivative **21**, which afforded 6a-*O*-methylthiomethyl derivative **22** on treatment with DMSO/acetic anhydride/acetic acid. Treatment of **22** with **21** in the presence of NIS/TfOH as promoter furnished the methylene-bridged dimer **23** in 73% yield. Hydrogenolytic *O*-debenzylation and concomitant *O*-debenzylidenation provided target molecule **5**.

The linkage of a lactose and a Le^X moiety through a methylene bridge could readily be performed with 6a-*O*-methylthiomethyl lactose derivative **22** and 6a-*O*-unprotected Le^X derivative **13** (Scheme 5). Treatment with the NIS/TfOH promoter system furnished the protected mixed dimer **24** in 82% yield. Hydrogenolytic *O*-debenzylation and *O*-debenzylidenation and subsequent *O*-deacetylation as described above afforded target molecule **6** in 82% yield.

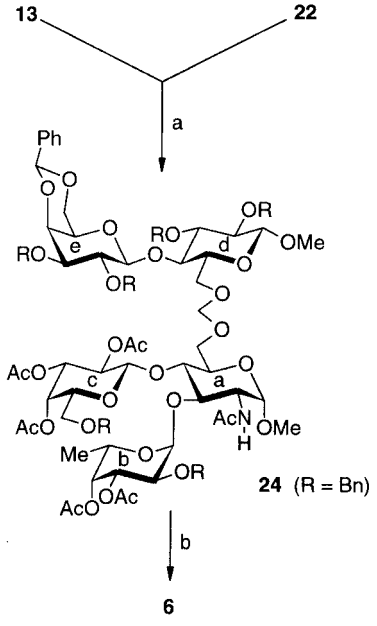
NMR Analysis

The analytical problems associated with the quantification of weak binding affinities are the practically inaccessible high concentrations at which glycosides form reasonable amounts of complexes. For example, only 5% of a dimeric complex is present at a concentration of 10 mM and an assumed affinity constant of $K_a = 5\ M^{-1}$. The expected concentration of a trimeric complex formed by two Le^X trisaccharides and a calcium ion would be even less. At mM concentrations, the trimeric complex $[Le^X] + [Ca^{++}] + [Le^X] \rightleftharpoons [Le^X{\cdot}Ca^{++}{\cdot}Le^X]$ would be disfavoured, but covalent tethering could shift the equilibrium $[Le^X{-}Le^X] + [Ca^{++}] \rightleftharpoons [Le^X{-}Le^X{\cdot}Ca^{++}]$ towards the side of the complex. Covalently linked Le^X trisaccharides would be expected to exhibit increased calcium binding affinities, thus making the complex accessible for NMR analysis (Figure 1).

For entropic reasons, the linkage must not be too long and flexible. An estimate of the amount of energy necessary



Scheme 4. Synthesis of lactose dimer **5**: a) ref. 31. b) TDS-Cl, Im, DMF (73 %). c) BnBr, NaH, TBAI, DMF (93 %). d) TBAF, THF (97 %). e) DMSO, Ac_2O, HOAc (59 %). f) NIS, TfOH, 4-Å mol. sieves, 0 °C, CH_2Cl_2 (73 %). g) Pd/C, H_2 (79 %)



Scheme 5. Synthesis of mixed dimer **6**: a) NIS, TfOH, 4-Å mol. sieves, 0 °C, CH_2Cl_2 (82 %). b) Pd/C, H_2; $MeNH_2$, EtOH (82 %)

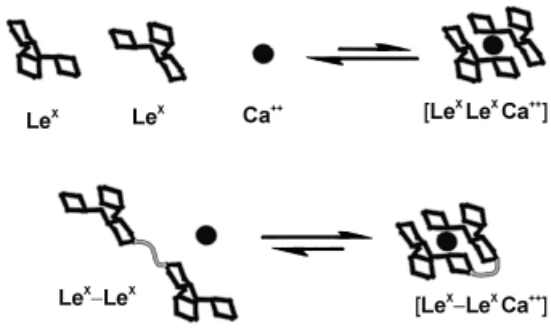


Figure 1. Schematic representation of cooperative calcium binding by two Le^X trisaccharide moieties; the trimeric complex is disfavoured in isotropic solution (above), while the calcium complex of a bivalent bis-Le^X hexasaccharide is favoured for entropic reasons; details are described in the text

to freeze the torsional entropy of a triethylene glycol linker is about 10 kcal/mol.[32] Complexation as weak as expected for Le^X and calcium could not compensate for a large torsional entropy term. On the other hand, neither must the tether between the trisaccharide moieties be too rigid, in order to permit the synergistic binding of calcium ions. For these reasons, compounds **2** and **3** were selected and synthesised as bivalent Le^X-Le^X dimers of the above formula. The identical number of six single bonds between the pyranose rings of GlcNAc in **2** and in **3** was chosen so as to ensure similar entropic contributions for the calcium binding.

The solvent has an important influence on the cation binding. Appreciable affinities between polyols and calcium ions in water are observed only for *ax-eq-ax* hydroxy-substituted pyranose rings.[33] However, the Le^X pentasaccharide shows no detectable affinity for calcium ions in water. Alcohols as solvents can increase the affinities between polyols and cations, one reason for this being the weaker solvation of calcium ions in alcohols than in aqueous solutions. Thus, even weak affinities between hydroxy groups and calcium ions become detectable in methanol, and a minimum affinity for calcium ions independent of the structure of the sugar is observed. Such unspecific binding between polyol structures and calcium ions has affinity constants K_a of below 10 M^{-1}. The Le^X pentasaccharide has recently been studied in methanol, and two complexing regions multiplying to an affinity constant of approximately 30 M^{-1} were identified.[34]

In our studies, ^1H NMR resonances shifted in a range between 20 and 200 Hz on addition of calcium chloride to solutions of compounds **1**−**6** in [D_4]methanol. Large signal chemical shift variations correspond with a small numerical error, which is not quantified here because the more significant error is the assumption of 100% complexation at the highest calcium concentrations. Large, nonphysiological amounts of calcium were added to overcome this drawback of NMR titrations of low-affinity interactions.

The calcium affinity of **1**, weak in methanol (K_a = 6 M^{-1}) and undetectable in water, multiplied to one of the highest affinities measured for a neutral oligosaccharide in **2** (K_a =

55 M^{-1}), assembled from two Le^X trisaccharides covalently linked through GlcNAc-*O*-6. The affinity of **2** as a receptor for calcium ions was even observable in water (K_a = 5−10 M^{-1}). The Le^X trisaccharide moieties cooperatively interacted in **2** to form a new receptor site with a significantly enhanced affinity for calcium ions. The two Le^X trisaccharides showed this cooperativity of calcium binding in isotropic solution and proved the homophilic carbohydrate-carbohydrate binding by spectroscopic methods.[35]

Figure 2 compares the ^1H chemical shift variations of **1** and **2**. The resonance signals of **1** shifted to lower field. The resonance signals of **2** exhibited a double-exponential dependence on the calcium concentration, different from the single-exponential dependence of **1**. (The residual solvent signals are included in Figure 2 to make this difference more visible.) In **2**, the first calcium-binding site was saturated below 20 equivalents of calcium chloride, and unspecific binding began at higher calcium concentrations. The occupation of secondary binding sites in this concentration range is separated in a Scatchard plot (Figure 3).[36] The bound fraction ν was determined from the value of the ^1H chemical shift and the amount of dissolved calcium [Ca^{++}] calculated from the total calcium chloride in solution. A linear extrapolation to ν = 0 yielded the binding affinity K_a. In addition to the low-field shifts, several proton signals of **2** moved to higher field or inverted their direction during the titration (Figure 2). The chemical shift variations of **2** proved that a conformational change was occurring together with the complexation of calcium ions. NOE studies revealed the relative orientations of the two trisaccharide moieties in the complex.[18] In the presence of calcium ions, additional NOEs became visible, identifying the hydrophobic contacts between the two Le^X trisaccharides. The 1,3-propanediol tether between the anomeric positions of two Le^X trisaccharides in compound **3** (K_a = 17 M^{-1}) did not support cooperative interaction of the two building blocks, although the separation was, as in **2**, again by six rotatable single bonds.

The significance of the bivalent calcium binding by **2** becomes more evident on comparison with other sugar-derived bivalent calcium receptors, of which several have been synthesised during this investigation. As mentioned above, the calcium affinities of neutral glycosides are generally very weak; lactose, for example, is a weak calcium binder that has been studied for its presumed involvement in calcium resorption from milk.[37] The monomeric lactoside **4** has an affinity for calcium ions in methanol (**4**, K_a = 7 M^{-1}) not surpassed by that of two covalently tethered lactosides (**5** K_a = 8 M^{-1}).[38] The affinity of the bis-lactoside **5** for calcium equals the affinity of lactoside **4** for calcium ions (i.e., the average affinity per lactose is divided by approximately two). Lactose lacks cooperativity of calcium binding, and so the affinity of the bivalent receptor **5** is therefore much lower than the multiplied monomeric affinities ($K_{bi} << K_{mono} \cdot K_{mono}$; $\Delta G_{bi} > \Delta G_{mono} + \Delta G_{mono}$), through its avoidance of the accumulation of cationic charges. Although crystal structures with selective coordination of lact-

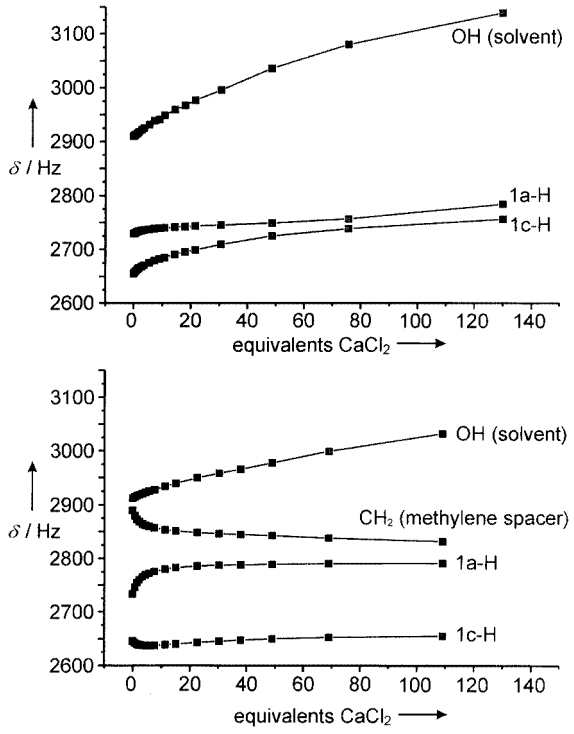


Figure 2. NMR titration of **1** (above, 10 mm) and **2** (below, 5 mm) with calcium chloride in methanol (a = GlcNAc, c = fucose); chemical shift variations [Hz] are plotted against the equivalents of calcium added; the low-field migrations of the chemical shifts of **1** and the single-exponential dependence on the calcium concentration are characteristics of unselective binding of the metal ion, as observable for numerous polyols; compound **2** behaves differently, with some signals migrating to higher field or reversing their direction during the titration; a change of the average conformation of **2** occurs upon calcium binding and produces the observed complex chemical shift dependence

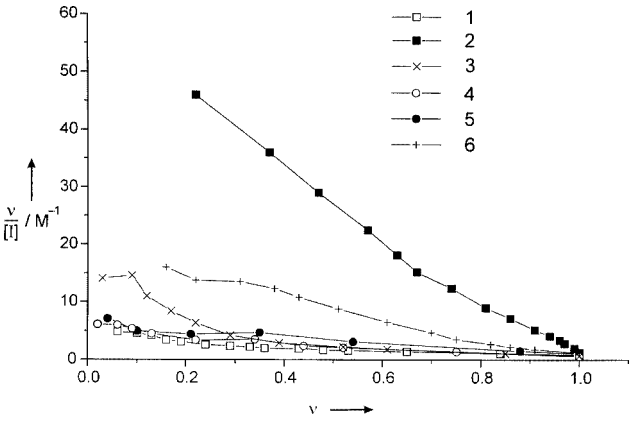


Figure 3. Scatchard plots of compounds **1**−**6**; the percentage of complexed carbohydrate (ν) is plotted against the ratio of ν to the concentration of free cation **I**; the affinity constant K_a [M^{-1}] is directly obtained at the *y*-axis after linear interpolation to ν = 0; the weak affinities with K_a below 10 M^{-1} are slightly overestimated because full complexation (ν = 1) is not reached without secondary effects playing a role due to the large excess of calcium chloride; the experimental error is small for **2**, where a straight line is observed in the Scatchard plot; the angle at ν = 0.65 indicates the additional unspecific binding at high calcium concentrations

ose to calcium salts are known,[39] lactose binds calcium only weakly in isotropic solution. The hybrid oligosaccharide of lactose and Le^X (**6**, $K_a = 20\ M^{-1}$) exhibits a calcium affinity weaker than the affinity observed for **2**.

Conclusion

Methylene-type spacers were inserted between two Le^X trisaccharide moieties to obtain the bivalent calcium receptors **2** and **3**. Covalent tethering was expected to mimic the preorientation of carbohydrate head groups on the surface of biological membranes. Only in the case of **2** did the oligovalency result in a cooperativity of cation binding in methanol, with a K_a of approx. $55\ M^{-1}$. Compound **2** bound calcium ions with the multiplied monomeric affinities of **1** ($K_a \approx 7-8\ M^{-1}$). The binding affinities depend on the environment and therefore cannot be translated directly into a biological membrane context. However, **2** allows direct NOE observation of the molecular contact surface between the two Le^X moieties and the identification of their cross-shaped dimerisation.[18]

Experimental Section

General Remarks: Solvents were purified by standard procedures. Flash chromatography was performed on J. T. Baker 60 silica gel (0.040−0.063 mm) or J. T. Baker RP-18 silica gel (40 μm) at a pressure of 0.4 bar. Thin layer chromatography was performed on Merck $60F_{254}$ silica gel plastic plates or Merck HPTLC $60F_{254}$ silica gel glass plates; spots were viewed by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL) and heating at 150 °C. Optical rotations were measured on a Perkin−Elmer 241 polarimeter in a 1 dm cell at 22 °C. NMR measurements were recorded at 22 °C on a Bruker AC250 Cryospec or a Bruker DRX600. TMS or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, δ = 7.26; D_2O, δ = 4.63 ppm. The carbohydrate monomers were assigned in alphabetical order, beginning from the aglycon, based in part on carbon−proton shift-correlation heteronuclear multiple quantum coherence (HMQC). MALDI-mass spectra were recorded on a Kratos Kompact MALDI I instrument with a 2,5-dihydroxybenzoic acid matrix.

NMR Spectroscopy: All spectra were acquired at 600 MHz (^1H) and are referenced to the residual CD_2HOD at δ = 3.3 ppm. Calcium chloride was added in a stepwise manner to NMR tubes containing individual solutions of the oligosaccharides **1**−**6** in [D_4]methanol. To compensate for dilution effects, the stock solution of calcium chloride in [D_4]methanol contained the same concentration of the oligosaccharide as the NMR tube. Solid calcium chloride was added only at the highest molar ratios. ^1H NMR spectra were recorded after equilibration times of several minutes between 0 and approx. 150 equivalents of calcium in order to follow the entire range between, until quantitative calcium complexation. The complete assignment of the ^1H and ^{13}C resonance signals was obtained from homo- and heteronuclear 2D spectra. The anomeric protons and well separated ring protons were taken for analysis. Binding affinities were taken from Scatchard plots as described in.[36] Compound **2** was investigated at 5 mm and at 20 mm: no significant differences were observed. The NMR titration of **2** in D_2O was performed at a concentration of 10 mm.

Methyl *O*-(3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (9): A suspension of acceptor **8**[19] (5.00 g, 15.5 mmol) in dry CH_2Cl_2 (120 mL) was cooled to −10 °C and ultrasonificated. TMSOTf (560 μL, 3.1 mmol, 0.2equiv.) was then added. A solution of donor **7**[20] (8.96 g, 18.6 mmol, 1.2 equiv.) in dry CH_2Cl_2 (20 mL) was slowly added to this reaction mixture over a period of 30 min, and the ice-bath was then removed. After an additional 30 min, the reaction mixture was neutralised with NEt_3 and concentrated in vacuo. Flash chromatography (petroleum ether/ethyl acetate, 1:3) gave **9** (8.49 g, 84%) as a colourless foam. TLC (petroleum ether/ethyl acetate, 1:3): $R_f = 0.34$. $[\alpha]_D = -18.0$ ($c = 1.0$, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ = 0.95 (d, $J_{5,6} = 6.5$ Hz, 3 H, 6b-CH_3), 1.64 (s, 3 H, $NCOCH_3$), 1.99, 2.13 (2 s, 6 H, 2 $COCH_3$), 3.37 (s, 3 H, OCH_3), 3.67 (dd, $^3J_{2,3} = {}^3J_{3,4} = 8.9$ Hz, 1 H, 3a-H), 3.77−3.81 (m, 2 H, HMQC: 3.78 (6a-H), 3.81 (4a-H), 3.86 (dd, $^3J_{1,2} = 3.4$, $^3J_{2,3} = 10.5$ Hz, 1 H, 2b-H), 4.08−4.12 (m, 2 H, 2a-H, 5a-H), 4.30 (dd, $^3J_{5,6} = 3.7$, $^2J_{6,6} = 9.2$ Hz, 1 H, 6′a-H), 4.33 (q, 1 H, 5b-H), 4.57, 4.69 (2 d, $^2J_{6,6} = 11.0$ Hz, 2 H, CH_2Ph), 4.94 (d, $^3J_{1,2} = 2.7$ Hz, 1 H, 1a-H), 5.18 (d, $^3J_{3,4} = 3.7$ Hz, 1 H, 4b-H), 5.25 (d, $^3J_{1,2} = 3.4$ Hz, 1 H, 1b-H), 5.32 (dd, $^3J_{2,3} = 10.5$, $^3J_{3,4} = 3.4$ Hz, 1 H, 3b-H), 5.59 (s, 1 H, CHPh), 6.33 (d, $^3J_{2,N} = 6.3$ Hz, 1 H, NH), 7.32−7.53 (m, 10 H, 2 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$): δ = 15.7 (6b-C), 20.6, 20.7 (2 $CO\mathit{C}H_3$), 22.6 ($NHCO\mathit{C}H_3$), 53.9 (2a-C), 55.3 (OCH_3), 62.9 (4a-C), 65.5 (5b-C), 68.9 (6a-C), 69.9 (3b-C), 71.6 (4b-C), 74.0 (CH_2Ph), 75.1 (5a-C), 75.3 (2b-C), 81.8 (3a-C), 98.4 (1b-C), 98.7 (1a-C), 101.2 (CHPh), 125.9−137.3 (2 C_6H_5), 169.7, 170.5, 170.6 (3 COMe). MALDI-MS: m/z = 666.2 [M + Na^+], 682.2 [M + K^+]. $C_{33}H_{41}NO_{12}\cdot 0.5H_2O$ (652.69): calcd. C 60.73, H 6.49, N 2.15; found C 60.60, H 6.54, N 1.97.

Methyl *O*-(3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (10): A solution of **9** (1.73 g, 2.65 mmol) in dry CH_2Cl_2 (20 mL) was treated with ethanethiol (1.2 mL, 15.9 mmol) and *p*-TsOH (100 mg, 530 μmol) and stirred overnight. After neutralisation with NEt_3, the solution was concentrated in vacuo and purified by flash chromatography (toluene/acetone, 1:1) to afford **10** (1.33 g, 90%) as a colourless foam. TLC (toluene/acetone, 1:1): $R_f = 0.20$. $[\alpha]_D = +6.6$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.15 (d, $^3J_{5,6} = 6.5$ Hz, 3 H, 6b-CH_3), 1.51 (s, 3 H, $NCOCH_3$), 2.01, 2.16 (2 s, 6 H, 2 $COCH_3$), 3.38 (s, 3 H, OCH_3), 3.58−3.96 (m, 7 H, 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 2b-H), 4.36 (q, 1 H, 5b-H), 4.64, 4.70 (2 d, ^2J = 11.2 Hz, 2 H, CH_2Ph), 5.06 (d, $^3J_{1,2} = 3.7$ Hz, 1 H, 1b-H), 5.10 (d, $^3J_{1,2} = 3.2$ Hz, 1 H, 1a-H), 5.26−5.33 (m, 2 H, 3b-H, 4b-H), 6.08 (d, $^3J_{2,N} = 4.8$ Hz, 1 H, NH), 7.27−7.40 (m, 5 H, C_6H_5). $C_{26}H_{37}NO_{12}\cdot 0\cdot 25H_2O$ (560.08): calcd. C 55.76, H 6.75, N 2.50; found C 55.81, H 6.74, N 2.17.

Methyl *O*-(3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-2-deoxy-6-*O*-(2,2,2-trichloroethoxycarbonyl)-α-D-glucopyranoside (11): A solution of **10** (251 mg, 325 μmol) in dry CH_2Cl_2 (15 mL) and dry pyridine (100 μL) was cooled to 0 °C. Troc-Cl (79 μL, 583 μmol, 1.3equiv.) was then added, and the reaction mixture was allowed to warm to room temp. and stirred overnight. After addition of saturated NH_4Cl solution and extraction with CH_2Cl_2 (3 × 50 mL), the combined organic layers were dried with sodium sulfate and concentrated in vacuo to give **11** (305 mg, 93%) as a colourless foam after flash chromatography (toluene/acetone, 1:1). TLC (toluene/acetone, 2:1): $R_f = 0.40$. $[\alpha]_D = +5.9$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.13 (d, $^3J_{5,6} = 6.5$ Hz, 3 H, 6b-CH_3), 1.49 (s, 3 H, $NCOCH_3$), 1.98, 2.14

(2 s, 6 H, 2 $COCH_3$), 3.36 (s, 3 H, OCH_3), 3.48−3.94 (m, 5 H, 2a-H, 3a-H, 4a-H, 5a-H, OH), 4.33 (q, 1 H, 5b-H), 4.41 (dd, $^3J_{5,6}$ = 5.3, $^2J_{6,6}$ = 11.6 Hz, 1 H, 6a-H), 4.54−4.70 (m, 3 H, 6′a-H, CH_2CCl_3), 4.74, 4.80 (2 d, ^2J = 11.9 Hz, 2 H, CH_2Ph), 5.02 (d, $^3J_{1,2}$ = 3.6 Hz, 1 H, 1b-H), 5.07 (d, $^3J_{1,2}$ = 3.2 Hz, 1 H, 1a-H), 5.24−5.30 (m, 2 H, 3b-H, 4b-H), 6.01 (d, $^3J_{2,N}$ = 5.4 Hz, 1 H, NH), 7.27−7.38 (m, 5 H, C_6H_5). $C_{29}H_{38}Cl_3NO_{14}$ (730.98): calcd. C 47.65, H 5.24, N 1.92; found C 47.51, H 5.34, N 1.68.

Methyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranoside (13): Acceptor **11** (154 mg, 211 μmol) and donor **12**[23] (138 mg, 255 μmol, 1.2equiv.) were dissolved in dry CH_2Cl_2 (3 mL) and cooled to 0 °C. TMSOTf (9.2 μL, 51 μmol, 0.3equiv.) was then added, the ice-bath was removed, and the mixture was stirred for 4 h. After neutralisation with NEt_3, the solvent was removed under reduced pressure. Flash chromatography (toluene/acetone, 4:1) gave impure trisaccharide [TLC (toluene/acetone, 4:1): R_f = 0.34], which was dissolved in THF/AcOH (2:1, 9 mL). After addition of activated zinc (250 mg), the mixture was stirred for 2 h at room temperature with sonification (15 min) and then filtered and washed with tetrahydrofuran (150 mL). After concentration of the solution under reduced pressure, the residue was coevaporated with toluene (3 ×) and purified by flash chromatography (toluene/acetone, 2:1 to 3:2) to give **13** (105 mg, 54%) as a colourless foam. TLC (toluene/acetone, 1:1): R_f = 0.34. $[\alpha]_D$ = −15.1 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.14 (d, $^3J_{5,6}$ = 6.5 Hz, 3 H, 6b-CH_3), 1.81, 1.90, 1.96, 2.05, 2.07, 2.11 (6 s, 18 H, 6 $COCH_3$), 3.32 (s, 3 H, OCH_3), 3.51 (m, 1 H, 5a-H), 3.69 (dd, $^3J_{5,6}$ = 7.6, $^2J_{6,6}$ = 9.8 Hz, 1 H, 6c-H), 3.76−3.87 (m, 5 H, HMQC: 3.77 (6′c-H), 3.78 (6a-H), 3.82 (6′a-H), 3.86 (2b-H), 3.86 (5c-H), 3.92 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.5 Hz, 1 H, 4a-H), 3.98 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ ≈ 9.7 Hz, 1 H, 3a-H), 4.24 (ddd, $^3J_{1,2}$ = 3.8, $^3J_{2,N}$ = 8.8, $^3J_{2,3}$ = 10.3 Hz, 1 H, 2a-H), 4.44 (d, ^2J = 11.6 Hz, 1 H, C*H*HPh), 4.48, 4.55 (2 d, ^2J = 11.9 Hz, 2 H, CH_2Ph), 4.68−4.76 (m, 4 H), HMQC: 4.68 ($^3J_{1,2}$ = 3.8 Hz, 1a-H), 4.72 ($^3J_{1,2}$ = 7.9 Hz, 1c-H), 4.74 (5b-H), C*H*HPh], 5.04 (dd, $^3J_{2,3}$ = 10.3, $^3J_{3,4}$ = 3.5 Hz, 1 H, 3c-H), 5.08 (dd, $^3J_{2,3}$ = 10.3, $^3J_{1,2}$ = 7.8 Hz, 1 H, 2c-H), 5.23 (dd, $^3J_{2,3}$ = 10.5, $^3J_{3,4}$ = 3.5 Hz, 1 H, 3b-H), 5.27 (d, $^3J_{3,4}$ = 3.7 Hz, 1 H, 4b-H), 5.33 (d, $^3J_{1,2}$ = 3.7 Hz, 1 H, 1b-H), 5.49 (d, $^3J_{3,4}$ = 3.5 Hz, 1 H, 4c-H), 6.98 (d, $^3J_{2,N}$ = 8.7 Hz, 1 H, NH), 7.25−7.32 (m, 10 H, 2 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 15.91 (6b-C), 53.70 (2a-C), 55.26 (OCH_3), 60.55 (6a-C), 64.60 (5b-C), 67.15 (6c-C), 67.49 (4c-C), 69.87 (3b-C, 2c-C), 71.24 (3c-C), 71.26 (5a-C), 71.85 (4b-C), 72.66 (5c-C), 74.03 (3a-C), 74.14 (2b-C), 74.22 (4a-C), 97.72 (1b-C), 98.16 (1a-C), 100.09 (1c-C). $C_{45}H_{59}NO_{20}$ (933.96): calcd. C 57.87, H 6.37, N 1.50; found C 57.54, H 6.37, N 1.21.

Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranoside (1): Compound **13** (215 mg, 230 μmol) was dissolved in methanol (6 mL) and acetic acid (0.1 mL). Palladium on carbon (50 mg, 10% Pd) was added, and the solution was stirred vigorously under a hydrogen atmosphere for 2 h [TLC ($CHCl_3$/methanol, 9:1): R_f = 0.30]. The catalyst was filtered off and washed with methanol. After evaporation in vacuo, the residue was dissolved in methylamine (33% in ethanol, 20 mL) and stirred for 90 min. The mixture was concentrated in vacuo and purified by flash chromatography ($CHCl_3$/methanol/water, 65:35:8 to 60:40:10) to give **1** (109 mg, 82%) as a colourless powder after lyophilization from water. HPTLC ($CHCl_3$/methanol/water, 60:40:10): R_f = 0.20. $[\alpha]_D$ = +11.3 (*c* = 1.0, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 1.18 (d, $^3J_{5,6}$ = 6.6 Hz, 3 H, 6b-CH_3), 2.03 (s, 3 H, $NCOCH_3$), 3.40 (s, 3 H, OCH_3), 3.51 (dd, $^3J_{1,2}$ = 7.6, $^3J_{2,3}$ = 4.1 Hz, 1 H, 2c-H), 3.51 (m, 1 H, 5c-H), 3.65−3.75 (m, 4 H, 2b-H, 3c-H, 6c-H, 6′c-H), 3.80 (d, $^3J_{3,4}$ = 2.6 Hz, 1 H, 4b-H), 3.85 (m, 1 H, 5a-H), 3.89−3.91 (m, 3 H, 6a-H, 3b-H, 4c-H), 3.94−3.98 (m, 3 H, 3a-H, 4a-H, 6′a-H), 4.19 (dd, $^3J_{1,2}$ = 3.3, $^3J_{2,3}$ = 10.0 Hz, 1 H, 2a-H), 4.47 (d, $^3J_{1,2}$ = 7.8 Hz, 1 H, 1c-H), 4.68 (d, $^3J_{1,2}$ = 3.4 Hz, 1 H, 1a-H), 4.85 (q, $^3J_{5,6}$ = 6.5 Hz, 1 H, 5b-H), 5.08 (d, $^3J_{1,2}$ = 3.9 Hz, 1 H, 1b-H). $C_{21}H_{37}NO_{15}$·$2H_2O$ (579.55): calcd. C 43.52, H 7.13, N 2.42; found C 43.31, H 6.81, N 2.05.

Methyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-6-*O*-methylthiomethyl-α-D-glucopyranoside (14). Procedure A: A solution of **13** (211 mg, 226 μmol), dimethyl sulfide (170 μL, 2.3 mmol) and lutidine (26 μL, 226 μmol) in dry acetonitrile (1 mL) was cooled to 0 °C, and dibenzoyl peroxide (219 mg, 904 μmol) was then added over 30 min. The ice-bath was removed, and the reaction mixture was stirred for 2 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (200 mL) and washed with saturated $NaHCO_3$ solution (50 mL). The organic layer was dried and concentrated in vacuo. Flash chromatography (toluene/acetone, 3:1 + 1% NEt_3) afforded **14** (200 mg, 89%) as a colourless solid.

Procedure B: A mixture of acetic anhydride/acetic acid [5.6:1 (v,v), 0.8 mL] was added to a solution of **13** (291 mg, 312 μmol) in dimethyl sulfoxide (1 mL).[24] The reaction mixture was stirred overnight and worked up as described above to give **14** (256 mg, 83%). TLC (toluene/acetone, 2:1): R_f = 0.31. $[\alpha]_D$ = +1.1 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.15 (d, $^3J_{5,6}$ = 6.5 Hz, 3 H, 6b-CH_3), 1.86, 1.89, 1.97, 2.07, 2.09, 2.11, 2.20 (6 s, 18 H, 5 $COCH_3$, SCH_3), 3.34 (s, 3 H, OCH_3), 3.36−5.52 (m, 25 H, 1a-H, 2a-H, 3a-H, 4a-H, 5a-H, 6a-H, 6′a-H, 1b-H, 2b-H, 3b-H, 4b-H, 5b-H, 1c-H, 2c-H, 3c-H, 4c-H, 5c-H, 6c-H, 6′c-H, OCH_2S, 2 CH_2Ph), 5.92 (d, $^3J_{2,N}$ = 9.2 Hz, 1 H, NH), 7.23−7.38 (m, 10 H, 2 C_6H_5). MALDI-MS: *m*/*z* = 1033.6 [M + Na^+], 1049.8 [M + K^+]. $C_{47}H_{63}NO_{20}S$ (994.07): calcd. C 56.79, H 6.39, N 1.41; found C 56.72, H 6.28, N 1.05.

Formaldehyde Bis[methyl *O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranosid-6-yl] Acetal (15): A solution of acceptor **13** (118 mg, 126 μmol), donor **14** (251 mg, 252 μmol) and *N*-iodosuccinimide (80 mg, 354 μmol) in dry CH_2Cl_2 (4 mL) in the presence of molecular sieves (AW-300) was stirred for 15 min at room temperature. TfOH (4 μL, 46 μmol) was then added and the solution was stirred for 30 min. After addition of NEt_3 and CH_2Cl_2 (200 mL), filtration and washing with dilute $Na_2S_2O_3$ solution, the organic layer was dried and concentrated in vacuo. Purification by flash chromatography (toluene/acetone, 1:1 + 1% NEt_3; long column) afforded **15** (138 mg, 58%) as a colourless solid. TLC (toluene/acetone, 1:1): R_f = 0.40. $[\alpha]_D$ = −6.0 (*c* = 0.5, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.12 (d, $^3J_{5,6}$ = 6.5 Hz, 6 H, 6b-CH_3), 1.83, 1.86, 1.93, 2.02, 2.07, 2.81 (6 s, 36 H, $COCH_3$), 3.29 (s, 6 H, OCH_3), 3.56 (m, 2 H, 5a-H), 3.66−3.98 (m, 16 H), HMQC: 3.71 (6c-H), 3.74 (6a-H), 3.78 (6′c-H), 3.82 (2b-H), 3.85 (6′a-H), 3.85 (5c-H), 3.94 (3a-H), 3.94 (4a-H), 4.29 (m, 2 H, 2a-H), 4.40−4.79 (m, 16 H), HMQC: 4.61 (1a-H), 4.68 (1c-H), 4.77 (5b-H), 4.77 (OCH_2O), CH_2Ph], 5.00 (dd, $^3J_{2,3}$ = 10.3, $^3J_{3,4}$ = 3.4 Hz, 2 H, 3c-H), 5.06 (dd, $^3J_{2,3}$ = 10.2, $^3J_{1,2}$ = 8.0 Hz, 2 H, 2c-H), 5.20 (dd, $^3J_{2,3}$ = 10.5, $^3J_{3,4}$ = 3.3 Hz, 2 H, 3b-H), 5.28 (d, $^3J_{3,4}$ = 3.3 Hz, 2 H, 4b-H), 5.32 (d, $^3J_{1,2}$ = 3.5 Hz, 2 H, 1b-H), 5.47 (d, $^3J_{3,4}$ = 2.8 Hz, 2 H, 4c-H), 5.92 (d, $^3J_{2,N}$ = 9.2 Hz, 2 H, NH), 7.21−7.29 (m, 20 H, C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 16.3 (6b-C), 53.8 (2a-C),

55.6 (OCH_3), 64.7 (5b-C), 65.0 (6a-C), 67.4 (6c-C), 67.7 (4c-C), 70.1 (2c-C), 70.2 (3b-C), 71.3 (5a-C), 71.6 (3c-C), 72.3 (4b-C), 73.0 (5c-C), 74.3 (3a-C, 4a-C), 96.2 (OCH_2O), 97.8 (1b-C), 98.4 (1a-C), 100.4 (1c-C). MALDI-MS: m/z = 1899.9 [M + Na^+]. $C_{91}H_{118}N_2O_{40}\cdot H_2O$ (1897.94): calcd. C 57.59, H 6.37, N 1.48; found C 57.53, H 6.53, N 1.30.

Formaldehyde Bis[methyl *O*-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranosid-6-yl] Acetal (2): Palladium on carbon (100 mg, 10% Pd) was added to a solution of **15** (131 mg, 69.0 μmol) in methanol (6 mL) and acetic acid (0.1 mL), and the solution was stirred vigorously under a hydrogen atmosphere for 2 h [TLC ($CHCl_3$/methanol, 12:1): R_f = 0.30]. After filtration and concentration under reduced pressure, the residue was dissolved in dry methanol, sodium methoxide (30 mg) was added, and the solution was stirred overnight. The solution was then neutralised with Amberlite IR120 (H^+) and filtered, and the solvents were evaporated. Purification by flash chromatography (ethyl acetate/2-propanol/water, 1:1:1) afforded **2** (52 mg, 69%) as a colourless powder after lyophilization from water. TLC (ethyl acetate/2-propanol/water, 1:1:1): R_f = 0.24. $[\alpha]_D$ = +7.9 (c = 1.0, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.17 (d, $^3J_{5,6}$ = 6.5 Hz, 6 H, 6b-CH_3), 1.96 (s, 6 H, $COCH_3$), 3.30 (s, 6 H, OCH_3), 3.44 (dd, $^3J_{5,6}$ = $^3J_{5,6'}$ ≈ 6.0 Hz, 2 H, 5c-H), 3.51−3.52 (m, 2 H, 2c-H, 3c-H), 3.60 (dd, $^3J_{1,2}$ = 3.9, $^3J_{2,3}$ = 10.2 Hz, 2 H, 2b-H), 3.67 (dd, $^3J_{5,6}$ = 5.1, $^2J_{6,6}$ = 11.4 Hz, 2 H, 6c-H), 4.71 (d, $^3J_{3,4}$ = 2.3 Hz, 2 H, 4b-H), 3.76 (dd, $^3J_{5,6'}$ = 6.9, $^2J_{6,6}$ = 11.3 Hz, 2 H, 6′c-H), 3.81−3.86 (m, 8 H), HMQC: 3.80 (4c-H), 3.82 (5a-H), 3.83 (3b-H), 3.86 (6a-H), 3.90 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ = 9.5 Hz, 2 H, 3a-H), 4.09 (dd, $^3J_{5,6'}$ = 3.4, $^2J_{6,6}$ = 11.4 Hz, 2 H, 6′a-H), 4.25 (dd, $^3J_{1,2}$ = 3.4, $^3J_{2,3}$ = 10.4 Hz, 2 H, 2a-H), 4.41 (m, 2 H, 1c-H), 4.55 (d, $^3J_{1,2}$ = 3.3 Hz, 2 H, 1a-H), 4.81 (s, 2 H, OCH_2O), 4.84 (m, 2 H, 5b-H), 5.01 (d, $^3J_{1,2}$ = 3.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (151 MHz, CD_3OD): δ = 16.62 (6b-C), 22.70 ($CO\mathit{C}H_3$), 55.15 (2a-C), 55.62 (OCH_3), 62.80 (6c-C), 67.28 (6a-C), 67.59 (5b-C), 69.91 (4c-C), 70.02 (2b-C), 71.26 (3b-C), 72.47 (5a-C), 72.88 (2c-C), 73.70 (4b-C), 74.57 (3a-C), 74.79 (3c-C), 75.61 (4a-C), 76.80 (5c-C), 97.56 (OCH_2O), 100.23 (1a-C), 100.48 (1b-C), 104.12 (1c-C), 173.81 (COMe). MALDI-MS: m/z = 1123.1 [M + Na^+], 1139.5 [M + K^+]. $C_{43}H_{74}N_2O_{30}$ (1099.05).

1,3-Propylene Bis-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (17): Donor **16**[30] (345 mg, 275 μmol, 2.75 equiv.) and propane-1,3-diol (7.2 μL, 100 μmol) were dissolved in dry CH_2Cl_2 (4 mL) in the presence of molecular sieves (AW-300). TMSOTf (9.2 μL, 51 μmol, 0.3equiv.) was then added at room temp., and the mixture was stirred for 30 min. After neutralisation with NEt_3 the solvent was removed under reduced pressure. Flash chromatography (toluene/acetone, 4:1) gave impure trisaccharide, which was dissolved in THF/Ac_2O/AcOH (6:2:1, 9 mL). After addition of activated zinc (150 mg), the mixture was stirred for 3 h at room temperature and then filtered and washed with CH_2Cl_2 (150 mL). After careful addition of saturated $NaHCO_3$ solution, the mixture was stirred vigorously for 3 h. The organic layer was separated and dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. Purification by flash chromatography (toluene/acetone, 2:1) furnished **17** (124 mg, 63%) as a colourless foam. TLC (toluene/acetone, 2:1): R_f ≈ 0.14. $[\alpha]_D$ = −47.6 (c = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.22 (d, $^3J_{5,6}$ = 6.8 Hz, 6 H, 6b-CH_3), 1.75 (m, 2 H, $CH_2C\mathit{H}_2CH_2$), 1.86, 1.92, 1.93, 2.02, 2.07, 2.09, 2.17 (7 s, 42 H, 14 $COCH_3$), 3.30 (dt, ^2J = ^3J ≈ 7.9 Hz, 2 H, OCH_2), 3.39 (ddd, $^3J_{1,2}$ = $^3J_{2,3}$ = $^3J_{2,N}$ ≈ 9.7 Hz, 2 H, 2a-H), 3.65 (m, 2 H, 5a-H), 3.70 (dd, $^3J_{4,5}$ = $^3J_{5,6}$ = 7.1 Hz, 2 H, 5c-H), 3.88−3.94 (m, 6 H), HMQC: 3.89 (OCH_2), 3.92 (2b-H), 3.93 (3a-H), 4.00 (dd, $^3J_{3,4}$ = $^3J_{4,5}$ = 9.5 Hz, 2 H, 4a-H), 4.18 (d, $^3J_{1,2}$ = 8.3 Hz, 2 H, 1a-H), 4.21−4.30 (m, 4 H), HMQC: 4.26 (6a-H), 4.28 (6c-H), 4.59−4.64 (m, 6 H), HMQC: 4.61 (6′c-H), 4.62 (1c-H), CHHPh], 4.74 (d, ^2J = 12.2 Hz, 2 H, CHHPh), 4.84 (dd, $^3J_{2,3}$ = 10.4, $^3J_{3,4}$ = 3.5 Hz, 2 H, 3c-H), 4.89 (d, $^2J_{6,6}$ = 11.0 Hz, 2 H, 6′a-H), 5.00 (qu, $^3J_{5,6}$ = 6.6 Hz, 2 H, 5b-H), 5.10 (dd, $^3J_{1,2}$ = 8.2, $^3J_{2,3}$ = 10.3 Hz, 2 H, 2c-H), 5.24 (dd, $^3J_{2,3}$ = 10.7, $^3J_{3,4}$ = 3.3 Hz, 2 H, 3b-H), 5.34 (d, $^3J_{3,4}$ = 3.5 Hz, 2 H, 4c-H), 5.36 (d, $^3J_{3,4}$ = 3.4 Hz, 2 H, 4b-H), 5.48 (d, $^3J_{1,2}$ = 3.8 Hz, 2 H, 1b-H), 7.24−8.05 (m, 20 H, 4 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 15.89 (6b-C), 28.41 ($CH_2\mathit{C}H_2CH_2$), 60.56 (6c-C), 60.94 (2a-C), 62.18 (6a-C), 64.24 (5b-C), 65.55 (OCH_2), 66.59 (4c-C), 68.91 (2c-C), 70.74 (3b-C), 71.04 (3c-C, 5c-C), 72.16 (4b-C), 73.19 (5a-C), 73.29 (2b-C), 73.77 (CH_2Ph), 74.54 (4a-C), 74.99 (3a-C), 97.84 (1b-C), 100.42 (1c-C), 102.04 (1a-C). $C_{95}H_{118}N_2O_{44}\cdot H_2O$ (2009.98): calcd. C 56.77, H 6.02, N 1.39; found C 56.79, H 6.01, N 1.15.

1,3-Propylene Bis-*O*-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranoside (3): Palladium on carbon (100 mg, 10% Pd) was added to a solution of **17** (212 mg, 105 μmol) in methanol (30 mL) and acetic acid (2 drops), and the solution was stirred vigorously under a hydrogen atmosphere overnight. After filtration and concentration under reduced pressure, the residue was dissolved in dry methanol (50 mL), sodium methoxide (30 mg) was added, and the mixture was stirred overnight. The solution was neutralised with Amberlite IR120 (H^+) and filtered, and the solvents were evaporated. Purification by flash chromatography (RP-18; water/methanol, 1:0 → 9:1) afforded **3** (94 mg, 79%) as a colourless powder after lyophilization from water. TLC-RP18 (methanol/water, 1:1): R_f ≈ 0.86. $[\alpha]_D$ = −73.6 (c = 1.0, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.17 (d, $^3J_{5,6}$ = 6.8 Hz, 6 H, 6b-CH_3), 1.78 (t, 2 H, $CH_2C\mathit{H}_2CH_2$), 1.97 (s, 6 H, $COCH_3$), 3.30 (s, 6 H, OCH_3), 3.41−3.59 (m, 10 H), HMQC: 3.42 (5a-H), 3.45 (5c-H), 3.46 (3c-H), 3.50 (2c-H), 3.54 ($CH_2(C\mathit{H}H)_2$]], 3.63 (dd, $^3J_{1,2}$ = 3.9, $^3J_{2,3}$ = 10.2 Hz, 2 H, 2b-H), 3.66 (dd, $^3J_{5,6}$ = 5.2, $^2J_{6,6}$ = 11.4 Hz, 2 H, 6c-H), 3.71 (d, $^3J_{3,4}$ = 3.3 Hz, 2 H, 4b-H), 3.76 (dd, $^3J_{5,6'}$ = 6.9, $^2J_{6,6}$ = 11.4 Hz, 2 H, 6′c-H), 3.80 (d, $^3J_{3,4}$ = 3.3 Hz, 2 H, 4c-H), 3.81−3.95 (m, 14 H), HMQC: 3.81 (2a-H), 3.86 (3b-H), 3.87 ($CH_2(C\mathit{H}H)_2$], 3.87 (3a-H), 3.87 (4a-H), 3.92 (6a-H), 3.92 (6′a-H), 4.43 (d, $^3J_{1,2}$ = 7.5 Hz, 2 H, 1c-H), 4.47 (d, $^3J_{1,2}$ = 8.1 Hz, 2 H, 1a-H), 4.81 (m, 2 H, 5b-H), 5.02 (d, $^3J_{1,2}$ = 4.0 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (151 MHz, CD_3OD): δ = 16.62 (6b-C), 22.19 ($CO\mathit{C}H_3$), 31.13 ($CH_2\mathit{C}H_2CH_2$), 57.52 (2a-C), 61.45 (6a-C), 62.76 (6a-C), 67.06 ($CH_2(\mathit{C}H)_2$], 67.70 (5b-C), 69.95 (4c-C), 70.05 (2b-C), 71.24 (3b-C), 72.79 (2c-C), 73.70 (4b-C), 74.91 (3c-C), 75.27 (3a-C), 76.53 (4a-C), 76.63 (5c-C), 77.41 (5a-C), 100.32 (1b-C), 102.39 (1a-C), 103.86 (1c-C), 173.75 (COMe). MALDI-MS: m/z = 1124.6 [M + Na^+], 1140.5 [M + K^+]. $C_{43}H_{74}N_2O_{30}\cdot 2H_2O$ (1135.08): calcd. C 45.50, H 6.82, N 2.47; found C 45.18, H 6.90, N 1.99.

Methyl *O*-(4,6-Di-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-6-*O*-thexyldimethylsilyl-β-D-glucopyranoside (19): TDSCl (143 μL, 730 μmol) was added at 0 °C to a suspension of **18**[31] (270 mg, 608 μmol) and imidazole (71 mg, 910 μmol) in dry dimethylformamide (5 mL). The mixture was allowed to warm to room temp. and stirred overnight. After addition of saturated NaCl solution, the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the organic layer was dried with Na_2SO_4. Evaporation of the solvent under reduced pressure and purification of the residue by flash chromatography ($CHCl_3$/methanol, 19:1) afforded **19** (262 mg, 73%) as a colourless foam. TLC ($CHCl_3$/methanol, 9:1): R_f = 0.34.

$[\alpha]_D = -25.0$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.11, 0.12 (2 s, 6 H, $Si(CH_3)_2$], 0.84−0.89 [m, 12 H, $C(CH_3)_2$], 1.57−1.68 (m, 1 H, CH), 3.36−4.43 (m, 17 H, 1a-H, 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 1b-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, OCH_3), 5.53 (s, 1 H, CHPh), 7.36−7.50 (m, 5 H, C_6H_5). $C_{28}H_{46}O_{11}Si\cdot 0{\cdot}25H_2O$ (591.25): calcd. C 56.88, H 7.93; found C 56.72, H 7.67.

Methyl *O*-(2,3-Di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-β-D-glucopyranoside (20): Sodium hydride (50 mg, 2.1 mmol) and TBAI (≈ 10 mg) were added to a solution of **19** (203 mg, 343 μmol) in dry dimethylformamide (4 mL). The mixture was cooled to 0 °C, benzyl bromide (205 μL, 1.73 mmol) was added, and the mixture was allowed to warm to room temp. and stirred for 3 h. After careful addition of methanol, the reaction mixture was diluted with diethyl ether (200 mL) and washed with saturated $(NH_4)_2CO_3$ solution (2 × 50 mL) and water (50 mL). The organic layer was dried with Na_2SO_4, evaporated under reduced pressure and purified by flash chromatography (petroleum ether/ethyl acetate, 9:1 to 4:1) to afford **20** (305 mg, 93%) as a colourless foam. TLC (petroleum ether/ethyl acetate, 4:1): $R_f = 0.58$. $[\alpha]_D = +8.5$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.04, 0.05 (2 s, 6 H, $Si(CH_3)_2$], 0.79−0.85 [m, 12 H, $C(CH_3)_2$], 1.55−1.61 (m, 1 H, CH), 3.10−5.30 (m, 25 H, 1a-H, 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 1b-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, 4 CH_2Ph, OCH_3), 5.51 (s, 1 H, CHPh), 7.16−7.57 (m, 25 H, 5 C_6H_5). $C_{56}H_{70}O_{11}Si$ (947.25): calcd. C 71.01, H 7.45; found C 71.08, H 7.45.

Methyl *O*-(2,3-Di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-glucopyranoside (21): nBu$_4$NF (1 M in tetrahydrofuran, 320 μL) was added at room temperature to a solution of **20** (255 mg, 269 μmol) in dry tetrahydrofuran (10 mL), and the mixture was stirred for 16 h. Silica gel was added, and the solvent was removed under reduced pressure. Purification was achieved by flash chromatography (petroleum ether/ethyl acetate, 1:1 to 2:3), to afford **21** (213 mg, 97%) as a colourless foam. TLC (petroleum ether/ethyl acetate, 1:1): $R_f = 0.29$. $[\alpha]_D = +1.5$ ($c = 1.0$, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.83 (dd, $^3J_{OH,6} = 9.4$, $^3J_{OH,6'} = 4.4$ Hz, 1 H, OH), 3.14 (s, 1 H, 5b-H), 3.17 (m, 1 H, 5a-H), 3.28 (dd, $^3J_{1,2} = {}^3J_{2,3} = 8.5$ Hz, 1 H, 2a-H), 3.46 (s, 3 H, OCH_3), 3.49 (dd, $^3J_{2,3} = 9.4$, $^3J_{3,4} = 3.5$ Hz, 1 H, 3b-H), 3.55 (dd, $^3J_{2,3} = {}^3J_{3,4} = 9.0$ Hz, 1 H, 3a-H), 3.70−3.85 (m, 5 H), HMQC: 3.72 (6a-H), 3.73 (2b-H), 3.79 (6b-H), 3.81 (4a-H), 3.84 (6′a-H), 4.00 (d, $^3J_{3,4} = 3.8$ Hz, 1 H, 4b-H), 4.13 (dd, $^2J_{6,6} = 12.3$, $^3J_{5,6} < 1$ Hz, 1 H, 6′b-H), 4.26 (d, $^3J_{1,2} = 7.6$ Hz, 1 H, 1a-H), 4.49 (d, $^3J_{1,2} = 7.9$ Hz, 1 H, 1b-H), 4.64−4.80 (m, 7 H, 7 C*H*HPh), 5.09 (d, $^2J = 10.7$ Hz, 1 H, C*H*HPh), 5.38 (s, 1 H, CHPh), 7.09−7.45 (m, 25 H, 5 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 57.23 (OCH_3), 60.98 (6a-C), 66.49 (5b-C), 68.96 (6b-C), 73.73 (4b-C), 75.26 (5a-C), 77.55 (4a-C), 78.87 (2b-C), 79.88 (3b-C), 81.95 (2a-C), 82.69 (3a-C), 103.23 (1b-C), 104.68 (1a-C). $C_{48}H_{52}O_{11}\cdot 0{\cdot}75H_2O$ (818.44): calcd. C 70.44, H 6.59; found C 70.46, H 6.39.

Methyl *O*-(2,3-Di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-methylthiomethyl-β-D-glucopyranoside (22): A mixture of acetic anhydride/acetic acid [5.6:1 (v,v), 1.2 mL] was added to a solution of **21** (406 mg, 504 μmol) in dimethyl sulfoxide (1.5 mL).[24] The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed with saturated $NaHCO_3$ solution (50 mL). The organic layer was dried and concentrated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 2:1) afforded **22** (257 mg, 59%) as colourless crystals. TLC (petroleum ether/ethyl acetate, 2:1): $R_f = 0.42$. M. p. 150−152 °C. $[\alpha]_D = +22.3$ ($c = 1.0$, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 2.04 (s, 3 H, SCH_3), 3.21−4.92 (m, 26 H, 1a-H, 2a-H, 3a-H, 4a-H, 5a-H, 2 6a-H, 1b-H, 2b-H, 3b-H, 4b-H, 5b-H, 2 6b-H, 7 C*H*HPh, OCH_2S, OCH_3), 5.17 (d, $^2J = 10.8$ Hz, 1 H, C*H*HPh), 5.49 (s, 1 H, CHPh), 7.18−7.55 (m, 25 H, 5 C_6H_5). $C_{50}H_{56}SO_{11}$ (865.05): calcd. C 69.42, H 6.52; found C 69.44, H 6.36.

Formaldehyde Bis[methyl *O*-(2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-glucopyranosid-6-yl] Acetal (23): A solution of acceptor **21** (176 mg, 215 μmol), donor **20** (242 mg, 280 μmol) and NIS (77 mg, 344 μmol) in dry CH_2Cl_2 (5 mL) in the presence of molecular sieves (AW-300) was stirred for 30 min at room temperature. After the mixture had been cooled to 0 °C, TfOH (≈ 0.5 μL) was added, and the solution was stirred for 30 min. After addition of NEt_3 and CH_2Cl_2 (200 mL), filtration and washing with dilute $Na_2S_2O_3$ solution, the organic layer was dried and concentrated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 1:1; long column) afforded **23** (254 mg, 73%) as a colourless solid. TLC (petroleum ether/ethyl acetate, 1:1): $R_f = 0.30$. $[\alpha]_D = +23.1$ ($c = 1.0$, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ = 3.18 (s, 2 H, 5b-H), 3.37−3.40 (m, 4 H), HMQC: 3.37 (5a-H), 3.38 (2a-H), 3.51 (s, 6 H, OCH_3), 3.57 (dd, $^3J_{2,3} = 9.7$, $^3J_{3,4} = 3.8$ Hz, 2 H, 3b-H), 3.64 (dd, $^3J_{2,3} = {}^3J_{3,4} \approx 8.8$ Hz, 2 H, 3a-H), 3.80−3.87 (m, 10 H), HMQC: 3.81 (6a-H), 3.81 (2b-H), 3.85 (4a-H), 3.86 (6′a-H), 3.86 (6b-H), 4.06 (dd, $^3J_{3,4} = 3.8$ Hz, 2 H, 4b-H), 4.22 (d, $^2J_{6,6} = 12.6$, $^3J_{5,6} < 1$ Hz, 2 H, 6′b-H), 4.30 (d, $^3J_{1,2} = 7.9$ Hz, 2 H, 1a-H), 4.55 (d, $^3J_{1,2} = 7.9$ Hz, 2 H, 1b-H), 4.58 (s, 2 H, OCH_2O), 4.71−4.88 (m, 14 H, C*H*HPh), 5.17 (d, $^2J = 10.9$ Hz, 2 H, C*H*HPh), 5.45 (s, 2 H, CHPh), 7.19−7.34 (m, 50 H, 10 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 56.94 (OCH_3), 66.29 (6a-C), 66.41 (5b-C), 68.98 (6b-C), 73.61 (4b-C), 74.95 (5a-C), 77.68 (2b-C), 78.90 (4a-C), 80.08 (3b-C), 81.87 (2a-C), 82.96 (3a-C), 96.39 (OCH_2S), 102.86 (1b-C), 104.47 (1a-C). $C_{97}H_{104}O_{22}$ (1621.88): calcd. C 71.83, H 6.46; found C 71.54, H 6.47.

Formaldehyde Bis[methyl *O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosid-6-yl] Acetal (5): Palladium on carbon (160 mg, 10% Pd) was added to a solution of **23** (269 mg, 166 μmol) in methanol (20 mL) and acetic acid (2 drops), and the solution was stirred vigorously under a hydrogen atmosphere overnight. After filtration and washing through with hot methanol, silica gel was added and the suspension was evaporated under reduced pressure. The residue was purified by flash chromatography ($CHCl_3$/methanol/water, 60:40:10 to 60:45:10) to give **5** (100 mg, 79%) as a colourless powder after lyophilization from water. HPTLC ($CHCl_3$/methanol/water, 60:40:10): $R_f = 0.25$. $[\alpha]_D = -3.9$ ($c = 1.0$, H_2O). ^1H NMR (600 MHz, D_2O): δ = 3.25 (dd, $^3J_{1,2} = {}^3J_{2,3} = 8.6$ Hz, 2 H, 2a-H), 3.47 (dd, $^3J_{1,2} = 7.7$, $^3J_{2,3} = 9.9$ Hz, 2 H, 2b-H), 3.50 (s, 6 H, OCH_3), 3.55−3.61 (m, 6 H), HMQC: 3.57, 3.58 (3a-H, 4a-H), 3.58 (3b-H), 3.62−3.72 (m, 8 H), HMQC: 3.63 (5b-H), 3.66 (5a-H), 3.67 (6b-H), 3.70 (6′b-H), 3.76 (dd, $^3J_{5,6} = 5.3$, $^2J_{6,6} = 11.4$ Hz, 2 H, 6a-H), 3.85 (d, $^3J_{3,4} = 3.5$ Hz, 2 H, 4b-H), 4.33 (dd, $^3J_{5,6'} = 2.0$, $^2J_{6,6} = 11.4$ Hz, 2 H, 6′a-H), 4.32 (d, $^3J_{1,2} = 7.8$ Hz, 2 H, 1b-H), 4.33 (d, $^3J_{1,2} = 8.1$ Hz, 2 H, 1a-H), 4.77 (s, 2 H, OCH_2O) ppm. ^{13}C NMR (151 MHz, D_2O): δ = 56.84 (OCH_3), 60.55 (6b-C), 65.90 (6a-C), 68.03 (4b-C), 70.44 (2b-C), 72.05 (3b-C), 72.26 (2a-C), 73.09 (5a-C), 73.89 (3/4a-C), 74.90 (5b-C), 78.01 (3/4a-C), 95.41 (OCH_2O), 102.62, 102.69 (1a-C, 1b-C). $C_{27}H_{48}O_{22}\cdot 2H_2O$ (760.69): calcd. C 42.63, H 6.89; found C 42.32, H 6.65.

Formaldehyde [Methyl *O*-(2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranosid-6-yl]-[methyl *O*-(2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galacto-pyr-

anosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-glucopyranosid-6-yl] Acetal (24): A solution of acceptor **13** (70 mg, 75 μmol), donor **22** (97 mg, 112 μmol) and NIS (30 mg, 135 μmol) in dry CH_2Cl_2 (3 mL) in the presence of molecular sieves (AW-300) was stirred for 30 min at room temperature. The mixture was cooled to 0 °C, TfOH (≈ 0.5 μL) was added, and the solution was stirred for 20 min. After addition of NEt_3 and CH_2Cl_2 (150 mL), filtration and washing with diluted $Na_2S_2O_3$ solution, the organic layer was dried and concentrated in vacuo. Purification by flash chromatography (toluene/acetone, 7:2) afforded **22** (108 mg, 82%) as a colourless solid. TLC (toluene/acetone, 3:1): R_f = 0.33. $[\alpha]_D$ = +1.5 (c = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.15 (d, $^3J_{5,6}$ = 6.4 Hz, 3 H, 6b-CH_3), 1.84, 1.89, 1.98, 2.05, 2.10, 2.11 (6 s, 18 H, 6 $COCH_3$), 3.22 (s, 3 H, OCH_3), 3.32 (s, 1 H, 5e-H), 3.39 (dd, $^3J_{1,2}$ = $^3J_{2,3}$ ≈ 8.5 Hz, 1 H, 2d-H), 3.43 (m, 1 H, 5d-H), 3.53 (m, 1 H, 5a-H), 3.54 (s, 3 H, OCH_3), 3.60 (dd, $^3J_{2,3}$ = 9.6, $^3J_{3,4}$ = 3.7 Hz, 1 H, 3e-H), 3.64 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ = 8.9 Hz, 1 H, 3d-H), 3.67 (m, 1H, 6a-H), 3.74−3.98 (m, 12 H), HMQC: 3.75 (6c-H), 3.79 (6′c-H), 3.79 (6d-H), 3.80 (5c-H), 3.81 (2e-H), 3.83 (4d-H), 3.84 (6′a-H), 3.86 (2b-H), 3.90 (6e-H), 3.95 (3a-H), 3.95 (4a-H), 3.97 (6′d-H), 4.12 (d, $^3J_{3,4}$ = 3.8 Hz, 1 H, 4e-H), 4.24 (d, $^2J_{6,6}$ = 11.4 Hz, 1 H, 6′e-H), 4.30 (ddd, $^3J_{1,2}$ = 4.0, $^3J_{2,3}$ = $^3J_{2,N}$ ≈ 9.4 Hz, 1 H, 2a-H), 4.33 (d, $^3J_{1,2}$ = 7.8 Hz, 1 H, 1d-H), 4.40−4.86 (m, 17 H), HMQC: 4.51 (1e-H), 4.57 (OC*H*HO), 4.62 (1a-H), 4.65 (1c-H), 4.68 (OCH*H*O), 4.78 (5b-H), 11 C*H*HPh], 4.99 (dd, $^3J_{2,3}$ = 10.3, $^3J_{3,4}$ = 3.6 Hz, 1 H, 3c-H), 5.07 (dd, $^3J_{1,2}$ = 8.0, $^3J_{2,3}$ = 10.3 Hz, 1 H, 2c-H), 5.21 (d, ^2J = 10.9 Hz, 1 H, C*H*HPh), 5.23 (dd, $^3J_{2,3}$ = 10.5, $^3J_{3,4}$ = 3.5 Hz, 1 H, 3b-H), 5.28 (d, $^3J_{3,4}$ = 3.7 Hz, 1 H, 4b-H), 5.34 (d, $^3J_{1,2}$ = 3.8 Hz, 1 H, 1b-H), 5.47 (s, 1 H, CHPh), 5.51 (d, $^3J_{3,4}$ = 3.7 Hz, 1 H, 4c-H), 5.94 (d, $^3J_{2,N}$ = 9.1 Hz, 1 H, NH), 7.17−7.54 (m, 35 H, 7 C_6H_5) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, excerpt): δ = 15.86 (6b-C), 53.51 (2a-C), 55.07 (OCH_3), 56.89 (OCH_3), 64.43 (5b-C, 6a-C), 65.80 (6d-C), 66.42 (5e-C), 66.99 (6c-C), 67.34 (4c-C), 68.98 (6e-C), 69.70 (5c-C), 73.39 (4e-C), 73.61, 73.75, 73.89 (3a-C, 4a-C, 2b-C), 74.83 (5d-C), 78.15 (4d-C), 78.90 (2e-C), 80.20 (3e-C), 81.90 (2d-C), 82.92 (3d-C), 95.48 (OCH_2O), 97.47 (1b-C), 98.02 (1a-C), 99.91 (1c-C), 103.21 (1e-C), 104.47 (1d-C). $C_{94}H_{111}NO_{31}$ (1750.90): calcd. C 64.48, H 6.39, N 0.80; found C 64.12, H 6.34, N 0.49.

Formaldehyde [Methyl *O*-(β-D-galactopyranosyl)-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-glucopyranosid-6-yl]-[methyl *O*-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosid-6-yl] Acetal (6): Compound **24** (162 mg, 92.5 μmol) was dissolved in methanol (20 mL) and acetic acid (2 drops). Palladium on carbon (42 mg, 10% Pd) was added, and the solution was stirred vigorously under a hydrogen atmosphere overnight. The catalyst was filtered off and washed with hot methanol. After evaporation in vacuo, the residue was dissolved in methylamine (33% in ethanol, 10 mL) and stirred overnight. The mixture was concentrated in vacuo and purified by flash chromatography ($CHCl_3$/methanol/water, 60:40:10 to 60:45:10) to give **6** (69.2 mg, 82%) as a colourless powder after lyophilization from water. HPTLC ($CHCl_3$/methanol/water, 60:40:10): R_f = 0.13. $[\alpha]_D$ = −0.3 (c = 1.0, CH_3OH). ^{1}H NMR (600 MHz, CD_3OD, 310 K): δ = 1.17 (d, $^3J_{5,6}$ = 6.6 Hz, 3 H, 6b-CH_3), 1.91 (s, 3 H, $COCH_3$), 3.23 (dd, $^3J_{1,2}$ = $^3J_{2,3}$ ≈ 8.3 Hz, 1 H, 2d-H), 3.38 (s, 3 H, 1a-OCH_3), 3.45 (dd, $^3J_{4,5}$ = $^3J_{5,6}$ ≈ 6.1 Hz, 1 H, 5c-H), 3.48−3.60 (m, 11 H), HMQC: 3.50 (3c-H), 3.51 (3d-H), 3.51 (2c-H), 3.51 (1d-OH_3), 3.53 (3e-H), 3.54 (2e-H), 3.56 (4d-H), 3.56 (5d-H), 3.60 (5e-H), 3.62 (dd, $^3J_{1,2}$ = 4.0, $^3J_{2,3}$ = 10.4 Hz, 1 H, 2b-H), 3.67−3.94 (m, 12 H), HMQC: 3.68 (6c-H), 3.71 (4b-H), 3.71 (6e-H), 3.77 (6′c-H), 3.77 (6′e-H), 3.81 (4c-H), 3.81 (5a-H), 3.83 (4e-H), 3.85 (3b-H), 3.87 (6a-H), 3.91 (6d-H), 3.92 (4a-H), 3.96 (dd, $^3J_{2,3}$ = $^3J_{3,4}$ ≈ 9.7 Hz, 1 H, 3a-H), 4.00 (dd, $^2J_{6,6}$ = 11.4, $^3J_{5,6'}$ = 1.7 Hz, 1 H, 6′d-H), 4.06 (dd, $^2J_{6,6}$ = 11.6, $^3J_{5,6'}$ = 3.6 Hz, 1 H, 6′a-H), 4.20 (d, $^3J_{1,2}$ = 7.8 Hz, 1 H, 1d-H), 4.24 (dd, $^3J_{1,2}$ = 3.3, $^3J_{2,3}$ = 10.2 Hz, 1 H, 2a-H), 4.33 (d, $^3J_{1,2}$ = 7.4 Hz, 1 H, 1e-H), 4.40 (d, $^3J_{1,2}$ = 6.6 Hz, 1 H, 1c-H), 4.55 (d, $^3J_{1,2}$ = 3.3 Hz, 1 H, 1a-H), 4.81 (dd, 2 H, OCH_2O), 4.84 (d, $^3J_{1,2}$ = 8.7 Hz, 1 H, 5b-H), 5.02 (d, $^3J_{1,2}$ = 3.8 Hz, 1 H, 1b-H) ppm. ^{13}C NMR (151 MHz, CD_3OD, 310 K): δ = 16.60 (6b-C), 26.33 ($CO*C*H_3$), 55.14 (2a-C), 55.66 (1a-OCH_3), 57.38 (1d-OCH_3), 62.49 (6c-C), 62.82 (6e-C), 67.21 (6a-C), 67.61 (5b-C), 67.94 (6d-C), 69.96, 69.99 (2b-C, 4c-C), 70.28 (4e-C), 71.25 (3b-C), 72.42 (5a-C), 72.62 (5d-C), 72.86 (3c-C), 73.70 (4b-C), 74.58, 74.66 (3a-C, 2d-C), 74.77, 74.82 (3d-C, 3e-C), 75.59 (4a-C, 4d-C), 76.41 (2c-C), 76.74 (5c-C), 77.08 (5e-C), 80.84 (2e-C), 97.56 (OCH_2O), 100.26 (1a-C), 100.38 (1b-C), 104.06 (1c-C), 105.20 (1d-C, 1e-C), 173.83 (COMe). MALDI-MS: m/z = 935.0 [M + Na^+], 951.3 [M + K^+]. $C_{35}H_{61}NO_{26}$ (911.85).

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