Synthesis of New Galβ1→4Fuc Segments Useful for Biological Investigations

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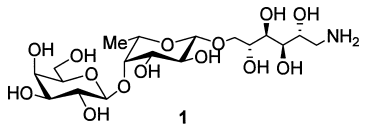
Useful segments (1, 2) for chemical probes embedded in a Galβ1→4Fuc unit were designed and prepared for characterizing sugar-binding proteins in *Caenorhabditis elegans*. Segment 1 with an amino group terminus was used as a recognition unit in affinity chromatography. It was revealed that some proteins (annexins and galectins) in *C. elegans* have an affinity for Galβ1→4Fuc.

Key words galectin; annexin; chemical probe; affinity chromatography

Galectins constitute the carbohydrate-recognition domain responsible for β-galactoside binding.[1—3] Galectins bind to cell-surface and extracellular-matrix glycans and thereby affect a variety of cellular processes (*e.g.*, immunity and tumor metastasis).[4] It is known that a Galβ1→4GlcNAc (*N*-acetyllactosamine) unit is the endogenous glycoepitope recognized by vertebrate galectins.[5—8] However, the existence of a glycan containing *N*-acetyllactosamine units has not been confirmed in *Caenorhabditis elegans*.[9—14] In the course of investigation of endogenous counterpart glycans of galectins of *C. elegans*, we found that the motif recognized by LEC-6, one galectin of *C. elegans*, is Galβ1→4Fuc, instead of Galβ1→4GlcNAc.[15—17] Other galectins of *C. elegans*, LEC-1 and LEC-10, were also found to have an affinity for Galβ1→4Fuc.[16,18] These findings indicate that the Galβ1→4Fuc unit is a principal disaccharide recognized by *C. elegans* galectins. Therefore, it appeared possible that Galβ1→4Fuc would serve as a recognition motif for other sugar-binding proteins in *C. elegans*. Although the sugar-binding abilities of various families of carbohydrate-binding proteins in *C. elegans*, such as galectins, C-type lectins, annexins *etc.*, have been demonstrated,[6,8,19—21] the Galβ1→4Fuc-binding ability of most of these proteins has not been reported. Synthetic probes containing Galβ1→4Fuc are regarded as good tools for characterizing sugar-binding proteins in *C. elegans*. In the present study, we describe the preparation of Galβ1→4Fuc derivatives with an amino group (**1**) or mercapto group (**2**) *via* a linker, which could be utilized as recognition units in probes for sugar-binding proteins (Fig. 1). Using compound **1**, we prepared a Sepharose derivative containing immobilized Galβ1→4Fuc and attempted to identify proteins adsorbed by this adsorbent. We found that various galectins and annexins have an affinity for Galβ1→4Fuc.



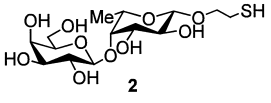


Fig. 1. Structures of the Galβ1→4Fuc Segment with an Amino Terminus (**1**) or a Mercapto Terminus (**2**)

Results and Discussion

For the synthesis of a Galβ1→4Fuc derivative with an amino terminus (**1**), we planned to utilize commercially available D-mannitol as a water-soluble spacer moiety (Chart 1). Primary hydroxyl groups of D-mannitol were protected as TBDMS (*tert*-butyldimethylsilyl) ethers to give the tetraol **3**.[17] Peracetylation of **3**, followed by selective deprotection of the TBDMS group, afforded the alcohol **5**.[17] To introduce the amino group, **5** was converted into a tosylate (**6**), which was subjected to azidation to give **7**. The TBDMS group of **7** was deprotected to form the agent **8**. In the presence of TMSOTf (trimethylsilyltrifluoromethane sulfonate), coupling of the acceptor **8** with the Galβ1→4Fuc donor **9**[17] proceeded smoothly to give only the β-linked product (**10**). Finally, deprotection of acetyl groups and successive reduction afforded the target **1**.

The synthesis of sulfanylethyl Galβ1→4Fuc **2** started from Galβ1→4Fuc **9**[17] by glycosylation with 2,2′-dithiodiethanol. Compared with the previous synthesis of thioalkyl glycosides,[22,23] using the disulfide acceptor 2,2′-dithiodiethanol provides easier access to the thiol terminus. The reaction proceeded smoothly to provide the disulfide (**12**) β-selectively. Reduction of **12** afforded the thiol (**13**) in good yield. Deacylation with sodium methoxide in MeOH afforded the desired target (**2**) efficiently (Chart 2).

After we synthesized the target segments (**1**, **2**), we used compound **1** as a recognition unit in affinity chromatography.[24] Compound **1** was immobilized onto NHS-activated Sepharose (GE Healthcare, St. Giles, U.K.), and the immobilized Galβ1→4Fuc adsorbent was packed in a disposable column. The extract of *C. elegans* was applied to this column to capture glycoproteins interacting with the Galβ1→4Fuc unit. Three annexins (NEX-1, -2, -3) in addition to galectins (LEC-1, -2, -4, -6, -9, -10, -12) were found to have affinity for Galβ1→4Fuc. Therefore, the immobilized Galβ1→4Fuc column proved to be useful for the capture of carbohydrate-binding proteins that interact with this unique sugar structure.

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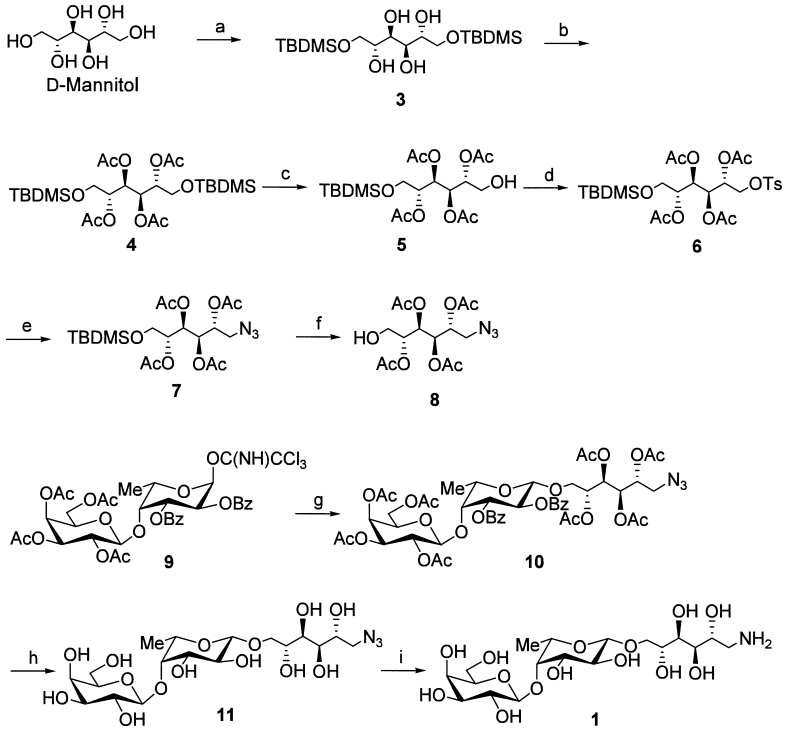
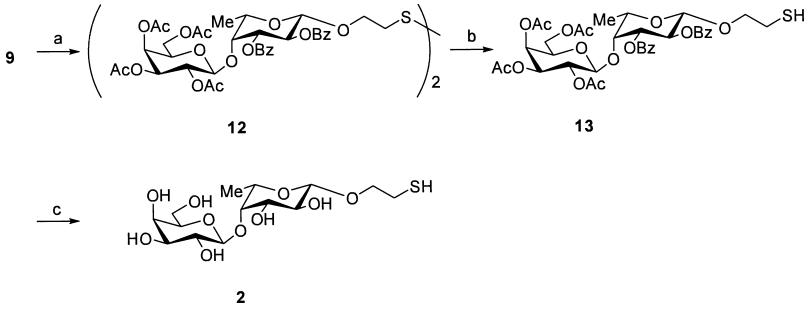


Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 79%; (b) Ac_2O, pyridine, rt, 88%; (c) 1% I_2-MeOH solution, rt, 42%; (d) *p*-TsCl, DMAP, pyridine, 60 °C, 72%; (e) NaN_3, DMF, 60 °C, 92%; (f) HF-pyridine, pyridine-THF, rt, 60%; (g) **8**, TMSOTf, CH_2Cl_2, MS4A, 0 °C, 70%; (h) NaOMe, MeOH, rt, 67%; (i) H_2, $Pd(OH)_2$, MeOH, rt, 99%.

Chart 1. Synthesis of Compound **1**



Reagents and conditions: (a) 2,2′-Dithiodiethanol, TMSOTf, CH_2Cl_2, MS4A, 0 °C, 84%; (b) Zn, HCl, EtOH, CH_2Cl_2, rt, 88%; (c) NaOMe, MeOH, rt, 96%.

Chart 2. Synthesis of Compound **2**

Because the terminus sulfanyl group of segment **2** is suitable for modification with amino acid residues in proteins, it could be used as a tool for preparing neoglycoconjugates.[25] An in-depth examination of this will be reported by our group in the future.

Conclusion

We synthesized two types of Galβ1→4Fuc-embedded segments (**1**, **2**) that could be utilized as recognition units in probes for sugar-binding proteins. We prepared an immobilized Galβ1→4Fuc adsorbent from compound **1** and identified proteins that have an affinity for Galβ1→4Fuc.

Experimental

General Procedures All reactions sensitive to air or moisture were conducted under an argon atmosphere. Materials were obtained from commercial suppliers. All anhydrous solvents were purified according to standard methods. The NMR spectra (^{1}H and ^{13}C) were determined on a JEOL 600 MHz (ECP-600) or 400 MHz (AL-400) spectrometer, using $CDCl_3$ (with tetramethylsilane (TMS) for ^{1}H-NMR and chloroform-*d* for ^{13}C-NMR as the internal reference) solution, unless the otherwise noted. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane as an internal standard and coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), broad (br). High resolution-mass spectra (HR-MS) were obtained on LCMS-IT-Time-of-Flight (TOF) (Shimadzu, Kyoto, Japan) for electrospray ionization (ESI). Sodium trifluoroacetate (TFA-Na) was used as internal standard for high resolution MS. Optical rotations were determined using a DIP-370 (Shimadzu, Kyoto, Japan) digital polarimeter in 100-mm cells and the sodium D line (589 nm) at room temperature in the solvent and concentration indicated. Infrared spectra (IR) were recorded on a JASCO Fourier Transform (FT)/IR-410 spectrometer using sodium chloride plates or potassium bromide pellets. Absorbance frequencies are recorded in reciprocal centimeters (cm^{-1}). Analytical thin layer chromatography was carried out using Merck silica gel 60 F_{254}. Column chromatography was carried out on Wakogel C-300 (45—60 μm), NH silicagel (Chromatorex® FujiSilysia

Chemical, 100—200 mesh). Reverse phase Column chromatography utilized ODS-SS10200T.

2,3,4,5-Tetra-*O*-acetyl-6-*O*-(*t*-butyldimethylsilyl)-1-*O*-(*p*-toluenesulfonyl)-D-mannitol (6) To a solution of **5** (1.2 g, 2.58 mmol) in pyridine (26 ml) were added *p*-TsCl (2.5 g, 12.9 mmol) and DMAP (158 mg, 1.29 mmol) at room temperature. The mixture was stirred at 60 °C for 12 h and water was added. After extraction with EtOAc, the organic phase was dried over Na_2SO_4, and the solvent was removed. The residue was purified by column chromatography (eluent hexane : EtOAc=4 : 1) to give **6** (1.2 g, 1.88 mmol, 72%) as a clear yellow oil. $[\alpha]_D^{24}$ −19.9 (c=1.0 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.76 (2H, d, J=8.4), 7.33 (2H, d, J=8.4), 5.42 (2H, m, H3, H4), 4.98 (1H, m, H5), 4.90 (1H, m, H2), 4.16 (1H, dd, J=3.0, 10.8, H1), 4.02 (1H, dd, J=4.2, 10.8, H1′), 3.69 (1H, dd, J=3.6, 11.4, H6), 3.57 (1H, dd, J=4.8, 11.4, H6′), 2.44 (3H, s, –PhC$\underline{H}_3$), 2.04 (3H, s, –COC$\underline{H}_3$), 2.03 (3H, s, –COC$\underline{H}_3$), 2.02 (3H, s, –COC$\underline{H}_3$), 1.99 (3H, s, –COC$\underline{H}_3$), 0.85 (9H, s, –Si(CH$_3$)$_2$C(C$\underline{H}_3$)$_3$), 0.00 (3H, s, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$), −0.01 (3H, s, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.0, 169.7, 169.4 (×2), 145.0, 132.6, 129.8, 128.1, 70.6 (C5), 67.6 (C2), 67.5 (C3), 67.4 (C4), 66.9 (C1), 61.3 (C6), 25.6 (×3, –Si(CH$_3$)$_2$C(C$\underline{H}_3$)$_3$), 21.0, 20.7, 20.6, 20.6, 18.1 (–Si(CH$_3$)$_2$C(CH$_3$)$_3$), −5.6 (×2, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$); IR (neat) 2955, 2930, 2857, 1752, 1370, 1217, 1030, 837 cm^{-1}; HR-MS (ESI) Calcd for $C_{27}H_{42}O_{12}SSi$ [M+Na]$^+$: 641.2058 Found 641.2043.

2,3,4,5-Tetra-*O*-acetyl-1-azide-6-*O*-(*t*-butyldimethylsilyl)-1-deoxy-D-mannitol (7) To a solution of **6** (793 mg, 1.28 mmol) in DMF (12.8 ml) was added NaN_3 (125 mg, 1.92 mmol) at room temperature. The suspension was stirred for 12 h at 60 °C. The mixture was diluted with H_2O and was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by column chromatography (hexane : EtOAc=1 : 3) to give **7** (574 mg, 1.17 mmol, 92%) as a clear oil. $[\alpha]_D^{24}$ −23.7 (c=1.0 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.43 (2H, m, H3, H4), 5.01 (1H, m, H2), 4.95 (1H, m, H5), 3.69 (1H, dd, J=3.6, 11.4, H6), 3.58 (1H, dd, J=5.4, 11.4, H6′), 3.47 (1H, dd, J=3.6, 13.2, H1), 3.26 (1H, dd, J=5.6, 13.2, H1′), 2.08 (3H, s, –COC$\underline{H}_3$), 2.07 (3H, s, –COC$\underline{H}_3$), 2.05 (3H, s, –COC$\underline{H}_3$), 2.04 (3H, s, –COC$\underline{H}_3$), 0.86 (9H, s, –Si(CH$_3$)$_2$C(C$\underline{H}_3$)$_3$), 0.01 (3H, s, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$), 0.00 (3H, s, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.7, 169.9, 169.8, 169.5, 70.7 (C5), 68.7 (C2), 68.3 (C3), 67.7 (C4), 61.5 (C6), 50.7 (C1), 25.7 (×3, –Si(CH$_3$)$_2$C(C$\underline{H}_3$)$_3$), 21.0, 20.8, 20.7, 20.7, 18.1 (–Si(CH$_3$)$_2$C(CH$_3$)$_3$), −5.6 (×2, –Si(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$); IR (neat) 2955, 2931, 2858, 2106, 1753, 1371, 1220, 1062, 1033, 839, 779 cm^{-1}; HR-MS (ESI) Calcd for $C_{20}H_{35}O_9N_3Si$ [M+Na]$^+$: 512.2035 Found 512.2044.

2,3,4,5-Tetra-*O*-acetyl-1-azido-1-deoxy-D-mannitol (8) **7** (270 mg, 0.55 mmol) was dissolved in pyridine (3.7 ml) and tetrahydrofuran (THF) (1.8 ml), then 30% HF-pyridine solution (1.84 ml) was added. After being stirred for 10 min, the mixture was poured into saturated $NaHCO_3$, and the desired compound was extracted with EtOAc, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography (Et_2O : CH_2Cl_2=1 : 1) to give **8** (124 mg, 0.33 mmol, 60%) as a clear oil. $[\alpha]_D^{24}$ 19.9 (c=1.0 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 5.45 (1H, dd, J=1.8, 9.6, H3), 5.38 (1H, dd, J=1.8, 9.6, H4), 5.15 (1H, m, H2), 4.83 (1H, m, H5), 3.73 (1H, br, H6), 3.52 (1H, br, H6′), 3.45 (1H, dd, J=3.6, 13.8, H1), 3.28 (1H, dd, J=6.0, 13.8, H1′), 2.48 (1H, br, OH), 2.15 (3H, s, –COC$\underline{H}_3$), 2.11 (6H, s, –COC$\underline{H}_3$), 2.10 (3H, s, –COC$\underline{H}_3$); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 171.6, 170.2, 169.8, 169.7, 69.9 (C5), 68.4 (C2), 68.2 (C3), 67.5 (C4), 60.5 (C6), 51.0 (C1), 21.0, 20.7, 20.7, 20.6; IR (neat) 2977, 2941, 2107, 1748, 1372, 1216, 1053 cm^{-1}; HR-MS (ESI) Calcd for $C_{14}H_{21}O_9N_3$ [M+Na]$^+$: 398.1170 found 398.1171.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-L-fucopyranosyl-(1→6)-2,3,4,5-tetra-*O*-acetyl-1-deoxy-1-azido-D-mannitol (10) A solution of **8** (88 mg, 0.23 mmol) and **9** (297 mg, 0.35 mmol) in dry CH_2Cl_2 (3.5 ml) was stirred with activated molecular sieves 4 A (180 mg) at room temperature for 1 h. Then the reaction mixture was cooled to 0 °C, and TMSOTf (32 μl, 0.18 mmol) was added. After 60 min, the reaction mixture was neutralized with saturated aq $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by column chromatography (toluene/EtOAc=2 : 1) to give **10** (173 mg, 0.16 mmol, 70%) as a clear oil. $[\alpha]_D^{24}$ −64.6 (c=0.3 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.03 (2H, dd, J=1.2, 8.4), 7.97 (2H, dd, J=1.2, 8.4), 7.51 (2H, m), 7.38 (4H, m), 5.61 (1H, dd, J=8.4, 10.8, Fuc H2), 5.39 (1H, dd, J=2.4, 7.8, H4), 5.32 (1H, dd, J=2.4, 8.4, H3), 5.26 (1H, dd, J=7.8, 10.8, Gal H2), 5.16 (1H, dd, J=0.6, 3.6, Gal H4), 5.10 (1H, dd, J=3.6, 10.8, Fuc H3), 5.02 (1H, m, H5), 4.98 (1H, m, H2), 4.93 (1H, dd, J=3.6, 10.8, Gal H3), 4.65 (1H, d, J=8.4, Fuc H1), 4.42 (1H, d, J=7.8, Gal H1), 4.19 (1H, d, J=3.6, Fuc H4), 3.77—3.84 (3H, m, H6, H6′, Fuc H5), 3.61 (1H, m, Gal H5), 3.41 (1H, dd, J=3.6, 13.8, H1), 3.22 (1H, dd, J=6.0, 13.8, H1′), 3.15 (2H, m, Gal H6, Gal H6′), 2.12 (3H, s, –COC$\underline{H}_3$), 2.07 (3H×2, s, –COC$\underline{H}_3$), 2.06 (3H, s, –COC$\underline{H}_3$), 2.04 (3H, s, –COC$\underline{H}_3$), 1.97 (3H, s, –COC$\underline{H}_3$), 1.93 (3H, s, –COC$\underline{H}_3$), 1.82 (3H, s, –COC$\underline{H}_3$), 1.37 (1H, d, J=6.0, Fuc Me); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.2, 170.1, 170.0×2, 169.8, 169.7, 169.3, 168.9, 166.0, 164.9, 133.2×2, 133.0×2, 130.1×2, 129.8×2, 128.3×2, 128.2×2, 101.8 (Fuc C1), 100.4 (Gal C1), 75.4 (Fuc C4), 73.1 (Fuc C3), 70.8 (Gal C3), 70.5 (Gal C5), 70.2 (C6), 69.2 (Gal C2), 68.8 (C5), 68.6 (C3), 68.6 (Fuc C4), 68.5 (C2), 67.9 (C4), 66.7 (Gal C4), 65.4 (Fuc C5), 60.3 (Gal C6), 50.5 (C1), 20.8, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5, 20.5, 16.0 (Fuc C6); IR (neat) 3025, 2984, 2939, 2106, 1748, 1371, 1282, 1219, 1111, 1071, 757, 714 cm^{-1}; HR-MS (ESI) Calcd for $C_{48}H_{57}O_{24}N_3$ [M+Na]$^+$: 1082.3224 Found 1082.3219.

β-D-Galactopyranosyl-(1→4)-β-L-fucopyranosyl-(1→6)-1-deoxy-1-azido-D-mannitol (11) To a solution of **10** (89 mg, 0.084 mmol) and NaOMe (68 mg, 1.26 mmol) in MeOH (0.28 ml) was stirred at room temperature for 12 h. Then the mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2/MeOH=1 : 1) to give **11** (29 mg, 0.06 mmol, 67%) as a clear oil. $[\alpha]_D^{23}$ −1.2 (c=0.3 in MeOH); ^{1}H-NMR (600 MHz, D_2O) δ: 4.32 (2H, m, Gal H1, Fuc H1), 3.92 (1H, m, H3), 3.88 (1H, d, J=3.0, Fuc H4), 3.74 (5H, m, H2, H4, H5, Gal H4, Fuc H5), 3.56 (7H, m, Gal H6, Gal H6′, Gal H5, Gal H3, Fuc H2, Fuc H3, H1), 3.40 (2H, m, GalH2, H6), 3.36 (1H, dd, J=6.6, 13.2, H1′), 3.29 (1H, dd, J=6.6, 12.6, H6′), 1.21 (3H, d, J=6.6, Fuc Me); ^{13}C-NMR (150 MHz, D_2O) δ: 103.4, 104.0, 79.8, 75.8, 73.0, 72.7, 72.0, 71.9, 71.5, 70.3, 70.2, 69.9, 69.3, 69.2, 67.2, 61.8, 54.6, 16.0; HR-MS (ESI) Calcd for $C_{18}H_{33}O_{14}N_3$ [M−H]$^-$: 514.1890 Found 514.1883.

β-D-Galactopyranosyl-(1→4)-β-L-fucopyranosyl-(1→6)-1-deoxy-1-amino-D-mannitol (1) To a solution of **11** (51 mg, 0.10 mmol) in MeOH (0.3 ml) was added $Pd(OH)_2$ (20.0 mg). The reaction was stirred for 2 h under a H_2 atmosphere and then filtered through cotton. Then the mixture was concentrated, and the residue was purified by column chromatography (EtOAc/2-propanol/H_2O=1 : 1 : 1) to give **1** (47 mg, 0.10 mmol, 99%) as a clear oil. $[\alpha]_D^{23}$ 0.9 (c=1.0 in H_2O); ^{1}H-NMR (600 MHz, D_2O) δ: 8.39 (2H, s, N$\underline{H}_2$), 4.40 (2H, m, Gal H1, Fuc H1), 4.01 (1H, m, H3), 3.96 (1H, br, Fuc H4), 3.86 (1H, br, Gal H4), 3.81 (4H, m, H2, H4, H5, Fuc H5), 3.65 (6H, m, Gal H6, Gal H6′ Gal H5, Gal H3, Fuc H2, Fuc H3), 3.47 (2H, m, Gal H2, H6), 3.36 (2H, m, H6′, H1), 3.00 (1H, dd, J=9.0, 12.6, H1′), 1.28 (3H, d, J=6.6, Fuc Me); ^{13}C-NMR (150 MHz, D_2O) δ: 103.4, 104.0, 79.8, 75.8, 73.1, 72.7, 72.0, 71.8, 71.6, 71.5, 69.8, 69.3, 69.2, 67.8, 67.3, 61.8, 43.2, 16.0; HR-MS (ESI) Calcd for $C_{18}H_{35}O_{14}N_1$ [M−H]$^-$: 488.1985 Found 488.1975.

Bis (2-Ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-L-fucopyranoside) Disulfide (12) To the solution of **9** (822 mg, 0.97 mmol) and 2,2′-dithiodiethanol (50 mg, 0.33 mmol) in dry CH_2Cl_2 (3.5 ml), containing activated molecular sieves 4A (40 mg), was stirred at 0 °C. TMSOTf (0.04 ml, 0.16 mmol) was added, and the mixture was stirred for 1 h, and then neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2, and the organic phase was dried, filtered, and concentrated. The residue was purified by silica gel chromatography (EtOAc/toluene=3 : 2) to yield **12** (414 mg, 0.81 mmol, 84%). $[\alpha]_D^{23}$ −110.9 (c=0.25 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.97 (2H×2, m), 7.91 (2H×2, m), 7.44 (2H×2, m), 7.31 (4H×2, m), 5.64 (1H, dd, J=8.4, 10.8, Fuc H2), 5.21 (1H×2, dd, J=7.8, 10.2, Gal H2), 5.53 (1H×2, dd, J=1.2, 3.6, Gal H4), 5.07 (1H×2, dd, J=3.6, 10.8, Fuc H3), 4.87 (1H×2, dd, J=3.6, 10.2, Gal H3), 4.63 (1H×2, d, J=8.4, Fuc H1), 4.36 (1H×2, d, J=7.8, Gal H1), 4.14 (1H×2, d, J=3.6, Fuc H4), 4.02 (1H×2, m, –C$\underline{H}_2$CH$_2$S), 3.76 (2H×2, m, –C$\underline{H}_2$CH$_2$S, Fuc H5), 3.55 (1H×2, m, Gal H5), 3.11 (2H×2, m, Gal H6, Gal H6′), 2.79 (1H×2, m, –CH$_2$C$\underline{H}_2$S), 2.76 (1H×2, m, –CH$_2$C$\underline{H}_2$S), 2.05 (3H×2, s, OAc), 2.02 (3H×2, s, OAc), 1.90 (3H×2, s, OAc), 1.86 (3H×2, s, OAc), 1.31 (3H×2, d, J=6.6, Fuc Me); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.2 (×2), 170.1 (×2), 170.0 (×2), 169.2 (×2), 166.1 (×2), 165.3 (×2), 133.2 (×2), 133.1 (×2), 130.1 (×2), 129.7 (×2), 128.3 (×2), 128.2 (×2), 101.8 (×2, Gal C1), 100.8 (×2, Fuc C1), 75.5 (×2, Fuc C4), 73.1 (×2, Fuc C3), 70.7 (×2, Gal C3), 70.5 (×2, Fuc C5), 70.2 (×2, Gal C5), 69.3 (×2, Gal C2), 68.5 (×2, Fuc C2), 66.7 (×2, Gal C4), 66.2 (×2, –C$\underline{H}_2$CH$_2$S), 60.3 (×2, Gal C6), 38.8 (×2, –CH$_2$C$\underline{H}_2$S), 20.8 (×2), 20.6 (×2), 20.6 (×2), 20.5 (×2), 16.1 (×2, Fuc C6); IR (neat) 2875, 1750, 1603, 1451, 1369, 1283, 1251, 1222, 1113, 1071, 1030, 757, 713 cm^{-1}; HR-MS (ESI) Calcd for $C_{72}H_{82}O_{32}S_2$ [M+Na]$^+$: 1545.4123 Found 1545.4123.

2-Sulfanylethyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-L-fucopyranoside (13) To 4.4 ml of ethanol/acetonitrile, 1 : 1 by volume, was added **12** (200 mg, 0.13 mmol). This was treated

with zinc dust (188 mg, 2.89 mmol) and 0.05 ml of concentrated HCl, and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was filtered to remove zinc and zinc salts, and the solvents were removed by rotary evaporation. The residue was dissolved in CH_2Cl_2, the resulting solution was washed once each with water, and the organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by column chromatography (toluene/EtOAc=1 : 2) to give **13** (176 mg, 0.23 mmol, 88%). $[\alpha]_D^{24}$ −103.9 (c=0.25 in $CHCl_3$); ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.04 (2H, dd, J=1.2, 8.4), 7.98 (2H, dd, J=1.2, 8.4), 7.51 (2H, m), 7.38 (4H, m), 5.7 (1H, dd, J=7.8, 10.2, Fuc H2), 5.28 (1H, dd, J=7.8, 10.2, Gal H2), 5.17 (1H, d, J=3.0, Gal H4), 5.13 (1H, dd, J=3.0, 10.2, Fuc H3), 4.94 (1H, dd, J=3.0, 10.2, Gal H3), 4.69 (1H, d, J=7.8, Fuc H1), 4.43 (1H, d, J=7.8, Gal H1), 4.2 (1H, d, J=3.0, Fuc H4), 3.98 (1H, m, –C$\underline{H}_2$CH$_2$SH), 3.83 (1H, m, Fuc H5), 3.67 (1H, m, –C$\underline{H}_2$CH$_2$SH), 3.62 (1H, m, Gal H5), 3.19 (2H, m, Gal H6, Gal H6′), 2.69 (1H, m, –CH$_2$C$\underline{H}_2$SH), 2.61 (1H, m, –CH$_2$C$\underline{H}_2$SH), 2.11 (3H, s), 2.09 (3H, s), 1.97 (3H, s), 1.93 (3H, s), 1.52 (1H, m, -S$\underline{H}$), 1.38 (3H, d, J=6.6, Fuc Me); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 170.2, 170.1, 170.0, 169.0, 166.1, 165.1, 133.2, 133.0, 130.1, 129.7, 128.3, 128.2, 101.8 (Gal C1), 100.9 (Fuc C1), 75.6 (Fuc C4), 73.2 (Fuc C3), 70.8 (Gal C3), 70.5 (Gal C5), 70.2 (Fuc C5), 69.9 (–$\underline{C}$H$_2$CH$_2$SH), 69.3 (Gal C2), 68.6 (Fuc C2), 66.7 (Gal C4), 60.3 (Gal C6), 24.5 (–CH$_2\underline{C}$H$_2$SH), 20.8, 20.6, 20.6, 20.5, 16.1 (Fuc C6); IR (neat) 2984, 2940, 2876, 2575, 1750, 1603, 1451, 1369, 1282, 1251, 1222, 1112, 1071, 1030, 756, 713 cm^{-1}; HR-MS (ESI) Calcd for $C_{36}H_{42}O_{16}S$ $[M+Na]^+$: 785.2086 Found 785.2089.

2-Sulfanylethyl β-D-Galactopyranosyl-(1→4)-β-L-fucopyranoside (2) To a solution of **13** (86 mg, 0.11 mmol) and NaOMe (43 mg, 0.79 mmol) in MeOH (2 ml) was stirred at room temperature for 12 h. Then the mixture was concentrated, and the residue was purified by reverse phase silica gel chromatography (H_2O/MeOH=1 : 1) to give **2** (42 mg, 0.11 mmol, 96%). $[\alpha]_D^{25}$ −3.7 (c=0.2 in CH_3OH); ^{1}H-NMR (600 MHz, CD_3OD) δ: 4.27 (1H, d, J=7.8, Fuc H1), 4.23 (1H, d, J=7.8, Gal H1), 4.03 (1H, m, –C$\underline{H}_2$CH$_2$SH), 3.82 (2H, m, –C$\underline{H}_2$CH$_2$SH, Fuc H4), 3.76 (1H, d, J=3.0, Gal H4), 3.72 (2H, m, Fuc H5, Gal H6), 3.64 (1H, dd, J=4.2, 11.4, Gal H6′), 3.58 (1H, dd, J=7.8, 10.2, Gal H2), 3.51 (1H, m, Gal H5), 3.39 (2H, m, Gal H3, Fuc H3), 3.39 (1H, dd, J=7.8, 10.2, Fuc H2), 2.94 (2H, t, J=6.6, –CH$_2$C$\underline{H}_2$SH), 1.32 (3H, d, J=6.6, Fuc Me); ^{13}C-NMR (150 MHz, CD_3OD) δ: 105.7 (Gal C1), 104.9 (Fuc C1), 80.9 (Fuc C4), 77.0 (Gal C5), 74.4 (Fuc C3), 74.1 (Gal C3), 73.1 (Fuc C2), 72.9 (Gal C2), 72.1 (Fuc C5), 70.3 (Gal C4), 69.5 (–$\underline{C}$H$_2$CH$_2$SH), 62.5 (Gal C6), 39.5 (–CH$_2\underline{C}$H$_2$SH), 16.7 (Fuc C6); HR-MS (ESI) Calcd for $C_{14}H_{26}O_{10}S$ $[M+Na]^+$: 409.1153 Found 409.1139.

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