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Note

Short synthesis of allyl 2-acetamido-2-deoxy-3,6-di-*O*-(α-L-fucopyranosyl)-β-D-glucopyranoside

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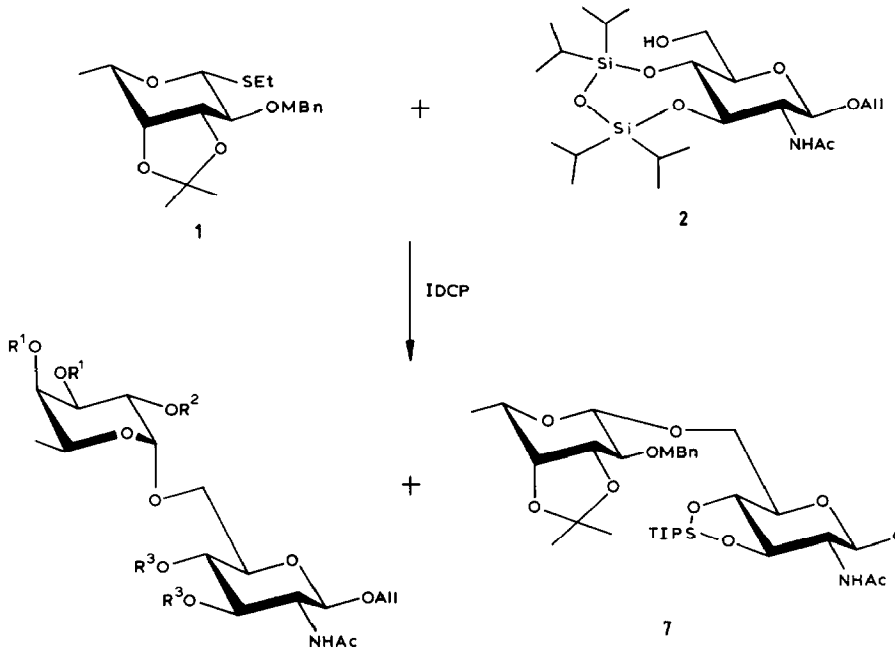
The presence of L-fucopyranosyl residues represents a common modification of glycoprotein N-glycans and contributes to their structural diversity. Recently, α-3,6-di-*O*-fucosylation of the innermost GlcNAc residue has been found within the N-glycan oligosaccharides of an insect glycoprotein, phospholipase A_2 (EC 3.1.1.4) from honeybee (*Apis mellifera*) venom[1]. α-(1 → 3)-Fucosylation of the asparagine-bound GlcNAc residue has, in addition, been identified as a potentially antigenic determinant[2]. As insect cells are being used for the production of recombinant proteins and glycoproteins, it has become important to investigate whether part structures, which are typically generated by these cells, could lead to adverse reactions upon administration of recombinant glycoproteins into vertebrate organisms. We have therefore set out to synthesize part structures of glycoprotein N-glycans bearing fucosyl residues in alternative linkages, in a form which provides access to haptens and immunogens for testing their immunogenic and antigenic potential.

The fucosyl donor **1**[3] was coupled with the previously described[4] glycosyl acceptor **2**, using various methods of thioglycoside activation. Whereas $CuBr_2$/DMF/Bu_4NBr[5] did not lead to significant formation of disaccharide derivatives, in situ activation with bromine[6] resulted in substantial degradation. Best results were obtained using iodonium ion-assisted glycosylation with iodonium di-*sym*-collidinium perchlorate (IDCP) in dichloromethane–diethyl ether[7] under "inverse conditions" (ref 8), which afforded the α- and β-(1 → 6)-linked disaccharide derivatives **3** and **7** in 64% yield (α/β ratio 2.2 : 1). The anomeric configuration of the fucopyranosyl residues was based on the observed values of the coupling constants $J_{1',2'}$ (3.5 Hz for **3**, 8.1 Hz for **7**). After separation of the isomers by silica gel chromatography, the silyl ether group was cleaved[9] by the action of 0.1 M Bu_4NF in tetrahydrofuran (THF), which gave the diol **4** in 82% yield. Removal of the 4-methoxybenzyl ether group by oxidation[10] with 2,3-di-

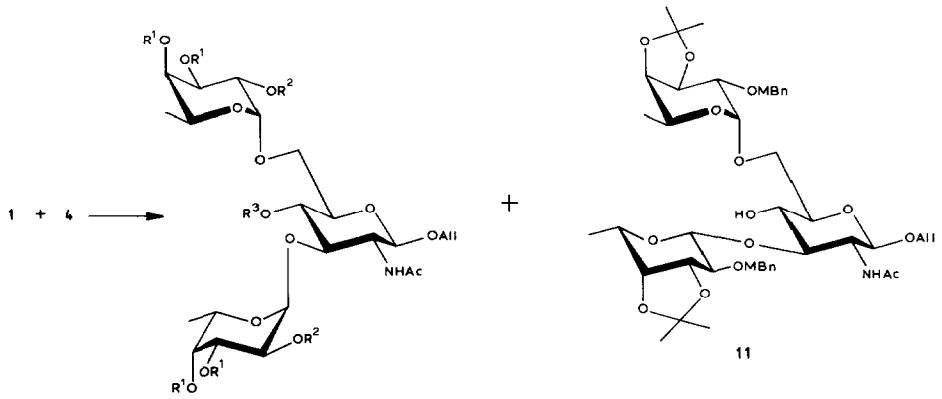


3 R^1 R^1 = Me_2C, R^2 = MBn, R^3 R^3 = TIPS
4 R^1 R^1 = Me_2C, R^2 = MBn, R^3 = H
5 R^1 = R^2 = R^3 = Ac
6 R^1 = R^2 = R^3 = H

MBn = 4-Methoxybenzyl
TIPS = 1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl

chloro-5,6-dicyano-1,4-benzoquinone (DDQ) resulted in a concomitant loss of the isopropylidene group. Subsequent *O*-acetylation (acetic anhydride–pyridine) furnished the per-*O*-acetylated disaccharide derivative **5** in 82% overall yield. Zemplén *O*-deacetylation finally gave the disaccharide allyl 2-acetamido-2-deoxy-6-*O*-(α-L-fucopyranosyl)-β-D-glucopyranoside (**6**) in quantitative yield.

Reaction of the diol **4** with one equivalent of the fucosyl donor **1** in dichloromethane–diethyl ether, promoted by IDCP, afforded regioselectively a 1:1 mixture of the α- and β-(1″ → 3)-linked trisaccharide derivatives **8** and **11** in 52% yield. The anomeric configuration was deduced from the coupling constants, $J_{1'',2''}$ (3.6 Hz for **8**; 8.2 Hz for **11**). The isomers were separated by column chromatography on silica gel. Blocking groups were removed from **8** by DDQ-oxidation, and *O*-acetylation then gave the crystalline trisaccharide derivative **9** in 64% yield, isolated by silica gel chromatography. A minor fraction containing a trisaccharide mixture with one remaining isopropylidene group was treated with 2% CF_3CO_2H in dichloromethane at −10°C. *O*-Acetylation afforded an additional portion of **9** (17%). The correct linkage of the second fucopyranosyl residue was confirmed by the downfield shift of the ^1H NMR signal attributable to H-4 of



the glucosamine residue (δ 4.89 ppm) in the per-*O*-acetylated derivative **9**. Zemplén *O*-deacetylation of **9** gave the target trisaccharide **10** in quantitative yield. The structure of **6** and **10** was confirmed by the ^1H and ^{13}C NMR spectroscopic data, which are based on H,H COSY, C,H COSY, and 1D NOE measurements. Immunochemical results obtained with glycoconjugates derived from the allyl glycosides will be published elsewhere.

EXPERIMENTAL

General methods.—These were as described previously[11].

Allyl O-*[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-α-*L*-fucopyranosyl]-(1 → 6)-2-acetamido-2-deoxy-3,4-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-glucopyranoside* (**3**) *and allyl* O-*[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-β-*L*-fucopyranosyl]-(1 → 6)-2-acetamido-2-deoxy-3,4-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-glucopyranoside* (**7**).—A suspension of **1** (731 mg, 2 mmol), iodonium di-*sym*-collidinium perchlorate (IDCP, 1 g, 2.1 mmol) and molecular sieves 4A (1 g) in 5:1 Et_2O–CH_2Cl_2 (10 mL) was stirred for 20 min at room temperature. After cooling to −20°C, a solution of **2** (500 mg, 0.99 mmol) in CH_2Cl_2 (3 mL) was added dropwise during 3 h under N_2. The reaction mixture was kept at room temperature for 20 h, diluted with EtOAc (50 mL), and filtered over Celite. The filtrate was washed with M aqueous $Na_2S_2O_3$ and satd aq $NaHCO_3$, then dried (Na_2SO_4). Concentration of the solution gave a syrup, which was chromatographed on silica gel (EtOAc–toluene 1:3 → 2:3). Pooling and evaporation of the fractions containing the faster moving isomer afforded **3** as a syrup (352 mg, 44%); $[\alpha]_D^{20}$ −30° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–7.26 (m, 2 H) and 6.85–6.83 (m, 2 H, arom.H), 5.85 (m, 1 H, –CH=), 5.52 (d, 1 H, $J_{NH,2} \sim 7.8$ Hz, NH), 5.24 (dq, 1 H,

$=CH_{2trans}$), 5.15 (dq, 1 H, $=CH_{2cis}$), 4.94 (d, 1 H, $J_{1,2}$ ~ 9.0 Hz, H-1), 4.83 (d, 1 H, $J_{1',2'}$ ~ 3.5 Hz, H-1'), 4.68 and 4.63 (AB system, 2 H, $J_{A,B}$ ~ 12.2 Hz, OCH_2), 4.31 (dd, 1 H, $J_{2',3'}$ ~ 7.8, $J_{3',4'}$ ~ 5.5 Hz, H-3'), 4.27 (m, 1 H, OCH_{2a}), 4.21 (dq, 1 H, $J_{4',5'}$ ~ 2.2, $J_{5',6'}$ ~ 6.7 Hz, H-5'), 4.09 (dd, 1 H, $J_{2,3}$ ~ 8.5 Hz, H-3), 4.06 (m, 1 H, OCH_{2b}), 4.04 (dd, 1 H, H-4'), 3.95 (dd, 1 H, $J_{5,6a}$ ~ 1.0, $J_{6a,6b}$ ~ −11.0 Hz, H-6a), 3.79 (s, 3 H, OCH_3), 3.66 (ddd, 1 H, H-5), 3.53 (m, 2 H, H-4, 6b), 3.50 (dd, 1 H, H-2'), 3.30 (ddd, 1 H, H-2), 1.96 (s, 3 H, Ac), 1.40 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 1.29 (d, 3 H, H-6'), and 1.02 [m, 28 H, 4 $SiCH(CH_3)_2$]. Anal. Calcd for $C_{40}H_{67}Si_2NO_{12}$: C, 59.30; H, 8.34; N, 1.73. Found: C, 59.43; H, 8.21; N, 1.67.

Further elution of the column afforded **7** (165 mg, 20%) as colorless crystals; mp 190°C (from EtOAc); $[\alpha]_D^{20}$ −14° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.33 (m, 2 H), and 6.87–6.85 (m, 2 H, arom. H), 5.85 (m, 1 H, -CH=), 5.56 (d, 1 H, $J_{NH,2}$ ~ 7.7 Hz, NH), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.13 (dq, 1 H, $=CH_{2cis}$), 5.02 (d, 1 H, $J_{1,2}$ ~ 8.5 Hz, H-1), 4.81 and 4.73 (AB system, 2 H, $J_{A,B}$ ~ 11.2 Hz, OCH_2), 4.35 (d, 1 H, $J_{1',2'}$ ~ 8.1 Hz, H-1'), 4.30 (m, 1 H, OCH_{2a}), 4.17 (dd, 1 H, $J_{2,3}$ ~ 9.9, $J_{3,4}$ ~ 8.0 Hz, H-3), 4.09 (dd, 1 H, $J_{2',3'}$ ~ 7.5, $J_{3',4'}$ ~ 5.6 Hz, H-3'), 4.06 (dd, 1 H, $J_{5,6a}$ ~ 5.5, $J_{6a,6b}$ ~ −11.0 Hz, H-6a), 4.06 (m, 1 H, OCH_{2b}), 3.96 (dd, 1 H, $J_{4',5'}$ ~ 2.0 Hz, H-4'), 3.91 (dd, 1 H, $J_{5,6b}$ ~ 1.5 Hz, H-6b), 3.78 (s, 3 H, OCH_3), 3.77 (dq, 1 H, $J_{5',6'}$ ~ 6.5 Hz, H-5'), 3.65 (dd, 1 H, $J_{4,5}$ ~ 9.4 Hz, H-4), 3.53 (ddd, 1 H, H-5), 3.33 (dd, 1 H, H-2'), 3.24 (ddd, 1 H, H-2), 1.96 (s, 3 H, Ac), 1.36–1.33 (2 s, 1 d, 9 H, 2 CH_3, H-6'), and 1.02 [m, 28 H, 4 $SiCH(CH_3)_2$]. Anal. Calcd for $C_{40}H_{67}Si_2NO_{12}$: C, 59.30; H, 8.34; N, 1.73. Found: C, 59.68; H, 8.08; N, 1.68.

Allyl O-[3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-α-L-fucopyranosyl]-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**4**).—A solution of **3** (268 mg, 0.33 mmol) and 1.1 M Bu_4NF in THF (0.14 mL) in dry THF (5 mL) was stirred for 4 h at room temperature. The solution was evaporated and the residue was purified by chromatography on silica gel (5:1 EtOAc–MeOH), which afforded **4** as a syrup (154 mg, 82%); $[\alpha]_D^{20}$ −100° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.29–7.27 (m, 2 H) and 6.87–6.84 (m, 2 H, arom. H), 5.91 (d, 1 H, NH), 5.86 (m, 1 H, =CH–), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 4.77 (br.s, 1 H, OH), 4.74 (d, 1 H, $J_{1',2'}$ ~ 3.5 Hz, H-1'), 4.74 and 4.61 (AB system, 2 H, $J_{A,B}$ ~ 12.0 Hz, OCH_2), 4.52 (d, 1 H, $J_{1,2}$ ~ 8.3 Hz, H-1), 4.30 (m, 1 H, OCH_{2a}), 4.30 (dd, 1 H, $J_{2',3'}$ ~ 7.8, $J_{3'4'}$ ~ 5.5 Hz, H-3'), 4.22 (dq, 1 H, $J_{4',5'}$ ~ 2.4, $J_{5',6'}$ ~ 6.7 Hz, H-5'), 4.04 (dd, 1 H, H-4'), 4.03 (m, 1 H, OCH_{2b}), 3.95 (dd, 1 H, $J_{5,6a}$ ~ 2.8, $J_{6a,6b}$ ~ −11.2 Hz, H-6a), 3.84 (br.s, 1 H, OH), 3.79 (s, 3 H, OCH_3), 3.72 (dd, 1 H, H-5), 3.71 (dd, 1 H, H-6b), 3.57 (dd, 1 H, $J_{3,4}$ ~ 9.0, $J_{2,3}$ ~ 9.0 Hz, H-3), 3.51 (dd, 1 H, H-2'), 3.49 (dd, 1 H, H-4), 3.46 (ddd, 1 H, H-2), 2.05 (s, 3 H, Ac), 1.42 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), and 1.30 (d, 3 H, H-6'). Anal. Calcd for $C_{28}H_{41}NO_{11} \cdot H_2O$: C, 57.42; H, 7.40; N, 2.39. Found: C, 57.85; H, 7.69; N, 2.26.

Allyl O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 6)-2-acetamido-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**5**).—A solution of **4** (31 mg) in 18:1 CH_2Cl_2–water (5 mL) was stirred with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 32 mg) for 15 h at room temperature. The solution was taken to dryness. A solution of

the residue in pyridine (5 mL) was treated with acetic anhydride (0.2 mL) for 5 h at room temperature. The solution was evaporated three times with addition of toluene (10 mL) and chromatographed on a column of silica gel (EtOAc). Pooling and evaporation of the main fraction gave **5** as a syrup (28 mg, 82%); $[\alpha]_D^{20}$ −61° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.87 (m, 1 H, =CH–), 5.43 (d, 1 H, $J_{NH,2}$ ~ 8.8 Hz, NH), 5.34 (dd, 1 H, $J_{2',3'}$ ~ 7.2, $J_{2',1'}$ ~ 3.3 Hz, H-2'), 5.31 (m, 1 H, H-4'), 5.30 (dd, 1 H, $J_{3,4}$ ~ 9.5 Hz, H-3), 5.30 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 5.13 (d, 1 H, H-1'), 5.08 (dd, 1 H, $J_{3',4'}$ ~ 3.7 Hz, H-3'), 5.03 (dd, 1 H, $J_{4,5}$ ~ 9.5 Hz, H-4), 4.71 (d, 1 H, $J_{1,2}$ ~ 8.3 Hz, H-1), 4.32 (m, 1 H, OCH_{2a}), 4.17 (dq, 1 H, $J_{4',5'}$ ~ 0.5, $J_{5',6'}$ ~ 6.5 Hz, H-5'), 4.08 (m, 1 H, OCH_{2b}), 3.85 (ddd, 1 H, $J_{2,3}$ ~ 10.6 Hz, H-2), 3.74 (dd, 1 H, $J_{5,6a}$ ~ 2.5, $J_{6a,6b}$ ~ −11.1 Hz, H-6a), 3.64 (ddd, 1 H, $J_{5,6b}$ ~ 5.2 Hz, H-5), 3.54 (dd, 1 H, H-6b), 2.16, 2.09, 2.03, 2.02, 1.99, 1.95 (6 s, 18 H, 6 Ac), and 1.13 (d, 3 H, H-6'). Anal. Calcd for $C_{27}H_{39}NO_{15}$: C, 52.51; H, 6.37; N, 2.27. Found: C, 52.63; H, 6.50; N, 2.17.

Allyl O-α-L-*fucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-*D-*glucopyranoside* (**6**). —A solution of **5** (16 mg) and 0.1 M methanolic NaOMe (0.5 mL) in dry MeOH (5 mL) was stirred for 3 h at room temperature. The pH of the solution was made neutral by addition of Dowex 50 (H^+) ion-exchange resin. Filtration and evaporation gave a syrup which was purified on a Bio-Gel P2 column (water). Yield: 10 mg (97%), amorphous powder; $[\alpha]_D^{20}$ −30° (*c* 0.4 water); ^1H NMR (D_2O): δ 5.89 (m, 1 H, =CH–), 5.31 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 4.95 (d, 1 H, $J_{1',2'}$ ~ 3.8 Hz, H-1'), 4.58 (d, 1 H, $J_{1,2}$ ~ 8.5 Hz, H-1), 4.34 (m, 1 H, OCH_2), 4.15 (dq, 1 H, $J_{5',4'}$ ~ 1.0 Hz, H-5'), 4.00 (dd, 1 H, $J_{6a,6b}$ ~ −11.9, $J_{6a,5}$ ~ 1.6 Hz, H-6a), 3.92 (dd, 1 H, $J_{3',2'}$ ~ 10.4, $J_{3',4'}$ ~ 3.4 Hz, H-3'), 3.82 (dd, 1 H, H-4'), 3.79 (dd, 1 H, H-2'), 3.79 (dd, 1 H, $J_{6b,5}$ ~ 5.1 Hz, H-6b), 3.73 (dd, 1 H, $J_{2,3}$ ~ 10.1 Hz, H-2); 3.59–3.52 (m, 3 H, H-5,3,4), 2.05 (s, 3 H, NHAc), 1.24 (d, 3 H, $J_{6',5'}$ ~ 6.6 Hz, H-6'); ^{13}C NMR: δ 175.54 (CO), 134.33 (=CH–), 119.04 ($=CH_2$), 101.15 (C-1), 100.21 (C-1'), 75.87 (C-5), 74.70 (C-3), 72.75 (C-4'), 71.45 (OCH_2), 70.74 (C-4), 70.44 (C-3'), 69.11 (C-2'), 68.09 (C-6), 67.65 (C-5'), 56.48 (C-2), 23.06 (CH_3), 16.26 (C-6'). Anal. Calcd for $C_{17}H_{29}NO_{10}$: C, 50.12; H, 7.17; N, 3.44. Found: C, 49.78; H, 7.25; N, 3.17.

*Allyl 2-acetamido-2-deoxy-3,6-di-*O*-[3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-*L*-fucopyranosyl]-β-*D*-glucopyranoside* (**8**) *and allyl 2-acetamido-2-deoxy-3-*O*-[3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-β-*L*-fucopyranosyl]-6-*O*-[3,4-*O*-isopropylidene-2-*O*-(4-methoxybenzyl)-α-*L*-fucopyranosyl]-β-*D*-glucopyranoside* (**11**).—A suspension of **1** (74 mg, 0.2 mmol), **4** (100 mg, 0.024 mmol), and molecular sieves 4A (2 g) in 1:1 CH_2Cl_2–Et_2O (9 mL) was stirred for 30 min at room temperature under N_2. IDCP (120 mg, 0.26 mmol) was added during 3 h in portions and stirring was continued for 3 h at room temperature. The suspension was diluted with CH_2Cl_2 (50 mL) and filtered over Celite. The filtrate was washed with M aqueous $Na_2S_2O_3$ and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Chromatography of the residue on silica gel (EtOAc) gave **8** as the faster migrating isomer. Yield: 42 mg (27%), colorless syrup; $[\alpha]_D^{20}$ −79° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$):

δ 7.29–7.25 (m, 4 H), 6.89–6.83 (m, 4 H, arom. H), 5.83 (m, 1 H, –CH=), 5.52 (d, 1 H, $J_{NH,2}$ ~ 7.3 Hz, NH), 5.23 (dq, 1 H, $=CH_{2trans}$), 5.14 (dq, 1 H, $=CH_{2cis}$), 4.86 (d, 1 H, $J_{1,2}$ ~ 7.9 Hz, H-1), 4.85 (d, 1 H, $J_{1',2'}$ ~ 3.5 Hz, H-1'), 4.82 (d, 1 H, $J_{1'',2''}$ ~ 3.6 Hz, H-1''), 4.72 and 4.59 (AB system, 2 H, $J_{A,B}$ ~ 11.8 Hz, OCH_{2a}), 4.70 and 4.63 (AB system, 2 H, $J_{A,B}$ ~ 12.0 Hz, OCH_2), 4.32 (dd, 1 H, $J_{2'',3''}$ ~ 7.7, $J_{3'',4''}$ ~ 5.5 Hz, H-3''), 4.30 (dd, 1 H, H-3'), 4.31–4.18 (m, 4 H, H-6a, 5', 5'', OCH_2), 4.03 (dd, 1 H, H-4''), 4.03 (m, 1 H, OCH_{2b}), 3.98 (dd, 1 H, H-6b), 3.98 (dd, 1 H, H-4'), 3.91 (br.dd, 2 H, H-3, OH), 3.80 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.70 (ddd, 1 H, H-5), 3.56 (dd, 1 H, $J_{2',3'}$ ~ 7.0 Hz, H-2'), 3.50 (dd, 1 H, H-2''), 3.50 (ddd, 1 H, H-4), 3.28 (ddd, 1 H, $J_{2,3}$ ~ 9.5 Hz, H-2), 1.76 (s, 3 H, Ac), 1.42, 1.41, 1.34, and 1.33 (4 s, 12 H, 4 CH_3), 1.30 (d, 3 H, $J_{6'',5''}$ ~ 6.7 Hz, H-6''), and 1.26 (d, 3 H, $J_{6',5'}$ ~ 6.6 Hz, H-6'). Anal. Calcd for $C_{45}H_{63}NO_{16}$: C, 61.84; H, 7.26; N, 1.60. Found: C, 61.41; H, 7.09; N, 1.47.

Further elution of the column furnished **11** as a syrup (39 mg, 25%); $[\alpha]_D^{20}$ −39° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.26–7.24 (m, 4 H), 6.87–6.81 (m, 4 H, arom. H), 5.86 (m, 1 H, –CH=), 5.81 (d, 1 H, $J_{NH,2}$ ~ 7.1 Hz, NH), 5.24 (dq, 1 H, $=CH_{2trans}$), 5.15 (dq, 1 H, $=CH_{2cis}$), 4.98 (d, 1 H, $J_{1,2}$ ~ 8.2 Hz, H-1), 4.80 (d, 1 H, $J_{1',2'}$ ~ 3.5 Hz, H-1'), 4.81 and 4.64 (AB system, 2 H, $J_{A,B}$ ~ 11.5 Hz, OCH_2), 4.68 and 4.61 (AB system, 2 H, $J_{A,B}$ ~ 12.0 Hz, OCH_2), 4.42 (d, 1 H, $J_{1'',2''}$ ~ 8.2 Hz, H-1''), 4.32 (dd, 1 H, $J_{2',3'}$ ~ 7.8, $J_{3',4'}$ ~ 5.6 Hz, H-3'), 4.28 (m, 1 H, OCH_{2a}), 4.18 (dq, 1 H, H-5'), 4.18 (dd, 1 H, $J_{2'',3''}$ ~ 7.0, $J_{3'',4''}$ ~ 5.5 Hz, H-3''), 4.17 (t, 1 H, H-3), 4.05 (m, 1 H, OCH_{2b}), 4.03 (dd, 1 H, $J_{4',5'}$ ~ 2.3 Hz, H-4'), 3.97 (dd, 1 H, $J_{4'',5''}$ ~ 2.0 Hz, H-4''), 3.91 (dd, 1 H, $J_{5,6a}$ ~ 2.0, $J_{6a,6b}$ ~ −11.5 Hz, H-6a), 3.83 (dq, 1 H, $J_{5'',6''}$ ~ 6.5 Hz, H-5''), 3.78 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.62 (dd, 1 H, $J_{5,6b}$ ~ 6.0 Hz, H-6b), 3.61 (d, 1 H, OH), 3.52 (ddd, 1 H, $J_{4,5}$ ~ 9.5 Hz, H-5), 3.49 (dd, 1 H, H-2'), 3.42 (dd, 1 H, H-2''), 3.41 (ddd, 1 H, H-4), 3.07 (ddd, 1 H, H-2), 1.97 (s, 3 H, Ac), 1.45 and 1.41 (2 s, 6 H, 2 CH_3), 1.37 (d, 3 H, H-6''), 1.37 and 1.35 (2 s, 6 H, 2 CH_3), and 1.30 (d, 3 H, H-6'). Anal. Calcd for $C_{45}H_{63}NO_{16}$: C, 61.84; H, 7.26; N, 1.60. Found: C, 61.98; H, 7.26; N, 1.52.

*Allyl 2-acetamido-4-*O*-acetyl-2-deoxy-3,6-di-*O*-(2,3,4-tri-*O*-acetyl-α-*L*-fuco-pyranosyl)-β-*D*-glucopyranoside* (**9**).—A solution of **8** (34 mg, 0.04 mmol) and DDQ (32 mg, 0.14 mmol) in 18:1 CH_2Cl_2–water (7 mL) was stirred for 24 h at room temperature. The solution was taken to dryness, pyridine (1.5 mL) and acetic anhydride (3 mL) were added, and stirring was continued for 6 h at room temperature. The solution was evaporated three times with addition of toluene (5 mL), and the residual syrup was purified by silica gel chromatography (3:1 hexane–EtOH) which gave **9** (22 mg, 64%) as colorless needles; mp 172°C (EtOAc); $[\alpha]_D^{20}$ −119° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.87 (m, 1 H, –CH=), 5.74 (d, 1 H, $J_{NH,2}$ ~ 7.3 Hz, NH), 5.29 (dq, 1 H, $=CH_{2trans}$), 5.21 (dq, 1 H, $=CH_{2cis}$), 5.37–5.07 (m, 8 H, H-1', 1'', 2', 2'', 3', 3'', 4', 4''), 5.02 (d, 1 H, $J_{1,2}$ ~ 7.9 Hz, H-1), 4.89 (dd, 1 H, $J_{3,4}$ ~ 9.6, $J_{4,5}$ ~ 8.2 Hz, H-4), 4.47 (dd, 1 H, $J_{2,3}$ ~ 9.3 Hz, H-3), 4.29 (m, 1 H, OCH_{2a}), 4.15 (dq, 2 H, $J_{5',6'}$ ~ 6.6, $J_{5'',6''}$ ~ 6.6 Hz, H-5', 5''), 4.06 (m, 1 H, OCH_{2b}), 3.67 (dd, 1 H, $J_{5,6a}$ ~ 2.3, $J_{6a,6b}$ ~ −10.8 Hz, H-6a), 3.57

(ddd, 1 H, $J_{5,6b}$ ~ 5.4 Hz, H-5), 3.52 (dd, 1 H, H-6b), 3.21 (ddd, 1 H, H-2), 2.15, 2.11, 2.09, 2.08, 2.05, 1.99, 1.98 (8 s, 24 H, 8 Ac), 1.13 (d, 3 H, H-6″), and 1.07 (d, 3 H, H-6′). Anal. Calcd for $C_{37}H_{53}NO_{21}$: C, 52.42; H, 6.30; N, 1.65. Found: C; 52.28; H, 6.15; N, 1.44.

A second crop of **9** was obtained as follows. The fraction eluted next in the chromatographic separation was evaporated, and the residue was dissolved in CH_2Cl_2 (3 mL) and treated at −10°C with aq 90% trifluoroacetic acid (0.1 mL) for 15 h. Triethylamine was then added (0.2 mL), and the solution was taken to dryness and acetylated with acetic anhydride (2 mL) in pyridine (1 mL) as described before. Column chromatography (EtOAc) afforded **9** (6 mg, 17%).

*Allyl 2-acetamido-2-deoxy-3,6-di-*O*-(α-*L*-fucopyranosyl)-β-*D*-glucopyranoside* (**10**). —A solution of **9** (14 mg, 0.016 mmol) and 0.1 M methanolic NaOMe (0.4 mL) in dry MeOH (3 mL) was stirred for 5 h at room temperature. The solution was made neutral by adding Dowex 50 (H^+) ion-exchange resin and filtered. The filtrate was evaporated and the residue was purified on a column of Bio-Gel P2 (water). Yield: 9.0 mg (quant.), amorphous powder; $[\alpha]_D^{20}$ −112° (*c* 0.4, water); ^{1}H NMR (D_2O): δ 5.91 (m, 1 H, –CH=), 5.31 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 5.00 (d, 1 H, $J_{1',2'}$ ~ 4.0 Hz, H-1′), 4.95 (d, 1 H, $J_{1'',2''}$ ~ 3.9 Hz, H-1″), 4.61 (d, 1 H, $J_{1,2}$ ~ 8.6 Hz, H-1), 4.35 (m, 1 H, OCH_{2a}), 4.35 (dq, 1 H, $J_{5',6'}$ ~ 6.6 Hz, H-5′), 4.19 (m, 1 H, OCH_{2b}), 4.17 (dq, 1 H, $J_{5'',6''}$ ~ 6.6 Hz, H-5″), 4.02 (dd, 1 H, $J_{6a,5}$ ~ 1.5, $J_{6a,6b}$ ~ −12.3 Hz, H-6a), 3.94 (dd, 1 H, $J_{3'',4''}$ ~ 3.4, $J_{3'',2''}$ ~ 10.4 Hz, H-3″), 3.87 (dd, 1 H, $J_{6b,5}$ ~ 5.3 Hz, H-6b), 3.86 (dd, 1 H, $J_{3',4'}$ ~ 3.3, $J_{3',2'}$ ~ 10.1 Hz, H-3′), 3.85 (dd, 1 H, $J_{2,3}$ ~ 10.3 Hz, H-2), 3.84 (dd, 1 H, $J_{4'',5''}$ ~ 1.0 Hz, H-4″), 3.82 (dd, 1 H, $J_{4',5'}$ ~ 1.0 Hz, H-4′), 3.81 (dd, 1 H, H-2″), 3.72 (dd, 1 H, H-2′), 3.69–3.64 (m, 3 H, H-3,4,5), 2.05 (s, 3 H, NHAc), 1.26 (d, 3 H, H-6″), 1.19 (d, 3 H, H-6′); ^{13}C NMR: 175.69 (CO), 134.38 (=CH), 119.11 ($=CH_2$), 100.99 (C-1,1′), 100.22 (C-1″), 81.39 (C-3), 75.90 (C-5), 72.82 (C-4′,4″), 71.55 (OCH_2), 70.51 (C-3′,3″), 69.50 (C-4), 69.18 (C-2″), 68.97 (C-2′), 68.18 (C-6), 67.89 (C-5′), 67.69 (C-5″), 56.25 (C-2), 23.18 (CH_3), 16.30 (C-6″), 16.15 (C-6′). Anal. Calcd for $C_{23}H_{39}NO_{14}$: C, 49.90; H, 7.10; N, 2.23. Found: 50.08; H, 7.03; N, 2.01.

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