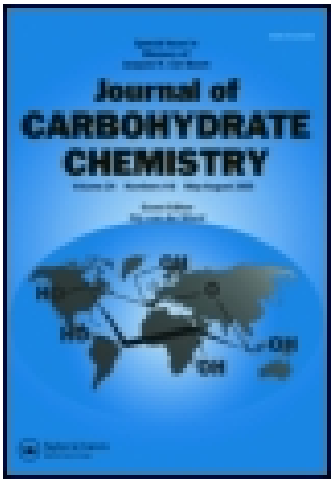

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Chemical Synthesis of (3-SO_3Na)GlcNAcβ1→3Fucβ1→OMe: The Oligosaccharide Involved in the Cell Aggregation of the Sponge Microciona Prolifera

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CHEMICAL SYNTHESIS OF (3-SO_3Na)GlcNAcβ1→3Fucβ1→OMe: THE OLIGOSACCHARIDE INVOLVED IN THE CELL AGGREGATION OF THE SPONGE *MICROCIONA PROLIFERA*

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ABSTRACT

The methyl glycoside, (3-SO_3Na)GlcNAcβ1→3Fucβ1→OMe, of a sulfated disaccharide that is involved in the species-specific reaggregation of dissociated cells of *Microciona prolifera*, was stereoselectively synthesized starting from L-fucose and 2-amino-2-deoxy-D-glucose.

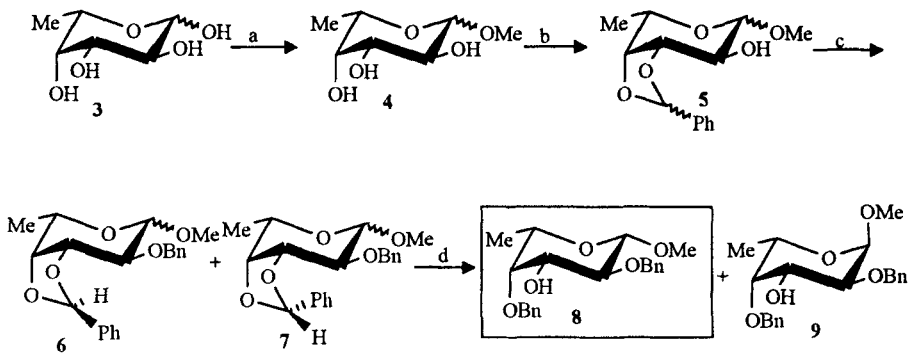
INTRODUCTION

The species-specific reaggregation of dissociated cells from the marine sponge *Microciona prolifera* has been used as a simple model for the study of the molecular and cellular interactions involved in tissue development and organization of higher animals.[1,2] The reaggregation of dissociated sponge cells depends on a large extracellular adhesion proteoglycan.[3,4] Biological studies have shown that the multiple low affinity carbohydrate-carbohydrate interactions of the proteoglycan themselves form the basis of cell reaggregation in the sponge system,[5,6] and two monoclonal antibodies, Block 1 and Block 2, against the glycans can selectively inhibit the aggregation of these proteoglycans. The

oligosaccharide units reacting with both antibodies have been recently characterized,[1,2] i.e., a trisaccharide containing pyruvate acetal,[1] (4,6-Pyr)Galβ1→4GlcNAcβ1→3Fuc for Block 1, and a sulfated disaccharide,[2] (3-SO_3Na) GlcNAcβ1→3Fuc (**1**) for Block 2. The identification of both structures outlines the structural requirements of antibody reactivity with the two reaggregation-associated oligosaccharide epitopes. In this paper, we wish to report the unambiguous chemical synthesis of the methyl glycoside **2** of the sulfated disaccharide **1**.

RESULTS AND DISCUSSION

Selectively protected β-L-fucoside **8**, an important intermediate for the synthesis of **2**, was prepared from L-fucose **3** according to reactions outlined in Scheme 1. Treatment of L-fucose with *p*-methylphenylsulfonic acid (TsOH) in refluxing methanol afforded quantitatively a mixture containing both α- and β-anomers (**4**) in a 1.2:1.0 ratio, the anomers being inseparable by column chromatography. This result differs from that reported[7] where only methyl α-L-fucoside was obtained under similar conditions. Compound **4** was then converted to the 3,4-benzylidene acetal **5** with TsOH and benzaldehyde dimethyl acetal in 57% yield. The separation of diastereoisomeric mixture **5** (containing all four diastereoisomers, 1.0:1.0:1.1:1.1 molar proportions) by column chromatography was difficult and thus the mixture was directly employed in the following reactions. On benzylation of **5**, diastereoisomeric products **6** (α:β=1.1:1.0) and **7** (α:β=1.1:1.0) were easily separated by column chromatography and obtained in 1.0:1.0 ratio. The stereochemistry of **6** and **7** was determined by comparison of their ^1H NMR data with that reported for analogous structures.[7,8] The reactions of **6** and **7** with diisobutylaluminum hydride (DIBAL) in dichloromethane were interesting, in that the reaction of **7** with DIBAL was very rapid and complete within 3 h to afford **8** and **9** (1.0:1.1) in 92% yield, but the reaction of **6** under the same conditions was too slow to be observed. This may be the result of steric interactions. We found that the reaction of **6** and DIBAL was promoted by boron trifluoride and accomplished within 0.5 h to afford **8** and **9** (1.0:1.1) in 93% yield. The phenomenon is now under further detailed investigations in this laboratory. The ring-opening reactions were regioselective and gave products having a

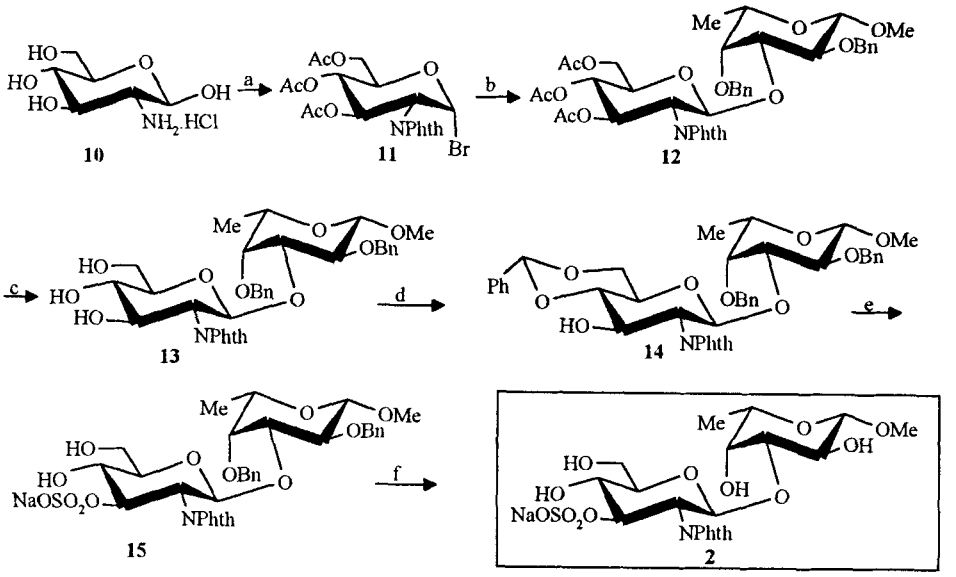


Conditions: a. MeOH, TsOH, reflux, 24 h; quant. b. $PhCH(OMe)_2$, TsOH, DMF, 45 °C, 5 h; 57% c. i. NaH, DMF, rt, 0.5 h; ii. BnBr, DMF, 10 h; 91% d. DIBAL, CH_2Cl_2, 0 °C, 3 h; 92%

Scheme 1

benzyl group on *O*-4 as reported.[7,8] The structures of **8** and **9** were further confirmed by comparison of their ^1H NMR spectra with those of corresponding derivatives, e.g. the *H*-3 signals of the acetylated products (δ: 4.87 and 5.22, respectively) of **8** and **9** shifted to downfield by 1.2 ppm compared to that of **8** (δ: 3.66) and **9** (δ: 4.07). Compounds **8** and **9** were easily separated by column chromatography.

The selectively protected glucosaminyl donor **11** was prepared according to the reported method.[9] Its coupling reaction with **8** was realized by a Koenigs-Knorr methodology to give disaccharide **12** in excellent yield (98%). The reaction was of high stereoselectivity and only the β-anomer ($J_{1',2'}$ = 8.5 Hz) was isolated. Deprotection of glucosaminyl residue in **12** and selective acetylation of the liberated amino group were carried out by a sequence of treatments without purification: hydrazinolysis of **12** with NH_2NH_2 in boiling ethanol for 6 h, followed by N-acetylation with acetic anhydride in pyridine overnight and finally O-deacetylation with potassium carbonate in methanol gave N-acetylated disaccharide **13** in 73% overall yield. The 4',6'-hydroxyl groups of **13** were then protected as the benzylidene acetal. The sulfation of 3'-OH in **14** was conducted with sulfur trioxide-pyridine complex in DMF at room temperature. The reaction was complete within 1 h, but during the following ion-exchange treatment of the resulted sulfate with Dowex-50W-Na^+, cleavage of the benzylidene group occurred to gave **15** as the product.



Conditions: a. i. NaOMe, rt, 10 min; ii. Dowex-H resin; iii. Phthalic anhydride, Et_3N, 50 °C, 2 h; iv. Ac_2O, Py., rt, 8 h; v. AcOH/HBr, 0 °C, 8 h, 40%. b. **8**, AgOTf, CH_2Cl_2, 4AMS, -40 °C to rt, 24 h; 98%. c. i. NH_2NH_2, 95% EtOH, reflux, 6 h; ii. Ac_2O, Py., rt, 10 h; iii. MeOH, K_2CO_3, rt, 2 h; 73%. d. $PhCH(OMe)_2$, DMF, Camphor sulfonic acid, 45 °C, 5 h; 86%. e. i. SO_3-Py., DMF, rt, 1 h; ii. Dowex-50-W-Na; 86%. f. 10%Pd-C, H_2, EtOH, quant.

Scheme 2

The substitution position of the sulfate group on **15** was confirmed by the downfield shift of its H-3' signal (δ: 4.51) compared to that of **13** (δ: 3.55) in the ^1H NMR spectrum (proton signals of **15** were assigned according to its ^1H-^1H COSY spectrum). Finally, hydrogenolysis of both benzyl groups on the fucosyl residue of **15** with 10% Pd-C in ethanol afforded the target compound **2** in quantitative yield.

In summary, the sulfated disaccharide, (3-SO_3Na)-GlcNAcβ1→3Fucβ1→OMe (**2**), which is involved in the species-specific reaggregation of dissociated cells of *Microciona prolifera*, was unambiguously prepared by chemical synthesis in a regio- and stereoselective manner.

EXPERIMENTAL

General methods. Optical rotations were measured with a Perkin-Elmer Model 241 MC Polarimeter at 25 °C. TLCs were performed on precoated plates of Silica Gel HF_{254} (0.5 mm, Qingdao, China) and detected by 10% sulfuric acid in methanol. Flash column chromatography was performed on Silica Gel H (400 mesh). ^1H NMR spectra were recorded at 300 or 600 MHz on a Bruker AM-300 or a Bruker AM-600 spectrometer with tetramethylsilane as the internal standard (unless otherwise specified). IR spectra were recorded on a Shimadzu IR-440 spectrophotometer, with potassium bromide disks for solid samples and films for liquid samples. Mass spectra were recorded on a VG QUATTRO MS instrument.

Methyl α- and β-L-Fucopyranoside (4). A mixture of L-fucose (10.0 g, 61.0 mmol, Aldrich), methanol (120 mL) and *p*-methylphenylsulfonic acid monohydrate (1.01 g, 6.1 mmol) was refluxed under nitrogen atmosphere for 24 h. The reaction mixture was treated with anhydrous sodium carbonate, and then concentrated *in vacuo*. The residue was chromatographed on a short silica-gel column to give a mixture of α- and β-L-fucopyranosides **4** (1.2:1.0 by ^1H NMR, 10.9 g, quant.) as a white solid: $[\alpha]_D$ -171.7° (*c* 0.8, water, lit.:[7] α-anomer, $[\alpha]_D$ -197.5°; lit.:[10] β-anomer, $[\alpha]_D$ +14.2°); IR (KBr) 3450 (OH) cm^{-1}; EIMS: *m/z* 179 $(M+1)^+$, 147 $(M-OMe)^+$.

Methyl 3,4-*O*-Benzylidene-α- and β-L-fucopyranoside (5). To a solution of **4** (3.23 g, 18.2 mmol) and benzaldehyde dimethyl acetal (4.8 mL, 1.6 mmol) in anhydrous dimethylformamide (DMF) (45 mL) was added *p*-methylphenylsulfonic acid monohydrate (0.360 g, 0.1 mmol). The solution was stirred at 50 °C under diminished pressure for 5 h, and then triethylamine (2 mL) was added to quench the reaction. The reaction mixture was poured into water, extracted with ethyl acetate, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate. Concentration followed by flash column chromatography of the residue afforded **5** (2.76 g, 57.1%, a mixture of all four diastereoisomers in 1.0:1.0:1.1:1.1) as a colorless syrup.

Methyl 2-*O*-Benzyl-3,4-*O*-benzylidene-α-, β-L-fucopyranoside (6 and 7). To a solution of **5** (2.64 g, 9.9 mmol) in anhydrous dimethylformamide (25 mL) was added sodium hydride (0.87 g, 60%) in portions at room temperature. Half an hour later,

benzyl bromide (2 mL, 2.9 g) was added and the resulting mixture was stirred at room temperature overnight. After addition of methanol (2 mL), the reaction mixture was poured into water and extracted with dichloromethane. The organic layer was washed with brine and water, then concentrated to afford a syrup, and subjected to flash column chromatography to give **6** (1.60 g, 46%) and **7** (1.60 g, 46%) as syrups. **6**: $[\alpha]_D$ -44.4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) α-anomer δ 7.4 (m, 10H, $2C_6H_5$), 5.95 (s, 1H, PhCH), 4.9~4.8 (m, 2H, $PhCH_2$), 4.71 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.65 (dd, 1H, $J_{2,3}$ = 8.0 Hz, $J_{3,4}$ = 5.1 Hz, H-3), 4.08~4.01 (m, 2H, H-4, H-5), 3.66 (dd, 1H, H-2), 3.41 (s, 3H, CH_3O), 1.35 (d, 3H, $J_{5,6}$ = 6.5 Hz, CH_3-6); β-anomer δ 7.4 (m, 10H, $2C_6H_5$), 5.94 (s, 1H, PhCH), 4.9~4.8 (m, 2H, $PhCH_2$), 4.49 (dd, 1H, $J_{2,3}$ = 6.6 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 4.29 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.1~4.0 (m, 1H, H-4), 3.80 (dq, 1H, $J_{4,5}$ = 1.8 Hz, $J_{5,6}$ = 6.6 Hz, H-5), 3.59 (s, 3H, CH_3O), 3.52 (dd, 1H, H-2), 1.43 (d, 3H, CH_3-6); EIMS: *m/z* 355 $(M-1)^+$, 325 $(M-OMe)^+$, 91 $(Bn)^+$. **7**: $[\alpha]_D$ -65.0° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) α-anomer δ 7.3 (m, 10H, $2C_6H_5$), 5.92 (s, 1H, PhCH), 4.8~4.65 (m, 2H, $PhCH_2$), 4.60 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 4.48 (dd, 1H, $J_{2,3}$ = 6.9 Hz, $J_{3,4}$ = 6.6 Hz, H-3), 4.20~4.16 (m, 2H, H-4, H-5), 3.54 (m, 1H, H-2), 3.41 (s, 3H, CH_3O), 1.42 (s, 3H, $J_{5,6}$ = 6.3 Hz, CH_3-6); β-anomer δ 7.5~7.1 (m, 10H, $2C_6H_5$), 5.92 (s, 1H, PhCH), 4.8~4.65 (m, 2H, $PhCH_2$), 4.32 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ = 6.5 Hz, H-3), 4.28 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.09 (dd, 1H, $J_{4,5}$ = 1.9 Hz, H-4), 3.94 (dq, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.56 (s, 3H, CH_3O), 3.46 (dd, 1H, H-2), 1.49 (s, 3H, CH_3-6); EIMS: *m/z* 355 $(M-1)^+$, 325 $(M-OMe)^+$, 279 $(M-Ph)^+$, 91 $(Bn)^+$.

Anal. Calcd for $C_{21}H_{24}O_5$ (356.42): C, 70.79; H, 6.74. Found: C, 70.70; H, 6.39.

Methyl 2,4-Di-*O*-benzyl-β-L-fucopyranoside (8) and Methyl 2,4-Di-*O*-benzyl-α-L-fucopyranoside (9). To a solution of **7** (0.82 g, 2.3 mmol) in dichloromethane (10 mL) was added diisobutylaluminum hydride (6 mL, 1M in hexane) dropwise in an ice-water bath. Three hours later, 5% HCl solution was added to quench the reaction. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and then concentrated. Flash column chromatography of the resulting residue afforded **8** (0.38 g, 45%) and **9** (0.39 g, 47%) as colorless syrups. **8** (crystallized on the shelf): $[\alpha]_D$ -19.8° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.5~7.2 (m, 10H, $2C_6H_5$), 4.95 and 4.66 (AB, 1H each, J = 11.4 Hz, $PhCH_2$), 4.82 and 4.72 (AB, 1H each, J = 11.6 Hz, $PhCH_2$), 4.23 (d,

1H, $J_{1,2}$ = 7.4 Hz, H-1), 3.66 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.57~3.55 (m, 2H, H-4, H-5), 3.57 (s, 3H, CH_3O), 3.54 (d, 1H, H-2), 1.23 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3-6); EIMS: *m/z* 359 $(M+1)^+$, 91$(Bn)^+$. **9**: $[\alpha]_D$ -56.8° (*c* 0.3, $CHCl_3$, lit.:[11] -68.1°); ^1H NMR ($CDCl_3$) δ 7.39~7.31 (m, 10H, $2C_6H_5$), 4.86 (AB, 2H, J = 12.7 Hz, $PhCH_2$), 4.68 (AB, 2H, J = 11.3 Hz, $PhCH_2$), 4.67 (d, 1H, $J_{1,2}$ = 3.3Hz, H-1), 4.07 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.89 (bq, 1H, $J_{5,6}$ = 6.4 Hz, $J_{4,5}$ < 0.5 Hz, H-5), 3.79 (dd, 1H, H-2), 3.64 (bd, 1H, H-4), 3.33 (s, 3H, CH_3O), 1.18 (d, 3H, CH_3-6); EIMS: *m/z* 359 $(M+1)^+$, 91 $(Bn)^+$.

Methyl 2,4-Di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-L-fucopyranoside (12). A mixture of the glycosyl acceptor **8** (0.38 g, 1.1 mmol), silver trifluoromethanesulfonate (0.36 g, 1.4 mmol), 2,4,6-trimethylpyridine (0.18 mL) and MS4A (0.10 g) in dichloromethane (2 mL) was stirred at room temperature under nitrogen for 0.5 h, then was cooled to -40 °C. To the stirred suspension was added the glycosyl donor **11**[9] (0.53 g, 1.1 mmol) in dichloromethane (2 mL). It was stirred at -40 °C for 2 h, then warmed up slowly to room temperature and stirred at room temperature overnight (8 h). The solution was diluted with ethyl acetate, filtered, concentrated, and the residue was subjected to flash column chromatography to afford **12** (0.64 g, 98% based on reacted **8**) as a white solid: $[\alpha]_D$ -37.6° (*c* 0.3, $CHCl_3$); IR (KBr) 1735 (OC=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.6~6.9 (m, 14H, $2C_6H_5$, C_6H_4), 5.76 (dd, 1H, $J_{2',3'}$ = 10.6 Hz, $J_{3',4'}$ = 9.6 Hz, H-3'), 5.70 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 5.22 (dd, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 9.6 Hz, H-4'), 4.78(AB, 2H, J = 11.3 Hz, $PhCH_2$), 4.45 and 4.15 (AB, 1H each, J = 11.4 Hz, $PhCH_2$), 4.43 (dd, 1H, $J_{2',3'}$ = 10.6 Hz, H-2'), 4.25 (dd, 1H, $J_{6'a,6'b}$ = 13.6 Hz, $J_{5',6'a}$ = 4.4 Hz, H-6'a), 4.21 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 3.98 (dd, 1H, $J_{5',6'b}$ = 2.3 Hz, H-6'b), 3.87 (dd, 1H, $J_{3,4}$ = 2.8 Hz, $J_{2,3}$ = 9.6 Hz, H-3), 3.79 (ddd, 1H, $J_{4',5'}$ = 9.6 Hz, H-5'), 3.65 (dd, 1H, H-2), 3.46 (s, 3H, CH_3O), 3.45 (bq, 1H, $J_{4,5}$ < 0.5 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.37 (bd, 1H, H-4), 2.03 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 1.83 (s, 3H, CH_3CO), 1.14 (d, 3H, CH_3-6). EIMS: *m/z* 667 $(M-1-OBn)^+$, 624 $(667-Ac)^+$, 418 (glucosaminyl)$^+$, 91 $(Bn)^+$.

Anal. Calcd for $C_{41}H_{45}NO_{14}$ (775.80): C, 63.48; H, 5.85; N, 1.80. Found: C, 63.76; H, 5.85; N, 1.47.

Methyl 3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-β-L-fucopyranoside (13). A solution of **12** (0.19 g, 0.25 mmol) and hydrazine monohydrate (0.5 mL) in 95% ethyl alcohol (10 mL) was refluxed for 6 h and then concentrated. After coevaporation with toluene, the resulting residue was mixed with pyridine (5 mL) and acetic anhydride (5 mL) which was stirred overnight (8 h) at room temperature. Pyridine and acetic anhydride were then removed *in vacuo* and the residue was purified by flash column chromatography to afford a solid, which was treated with methanol and potassium carbonate for 2 h. The solution was concentrated, and the obtained residue was purified by flash column chromatography to give **13** (0.10 g, 73% overall) as a white solid: $[\alpha]_D$ -38.4° (*c* 0.1, acetone); IR (KBr) 3460 (OH), 2900 (CH_3), 1655 (NC=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.8-7.2 (m, 10H, $2C_6H_5$), 4.90 and 4.64 (AB, 1H each, J = 11.4 Hz, $PhCH_2$), 4.82 and 4.42 (AB, 1H each, J = 11.4 Hz, $PhCH_2$), 4.70 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 4.21 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.04 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 3.7~3.6 (m, 2H, H-6a, H-4'), 3.55 (dd, 1H, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 7.4 Hz, H-3'), 3.5~3.1 (m, 6H, H-2', H-5', H-6b', H-2, H-4, H-5), 3.36 (s, 3H, CH_3O), 1.64 (s, 3H, CH_3CO), 1.09 (d, 3H, $J_{5,6}$ = 5.9 Hz, CH_3-6); EIMS: *m/z* 531 $(M+1-OMe)^+$, 204 $(glucosaminyl)^+$, 186 $(204-H_2O)^+$, 91 $(Bn)^+$.

Anal. Calcd for $C_{29}H_{39}NO_{10}$ (561.62): C, 62.02; H, 7.00; N, 2.49. Found: C, 61.48; H, 7.18; N, 2.52.

Methyl 3-*O*-(2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-β-L-fucopyranoside (14). A solution of **13** (50 mg, 0.089 mmol), benzaldehyde dimethyl acetal (0.10 mL) and camphorsulfonic acid (2 mg) in dimethylformamide (1.5 mL) was stirred for 5 h at room temperature. The reaction was then quenched with triethylamine (0.05 mL), diluted with water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and then concentrated. The resulting residue was purified with flash column chromatography to afford **14** (50 mg, 86%) as a white solid: $[\alpha]_D$ -39.6° (*c* 0.1, $CHCl_3$); IR (KBr) 3460 (OH), 2900 (CH_3), 1655 (NC=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.5~7.3 (m, 15H, $3C_6H_5$), 5.80 (bs, 1H, NH), 5.57 (s, 1H, PhCH), 5.00 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.92 and 4.72 (AB, 1H each, J = 11.7 Hz, $PhCH_2$), 4.80 and 4.73 (AB, 1H each, J = 11.7 Hz, $PhCH_2$), 4.30 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 4.26 (dd, 1H, $J_{3',4'} = J_{4',5'}$ = 8.1 Hz, H-4'),

4.22 (dd, 1H, $J_{5',6'a}$ = 4.2 Hz, $J_{6'a,6'b}$ = 10.7 Hz, H-6'a), 3.91 (dd, 1H, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 2,6 Hz, H-3), 3.8~3.4 (m, 5H, H-3', H-2', H-6'a, H-2, H-4), 3.53 (s, 3H, CH_3O), 3.32 (m, 2H, H-5, H-5'), 1.62 (s, 3H, CH_3CO), 1.29 (d, 3H, $J_{5,6}$ = 6.4 Hz, CH_3-6); EIMS: *m/z* 559 $(M+1-Bn)^+$, 91 $(Bn)^+$.

Anal. Calcd for $C_{36}H_{43}NO_{10}·H_2O$ (667.75): C, 64.76; H, 6.74; N, 2.09. Found: C, 64.89; H, 6.53; N, 1.64.

Methyl 3-*O*-(2-Acetamido-2-deoxy-3-*O*-sulfo-β-D-glucopyranosyl)-2,4-di-*O*-benzyl-β-L-fucopyranoside, sodium salt (15). To a solution of **14** (50 mg, 0.077 mmol) in DMF (1.5 mL) was added sulfur trioxide-pyridine (120 mg, 0.75 mmol), and the solution was stirred at room temperature for 1 h. Then, the reaction was quenched with methanol (0.1 mL) and DMF was evaporated *in vacuo*. The resulting residue was purified by ion-exchange column chromatography (Dowex 50w, Na^+) to afford **15** (50 mg, 86%) as a white solid: $[\alpha]_D$ -23.3° (*c* 0.1, acetone); IR (KBr) 3460 (OH), 2900 (CH_3), 1655 (NC=O) cm^{-1}; ^1H NMR (CD_3OD) δ 7.51-7.25 (m, 10H, Ph), 5.14 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 5.07 and 4.58 (AB, 1H each, J = 11.3 Hz, $PhCH_2$), 4.88 and 4.78 (AB, 1H each, J = 11.6 Hz, $PhCH_2$), 4.51 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 8.5 Hz, H-3'), 4.58 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.18 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 3.87 (bd, 1H, $J_{4,5}$ < 0.5 Hz, H-4), 3.84 (dd, 1H, H-2'), 3.79 (dd, 1H, $J_{5',6'a}$ = 1.0 Hz, $J_{6'a,6'b}$ = 11.7 Hz, H-6'a), 3.66 (bq, 1H, $J_{5,6}$ = 6.3 Hz, H-5), 3.61 (s, 2H, 2×OH), 3.60 (m, 1H, H-6'b), 3.59 (dd, 1H, H-2), 3.55 (dd, 1H, $J_{4',5'}$ = 8.4 Hz, H-4'), 3.43 (s, 3H, OMe), 3.40 (m, 1H, H-5'), 3.30 (s, 1H, NH), 1.80 (s, 3H, CH_3CO), 1.21 (d, 3H, CH_3-6). ESIMS: *m/z* 681 $(M+H_2O)^+$.

Methyl 3-*O*-(2-Acetamido-2-deoxy-3-*O*-sulfo-β-D-glucopyranosyl)-β-L-fucopyranoside, sodium salt (2). A suspension of **15** (30 mg, 0.045 mmol) and palladium on charcoal (10%, 20 mg) in ethanol (10 mL) was stirred for 80 h at room temperature (20 °C) under hydrogen (1 atm.). TLC (dichloromethane-methanol, 3:1) showed the disappearance of **15**. The suspension was filtered, concentrated to dryness, and the residue further purified by flash column chromatography (dichloromethane-methanol, 3:1) to afford **2** (21 mg, quant.) as a white solid: $[\alpha]_D$ -33° (*c* 0.1, water); IR (KBr) 3450 (OH), 1650 (NC=O) cm^{-1}; ^1H NMR (D_2O, with DOH as internal standard) δ 4.88 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 4.48 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.6 Hz, H-3'), 4.37 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 3.98 (dd, 1H, $J_{6'a,6'b}$ = 12.3 Hz, $J_{5',6'a}$ = 5.7 Hz, H-6'a), 3.91 (bd, 1H, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ <

0.5 Hz, H-4), 3.89 (dd, 1H, $J_{2,3}$ = 11.0 Hz, H-3,), 3.87 (dd, 1H, H-2'), 3.83 (dd, 1H, $J_{5',6'b}$ = 5.7 Hz, H-6'b), 3.80 (bq, 1H, $J_{5,6}$ = 6.5 Hz, H-5), 3.69 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 3.63 (dd, 1H, H-2), 3.60 (s, 3H, CH_3O), 3.57 (m, 1H, H-5'), 3.40 (s, OH), 2.28 (s, 1H, NH), 2.07 (s, 3H, CH_3CO), 1.32 (d, 3H, CH_3-6); ESIMS: *m/z* 500 $(M-1+H_2O)^+$.

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