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Synthesis of 2-acetamido-2-deoxy-3-*O*-sulfonato -D-galactopyranose sodium salt

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Introduction

T. Okuda et al. reported the molecular cloning and characterization of GalNAc 4-sulfotransferase.[1] In the work, the commercial monosulfated compound **1** and **2** (Figure 1) were used as the markers to characterize the products of the enzyme. We prepared 3-*O*-sulfated compound **3**, and also used it as one of the markers. Here we report the synthesis of **3** from D-galactosamine hydrochloride in detail, and discuss ^{1}H and ^{13}C NMR chemical shifts for the compound **1**, **2**, **3**, and GalNAc **4**.

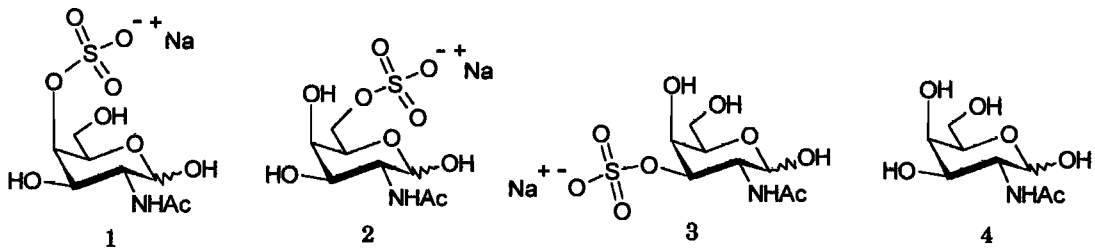
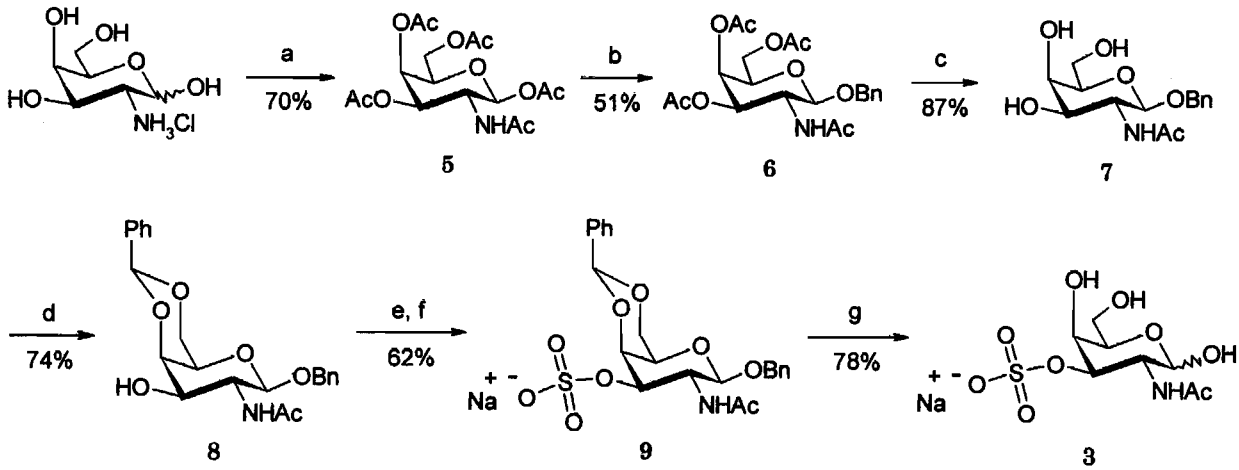


Figure 1. Structures of GalNAc(4SO$_4$) **1**, GalNAc(6SO$_4$) **2**, GalNAc(3SO$_4$) **3**, and GalNAc **4**.

Results and Discussion



Scheme 1. (a) Ac_2O, py, rt; (b) BnOH, $BF_3 \cdot OEt_2$, CH_2Cl_2, reflux; (c) 60% NaH, MeOH, rt; (d) benzaldehyde, $ZnCl_2$, rt; (e) py·SO_3, rt; (f) ion-exchange resin Na^+; (g) 5% Pd-C, 95% EtOH-H_2O, H_2, rt.

D-Galactosamine hydrochloride was treated with pyridine and acetic anhydride as the usual manner[2] to give the acetylated compound **5** in 70% yield as shown in Scheme 1. Next, the compound **5** was glycosylated with benzyl alcohol by utilizing boron trifluoride ethyl ether complex as a promoter to afford the benzyl β-*O* -glycoside **6** in 51% yield. The structure of **6** was established by ^{1}H NMR measurement. The signal for H -1 appeared as a doublet with a $J_{H-1,\ H-2}$ coupling of 8.3 Hz. The β-selective glycosylation was caused by neighboring group participation of 2-acetamido group.[3] The β-*O*-glycoside derivative **6** was de-*O*-acetylated with methanolic sodium methoxide (MeOH/NaOMe) at rt for 4 h to afford colorless needles of **7** in 87% yield.

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On the basis of ^{1}H NMR data, the compound **7** was confirmed to keep βconfiguration and had only one acetyl group attributed to 2-acetamido group. The signal for H-1 exhibited a $J_{H-1,\ H-2}$ coupling of 8.5 Hz,[4] and the signal for *N*-acetyl group appeared as a singlet at 1.80 ppm. The triol **7** was treated with an excess of benzaldehyde and 3 molar excess amounts of zinc(II) chloride to give 4,6-*O*-benzylidene derivative **8** in 74% yield.[5] The newly formed 6-membered ring involving benzylidene group is present in the chair conformation as indicated in Scheme 1. *O*-Sulfation of **8** with sulfur trioxide pyridine complex followed by ion exchange chromatography gave the sulfated compound **9** in 62% yield.[6] Under the reaction conditions, the selective 3-*O*-sulfation of **8** was accomplished without release of the 4,6-*O*-benzylidene group. Final hydrogenation of **9** with 5% Pd-C in 95% ethanol-water afforded the target molecule **3** in 78% yield. The compound **3** was used as one of the markers.[1]

^{1}H NMR data of the compound **1**, **2**, **3**, and **4** are summarized in Table 1. The proton signal for the position bonded to *O*-sulfonato group was shifted downfield relative to the corresponding signal for **4**. For example, the α-H-4 signal of 4-*O*-sulfated compound **1** was shifted downfield by 0.75 ppm relative to the corresponding signal in the spectra of **4**. The α-H-6 signal for 6-*O*-sulfated compound **2** appeared at 4.09 ppm more downfield than the corresponding signal for **4**. From the chemical shifts of the proton signal observed in the standard compounds, the downfield shift of α-H-3 signal for compound **3** by 0.75 ppm relative to the corresponding signal for **4** indicates that compound **3** bears sulfate group at position 3.

Table 1. ^{1}H NMR data (D_2O) of the compounds **1**-**4**.

	GalNAc(4SO$_4$) **1**	GalNAc(6SO$_4$) **2**	GalNAc(3SO$_4$) **3**	GalNAc **4**
αH-1	5.14	5.11	5.14	5.11
βH-1	4.56	4.53	4.68	4.52
αH-2	4.03	4.00	4.21	4.02
βH-2	3.75	3.75	3.88	3.76
αH-3	3.93	3.81	4.43	3.58
βH-3	3.71	3.60	4.27	3.57
αH-4	4.62	3.91	4.18	3.87
βH-4	4.56	3.86	4.12	3.82
αH-5	4.11	4.21	4.03	3.78
βH-5	3.68[a]	3.80	3.64	3.98
αH-6	3.66	4.09	3.65	3.62
βH-6	3.72	4.05	3.63	3.68

[a] Chemical shift was assigned by HMQC.

^{13}C NMR data of the compounds **1**-**4** are shown in Table 2. The carbon signal for the position bearing *O*-sulfonato group was shifted downfield relative to the corresponding signal for **4**. For example, the α-C-4 signal of **1** appeared at 79.71 ppm more downfield by 8.26 ppm than the corresponding signal of **4**. The C-6 signal for **2** was shifted downfield less than 3.5 ppm relative to the corresponding signal in **4**. Therefore, the downfield shift of α-C-3 signal for compound **3** by 4.47 ppm relative to the corresponding signal for **4** indicates that bears sulfate group at position **3**.

Table 2. ^{13}C NMR data (D_2O) of the compounds **1-4**.

	GalNAc(4SO$_4$) **1**	GalNAc(6SO$_4$) **2**[a]	GalNAc(3SO$_4$) **3**	GalNAc **4**
αC-1	93.81	91.00	94.13	93.86
βC-1	98.17	95.34	97.85	98.27
αC-2	53.51	50.17	51.18	53.12
βC-2	56.87	53.56	54.68	56.54
αC-3	69.36	72.72	78.47	74.00
βC-3	73.00	71.94	80.90	78.04
αC-4	79.71	68.25	69.96	71.45
βC-4	78.64	67.50	69.22	70.72
αC-5	72.80	68.41	73.23	70.26
βC-5	77.24	67.22	77.68	73.40
αC-6	64.04	67.58	63.97	64.09
βC-6	63.90	67.09	63.76	63.86

[a] Chemical shifts were assigned by HMQC.

Experimental Section

General method. ^1H and ^{13}C NMR spectra were recorded with a JEOL LA-400 spectrometer operating at 400 MHz and 100.4 MHz. Chemical shifts were referenced to TMS.

Preparation of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose (5).

D-Galactosamine hydrochloride (1.00 g, 4.64 mmol) was treated with Ac_2O (4.5 cm^3) and pyridine (7.0 cm^3) at rt for 5 h. The mixture was cooled to 10 ℃, then, was poured into water to liberate colorless powder. The powder was filtered and washed with water and ethanol to give compound **5** (1.27 g, 70%); ^1H NMR (400.0 MHz, $CDCl_3$) δ=1.92 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 2.15 (s, 3H, CH_3CO), 4.00 (td, 1H, $J_{5,6a}=J_{5,6b}=6.5$ Hz, $J_{4,5}=1.1$ Hz, H-5), 4.07 (dd, 1H, $J_{6a,6b}=11.2$ Hz, $J_{5,6a}=6.5$ Hz, H-6a), 4.15 (dd, 1H, $J_{6a,6b}=11.2$ Hz, $J_{5,6b}=6.5$ Hz, H-6b), 4.43 (dt, 1H, $J_{2,3}=11.3$ Hz, $J_{1,2}=9.1$ Hz, H-2), 5.06 (dd, 1H, $J_{2,3}=11.3$ Hz, $J_{3,4}=3.3$ Hz, H-3), 5.36 (dd, $J_{3,4}=3.3$ Hz, $J_{4,5}=1.1$ Hz, H-4), 5.38 (s, 1H, NH), 5.68 (d, 1H, $J_{1,2}=$ 9.1 Hz, H-1); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ=20.62 (q, CH_3), 20.65 (q, CH_3), 20.88 (q, CH_3), 23.31 (q, CH_3), 49.85 (d, C-2), 61.28 (t, C-6), 66.33 (d, C-4), 70.32 (d, C-3), 71.89 (d, C-5), 93.06 (d, C-1), 169.54 (s, C=O), 170.14 (s, C=O), 170.21 (s, C=O), 170.37 (s, C=O), 170.72 (s, C=O).

Preparation of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside (6).

A mixture of compound **5** (2.00 g, 5.13 mmol) and BnOH (1.06 cm^3, 10.24 mmol) and $BF_3\cdot OEt_2$ (1.56 cm^3, 12.31 mmol) in dry CH_2Cl_2 (10.0 cm^3) was refluxed for 5 h. The mixture was washed with aqueous solution of $NaHCO_3$, and water, then, dried over Na_2SO_4, and evaporated. The residue was recrystallized from CH_2Cl_2 -hexane to give compound **6** (1.16 g, 51%) as colorless crystals; ^1H NMR (400.0 MHz, $CDCl_3$) δ=1.89 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 3.87 (td, 1H, $J_{5,6a}=J_{5,6b}=6.7$ Hz, $J_{4,5}=$ 1.1 Hz, H-5), 4.06 (ddd, 1H, $J_{2,3}=11.2$ Hz, $J_{2,NH}=8.5$ Hz, $J_{1,2}=8.3$ Hz, H-2), 4.13 (dd, 1H, $J_{6a,6b}=11.5$ Hz, $J_{5,6a}=$ 6.7 Hz, H-6a), 4.19 (dd, 1H, $J_{6a,6b}=11.5$ Hz, $J_{5,6b}=6.7$ Hz, H-6b), 4.59 (d, 1H, $J_{gem}=12.2$ Hz, OCH(H)Ph), 4.64

(d, 1H, $J_{1,2}$=8.3 Hz, H-1), 4.89 (d, 1H, J_{gem}=12.2 Hz, OCH(H)Ph), 5.18 (dd, 1H, $J_{2,3}$=11.2 Hz, $J_{3,4}$=3.4 Hz, H-3), 5.22 (d, 1H, $J_{2,NH}$=8.5 Hz, NH), 5.33 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=1.1 Hz, H-4), 7.27-7.36 (m, 5H, arom. H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ=20.63 (q, CH_3), 20.68 (q, CH_3), 23.39 (q, CH_3), 51.41 (d, C-2), 61.50 (t, C-6), 66.75 (d, C-4), 69.97 (d, C-3), 70.71 (C-5+O$\underline{C}H_2$Ph), 99.73 (d, C-1), 128.04 (d, arom. CH), 128.07 (d, arom. CH), 128.46 (d, arom. CH), 136.93 (s, arom. C), 170.21 (s, C=O), 170.26 (s, C=O), 170.42 (s, C=O), 170.49 (s, C=O).

Preparation of benzyl 2-acetamido-2-deoxy-β-D-galactopyranoside (7).

Compound **6** (0.30 g, 0.68 mmol) was dissolved in dry MeOH (6.0 cm^3) and treated with 60% NaH (1 mg) at rt. After a few minute, colorless powder separated out. After stirring for 4 h, the mixture was neutralized by acetic acid. The crystals were filtered and washed with MeOH to give compound **7** (0.18 g, 87%); ^{1}H NMR (400.0 MHz, DMSO-d_6) δ=1.80 (s, 3H, CH_3CO), 3.42-3.45 (m, 1H, H-5), 3.45 (d, 1H, $J_{2,3}$=9.3 Hz, H-3), 3.54 (d, 2H, $J_{5,6}$=4.9 Hz, H-6), 3.65 (s, 1H, H-4), 3.80 (q, 1H, $J_{1,2}$=$J_{2,3}$=$J_{2,NH}$=9.3 Hz, H-2), 4.32 (d, 1H, $J_{1,2}$=9.3 Hz, H-1), 4.49 (d, 1H, J_{gem}=12.3 Hz, OC$\underline{H}$(H)Ph), 4.50-4.54 (m, 1H, C-4-OH), 4.55-4.63 (m, 2H, C-3-OH+C-6-OH), 4.76 (d, 1H, J_{gem}=12.3 Hz, OCH($\underline{H}$)Ph), 7.23-7.34 (m, 5H, arom. H), 7.65 (d, 1H, $J_{2,NH}$=9.3 Hz, NH); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ=23.07 (q, CH_3), 52.00 (d, C-2), 60.48 (t, C-6), 67.49 (d, C-4), 69.23 (t, O$\underline{C}H_2$Ph), 71.33 (d, C-3), 75.37 (d, C-5), 101.11 (d, C-1), 127.14 (d, arom. CH), 127.20 (d, arom. CH), 128.07 (d, arom. CH), 138.24 (s, arom. C), 169.34 (s, C=O).

Preparation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (8).

A mixture of compound **7** (0.19 g, 0.61 mmol) and $ZnCl_2$ (0.25 g, 1.83 mmol) in benzaldehyde (4.0 cm^3, 39.4 mmol) was stirred for 11 h at rt. The mixture was poured into water (10 cm^3), and colorless powder separated out. The powder was filtered out, washed with Et_2O to give compound **8** (0.18 g, 74%); ^{1}H NMR (400.0 MHz, CD_3OD) δ=1.94 (s, 3H, CH_3CO), 3.55 (d, 1H, $J_{5,6a}$=$J_{5,6b}$=1.6 Hz, H-5), 3.77 (dd, 1H, $J_{2,3}$=10.8 Hz, $J_{3,4}$=3.4 Hz, H-3), 4.07 (dd, 1H, $J_{2,3}$=10.8 Hz, $J_{1,2}$=8.5 Hz, H-2), 4.17 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6a}$=1.6 Hz, H-6a), 4.21 (d, 1H, $J_{3,4}$=3.4 Hz, H-4), 4.26 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6b}$=1.6 Hz, H-6b), 4.56 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 4.61 (d, 1H, J_{gem}=12.1 Hz, OC$\underline{H}$(H)Ph), 4.90 (d, 1H, J_{gem}=12.1 Hz, OCH($\underline{H}$)Ph), 5.65 (s, 1H, CH), 7.23-7.38 (m, 8H, arom. H), 7.56-7.58 (m, 2H, arom. H); ^{13}C NMR (100.4 MHz, CD_3OD) δ=23.02 (q, CH_3), 54.18 (d, CH), 68.14 (d, CH), 70.28 (t, CH_2), 71.62 (d, CH), 71.65 (t, CH_2), 76.89 (d, CH), 102.03 (d, CH), 102.49 (d, CH), 127.71 (d, arom. CH), 128.70 (d, arom. CH), 128.85 (d, arom. CH), 129.01 (d, arom. CH), 129.35 (d, arom. CH), 129.91 (d, arom. CH), 139.19 (s, arom. C), 139.70 (s, arom. C), 174.04 (s, C=O).

Preparation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-sulfonato-β-D-galactopyranoside sodium salt (9).

Compound **8** (20 mg, 0.05 mmol) was treated with SO_3·py (42 mg, 0.26 mmol) at 45 ℃ for 14.5 h. After addition of MeOH, the mixture was subjected to ion-exchange resin Na^+. The residue was purified by silica gel column chromatography to give compound **9** (12.8 mg, 62%); ^{1}H NMR (400.0 MHz, CD_3OD) δ=1.94 (s, 3H, CH_3CO), 3.60 (d, 1H, $J_{5,6a}$=$J_{5,6b}$=1.7 Hz, H-5), 4.17 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6a}$=1.7 Hz, H-6a), 4.25 (dd, 1H, $J_{6a,6b}$=12.4 Hz, $J_{5,6b}$=1.7 Hz, H-6b), 4.25 (dd, 1H, $J_{2,3}$=11.1 Hz, $J_{1,2}$=8.3 Hz, H-2), 4.48 (dd, 1H, $J_{2,3}$=11.1 Hz, $J_{3,4}$=3.3 Hz, H-3), 4.60 (d, 1H, $J_{3,4}$=3.3 Hz, H-4), 4.62 (d, 1H, J_{gem}=12.2 Hz, OC$\underline{H}$(H)Ph), 4.68 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 4.90 (d, 1H, J_{gem}=12.2 Hz, OCH($\underline{H}$)Ph), 5.64 (s, 1H, CH), 7.24-7.36 (m, 8H, arom. H), 7.53-7.56 (m, 2H, arom. H); ^{13}C NMR (100.4 MHz, CD_3OD) δ=23.14 (q, CH_3), 51.86 (d, C-2), 67.98 (d, C-5), 70.26 (t, C-6), 71.76 (t, O$\underline{C}H_2$Ph), 75.24 (d, C-4), 76.64 (d, C-3), 102.25 (d, $\underline{C}$HPh), 102.27 (d, C-1), 127.66 (d, arom. CH), 128.66 (d, arom. CH), 128.86 (d, arom. CH), 128.92 (d, arom. CH), 129.32 (d, arom. CH), 129.79 (d, arom. CH), 139.16 (s, arom. C), 139.68 (s, arom. C), 173.88 (s, C=O).

Preparation of 2-acetamido-2-deoxy-3-*O*-sulfonato-D-galactopyranose sodium salt (3).

Compound **9** (12.8 mg, 0.031 mmol) was dissolved in 95% EtOH-H_2O (4.0 cm^3) and 5% Pd-C (25.0 mg) was added. Hydrogenolysis was carried out under H_2 atmosphere for 24 h at rt. The mixture was filtered (Celite), and concentrated. The residue was purified by gel-filtration to afford compound **3** (7.8 mg, 78%); ^{1}H NMR (400.0 MHz, D_2O) δ=1.90 (s, 3H, β-$COCH_3$), 1.92 (s, 3H, α-$COCH_3$), 3.59-3.68 (m, β-H-5+α-H-6+β-H-6),

3.88 (dd, 1H, $J_{2,3}$=11.0 Hz, $J_{1,2}$=8.5 Hz, β-H-2), 4.03 (t, 1H, $J_{5,6}$=6.2 Hz, α-H-5), 4.12 (d, 1H, $J_{3,4}$=3.2 Hz, β-H-4), 4.18 (d, 1H, $J_{3,4}$=3.0 Hz, α-H-4), 4.21 (dd, 1H, $J_{2,3}$=11.2 Hz, $J_{1,2}$=3.7 Hz, α-H-2), 4.27 (dd, 1H, $J_{2,3}$=11.0 Hz, $J_{3,4}$=3.2 Hz, β-H-3), 4.43 (dd, 1H, $J_{2,3}$=11.2 Hz, $J_{3,4}$=3.0 Hz, α-H-3), 4.68 (d, 1H, $J_{1,2}$=8.5 Hz, β-H-1), 5.14 (d, 1H, $J_{1,2}$=3.7 Hz, α-H-1); ^{13}C NMR (100.4 MHz, D_2O) δ=24.92 (q, α-CH_3), 25.13 (q, β-CH_3), 51.18 (d, α-C-2), 54.68 (d, β-C-2), 63.76 (t, β-C-6), 63.97 (t, α-C-6), 69.22 (d, β-C-4), 69.96 (d, α-C-4), 73.23 (d, α-C-5), 77.68 (d, β-C-5), 78.47 (d, α-C-3), 80.90 (d, β-C-3), 94.13 (d, α-C-1), 97.85 (d, β-C-1), 177.53 (s, α-C=O), 177.76 (s, β-C=O).

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