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Note

Synthesis of benzyl *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside and benzyl *O*-(β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-6-*O*-methyl-α-D-galactopyranoside as specific acceptors for sialyltransferases ☆

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In our laboratory, we are currently involved in the synthesis of specific acceptors for sialyltransferases (STs). GalNAc: α-(2 → 6) STs catalyze the transfer of a sialic acid to 0-6 of GalNAc, and Gal: α-(2 → 3) STs are involved in the synthesis of oligosaccharides containing sialic acid linked α-(2 → 3) to Gal residues. Based on the specificities of different acceptor-substrates and differences in biochemical properties between enzymes from different sources, at least 12 different sialyltransferases (STs) are known [2–5].

In order to achieve a specific, quantitative determination of individual STs, we embarked on a synthetic program to obtain well defined, low molecular weight oligosaccharides capable of acting as acceptors for a single enzyme, even in the presence of other related enzymes. The present communication demonstrates that 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl bromide (**2**) is an effective glycosylating reagent for the synthesis of benzyl *O*-(3-*O*-methyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**7**) and benzyl *O*-(3-*O*-methyl-β-D-galactopyranosyl)-

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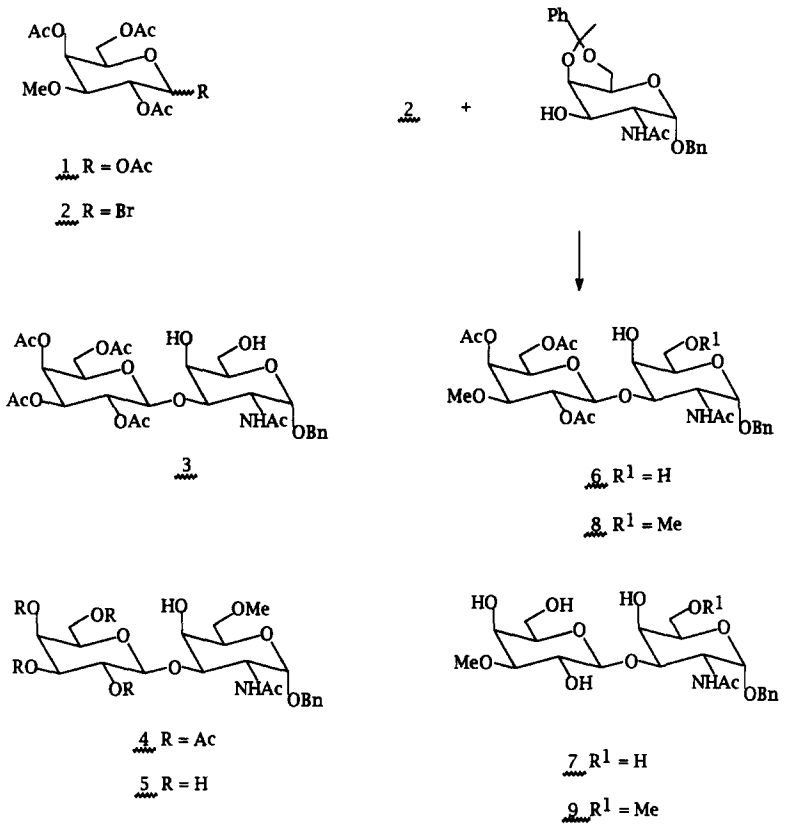

Scheme 1.

(1 → 3)-2-acetamido-2-deoxy-6-*O*-methyl-α-D-galactopyranoside (**9**). We have also accomplished the synthesis of benzyl *O*(β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-6-*O*-methyl-α-D-galactopyranoside (**5**). Compounds **5** and **7** should serve as ideal acceptors in measuring the *O*-glycan related α-(2 → 3) and α-(2 → 6) STs in tumors, other malignant tissues, as well as in cultured human cells for the purpose of studying biosynthetic pathways. Compound **9** may serve as an inhibitor in the study of this class of enzymes.

Methylation of benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**3**) [6] with trimethyloxonium tetrafluoroborate-2,6-di-(*tert*-butyl)-4-methyl pyridine [7] in dichloromethane gave the 6-*O*-methyl derivative **4** in 82% yield. Zemplen transesterification of **4** furnished compound **5** in 75% yield. The ^{13}C NMR spectrum of amorphous **5** was in agreement with the structure assigned (see Experimental section).

A common intermediate, namely, benzyl *O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**6**) was used for the synthesis of **7** and **9**.

2,4,6-Tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl bromide **2**, obtained through acetolysis of methyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranoside [8] (**1**), followed by treatment with 31% HBr in glacial acetic acid, was the key glycosyl donor. Regioselective methylation [9,10] of methyl α-D-galactopyranoside followed by acetoly-

sis with 1% H_2SO_4 in acetic anhydride provided compound **1** in 72% yield. Glycosylation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside with bromide **2** in 1:1 benzene–nitromethane and in the presence of $Hg(CN)_2$, followed by removal of the acetal ring with 70% aqueous acetic acid, produced the diol **6** in 77% yield. *O*-Deacetylation of **6** with methanolic sodium methoxide afforded, in 90% yield, the desired disaccharide **7**. A similar methylation of **6** with trimethyloxonium tetrafluoroborate-2,6-di-(*tert*-butyl)-4-methyl pyridine and processing in a manner analogous to that described for **3** (to give **5**) afforded compound **9** in 51% yield. The ^{13}C NMR spectra and FABS (see Experimental section) of both **7** and **9** were in agreement with the structures assigned.

In the ^{13}C NMR spectra of compounds **5** and **9**, the C-6 resonance of the GalNAcα-OBn residue exhibited a downfield shift (δ 67.76 and 67.73), thus confirming this position as the site of methylation. Similarly, the resonance of C-3 of the Gal residue in compounds **7** and **9** displayed a downfield shift at δ83.03 confirming this position as the site of methylation in these compounds.

1. Experimental

General methods. — Optical rotations were measured at ~ 25°C with a Perkin–Elmer 241 Polarimeter. TLC was conducted on glass plates, precoated with a 0.25 mm layer of Silica Gel 60F-254 (Analtech GHLF uniplates). The compounds were visualized by exposure to UV light and/or by spraying with 5% H_2SO_4 in EtOH and charring. The silica gel used for column chromatography was Baker Analyzed (60–200 mesh). NMR spectra were recorded at ~ 25°C; ^1H NMR spectra were obtained with a Varian EM-390 (90 MHz) and with a Bruker AM-400 (400 MHz), and ^{13}C NMR spectra with a Bruker AM-400 spectrometer operating at 100.6 MHz for ^{13}C. All chemical shifts are referenced to tetramethylsilane (TMS). Solutions in organic solvents were generally dried with anhyd Na_2SO_4. Dichloromethane, DMF and benzene were kept dry over 4 Å molecular sieves. Elemental analyses were performed by the Robertson Laboratory, Madison, New Jersey.

1,2,4,6-Tetra-O-*acetyl-3*-O-*methyl*-D-*galactopyranose* (1). — A solution of methyl 3-*O*-methyl-α-D-galactopyranoside (10 g) in acetic anhydride (150 mL) containing ~ 1% by volume of concd H_2SO_4 was stirred for 6 h at room temperature. The mixture was then diluted with dichloromethane (700 mL) and successively washed with water, saturated $NaHCO_3$ and water, dried and concentrated under reduced pressure. The residue was applied to a column of silica gel and eluted with 30% ethyl acetate in hexane to give **1** (11.5 g, 71.6%); $[\alpha]_D$ +109 (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ6.29 (d, 0.9 H, *J* ~ 4 Hz), 3.40 (bs, 3 H, OMe), 2.17–2.03 (cluster of s, 12 H, 4 × OAc).

Anal. Calcd for $C_{14}H_{22}O_9$: C, 50.29; H, 6.63. Found: C, 50.15; H, 6.59.

2,4,6-Tri-O-*acetyl-3*-O-*methyl*-α-D-*galactopyranosyl bromide* (2). — 1,2,4,6-Tetra-*O*-acetyl-3-*O*-methyl-D-galactopyranose (30 g) in methylene chloride (300 mL) and acetic anhydride (15 mL) was cooled (0°C, bath) and treated with 31% HBr in glacial acetic acid (200 mL), and the mixture stirred for 2 h at 0°C. The mixture was diluted with methylene chloride (200 mL), and successively washed with cold water, cold saturated $NaHCO_3$ and cold water, dried and evaporated. The residue was dissolved in methylene chloride, and addition of ether–hexane resulted in the precipitation of **2** (21 g, 59%), a white powder; $[\alpha]_D$ +202 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ5.62 (d, *J* = 4

Hz, 1 H, H-1), 5.52 (d, $J = 3$ Hz, 1 H, H-4), 4.87 (dd, $J = 4.5$ Hz, 1 H, H-3), 3.43 (s, 3 H, OMe), 2.13, 2.10 and 2.07 (each s, 9 H, 3 × OAc).

Benzyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-6*-O-*methyl-α*-D-*galactopyranoside* (**4**). — To a cold (0°, bath) and stirred solution of benzyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (1.2 g, 1.9 mmol) in methylene chloride (30 mL) was added 2,6-di-(*tert*-butyl)-4-methylpyridine (0.8 g, 3.9 mmol) and trimethyloxonium tetrafluoroborate (0.4 g, 2.7 mmol). Stirring was continued for 5 h at the same temperature. The mixture was diluted with methylene chloride and successively washed with saturated aqueous $NaHCO_3$, water, dried and concentrated under reduced pressure. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 3–5% methanol in chloroform. The earlier fractions contained the faster-migrating compound **4**. On concentration these fractions afforded a solid (0.75 g, 82% on the basis of **3** reacted); $[\alpha]_D$ +85° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.26 (m, 5 H, aromatic), 5.46 (d, $J = 9.6$ Hz, 1 H, NH), 5.36 (d, $J = 3.4$ Hz, 1 H, H-4′), 5.18 (dd, $J = 7.9$ Hz, 1 H, H-2′), 4.98 (d, $J = 3.5$ Hz, 1 H, H-1), 4.94 (dd, 1 H, H-3′), 4.59 (d, $J = 7.8$ Hz, 1 H, H-1′), 3.41 (s, 3 H, OMe), 2.15, 2.05, 2.03, 1.97 (each s, 12 H, 4 × OAc), 1.92 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ 101.59 (C-1′), 97.42 (C-1), 78.09 (C-3), 68.43 (C-6), 61.43 (C-6′), 59.33 (OMe), 47.84 (C-2).

Anal. Calcd for $C_{30}H_{41}NO_{15}$: C, 54.95; H, 6.30; N, 2.14. Found: C, 54.82; H, 6.43; N, 1.94.

Later fractions contained pure unreacted compound **3** (0.3 g).

Benzyl O-*(β*-D-*galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-6*-O-*methyl-α*-D-*galactopyranoside* (**5**). — Compound **4** (0.7 g) was stirred in 0.05 *M* methanolic sodium methoxide (50 mL) for 16 h at room temperature. The solution was deionized with Amberlite IR-120 (H^+) cation-exchange resin, filtered and concentrated under reduced pressure. After purification over a silica gel column with $CHCl_3$–MeOH–H_2O (13 : 6 : 1) as the eluent, **5** (0.39 g, 75%) was obtained as an amorphous solid, $[\alpha]_D$ +125° (*c* 1.2, DMSO); ^{1}H NMR [$(CD_3)_2SO$]: δ 7.63 (d, $J = 8.1$ Hz, 1 H, NH), 7.36–7.35 (m, 5 H, aromatic), 4.80 (d, $J = 3.5$ Hz, 1 H, H-1), 3.27 (s, 3 H, OMe), 1.82 (s, 3 H, NAc); ^{13}C NMR: δ 103.76 (C-1′), 96.63 (C-1), 75.64 (C-3), 75.38 (C-5′), 73.37 (C-5), 71.99 (C-3′), 69.37 (C-2′), 68.35 (C-4), 68.23 (C-4′), 67.76 (C-6), 60.60 (C-6′), 58.40 (OMe), 48.45 (C-2), 22.76 (NAc); *m/z*: 488.2 $(M + H)^+$, 486.5 $(M - H)^-$.

Anal. Calcd for $C_{22}H_{33}NO_{11}$: C, 54.20; H, 6.82; N, 2.87. Found: C, 53.94; H, 7.11; N, 2.84.

Benzyl O-*(2,4,6-tri*-O-*acetyl-3*-O-*methyl-β*-D-*galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α*-D-*galactopyranoside* (**6**). — A stirred mixture of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (1.6 g, 4 mmol) and powdered $Hg(CN)_2$ (2.0 g, 8 mmol) in 1 : 1 benzene–nitromethane (100 mL) was boiled until 20 mL of the solvent had distilled off. The temperature was then adjusted to 45°C, and a solution of 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-galactopyranosyl bromide (3.0 g, 7.8 mmol) in benzene (30 ml) was added and stirring was continued for 16 h at room temperature. The mixture was diluted with benzene and washed with saturated aqueous $NaHCO_3$, 10% aqueous KI solution and water, dried and evaporated to a syrup. This crude compound in 70% aqueous acetic acid (100 mL) was stirred for 2 h at ~ 90°C. Acetic acid was evaporated under reduced pressure, the last traces being removed by co-evaporation with several added portions of toluene to leave a residue that was purified on a column of silica gel using a solvent gradient consisting of 3–5% methanol

in chloroform, yielding after evaporation of fractions amorphous product **6** (1.9 g, 77%); $[\alpha]_D$ +103° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ7.73–7.31 (m, 5 H, aromatic), 5.59 (d, J = 9.3 Hz, 1 H, NH), 5.45 (d, J = 3.1 Hz, 1 H, H-4′), 4.99 (d, J = 3.5 Hz, 1 H, H-1), 4.55 (d, J = 7.9 Hz, 1 H, H-1′), 3.34 (s, 3 H, OMe), 2.13, 2.08 and 2.04 (each s, 9 H, 3 × OAc), 1.94 (s, 3 H, NAc); ^{13}C NMR ($CDCl_3$): δ101.46 (C-1′), 97.48 (C-1), 79.37 (C-3′), 77.64 (C-3), 62.72 (C-6′), 61.97 (C-6), 57.98 (OMe), 47.89 (C-2).

Anal. Calcd for $C_{28}H_{39}NO_{14}$: C, 54.80; H, 6.41; N, 2.28. Found: C, 55.11; H, 6.29; N, 2.14.

Benzyl O-(3-O-methyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**7**). — *O*-Deacetylation of compound **6** (0.5 g) with 0.025 *M* methanolic sodium methoxide afforded compound **7** (0.36 g, 90%); $[\alpha]_D$ +127° (*c* 1.0, Me_2SO); ^{1}H NMR [$(CD_3)_2SO$]; δ7.60 (d, J = 8.0 Hz, 1 H, NH), 7.37–7.34 (m, 5 H, aromatic), 4.79 (d, J = 3.6 Hz, 1 H, H-1), 3.32 (s, 3 H, OMe), 1.82 (s, 3 H, NAc); ^{13}C NMR: δ103.64 (C-1′), 96.40 (C-1), 83.03 (C-3′), 75.83 (C-3), 75.12 (C-5′), 71.45 (C-5), 69.62 (C-2′), 68.02 (C-4), 67.36 (C-4′), 60.68 (C-6), 60.40 (C-6′), 56.15 (OMe), 48.48 (C-2), 22.63 (NAc); m/z: 488.3 $(M + H)^+$, 486.4 $(M - H)^-$.

Anal. Calcd for $C_{22}H_{33}NO_{11}$: C, 54.20; H, 6.82; N, 2.87. Found: C, 54.11; H, 6.91; N, 2.91.

Benzyl O-(3-O-methyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-6-O-methyl-α-D-galactopyranoside (**9**). — Methylation of compound **6** (1.2 g), followed by *O*-deacetylation as described for the preparation of **5**, afforded compound **9** (0.5 g, 51%) after silica gel column chromatography (solvent gradient consisting of 15–20% methanol in chloroform); $[\alpha]_D$ +121° (*c* 1.6, Me_2SO); ^{1}H NMR [$(CD_3)_2SO$]: δ7.61 (d, J = 7.9 Hz, 1 H, NH), 7.38–7.35 (m, 5 H, aromatic), 4.80 (d, J = 3.5 Hz, 1 H, H-1), 3.32 and 3.27 (each s, 6 H, 2 × OMe), 1.81 (s, 3 H, NAc); ^{13}C NMR: δ103.64 (C-1′), 96.58 (C-1), 83.03 (C-3′), 75.54 (C-3), 75.16 (C-5′), 71.93 (C-5), 69.60 (C-2), 69.33 (C-4), 68.27 (C-4′), 67.73 (C-6), 60.45 (C-6′), 58.34 and 56.17 (2 × OMe), 48.37 (C-2), 22.63 (NAc); m/z: 502.3 $(M + H)^+$, 500.5 $(M - H)^-$.

Anal. Calcd for $C_{23}H_{35}NO_{11}$: C, 55.08;H, 7.03; N, 2.79. Found: C, 54.94; H, 7.10; N, 2.64.

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