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Synthesis of Methyl 2-Acetamido-2-Deoxy-3-*O*-(β-D-Galactopyranosyl)-α-D-Galactopyranoside from a Corresponding Thioglycoside Derivative

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The title disaccharide glycoside was synthesized by halide ion-promoted glycosidation, using methanol and the disaccharide bromide derived from methyl 2-azido-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside. This derivative in turn was prepared by silver triflate-promoted condensation of monosaccharide derivatives.

In *O*-linked carbohydrate chains of glycoproteins, 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-galactopyranose occurs as a structural unit, usually glycosidically α-linked to serine or threonine. Several syntheses of this disaccharides [1] and its derivatives [2-6] have been reported. We now report synthesis of the protected Gal-GalNAc derivative **5** and its conversion into the disaccharide methyl glycoside **6**.

Results and Discussion

Treatment of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-galactopyranosyl bromide [7] with sodium thioacetate [8] in *N,N*-dimethyl formamide gave the 1-thioacetyl derivative **1** in 61% yield. *S*-Deacetylation of **1** with methanolic sodium methoxide at -30°C gave the corresponding 1-thiol, which was directly alkylated with methyl iodide to give **2** in 94% yield. Deacetylation of **2** with methanolic sodium methoxide and subsequent treatment of the product with α,α-dimethoxytoluene and *p*-toluenesulfonic acid in acetonitrile gave the 4,6-benzylidene derivative **3** in 91% yield. Glycosidation of **3** with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide [9], using silver triflate as promoter, gave the disaccharide 1-thioglycoside **4** in 55% yield. This derivative is a suitable block that can be used in synthesis of higher oligosaccharides containing the Gal-GalNAc structure, as exemplified in the following paper [10]. It can also be used directly in glycosidation of alcohols to produce alkyl Gal-GalNAc glycosides. As an example of

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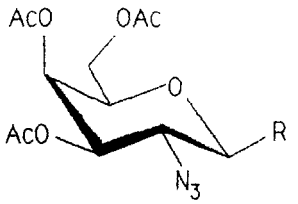
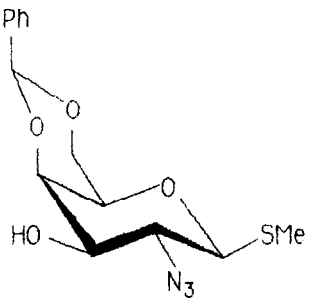


1 : R = SCOMe

2 : R = SMe



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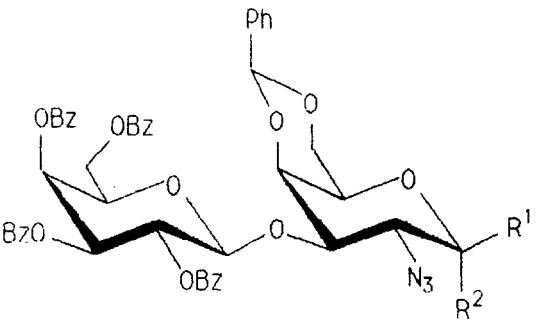
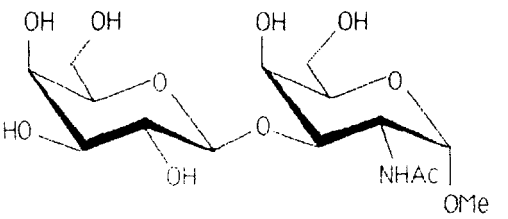


4 : R^1 = SMe R^2 = H

5 : R^1 = H R^2 = OMe



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this, compound **4** was treated with bromine in dichloromethane, and the resulting bromide was directly reacted with methanol and tetraethylammoniumbromide [11] to give the α-methyl glycoside **5** in 88% yield. Treatment of **5** with, successively, methanolic sodium methoxide, 80% aqueous acetic acid, hydrogen/palladium on carbon, and acetic anhydride in methanol gave the deprotected disaccharide methyl glycoside **6** (52% overall yield).

Experimental

General Methods

Melting points are corrected. Concentrations were performed at 1-2 kPa at <40°C (bath). Optical rotations were recorded for 0.5-1.0% solutions in chloroform, unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25°C for solutions in C^2HCl_3 unless otherwise stated using JEOL FX 100, JEOL GX 400, or Bruker AM 500 instruments. The following reference signals were used: C^2HCl_3 δ 77.17 (^{13}C in C^2HCl_3); $CHCl_3$ δ 7.26 (^1H in C^2HCl_3); dioxane δ 67.4 (^{13}C in ^2H_2O); Me_2CO δ 2.225 (^1H in ^2H_2O).

Only selected data are reported. All ^1H assignments were corroborated by 2-D COSY experiments. TLC was performed on silica gel F_{254} (Merck, Darmstadt, W. Germany) with detection by UV light when applicable or by charring with sulfuric acid. Column chromatography was performed on silica gel 60 (0.04-0.063 mm, Merck) with loadings in the range 1/25-1/100 and elution with toluene/ethyl acetate mixtures unless otherwise stated. Organic solutions were dried over $MgSO_4$. Molecular sieves (4Å, Merck) were desiccated in a vacuum at 300°C overnight and ground immediately before use. Dowex-50 (H^+) ion exchange resin was washed extensively with methanol before use.

*1-S-Acetyl 3,4,6-Tri-O-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranose (**1**)*

Potassium thioacetate (0.20 g) was added to a stirred solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide [7] (0.30 g) in *N,N*-dimethyl formamide (2.0 ml). After 1 h, dichloromethane was added and the mixture was washed with water. The organic layer was concentrated and purified by column chromatography. The main fraction was syrupy **1** (0.18 g, 61%), $[\alpha]_D$ +28°. NMR data: ^{13}C, δ 20.6 (CH_3COO), 30.8 (CH_3COS), 59.3 (C-2), 61.2 (C-6), 66.6, 73.1, 75.0 (C-3,4,5), 81.3 (C-1); ^1H, δ 5.09 (d, $J_{1,2}$ 7.4 Hz, H-1).

*Methyl 3,4,6-Tri-O-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside (**2**)*

Sodium methoxide (0.5 M, 1.0 ml) was added, at -30°C, to a stirred solution of **1** (0.16 g) in methanol (10 ml). After 30 min, iodomethane (0.05 ml) was added, and the solution was concentrated. The residue was partitioned between water and dichloromethane, and the organic layer was dried and concentrated. Crystallization of the residue from ethyl acetate/hexane gave **2** (0.13 g, 94%), m.p. 121-122°C, $[\alpha]_D$ -56°. NMR data: ^{13}C, δ 12.6 (SCH_3), 20.6 (CH_3COO), 60.1, 61.5, 66.6, 72.9, 74.4 (C-2,3,4,5,6), 84.9 (C-1); ^1H, δ 4.32 (d, $J_{1,2}$ 10.2 Hz, H-1).

Analytical data. Calculated for $C_{13}H_{19}N_3O_7S$: C, 43.2; H, 5.3; N, 11.6; S, 8.9. Found: C, 43.3; H, 5.3; N, 11.5; S, 8.7.

*Methyl 2-Azido-4,6-O-benzylidine-2-deoxy-1-thio-β-D-galactopyranoside (**3**)*

A suspension of **2** (4.42 g) in methanolic sodium methoxide (0.05 M, 50 ml) was stirred at room temperature for 2 h, then neutralized with Dowex-50 (H^+) resin, and concentrated. The residue was mixed with acetonitrile (50 ml), α,α-dimethoxytoluene (2.4 g), and *p*-toluenesulfonic acid (50 mg) and stirred overnight at room temperature. Then triethylamine (1 ml) was added, and the mixture was concentrated. The residue was purified by column chromatography to give **4** (3.62 g, 91%). Crystallized from ethyl acetate/hexane, it had an m.p. 107-108°C, $[\alpha]_D$ -29°. NMR data: ^{13}C, δ 11.6 (SCH_3), 62.6, 69.1, 69.8, 72.9, 74.7 (C-2,3,4,5,6), 83.6 (C-1), 101.3 (PhCH).

Analytical data. Calculated for $C_{14}H_{17}N_3O_4S$: C, 52.0; H, 5.3; N, 13.0. Found: C, 51.9; H, 5.3; N, 12.9.

*Methyl 2-Azido-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside (**4**)*

A mixture of **3** (0.35 g), tetra-*O*-benzoyl-β-D-galactopyranosyl bromide [9] (0.88 g), dichloromethane (10 ml), and molecular sieves was stirred at -20°C while a solution of silver triflate (0.38 g) and 2,4,6-trimethylpyridine (0.12 ml) in dichloromethane/toluene (5/2 by vol, 7 ml) was added. After 20 min, pyridine (1 ml) was added, and the mixture was diluted with dichloromethane and filtered. The filtrate was washed with, successively, aqueous sodium thiosulfate, water, 1 M sulfuric acid, and aqueous sodium bicarbonate, then concentrated. The residue was purified by column chromatography to give syrupy **4** (0.56 g, 55%), $[\alpha]_D$ +60°. NMR data: ^{13}C δ 10.9 (SCH_3), 60.4, 62.6, 68.3, 69.0, 69.8, 69.9, 71.7, 72.0, 75.2, 81.1 (C-2,3,4,5,6, C-2',3',4',5',6'), 83.9 (C-1), 100.9 (PhCH), 102.8 (C-1'); ^1H, δ 2.20 (s, SCH_3), 3.22 (broad s, H-5), 3.54 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.2 Hz, H-3), 3.70 (dd, $J_{5,6}$ 1.3, $J_{6a,6b}$ 12.5 Hz, H-6a), 3.87 (dd, $J_{1,2}$ 9.9 Hz, H-2), 4.15 (d, H-1), 4.21 (dd, $J_{5,6}$ 1.2 Hz, H-6b), 4.34 (d, H-4), 4.40 (m, H-6'a, 6'b), 4.80 (dd, $J_{5',6'a}$ 6.4, $J_{5',6'b}$ 10.5 Hz, H-5'), 5.16 (d, $J_{1',2'}$, 7.9 Hz, H-1'), 5.48 (s, PhCH), 5.60 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3'), 5.90 (dd, H-2'), 6.00 (d, H-4').

*Methyl 2-Azido-3-O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (**5**)*

A solution of bromine (0.056 ml) in dichloromethane (10 ml) was added dropwise to a stirred mixture of **4** (0.93 g) and molecular sieves in dichloromethane (10 ml). After 15 min, cyclohexene (0.25 ml) was added (to destroy excess bromine), followed by a mixture of tetraethylammonium bromide (0.50 g), molecular sieves, and dichloromethane (10 ml). After 2 min, methanol (2.0 ml) was added, and the mixture was stirred for 5 h, then filtered, washed with water, and concentrated. The residue was purified by column chromatography to give syrupy **5** (0.80 g, 88%) as the main fraction, $[\alpha]_D$ +223°. NMR data: ^{13}C, δ 55.7 (OCH_3), 59.0 (C-2), 62.6, 63.0, 68,3, 69.0, 69.6, 71.7, 72.2, 76.0, 76.6 (C-3,4,5,6, C-2',3',4',5',6'), 99.6 (C-1), 100.7 (PhCH), 102.8 (C-1'); ^1H, δ 4.83 (d, *J* 3,6 Hz, H-1), 5.13 (d, *J* 8.0 Hz, H-1').

*Methyl 2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranoside (**6**)*

Methanolic sodium methoxide (0.05 M, 10 ml) was added to a solution of **5** (0.70 g) in chloroform (3 ml). After 30 min, the mixture was neutralized with Dowex-50 and concentrated. The residue was dissolved in 80% aqueous acetic acid and heated to 90°C for 30 min, then concentrated. The residue was partitioned between diethyl ether and water. The aqueous layer was adjusted to 20 ml, mixed with Pd/C (10%, 0.075 g) and hydrogenated at 300 kPa overnight. Methanol (10 ml) was added, then acetic anhydride in portions (3 × 2 ml, 1 h intervals). After 4 h, the mixture was filtered and concentrated. The residue was purified by gel filtration on a column of Bio-Gel P-2. Elution with water gave **6** (0.16 g, 52%) as the main fraction. Crystals were obtained from methanol, m.p. 228-230°C, $[\alpha]_D$ +65° (*c* 0.2, water). NMR data: ^{13}C, δ 23.0 (CH_3CONH), 49.5 (C-2), 56.0 (OCH_3) 61.9, 62.2 (C-6, C-6'), 69.5, 69.7, 71.4, 71.5, 73.5, 75.9, 78.2, (C-3,4,5, C-2',3',4',5'), 99.3 (C-1), 105.6 (C-1'), 175.5 (CH_3CONH); ^1H, δ 2.02 (s, CH_3CONH), 3.39 (s, OCH_3), 3.51 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.9 Hz, H-2'), 3.61 (dd, $J_{3',4'}$ 3.5 Hz, H-3'), 3.65 (m, H-5'), 3.71-3.80 (m, H-6a,b, H-6'a,b), 3.91 (dd, $J_{4',5'}$ 0.9 Hz, H-4'), 3.96 (m, H-5), 4.01 (dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.3 Hz, H-3), 4.23 (dd, $J_{4,5}$ 0.7 Hz, H-4), 4.33 (dd, $J_{1,2}$ 3.8 Hz, H-2), 4.45 (d, H-1'), 4.78 (d, H-1).

Analytical data. Calculated for $C_{15}H_{27}NO_{11}.H_2O$: C, 43.4; H, 7.0; N, 3.4. Found: C, 42.9; H, 7.0; N, 3.2.

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