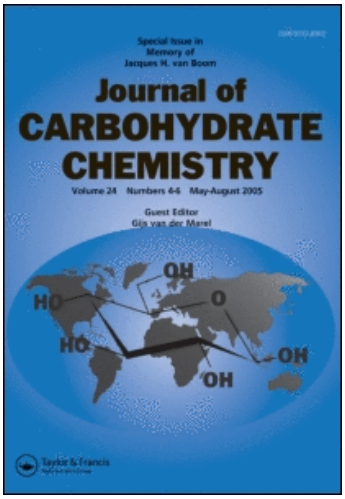

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SYNTHESIS OF METHYL 2-ACETAMIDO-2-DEOXY-5,6-O-ISOPROPYLIDENE-β-D-GALACTOFURANOSIDE AND THE TF-ANTIGENIC DISACCHARIDE

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ABSTRACT

Methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside was prepared in one step from 2-acetamido-2-deoxy-D-galactose and 2,2-dimethoxypropane in good yield. Condensation of this compound with acetobromogalactose using mercury (II) cyanide as catalyst followed by removal of the protecting groups from the resulting disaccharide derivative yielded the Thomsen-Friedenreich (TF) antigenic disaccharide.

INTRODUCTION

It has been reported by Hasegawa *et al.* that when *N*-acetylglucosamine was allowed to react with 2,2-dimethyoxypropane in the presence of *p*-toluenesulfonic acid using dioxane as solvent at 65-70 °C, 2-acetamido-2-deoxy-3,4:5,6-di-*O*-isopropylidene-aldehydo-D-*galacto*-dimethyl acetal was obtained in good yield. An identical derivative of *N*-acetylgalactosamine, however, gave a different product which was then utilized for the synthesis of the TF-disaccharide.

RESULTS AND DISCUSSION

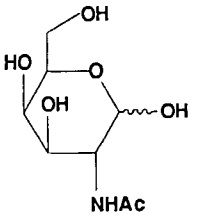
2-Acetamido-2-deoxy-D-galactose (**1**), when treated with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid in dioxane at 65-70 °C for 3 h, gave two products. The major product was characterised by ^{1}H NMR and methylation as methyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (**2**), obtained in 72% yield, together with a minor amount (20%) of another isopropylidene derivative (**3a**). While **2** was also reported [2] to be formed when 2-acetamido-2-deoxy-D-galactose was allowed to react with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid using

N,N-dimethylformamide as solvent, but the yield was low. The isopropylidene derivative **3a** was characterised as methyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranoside from its ^{1}H NMR spectrum and also from the identity of its tosyl derivative (**3b**) with methyl 2-acetamido-2-deoxy-3,4-*O*-isopropylidene-6-*O*-tosyl-α-D-galactopyranoside as reported earlier.[3]

Compound **2** was allowed to condense with acetobromogalactose [4] in the presence of mercury (II) cyanide and molecular sieves (4A) in benzene-nitromethane (1:1) as solvent to give the disaccharide derivative **6** in 30% yield. Removal of the protecting groups and acetolysis of the methyl glycofuranosidic linkage furnished the TF-antigenic disaccharide **8**. The low yield of the final condensation product could not be clearly understood. However, this report may be significant considering the simplicity of the method used and the biological importance[5] of the TF-antigenic disaccharide.

The disaccharide was characterised by comparing its ^{1}H NMR data with those in the literature[6] and also by performing hemagglutination-inhibition assays[7] of jackfruit and peanut lectins with standard TF-disaccharide and the one synthesised by us.

The minimum disaccharide concentration necessary for visible inhibition of the jackfruit lectin was 10 M[8] and that of peanut lectin was 0.185 mM[9] while the minimum concentration required for the standard TF-disaccharide was 0.16 mM.



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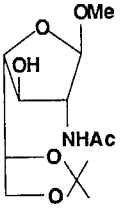

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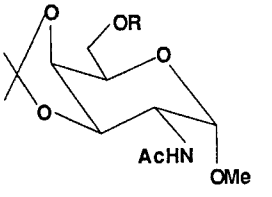
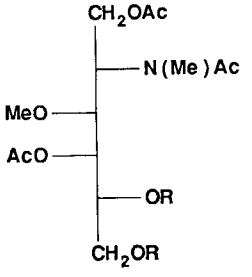


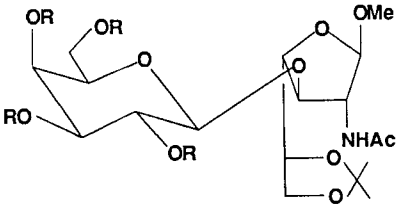
3a R = H

b R = Ts



4 R = Ac

5 R = Me



6 R = Ac

7 R = H

β-D-GaL-(1-->3)-D-GalNAc

EXPERIMENTAL

General methods. All reactions were monitored by TLC using Silica Gel G (Merck). Column chromatography was performed on 100-120 mesh Silica Gel (SRL, India). Optical rotations were measured at 589 nm with a Perkin-Elmer Model 241 MC Spectropolarimeter. NMR spectra were taken with a Jeol Model FX-100 spectrometer using TMS as an internal standard and chloroform-d or methanol-d as the solvent. The mass spectra were taken on a Hewlett-Packard Model 5988A mass spectrometer.

Methyl 2-Acetamido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (2). 2-Acetamido-2-deoxy-D-galactose (**1**, 750 mg, 3.39 mmol) was treated with 2,2-dimethoxypropane (3 mL) and *p*-toluenesulfonic acid (105 mg) at 65-70 °C in dioxane (7.5 mL) for 3 h. The reaction was quenched with triethylamine and solvent was removed under reduced pressure. Column chromatography (ethyl acetate) of the syrupy residue gave **2** (670 mg, 72%) and **3** (192 mg, 20%). Compound **2** had $[\alpha]_D$ 35 -31.2° (c 3.05, chloroform), [Lit.[2] $[\alpha]_D$ -38.5°]; ^{1}H NMR δ 1.4 and 1.46 (2s, 6H, CMe_2) 1.92 (s, 3H, NAc), 3.38 (s, 3H, OMe), 4.85 (s, 1H, H-1).

Anal. Calcd for $C_{12}H_{21}O_6N$: C, 52.4; H, 7.69; N, 5.09. Found: C, 52.3; H, 7.61; N, 5.15.

Compound **3a** had mp 159-160 °C and $[\alpha]_D^{25}$ + 178° (c 1, EtOH). [Lit.[3] mp 160-162 °C and $[\alpha]_D^{25}$ + 178.4°]. Tosyl derivative of **3a** had mp 171-172 °C and [α] + 105° (*c* 0.5, $CHCl_3$). ^{1}H NMR data: δ 1.32 and 1.55 (2s, 6H, CMe_2), 2.00 (s, 3H, NAc), 3.35 (s, 3H, MeO), 4.7 (d, 1H, H-1). Methylation of **2** and characterization of the corresponding alditol acetate (**4**) by mass spectrometry was carried out in the usual way. In another experiment the isopropylidene substituent was removed from **2** by heating it with 50% acetic acid at 50 °C for 1 h and the resulting methyl furanoside was methylated and analysed by mass spectrometry as its alditol acetate (**5**). Mass spectral data for compounds **4** and **5** are given in the table.

TABLE: Mass fragments of compounds **4** and **5**.

Compound	Peaks at m/e
4	74, 87, 89, 116 (base peak), 142, 158, 201, 261
5	45, 59, 74, 89, 101, 116 (base peak) 142, 145, 158, 173, 205, 318

Methyl 2-Acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-5,6-*O*-isopropylidene-β-D-galactofuranoside (6). Compound **2** (390 mg, 1.418 mmol) was dissolved in dry nitromethane-benzene (1:1, 7 mL). To this was added 4A molecular sieve and mercury (II) cyanide (800 mg, 3.16 mmol) and the mixture was stirred in argon atmosphere at r.t. After 30 minacetobromogalactose (885 mg, 2.15 mmol) in nitromethane-benzene (1:1, 3 mL) was added dropwise by means of a syringe. The reaction mixture was stirred for 24 h at r.t. The mixture was diluted with dichloronethane (30 mL) and filtered through a bed of celite. The filtrate and washings (60 mL) were washed with 10% potassium iodide, saturated sodium bicarbonate and water in succession. The organic layer was dried (Na_2SO_4), concentrated to a syrup and then purified by column chromatography. Elution of the column with 20% EtOAc-Et_2O furnished compound **6** (250 mg, 30%); $[\alpha]_D^{35}$-11° (*c* 1.96, $CHCl_3$). ^1H NMR data: δ 1.42 and 1.46 (2s, 6H, CMe_2), 1.96-2.1 (15 H, NAc and OAc), 3.4 (s, 3H, OMe), 4.8 (s, 1H, H-1) and 4.5 (d, 1H, J = 10 Hz, H-1).

Anal. Calcd for $C_{26}H_{39}O_{15}N$: C, 51.56; H, 6.49; N, 2.31. Found: C, 52.16; H, 6.66; N, 2.76.

Methyl 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-5,6-*O*- isopropylidene-β-D-galactofuranoside (7). Compound **6** (235 mg) was deacetylated with sodium methoxide solution (5 mL, 0.05 M) for 6 h at r.t. The mixture was then decationised with Dowex 50 W x 8 (H^+) resin and concentrated to dryness to give **7** (129 mg); $[\alpha]_D^{35}$-57.3° (*c* 2.48, water).

2-Acetamido-2-deoxy-(β-D-galactopyranosyl)-D-galactose (8). Compound **7** (88 mg) was heated with 50% acetic acid (5 mL) at 80 °C for 2.5 h. Acetic acid was removed by evaporation under reduced pressure at r.t. The residue was paper chromatographed (ethyl acetate-pridine-water 8:2:1) and the major slower moving spot was collected as compound **8** $[\alpha]_D^{35}$ + 34.5° (*c* 0.3, water) (equilibrium). [Lit.[11] + 31.0° (equilibrium)]. ^1H NMR data: δ 1.98 (s, NAc), 3.5-4.5 (ring protons), 4.7 (d, 8 Hz, H-1), 5.2 (d, 4 Hz, H-1).

Anal. Calcd for $C_{14}H_{25}O_{11}N$: C, 43.9; H, 6.57. Found: C, 43.6; H, 6.81.

Hemagglutination-inhibition study[7]. To a two fold serial dilution of the test solution containing the disaccharide (25 μL) was added the lectin solution buffer (25 μL). After incubation for 2 h at 22 °C, 25 μL of a 2% suspension of erythrocytes (normal for jackfruit lectin and neuraminidase treated for peanut lectin) were added, and the mixture was stored for 1 h at 22 °C. The minimum dilution of the disaccharide solution showing visible inhibition was determined.

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