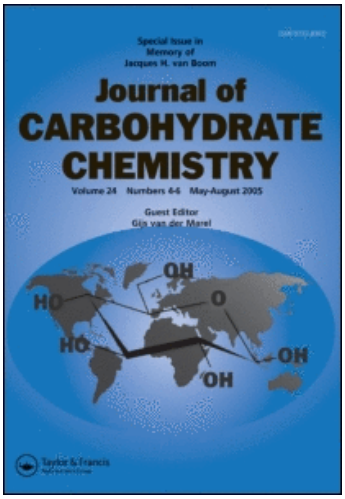

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Gupta, Dipti and Surolia, Avadhesha(1992) 'Synthesis of Specifically Deuteriated Derivatives of D-Galactose and D-Galactosamine', Journal of Carbohydrate Chemistry, 11: 2, 171 — 182
To link to this Article: DOI: 10.1080/07328309208017798
URL: http://dx.doi.org/10.1080/07328309208017798

J. CARBOHYDRATE CHEMISTRY, 11(2), 171-182 (1992)

SYNTHESIS OF SPECIFICALLY DEUTERIATED DERIVATIVES OF D-GALACTOSE AND D-GALACTOSAMINE

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Received November 29, 1990 - Final Form October 24, 1991

ABSTRACT

Simple and convenient methods for introducing deuterium label at C-3 and C-6 position of N-acetyl-D-galactosamine and D-galactose, respectively, are described. For the synthesis of 2-acetamido-2-deoxy-D-3-[^{2}H] galactopyranose, benzyl 2-acetamido-2-deoxy-4,6-O-benzylidene-α-D-galactopyranoside was oxidized with dimethyl sulfoxide- acetic anhydride and the product was reduced with sodium borodeuteride to introduce the deuterium at C-3. After benzylidene reduction, the mixture was subjected to hydrogenolysis and purified by column chromatography. 1,2:3,4-di-O-isopropylidene-α-D-galactopyranoside was oxidized followed by reduction with sodium borodeuteride and deprotection to yield D-6-[^{2}H] galactose.

INTRODUCTION

It is evident from studies of the composition and properties of normal and malignant cell surfaces, that the latter expresses antigens which are unique to these cells and different from those expressed by their normal counterparts.[1-2] It has been shown that most of these differences are associated with the carbohydrate portion of the cell surface glycoprotein and glycolipids.[3] These membrane components are involved in numerous biological processes,[4-5] including cell-cell recognition, cell differentiation,[6] antigenicity, viral and bacterial infection and

metastasis.[7] On the plasma membrane, these structures appear to function as receptors for biomolecules that initiate the aforementioned processes. Most of these biological processes are based on carbohydrate-protein interactions.[8-11] Lectins, a class of carbohydrate binding proteins of non-immune origin, are excellent models for studying the molecular basis of carbohydrate-protein interactions. In addition, they are useful probes to monitor the changes occurring on the cell surface during physiological and pathological processes, for characterizing glycoproteins and glycolipids, for histochemical studies of cells and tissues and for tracing neuronal pathways.

Nuclear Magnetic Resonance (NMR) spectroscopy is a convenient method to study such interactions[12-18] and has been widely used to obtain information about the dynamics of such reactions. Nuclei such as ^{19}F, ^{13}C, and ^{2}H are preferred over ^{1}H for such studies, because of simplicity of the spectra obtained and consequent ease of interpretation.[19-21]

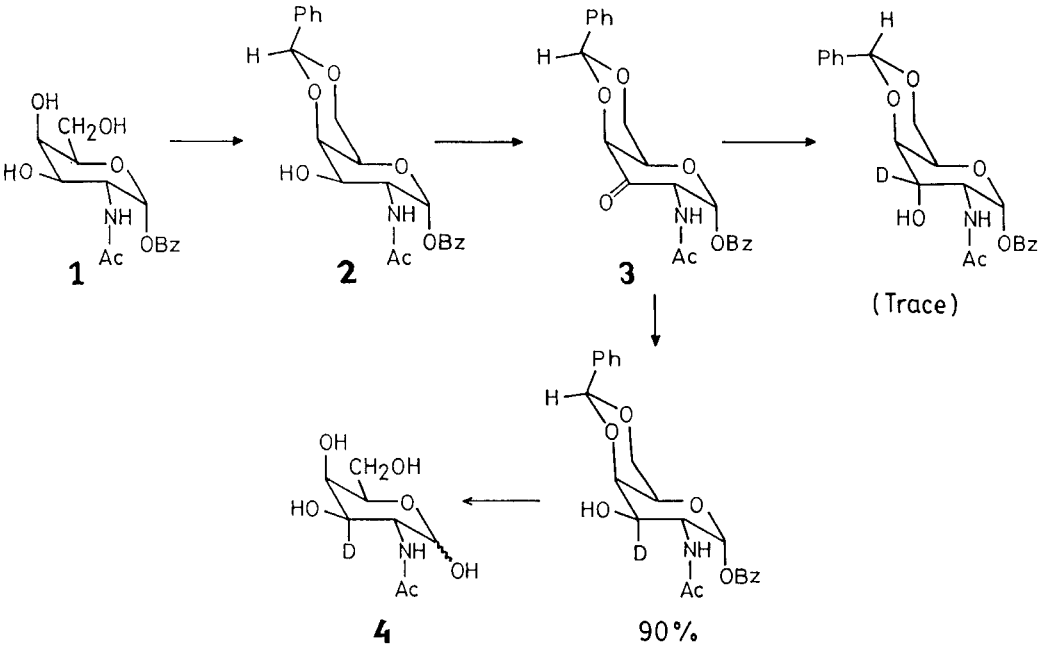
Deuterium NMR has been of tremendous value in the study of the dynamics of molecules at membrane surfaces. The deuterium nuclear quadrupole moment is of special significance because deuterium relaxation occurs almost exclusively via the quadrupole mechanism. Thus, a change in deuterium relaxation will reflect the change of correlation time of the deuterium nucleus in a ligand, arising from its interaction with macromolecules. Hence, ligands containing deuterium at different positions are useful as they would yield information about the extent of involvement of these positions in the binding events. Since the carbohydrates terminally located at the cell surface are mainly D-galactose[22] and sialic acid,[23] we have synthesized deuteriated derivatives of N-acetyl-D-galactosamine and D-galactose, in order to study their interactions with lectins, that recognize them.

RESULTS AND DISCUSSION

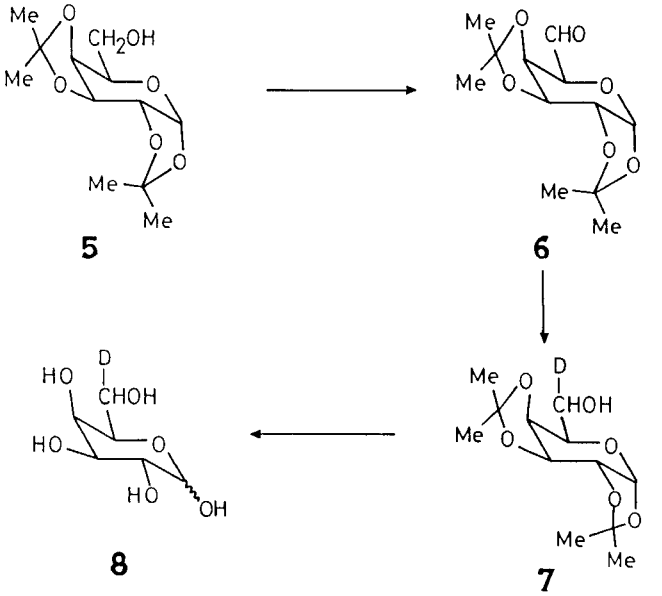
The chemical reactions described herein provide a simple and economical means to synthesize 2-acetamido-2-deoxy-3-[^{2}H]-D-galactosamine and 6-[^{2}H]-D-galactose. The synthesis of 2-

acetamido-2-deoxy-3-[^{2}H]-D-galactose was accomplished starting from benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside **1**. This derivative was chosen in order to generate the corresponding reducing sugar under the mild conditions of hydrogenolysis. Compound **1** was prepared from 2-acetamido-2-deoxygalactose by the treatment of the latter with a solution of gaseous hydrogen chloride in benzyl alcohol at 70 °C. The amorphous product was converted to pure and crystalline benzyl 2-acetamido-2-deoxy-4,6-O-benzylidene-α-D-galactopyranoside **2** by $ZnCl_2$ -catalyzed benzylidination with benzaldehyde, followed by crystallization. Oxidation of **2** with dimethyl sulfoxide-acetic anhydride[24] gave benzyl 2-acetamido-2-deoxy-4,6-O-benzylidene-α-D-galactopyranoside-3-ulose **3** in high yield (90%). After purification by crystallization, **3** was reduced with sodium borodeuteride in methanol and subsequent deprotection with 70% acetic acid and catalytic hydrogenation gave a mixture containing mainly 2-acetamido-2-deoxy-D-3-[^{2}H] galactopyranoside in 90% yield and only traces of one more compound. The resultant syrup was subjected to column chromatography to afford pure 2-acetamido-2-deoxy-D-3-[^{2}H] galactopyranoside **4**. Traces of the other compound could not be characterized. It was presumably the gulo epimer. It was surprising to find that the deuterium was mainly incorporated at the axial position (90%). The best explanation for the observed stereochemistry is that attack from the α-face results in a product which lacks a destabilizing 1,3 diaxial interaction which would occur on reduction from the β-face, thus the most stable epimer, i.e., the galacto epimer will predominate.

1,2:3,4-Di-O-isopropylidene-α-D-galactopyranoside **5** is the key intermediate in the preparation of several D-galactose analogs modified at C-6. For synthesis of D-6-[^{2}H] galactose, **5** was oxidized with dimethyl sulfoxide-acetic anhydride, to give 1,2:3,4-di-O-isopropylidene-α-D-galacto-hexodialdo-1,5-pyranose **6**, which was reduced with sodium borodeuteride in methanol at room temperature to yield 1,2:3,4-di-O-isopropylidene-α-D-6-[^{2}H] galactose **7** in almost quantitative yield. Deprotection with 85% aqueous acetic acid gave D-6-[^{2}H] galactose **8**, which was purified by column chromatography. Alternatively, **6** may be prepared from D-



Scheme 1



Scheme 2

Table 1. ^{1}H NMR spectral data[a] for Glycopyranoses and Compound **4** & **8** (δ in ppm)

Sugar	H-1	H-2	H-3	H-4	H-5	H-6	H6'	NAc
α-D-GalNAc	5.29	4.20	3.96	4.05	4.12	3.79	3.79	2.01
β-D-GalNAc	4.69	3.90	3.75	3.99	3.71	3.83	3.83	2.01
4	5.18	4.19	-	4.05	4.10	3.80	3.80	2.00
	4.73	3.90	-	4.00	3.70	3.84	3.84	2.00
α-D-Gal	5.23	3.79	3.81	3.94	4.03	3.68	3.70	
β-D-Gal	4.54	3.45	3.59	3.88	3.62	3.64	3.73	
8	5.21	3.78	3.82	3.93	4.03	-[b]	-[b]	
	4.54	3.46	3.58	3.88	3.61	-[b]	-[b]	

a. Obtained for solution in deuterium oxide (Internal TMS: δ0.0)

b. A very broad peak at δ 3.66-3.72 appeared due to equal deuteration at position C-6 & C-6' and deuterium quadrupolar relaxation.

galactose by oxidation with D-galactose oxidase.[25-27] Since the yields obtained by the galactose oxidase method were not reported, it is not possible to compare the results of both procedures. Compound **4** and **8** were characterized by ^{1}H NMR spectroscopy. Spectra of authentic N-acetyl-D-galactosamine and D-galactose were similar to those obtained for **4** and **8** except for the ^{1}H signals lost due to deuterium substitution (Table 1). Appearance of a single peak in deuterium NMR confirmed that deuteration is occurring at a single carbon atom as expected (Figs. 1-2). The sites of deuteration were further confirmed by the comparison of ^{13}C NMR shifts of 2-acetamido-2-deoxy-D-galactose and D-galactose with their deuteriated derivatives (Table 2). ^{13}C NMR spectra of **4** shows that substitution of deuterium for hydrogen causes disappearance of signal attributable to C-3 of α and β isomer of

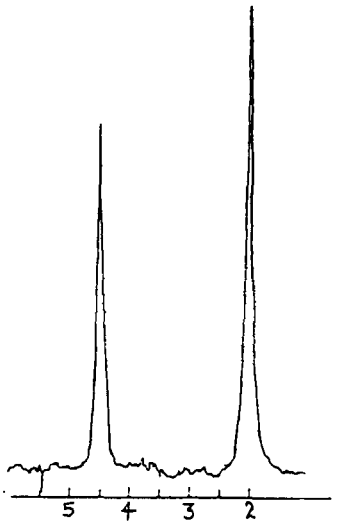


FIG.1. ^{2}H NMR spectra of **4** in H_2O. The resonance at high field (2.0 ppm) is due to internal standard, CD_3CN.

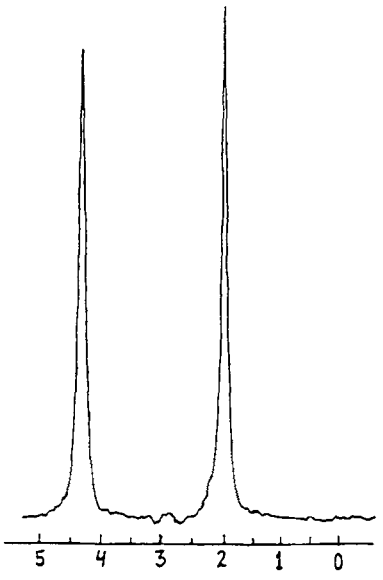


FIG.2. ^{2}H NMR spectra of **8** in H_2O. The resonance at high field (2.0 ppm) is due to internal standard, CD_3CN.

Table 2. ^{13}C NMR spectral shifts[a] for Glycopyranoses and Compound **4** & **8**

Sugar	C-1	C-2	C-3	C-4	C-5	C-6	CO	CH_3
α-D-GalNAc	91.95	51.40	68.40	69.86	71.36	62.40	23.2	176.1
β-D-GalNAc	96.29	54.80	72.01	69.00	76.00	62.00	23.4	175.8
4	92.00	51.30	-	70.00	71.40	62.50	23.0	176.0
	96.40	54.90	-	69.00	76.00	62.10	23.3	175.8
α-D-Gal	93.18	69.35	70.13	70.28	71.30	62.04		
β-D-Gal	97.37	72.96	73.78	69.29	75.93	61.84		
8	93.20	69.35	70.10	70.31	71.40	-		
	97.40	72.95	73.80	69.29	76.00	-		

a. In ppm from external tetramethylsilane.

2-acetamido-2-deoxy-D-galactose. Similar disappearance of signal due to C-6 was observed in compound **8** also.

This methodology, with adequate safety precautions, may be used to prepare the corresponding tritiated galactose analogs.

EXPERIMENTAL

General Methods: Melting points are uncorrected. Reactions were monitored by TLC on silica gel. Plates were developed with a H_2SO_4 -in-MeOH spray at 100 °C. Column chromatography was performed on silica gel (Merck, 100-200 mesh). Samples were concentrated using rotary evaporator at 40 °C. Silica gel (Merck, 100-200 mesh) was used for flash column chromatography. $C^2H_3O^2H$ (99.5%) and $(C^2H_3)_2CO$ were purchased from Sigma Chemical Company, USA. Sodium borodeuteride was purchased from Merck. Resins were dried properly after regeneration and before use. All solvents were distilled from appropriate drying agents. ^{1}H NMR spectra were recorded on a Bruker WH-270 spectrometer

operating in the pulsed Fourier transform mode and spectra were obtained in D_2O and $CDCl_3$ with Me_4Si as the internal standard. ^{13}C NMR spectra were recorded on the same spectrometer operating at 67.8 MHz in the pulsed Fourier transform mode with complete proton decoupling chemical shifts.

2-Acetamido-2-deoxy-D-galactose. D-Galactosamine hydrochloride (1 g) in 23 mL of water and 2.5 mL of methanol was stirred for 90 min at 4 °C with DOWEX 1 (28 mL, carbonate form) and acetic anhydride (0.60 mL). The mixture was filtered and the resin was washed with water. Washings were passed through a column containing Amberlite IR-120 resin (H^+ form, 7 mL) and washed with water. The colorless effluent and washings were concentrated to dryness *in vacuo* at 45 °C. The 2-acetamido-2-deoxy-D-galactose crystallized during the evaporation as colorless web-shaped crystals (920 mg, 73.6%). Rf=0.75 (CH_2Cl_2 :MeOH::1:1,v/v) mp 164 °C, lit.[28] mp 163-164 °C.

Benzyl 2-Acetamido-2-deoxy-4,6-O-benzylidene-α-D-galacto pyranoside (2). N-acetyl-D-galactosamine (500 mg) was stirred overnight at 70 °C with 2% solution of gaseous hydrogen chloride in benzyl alchohol. The reaction mixture was cooled in an ice bath, an excess of dry ether was added and benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside **1** precipitated. The precipitate was isolated by filtration, was washed thoroughly with dry ether and dried to give a white compound with mp 185 °C, $[\alpha]_D$ +190°(*c* 1.0, H_2O). It gave a single spot on TLC, Rf= 0.33 (CH_2Cl_2 : MeOH :: 5.5: 4.5). This material was shaken for 24 hrs with freshly distilled benzaldehyde and fused zinc chloride (1.0 g). The clear solution obtained was shaken vigorously with an excess of cold water and n-pentane. The white precipitated product was separated by filtration, washed thoroughly with water and pentane, and dried. Crystallization from 60% pyridine in water gave white needles of mp 245 °C, yield 375 mg (40%). Recrystallization from same solvent mixture raised the mp to 246 °C, $[\alpha]^{28}$ +219° (*c* 2.12, pyridine).

Anal. Calcd for $C_{22}H_{25}NO_6.H_2O$: C, 63.35; H, 6.34. Found C, 63.25; H, 6.60.

Benzyl 2-Acetamido-2-deoxy-4,6-O-benzylidene-α-D-hexapyranoside-3-ulose (3). 500 mg of 2 in DMSO (20 mL) and acetic anhydride (10 mL) was stirred and allowed to stand at room temperature for 48 hrs. The reaction mixture was poured into a saturated sodium bicarbonate solution which caused the precipitation of crude 3. The precipitate was filtered and washed extensively with cold water and dried in vacuo over P_2O_5. Recrystallization of residual 3 from chlorofom-acetone gave white crystalline 3 (88%). mp 168 °C; Rf= 0.50 (C_6H_{14}; CH_2Cl_2:: 3:7, v/v); νmax 1742 (C=O) and 1657 cm^{-1} (-$NHCOCH_3$).

Anal. Calcd for $C_{22}H_{23}O_6N$ C,66.49; H,5.83; N 3.52 found C, 66.20; H, 5.55; N, 3.35.

2-Acetamido-2-deoxy-3-[^{2}H]-D-galactose (4): Crystalline 3, (500 mg) was stirred in methanol (15 mL) with sodium borodeuteride (150 mg) for 20 hrs at room temperature. During the reduction, insoluble ketone 3, dissolved. The solution was concentrated to give a solid residue. The residue was dissolved in chloroform and then was washed with water and dried over anhydrous sodium sulphate. Removal of sodium sulphate by filtration and concentration of the chloroform solution gave a solid residue. TLC analysis of the residue showed complete disappearance of the 3 and appearance of benzyl 2-acetamido-2-deoxy-4,6-O-benzylidene-α-D-3-[^{2}H] galactopyranoside.

The benzylidene group was removed by heating the residue at 100 °C in 75% acetic acid until all the residue dissolved. The resulting solution was evaporated to dryness and co-evaporated a number of times with water and toluene. The residue was dissolved in ethanol-water and hydrogenated over Pd-C for 24 hrs at room temperature. The solution was then filtered and evaporated to dryness. The syrupy epimeric mixture of 2-acetamido-2-deoxy-D-3-[^{2}H] hexoses contained mainly galacto isomer and traces of presumably gulo isomer. Column chromatography of this syrup with ethyl acetate-methanol as eluant gave 4, a syrup which was shown to be pure by TLC and paper chromatography and was used for recording the NMR spectrum without crystallization. Rf=0.72 (CH_2Cl_2:MeOH::1:1,v/v) ; Rf=0.34 (CH_2Cl_2:MeOH::1.5:1)

Anal. Calcd for $C_8H_{15}O_6N$: C,43.49; H,6.79;N, 6.33 ,Found C, 43.35; H, 6.76 ; N,6.32. Compound 5 was prepared by the method of Schmidt.[29]

1,2:3,4-Di-O-isopropylidene-α-D-galacto-hexodialdo-1,5-pyranose (6). Compound 5 (2 g) was dissolved in dimethyl sulfoxide (40 mL) and to this solution acetic anhydride (20 mL) was added. The reaction mixture was kept at room temperature for 48 hours and monitored on TLC in solvent system (hexane:ether::1:1, v/v). After 40 hours no trace of 5 was detected and the reaction mixture was poured into ice cold water. After 10 minutes, the aqueous phase was decanted from an oil which had separated at bottom of flask. The oil was dissolved in chloroform and the chloroform solution was washed with water. The aqueous washings were extracted with chloroform. The combined organic phases were dried over anhydrous sodium sulphate, filtered and concentrated to colorless syrup (1.75 g). This syrup was distilled in vacuo to give **6** as a colorless liquid. Bp 104 °C, lit.[30]104-106 °C. IR (KBr) 1737 cm^{-1}.

6-[^{2}H]-D-galactose. Compound **6** (1.6 g) was stirred in methanol (50 mL) with sodium borodeuteride (400 mg) for 16 hrs at room temperature. The solution was evaporated in vacuo to a solid residue. The residue was heated for 6 hours at 90 °C in 70% aqueous acetic acid. The solution was evaporated to give a solid residue. Borates were removed by co-distillation with methanol. Water was added to the solid residue, solution extracted with chloroform and colorless aqueous phase evaporated to give pure **8**.

Anal. Calcd for $C_6H_{14}O_6$: C,39.56; H,7.69 Found: C,76.32;H, 7.65.

ACKNOWLEDGEMENT:

This work was supported by a grant from Department of Science and Technology (India), Integrated Long Term Programme.

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