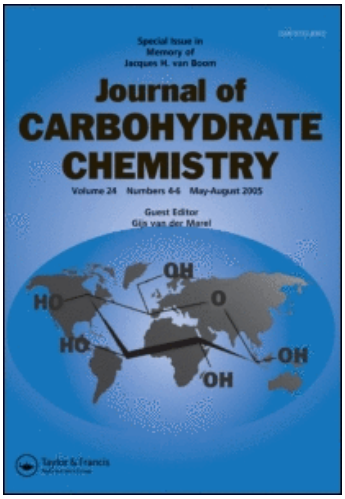


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of 6'-Aminohexyl 2-Acetamido-2-deoxy-D-galactoside Isomers and a Unique Isomerization Catalyzed by Ion Exchange Resin

Reiko T. Lee[a]; Ting C. Wong[a]; Yuan Chuan Lee[a]
[a] Department of Biology and McCollum-Pratt, Institute The Johns Hopkins University, Baltimore, MD

To cite this Article Lee, Reiko T. , Wong, Ting C. and Lee, Yuan Chuan(1986) 'Synthesis of 6'-Aminohexyl 2-Acetamido-2-deoxy-D-galactoside Isomers and a Unique Isomerization Catalyzed by Ion Exchange Resin', Journal of Carbohydrate Chemistry, 5: 3, 343 — 357
To link to this Article: DOI: 10.1080/07328308608058840
URL: http://dx.doi.org/10.1080/07328308608058840

J. CARBOHYDRATE CHEMISTRY, 5(3), 343-357 (1986)

SYNTHESIS OF 6'-AMINOHEXYL 2-ACETAMIDO-2-DEOXY-D-GALACTOSIDE ISOMERS AND A UNIQUE ISOMERIZATION CATALYZED BY ION EXCHANGE RESIN[1]

Reiko T. Lee, Ting C. Wong, and Yuan Chuan Lee*

Department of Biology and McCollum-Pratt Institute
The Johns Hopkins University
Baltimore, MD 21218

Received April 28, 1986 - Final Form June 13, 1986

ABSTRACT

The 6-aminohexyl α- and β-pyranosides and β-furanoside of 2-acetamido-2-deoxy-D-galactose (N-acetyl-D-galactosamine) were prepared. N-Acetyl-D-galactosamine was reacted with acetyl chloride in the cold. The per-O-acetyl 1-chloro derivative was then reacted with 6-(N-trifluoroacetyl)aminohexanol in the presence of mercuric cyanide. Glycoside products were isolated by gel chromatography, de-O-acetylated, and de-N-trifluoroacetylated. The β-pyranoside, which is the major product, was obtained in 29% overall yield, as well as the β-furanoside.

The α-pyranoside was prepared in 70% overall yield by reacting 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-galactopyranose, which is a byproduct of the above synthetic scheme (22%), with 6-(N-trifluoroacetyl)aminohexanol in the presence of boron trifluoride etherate.

A strong cation exchange resin (Dowex 50X8, H^+ form) catalyzed interconversion of the β-furanoside and the β-pyranoside, with the latter predominating at equilibrium. The α-pyranoside was not produced in any appreciable amount in the Dowex-catalyzed isomerization. Although the allyl β-pyranoside and β-furanoside of N-acetyl-D-galactosamine also isomerized under

 0732-8303/86/0503-0343$3.50/0

similar conditions, the competing hydrolysis of the glycosidic bond occurred to a greater extent. A possible mechanism for the isomerization is discussed.

INTRODUCTION

We have previously reported facile methods for preparing ω-aminoalkyl glycosides of a number of mono- and di-saccharides[2-5]. These ω-aminoalkyl glycosides are versatile reagents that can be used in the preparation of cluster ligands[6] or gels containing specific carbohydrates[7,8]. The mammalian hepatic lectin specific for Gal/GalNAc currently under study in our laboratory has far stronger affinity for GalNAc than for Gal or lactose[9,10]. We have now developed practical methods for the preparation of 6-aminohexyl pyranosides of GalNAc which serve as building blocks of high affinity ligands to be used in various biochemical studies of the lectin.

RESULTS AND DISCUSSION

Two synthetic schemes were used for the preparation of three isomeric 6-(N-trifluoroacetyl)aminohexyl glycosides of GalNAc. Preparation of the β-pyranoside and β-furanoside involves the following steps: 1) formation of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl (and furanosyl) chloride (1) by modification of Horton's method[11], 2) conversion of the 1-chloro derivative to glycosides using 6-(N-trifluoroacetyl)-aminohexanol and mercuric cyanide, 3) sodium methoxide-catalyzed de-O-acetylation followed by de-N-trifluoroacetylation with triethylamine. Horton[11] developed a "one-pot" synthesis of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride by directly reacting GlcNAc with acetyl chloride. However, when this method was applied to GalNAc, considerable degradation resulted and the reaction mixture became discolored. By carrying

out the reaction briefly at room temperature and mostly in the cold in a tightly sealed flask, we were able to obtain the 1-chloro derivative in about 60% yield with little discoloration. A by-product, 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-galactopyranose (2), persisted in the reaction mixture for several days. The preparation containing both 1 and 2 was used as such for glycosylation. The by-product (2), which remained unchanged during the glycosylation reaction, was completely separated from the glycosides by gel filtration through a column of Sephadex LH-20, and was obtained in a crystalline form in about 22% yield.

The glycosylation reaction produced the β-pyranoside and the β-furanoside in a ratio of approximately 4 to 1. The isomeric products were separated by repeated gel filtration after de-O-acetylation. Alternatively the β-pyranoside can be obtained in a pure state as the per-O-acetylated product by fractional crystallization, leaving a mother liquor enriched in the per-O-acetylated β-furanoside. The isomers, after de-O-acetylation, interconvert in the presence of Dowex 50X8, with the β-pyranoside predominating at apparent equilibrium. Therefore, the following sequence of operation after glycosylation gave the best yield of the β-pyranoside: 1) fractional crystallization of the per-O-acetyl-β-pyranoside, 2) de-O-acetylation of the crystalline product (β-pyranoside only) as well as the mother liquor (β-pyranoside and β-furanoside), 3) Dowex-50 treatment of the de-O-acetylated mother liquor to increase the β-pyranoside, followed by separation by gel filtration (Sephadex G-25), 4) de-N-trifluoroacetylation of the combined β-pyranoside fractions. In this fashion we obtained 6'-aminohexyl 2-acetamido-2-deoxy-β-D-galactopyranoside in 29% overall yield.

The byproduct, 2, obtained above was utilized for the preparation of the α-pyranoside. Refluxing 2 and 6-(N-trifluoroacetyl)aminohexanol for 1.5 h in toluene or nitromethane in the presence of 0.1 M boron trifluoride etherate produced a glycoside mixture in ~90% yield. Examination by thin layer chromatography

(t.l.c.) in solvent D after de-O-acetylation indicated that the α-pyranoside was the major product (~70%), followed by the β-pyranoside (~10%) and the β-furanoside (<5%). Crystalline α-pyranoside (4αp) was obtained in 70% yield after gel filtration as described above. The boron trifluoride-catalyzed glycoside formation appeared to proceed via an oxazoline derivative. Detailed studies of this method will be presented elsewhere. Allyl glycosides were prepared by the Königs-Knorr type reaction (β-pyranoside and β-furanoside) and by the Fischer synthesis (α-pyranoside)[4]. Structural assignment of the glycoside isomers was made primarily from ^{1}H-NMR examination of per-O-acetylated derivatives[12]. ^{1}H-NMR parameters for per-O-acetylated derivatives of allyl and 6-(N-trifluoroacetyl)aminohexyl glycosides of GalNAc are presented in Table 1. It can be seen that in the pyranoside, the chemical shift of H5 (δ 3.9) is at a much higher field than that of H4 (δ 5.4), while the positions are reversed in the furanoside structure. The coupling constants, $J_{1,2}$, of 3.7 Hz and 8.9 Hz are those expected for pyranosides of the α- and β-configuration, respectively. A small $J_{1,2}$ (0.9 Hz) for the furanoside strongly suggests a β-configuration. In addition, a mild periodate oxidation followed by a specific determination of formaldehyde[13] was used to differentiate pyranosyl and furanosyl forms. Compounds 4βf and 5βf produced 0.7 and 0.88 mol of formaldehyde per mol, while 4βp produced only a negligible amount.

A strongly acidic cation exchanger (Dowex 50X8 200-400 mesh, H^+ form) catalyzed isomerization between the β-pyranoside and the β-furanoside. Although all the β-pyranosides and β-furanosides of N-acetyl-galactosamine we have tested appeared to undergo this isomerization, interconversion of the 6-(N-trifluoroacetyl)amino-hexyl β-furanoside and β-pyranoside (4βf and 4βp, respectively) proceeded most smoothly. Under Condition A (see EXPERIMENTAL) in which the sulfonic acid of Dowex 50 resin was equivalent to ca. 0.1 M if it existed as a homogeneous solution, 4βf and 4βp isomerized to an apparent equilibrium in 1-2 days, at which time the β-pyranoside and the β-furanoside represented about 95% and

TABLE 1

Chemical Shifts and Coupling Constants of Per-O-acetylated Glycosides

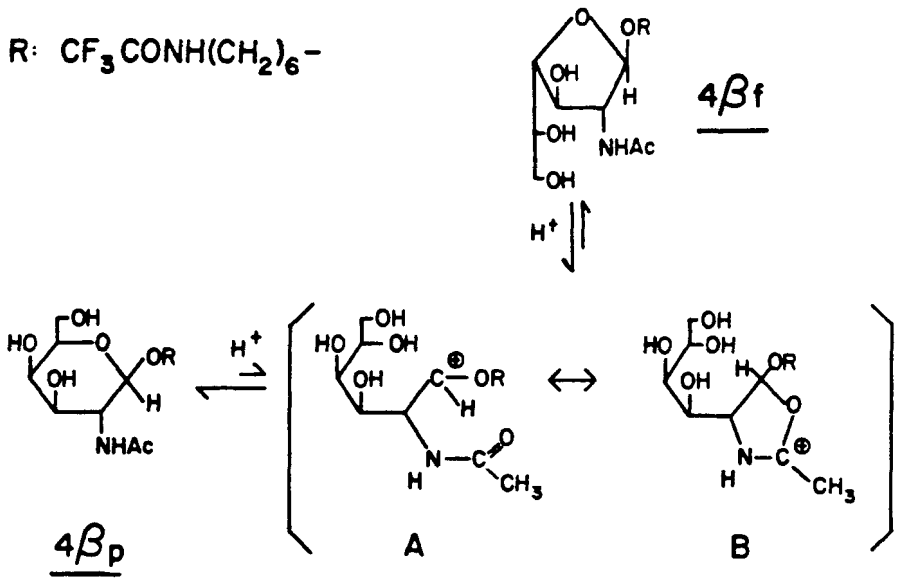
Sugar Structure	Aglycon	H1	$J_{1,2}$	H2	$J_{2,3}$	H3	$J_{3,4}$	H4	$J_{4,5}$	H5	H6
βp[a]	TFA-AH[b]	4.645	8.9	4.003	11.5	5.250	3.4	5.375	1.8	3.910	4.165
βp	Allyl	4.757	8.9	3.985	11.0	5.316	3.4	5.370	c	3.915	4.146
αp	TFA-AH	4.880	3.7	4.574	11.0	5.164	3.4	5.378	c	d	d
αp	Allyl	4.935	3.7	4.600	11.9	5.193	3.4	5.381	1.5	e	e
βf	TFA-AH	4.934	0.9	4.249	3.2	4.739	5.5	4.208	3.7	5.352	4.362, 4.219
βf	Allyl	4.990	0.9	4.411	2.4	4.758	5.2	4.221	4.3	5.374	4.359, 4.235

[a]Anomeric configuration is indicated by α and β; ring size by p(pyranose) and f(furanose). [b]N-(Trifluoroacetyl)aminohexyl. [c]Not determinable. [d]H5 and H6 signals overlap in the range of 4.076 to 4.163. [e]H5 and H6 signals overlap in the range of 4.059 to 4.213.

5%, respectively, of the total glycoside (as estimated by integration of the H1 signals in the ^{1}H-NMR spectrum), regardless of whether the process was started from 4βf or 4βp. Therefore, the isomerization was very much easier to observe from the β-furanoside than from the β-pyranoside. In fact, Dowex-treatment of 4βf sometimes produced a barely detectable amount of 4αp in addition to a large amount of 4βp. However the isomerization between 4αp and 4βp was never observed. In the presence of a larger amount of the Dowex resin (Condition B), the isomerization was faster but was accompanied by increased hydrolysis of the glycosidic bond. Inclusion of 67% isopropanol also did not alter the overall isomerization pattern. Acidity alone is not sufficient for the isomerization to occur, since 4βf and 4βp did not isomerize when 0.1 M p-toluenesulfonic acid was used instead of Dowex-50 resin. We speculate that the hydrophobic nature of the Dowex resin matrix, in addition to its acidic nature, is essential for the isomerization to proceed smoothly.

Two other 6-(N-trifluoroacetyl)aminohexyl glycosides, the β-pyranosides of N-acetyl-glucosamine and galactose, were also tested for isomerization (β-Furanosides of these sugars were not available to us). Using t.l.c. (which can detect as little as ca. 5% isomerization), we did not observe isomerization of these two β-glycosides. Under condition B, where a large amount of Dowex 50 was used, a small degree of hydrolysis of the glycosidic bond was observed for the N-acetyl-glucosamine glycoside, but not for the galactose glycoside. From such observations, we speculate that the 2-acetamido group plays an important role in the isomerization (hydrolysis).

We propose the mechanism as depicted in Scheme 1 for this isomerization (hydrolysis). Acid-catalyzed hydrolysis of glycosidic bonds is thought to proceed through formation of an acyclic or a cyclic carbonium ion. Of the two postulated carbonium ions, the latter is believed to be the favored intermediate in most cases[14]. In the present case of the Dowex-50 catalyzed isomerization (hydrolysis), however, the cyclic



SCHEME I

carbonium intermediate is not favored. This is supported by the following evidence. The aglycon is retained to a very high degree in aqueous media, and in a medium containing 67% isopropanol (condition C). In the process of the cyclic carbonium ion formation, the aglycon leaves as alcohol and once lost it cannot compete favorably against water or isopropanol for a nucleophilic attack on the carbonium ion. Secondly, a cyclic intermediate of a defined ring size will not permit furanoside--pyranoside isomerization. Instead, as depicted in Scheme 1, the alternative, acyclic carbonium ion (A), stabilized by participation of the 2-acetamido group (B), can explain our experimental results. The fact that the β-configuration was largely maintained during the 4βf-4βp isomerization may suggest that the intermediate B is the more important species in the isomerization. Initial attack on C-1 by the 2-acetamido group would directly form the intermediate B with a fixed C-1 configuration. Ring reclosure would form only

the β-glycosides. An alternative explanation is that a strong resin-glycoside interaction (hydrophobic interaction and/or sulfonate-carbonium ion interaction) effectively fixes the spatial orientation of the aglyconic group in both intermediates A and B, so that ring closure would occur predominantly from one side leading to the β-glycosides. The latter consideration also explains the predominance of isomerization over hydrolysis, as well as the ineffectiveness of p-toluenesulfonic acid as compared with the resin. Whether the small amount of hydrolysis observed resulted directly from A and B or from a small amount of a cyclic intermediate cannot be determined at this moment. A much faster rate of hydrolysis of the GalNAc glycoside compared with the GlcNAc glycoside agrees with the well-documented fact that glycosides of D-galacto configuration containing an axial hydroxyl group are hydrolyzed much faster than those of D-gluco configuration[15].

EXPERIMENTAL

Materials and Methods. N-Acetyl-D-galactosamine, acetyl chloride and Dowex 50X8 (H^+ form, 200-400 mesh) were obtained from Sigma Chem. Co.; triethylamine was from Aldrich Chemical Co. The preparation of 6-(N-trifluoroacetyl)aminohexanol and its conversion to β-glycopyranosides of N-acetyl-D-glucosamine and D-galactose have been described[5]. Preparation of the allyl and methyl glycosides of GalNAc will be described elsewhere.

All evaporation was done under reduced pressure and uncorrected melting points were determined with a Fisher-Johns apparatus. Elemental analyses were performed by Galbraith Laboratories, Inc., (Knoxville, TN). ^{1}H-NMR spectra were obtained with a Varian 400 MHz spectrometer. For determination of formaldehyde (produced from furanosides), the chromotropic acid method of Hanahan and Olley[13] was modified as follows: A 0.25 mL sample containing 10--150 nmol of glycol in a 13 x 100-mm tube was treated with 0.1 M $NaIO_4$ (0.1mL) and 1 M H_2SO_4 (0.1 mL) for

exactly 5 min at room temperature. To the solution were added 10% $NaHSO_3$ (0.1 mL) and the chromotropic acid reagent[13] (2.5 mL), and the mixture was heated in a boiling water bath for 30 min. After cooling a half-saturated thiourea solution (0.25 mL) was added and the absorbance was read at 570 nm. Concentration of solutions containing GalNAc glycosides was determined with the automated amino sugar analyzer[16] after acid hydrolysis. T.l.c. was done on 0.2-mm layers of silica gel 60 F_{254} precoated on aluminum sheets (E. Merck), using solvent A [1:4 (v/v) toluene--ethyl acetate], solvent B [9:4:2 (v/v) ethyl acetate--isopropanol--water], solvent C [3:2:1 (v/v) ethyl acetate--acetic acid--water], or solvent D [8:2:1 (v/v) ethyl acetate--isopropanol--water]. After the plates were dried, carbohydrate components were visualized by spraying with 15% sulfuric acid in 50% ethanol and heating at ~140°. The amino group was detected by spraying with 0.2% ninhydrin in 95% ethanol and heating the plate briefly. The N-trifluoroacetamido group was detected by the following steps: 1) spray the plate with 1 M NaOH and keep the plate for a few minutes at room temperature to de-N-trifluoroacetylate, 2) spray with glacial acetic acid to neutralize the excess alkali, and 3) spray with the ninhydrin reagent and heat it as described above.

2-Acetamido-3,4,6-tri-O-acetyl-1-chloro-1,2-dideoxy-α-D-galactopyranose (1). Finely ground N-acetyl-D-galactosamine (1g, 4.5 mmol) was suspended in 10 mL of acetyl chloride in a tightly sealed flask and stirred for about 2 h at room temperature. When the suspension appeared slightly thinner, the mixture was moved to the cold room and stirred for 2 days, at which time a clear solution had resulted. T.l.c. of the mixture in solvent A indicated about a 60% yield of 1 (R_F 0.41). The mixture was poured into 50 mL of cold dichloromethane and then into 80 mL of ice water. Layers were separated quickly and the organic layer was drained directly into 120 mL of cold saturated sodium bicarbonate. The mixture was carefully shaken and the organic layer was drained into a flask containing anhydrous sodium sulfate. When the solution became clear, it was filtered and the filtrate was

evaporated to a small volume under reduced pressure at room temperature. Diethyl ether (80 mL) and petroleum ether (b.p. 35--70°, 40 mL) were added in that order and the mixture was left in the cold overnight. The clear supernatant solution was decanted and the precipitate was washed with a mixture of 1:5 (v/v) diethyl ether--petroleum ether and dried for about 1 h in a desiccator. The product (1.05 g), which contained 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-D-galactopyranose (2) (R_F 0.15) in addition to 1, was used in the next reaction without purification.

6'-(N-Trifluoroacetyl)aminohexyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranoside (3βp) and 2-Acetamido-3,5,6-tri-O-acetyl-2-deoxy-β-D-galactofuranoside (3βf). To the flask containing amorphous 1 from the previous step were added 6-(N-trifluoroacetyl)hexanol (0.75 g, 3.5 mmol), Drierite (0.3 g), mercuric cyanide (0.88 g, 3.5 mmol) and 30 mL of a 1:1 (v/v) mixture of toluene--nitromethane, and the mixture was stirred overnight at room temperature. T.l.c. (solvent A) showed that 1 had been exhausted and a compound which could be charred and which also contained the trifluoroacetamido group (R_F 0.22) was produced. The reaction mixture was filtered, the filtrate evaporated and the residue was taken up in chloroform. The chloroform solution was washed twice with 1 M NaCl, once with 0.5 M KBr, and then dried as before with anhydrous sodium sulfate. The dried solution was evaporated to a syrup, dissolved in 15 mL of 95% ethanol and fractionated on a column (5x190 cm) of Sephadex LH-20 using 95% ethanol as eluant. Compounds 3 and 2 emerged in succession with complete separation. The isomers, 3βp and 3βf, overlapped almost completely both by t.l.c. and by gel filtration, with 3βf moving slightly ahead in t.l.c. and eluting slightly later than 3βp from the gel filtration column. The fractions containing 3βp and 3βf were evaporated to a syrup, suspended in a mixture of ethanol--diethyl ether--petroleum ether (5:2:2, v/v) to yield crystalline 3βp (0.31g, 0.59 mmol). The mother liquor was evaporated to yield ca. 1 g of amorphous solid (3βp and 3βf). The crystalline 3βp was recrystallized once from ethanol--diethyl

ether--petroleum ether (5:2:2, v/v); mp 137-138°. Overall yield of 3 (βp + βf) from GalNAc was ~45%. See Table 1 for ^{1}H-NMR data of 3βp and 3βf.

Anal. of 3βp. Calc: C, 48.70; H, 6.13; N, 5.16. Found: C, 48.59; H, 6.19; N, 5.15.

Fractions containing 2 were combined and evaporated to a syrup which crystallized upon standing, yielding 0.42g (1.08 mmol) of pure 2; mp 180-181° (lit.[17] 182-183°). ^{1}H-NMR data ($CDCl_3$): δ 1.94 (s, 3, $COCH_3$); 2.02 (s, 6, 2 $COCH_3$); 2.16 (s, 6, 2 $COCH_3$); 4.04-5.41 (m, 6, CH_2 and ring H); 6.20 (d, 1, anomeric H, J 3.7 Hz).

Anal. Calc: C, 49.35; H, 5.95; N, 3.60. Found: C, 48.91; H, 5.99; N, 3.56.

6'-(N-Trifluoroacetyl)aminohexyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (4βp) and 2-Acetamido-2-deoxy-β-D-galactofuranoside (4βf). Crystalline 3βp and the residue from the mother liquor were de-O-acetylated separately in dry methanol containing 0.01 M sodium methoxide for a few hours at room temperature. T.l.c. in solvent B showed that product 4βp (R_F 0.51) was produced exclusively from crystalline 3βp, while a syrupy residue from the mother liquor produced 4βp and a second component (4βf) of higher R_F (0.64) and of similar quantity (by both charring and NaOH--ninhydrin). The methanolic solution containing only 4βp was treated with Dowex 50X8 (H^+ form) in a small amount of water till neutral pH was obtained, filtered and evaporated to yield 4βp quantitatively as a white solid; mp 187-189°. The mixture containing both 4βp and 4βf was acidified with acetic acid, evaporated to ~5 mL and chromatographed on a column (5x220 cm) of Sephadex G-25 with 0.1 M acetic acid. The two isomers were eluted with much overlap, with 4βp eluting slightly ahead of 4βf. The fractions containing 4βp only or 4βf only were combined and evaporated. The fractions containing both isomers were combined and subjected to either another gel filtration or isomerization by Dowex 50X8 as described later.

6'-Aminohexyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (5βp) and 2-Acetamido-2-deoxy-β-D-galactofuranoside (5βf). Relatively pure 4βp (0.42g, 1 mmol) was de-N-trifluoroacetylated by dissolving it in 5 mL of an aqueous solution containing 10% triethylamine and 9% ethanol and leaving the solution overnight at room temperature. The reaction mixture was evaporated to a syrup, dissolved in 5 mL of 0.1 M acetic acid and fractionated on the Sephadex G-25 column as described above. The fractions containing 5βp (R_F 0.17 in solvent C) were combined and evaporated. The residue was dissolved in absolute ethanol, mixed with an equal volume of toluene and the mixture was evaporated again. Crystals obtained upon standing were trituated in 3:1 (v/v) ethanol--ether and filtered, yielding 0.32 g (1 mmol) of 5βp; mp 139-140°. By using the Dowex 50-catalyzed isomerization to increase the yield of the β-pyranoside (5βp), the final product 5βp (crystalline and amorphous solid) was obtained in 29% yield from GalNAc. ^{1}H-NMR data of 5βp (D_2O) : δ 1.36--1.66 [m, 8, $C(CH_2)_4C$]; 2.03 (s, 3, $COCH_3$); 2.97--3.94 (m, 10--11, 3 CH_2 and ring H); 4.44 (d, 1, anomeric H, J 8.5 Hz).

Similarly, 4βf was de-N-trifluoroacetylated to yield 5βf, which also had a higher R_F (0.27 in solvent C) than the β-pyranoside (5βp). As in the case of 4βf and 4βp, 5βp was eluted slightly ahead of 5βf from the Sephadex G-25 column, thus enabling partial fractionation at this stage as well. ^{1}H-NMR data of 5βf (D_2O): δ 1.35--1.61 [m, 8, $C(CH_2)_4C$]; 2.02 (s, 3, $COCH_3$); 2.80--4.16 (m, 10--11, 3 CH_2 and ring H); 4,974 (d, 1, anomeric H, J 2.4 Hz).

6'-(N-Trifluoroacetyl)aminohexyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (3αp). 6-(N-Trifluoroacetyl)-hexanol (0.41 g, 1.9 mmol) and 2 (0.39 g, 1 mmol) were dissolved in dry nitromethane (5 mL). Boron trifluoride etherate (0.1 mL) was added and the mixture was refluxed for 1.5 h. T.l.c. (solvent A) examination showed that 2 had been totally spent with concomitant production of one major product (3). The reaction mixture was evaporated; the residue was dissolved in chloroform

(~20 mL) and washed with 1 M NaCl (2 x 10 mL). The chloroform layer was dried (anhydrous sodium sulfate), filtered and evaporated. The residue was dissolved in absolute ethanol and decolorized. Evaporation of the ethanolic solution resulted in crude, solid 3αp (0.41 g, 0.76 mmol).

6'-(N-Trifluoroacetyl)aminohexyl 2-Acetamido-2-deoxy-α-D-galactopyranoside (4αp). The residue (mainly 3αp) obtained above was dissolved in dry methanol (10 mL) and the solution was made 5 mM in sodium methoxide. After a few hours at room temperature, t.l.c. (solvent D) examination of the mixture showed the presence of 4αp and 4βp in an approximately 9 to 1 ratio with a trace amount of 4βf. The mixture was evaporated after addition of 80% acetic acid (~50 μL). The residue was dissolved in 0.1 M acetic acid and purified on a column (2.5 x 136 cm) of Sephadex G-15 using 0.1 M acetic acid as eluant. Fractions containing the major product (4αp), eluted just after a small peak of 4βp, were combined and evaporated to produce 0.29 g of crystalline 4αp (70% overall yield); mp, 158-161°. ^{1}H-NMR data (D_2O): δ 1.36--1.60[m, 8, $C(CH_2)_4C$]; 2.04(S, 3, $COCH_3$); 3.31--4.15(m,10--11, 3 CH_2 and ring H); 4.896 (d, 1, anomeric H, J 4.0 Hz).

6'-Aminohexyl 2-Acetamido-2-deoxy-α-D-galactopyranoside (5αp). De-N-trifluoroacetylation was carried out as described above for the preparation of 5βp. A crystalline product was obtained from ethanol--ether. Mp 125-126°; R_F in solvent C, 0.30.

Dowex 50-Catalyzed Isomerization. Condition A: 1 mg each of test compounds was dissolved in 150 μL of water and treated with 7 mg of fresh Dowex 50X8 (200 - 400 mesh, H^+ form). Condition B: as above except that 50 mg of Dowex 50x8 was used. Condition C: as the condition A except that 67% isopropanol was used instead of water. Condition D: as the condition A except that Dowex 50 was omitted and p-toluenesulfonic acid was added to make 0.1 M in the mixture. The compounds tested were 4βf, 4βp, 4αp, 6-(N-trifluoroacetyl)aminohexyl β-glycosides of Gal and GlcNAc. Allyl β-pyranoside and β-furanoside of GalNAc and methyl β-pyranoside of GalNAc were also examined under the condition A. Progress of

reactions was monitored by t.l.c. in solvent D. The three 6-(N-trifluoroacetyl)aminohexyl glycosides had the following R_F values: 0.33 (4βp), 0.44 (4αp) and 0.48 (4βf). For the purpose of ^{1}H-NMR spectroscopy, reactions were carried out with five-fold larger amount of reactants and spectra were taken before and after the reaction.

ACKNOWLEDGMENT

The high-field NMR spectrometer used was acquired with the assistance of major instrumentation grants (JHU, NSF PCM 83-03176 and NIH RR 01934).

REFERENCES AND FOOTNOTE

1. Contribution No. 1316 from the McCollum-Pratt Institute, The Johns Hopkins University. Supported by U.S. Public Health Service National Institute of Health Research Grants AM9970 and CA 21901.

2. S. Chipowsky and Y.C. Lee, Carbohydr. Res., 31, 339 (1973).

3. R.T. Lee and Y.C. Lee, Carbohydr. Res., 34, 151 (1974).

4. R.T. Lee and Y.C. Lee, Carbohydr. Res., 37, 193 (1974).

5. P.H. Weigel, M. Naoi, S. Roseman, and Y.C. Lee, Carbohydr. Res., 70, 83 (1979).

6. R.T. Lee, P. Lin, and Y.C. Lee, Biochemistry, 23, 4255 (1984).

7. P.H. Weigel, R.L. Schnaar, S. Roseman, and Y.C. Lee, Methods Enzymol., 83, 294 (1982).

8. R.L. Schnaar, P.H. Weigel, S. Roseman, and Y.C. Lee, Methods Enzymol., 83, 306 (1982).

9. M. Sarkar, J. Liao, E.A. Kabat, T. Tanabe, and G. Ashwell, J. Biol. Chem., 254, 3170 (1979).

10. C.P. Stowell, R.T. Lee, and Y.C. Lee, Biochemistry, 19, 4904 (1980).

11. D. Horton, Methods Carbohydr. Chem., 6, 282 (1972).

12. D.R. Bundle and R.U. Lemieux, Methods Carbohydr. Chem., 7, 79 (1976).

13. D.J. Hanahan and J.N. Olley, J. Biol. Chem., 231, 813 (1958).

14. J.N. BeMiller, Adv. Carbohydr. Chem., 22, 25 (1967).

15. W.G. Overend, C.W. Rees, and J.S. Sequeira, J. Chem. Soc., 3429 (1962).

16. Y.C. Lee, Methods Enzymol., 28, 63 (1972).

17. C.S. Hudson and J.K. Dale, J. Amer. Chem. Soc., 38, 1431 (1916).