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SYNTHESIS AND CONFORMATIONAL STUDIES OF 2-β-CHLORO, 2-α-FLUORO, AND 2-β-FLUORO DERIVATIVES OF 2-DEOXY-*N*-ACETYL-NEURAMINIC ACID

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ABSTRACT

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-fluoro-D-*glycero*-α- and -β-D-*galacto*-2-nonulosonic acid methyl esters and the β-chloro analog were synthesized from *N*-acetylneuraminic acid. Their ^{1}H- and ^{13}C-n.m.r. spectra were completely assigned by using single-frequency decoupling, off-resonance decoupling, and spin-simulation programs. Bond angles estimated from the ^{1}H coupling-constants indicate that all of the compounds adopt the $^2C_5$(L) conformation with minor conformational differences in the C_3 side chain. 5-Acetamido-2,3,5-trideoxy-2-fluoro-D-*glycero*-α- and -β-D-*galacto*-2-nonulosonic acid and their methyl esters were also prepared.

INTRODUCTION

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (*N*-acetylneuraminic acid, AcNeu, **1**), is the most widely distributed of the sialic acids, which are significant in a large number of biological reactions and are important constituents of glycoproteins and glycolipids[1–6]. AcNeu is usually α-linked as the non-reducing end-group of an oligosaccharide chain. Many groups have investigated the synthesis of α-ketosides of AcNeu, with varying degrees of success[7–11]; our investigations have used the α- and β-glycosyl fluorides of peracetylated AcNeu. It was decided that conformational analyses of these two fluoro derivatives (**5** and **6**) and of the β-chloro analog (**4**) might help to predict the course of glycosylation reactions of these compounds. The fluoro derivatives **7** and **8** were also sought for use by others[12–15] as substrates in investigating the specificity and activities of neuraminidases.

RESULTS AND DISCUSSION

AcNeu (**1**) was isolated from edible bird's nest by a modification of the

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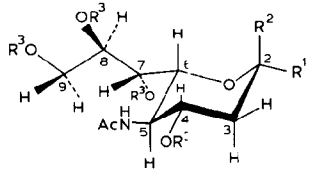

1 R^1 = CO_2H, R^2 = OH, R^3 = H
2 R^1 = CO_2Me, R^2 = OH, R^3 = H
3 R^1 = CO_2Me, R^2 = OAc, R^3 = Ac
4 R^1 = CO_2Me, R^2 = Cl, R^3 = Ac
5 R^1 = CO_2Me, R^2 = F, R^3 = Ac
6 R^1 = F, R^2 = CO_2Me, R^3 = Ac
7 R^1 = CO_2Me, R^2 = F, R^3 = H
8 R^1 = F, R^2 = CO_2Me, R^3 = H
9 R^1 = F, R^2 = CO_2Na, R^3 = H

method of Flashner *et al.*[16] in which the higher molecular-weight protein and peptides were removed by ultrafiltration. The method gave adequately pure **1** more rapidly and in higher yield than previously reported. The methyl ester[2] (**2**) and the pentaacetyl derivative[10] (**3**) were prepared as described previously. Treatment of (**3**) with hydrogen chloride in ether and acetyl chloride gave the glycosyl chloride **4** which, after chromatography, was isolated crystalline; it was stable for several months at −20° under nitrogen.

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-fluoro-D-*glycero*-α- and -β-D-*galacto*-2-nonulosonic acid methyl esters (**6** and **5**) were synthesized from the β-chloride **4** with silver fluoride in acetonitrile. The α anomer **6** was isolated in 50% yield and the β anomer **5** in 15% yield. The by-product of the reaction was mainly the 2,3-dehydro AcNeu derivative. The anomeric stereochemistry was initially assigned on the basis of the optical rotations of **5** (+13.3°) and **6** (−17.5°).

The β-fluoride **5** was needed in higher yield for exploring the synthesis of α-ketosides of AcNeu. The fluorination procedure of Olah *et al.*[17] using 70% hydrogen fluoride–pyridine was found to give **5** from **4** in 80% yield at 0° and in the absence of solvent.

The deacetylated products **7** and **8** were prepared from **5** and **6** by catalytic transesterification, and the deesterified fluoride **9** was prepared from **8** by using sodium hydroxide in methanol. These fluorides proved reasonably stable to the basic conditions of deacetylation and deesterification, and even survived reversed-phase l.c. with water to give the desired products in ~50% yield. Derivatives **7**, **8**, and **9** are intended as substrates for studying the activity and specificity of neuraminidases

The 360-MHz ^{1}H-n.m.r. spectra of compounds **4**, **5**, and **6** were essentially first-order. The proton assignments were based on published data[7,18,19] and by spin-decoupling experiments (see Table I). Methyl resonances of the *N*-acetyl

groups were found at δ 1.9 and of *O*-acetyl groups at δ 2.0–2.1. The axial H-3a resonated at δ 2.28 in compound **4** but in compounds **5** and **6** it was beneath the acetyl-proton resonances (δ 2.0). The H-3e signals for the β-chloride **4** and the β-fluoride **5** were at δ 2.8 and 2.7, respectively. The H-3a resonance has been used to differentiate anomeric ketosides of AcNeu in that H-3e of the β anomers lies at higher fields than in the α anomers[7].

The remaining protons (H-4,5,6,7,8,9, and 9′) were assigned by selective decoupling and by comparison with ^{1}H spectra of known ketosides of peracetylated AcNeu[7,8,19]. Table I shows that the chemical shifts for corresponding protons in each compound are approximately the same, with slight shifts caused by the fluorine or by the change from α to β.

The ^{1}H coupling-constants were determined either directly, by spin decoupling or, for overlapped signals, by a spin-simulation program (Varian). Coupling constants for the protons in compounds **5** and **6** that also showed proton–fluorine coupling were determined by using a modified LAOCN III spin-simulation program. As shown in Table II, the proton coupling-constants for corresponding protons is almost the same for compounds **4, 5,** and **6,** except for protons of the C_3 side chain (H-7,8,9, and 9′). As the substituent groups are the same in all compounds, with the exception of chlorine and fluorine, the overall conformations for each compound must be very similar.

The $J_{H,F}$ values for **5** and **6** may be used to assign the anomeric configuration. In **5**, $J_{3e,F}$ is 4.8 and $J_{3a,F}$ 24.0 Hz, indicating from the Karplus relationship that the angle between H-3e and F is ~60° and that between H-3a and F ~180° (β configuration). Compound **6** shows $J_{3a,F}$ = 10.0 and $J_{3c,F}$ = 8.6 Hz, indicating a 60° angle between fluorine and both H-3a and H-3e (α configuration).

The conformations of **4, 5,** and **6** were determined by using a modified

TABLE I

^{1}H CHEMICAL SHIFTS (δ) FOR COMPOUNDS **4, 5,** AND **6**

Compound	*N-H*	*H-3a*	*H-3e*	*H-4*	*H-5*	*H-6*	*H-7*	*H-8*	*H-9*	*H-9′*
4(β)	5.6	2.28	2.80	5.40	4.20	4.30	5.47	5.18	4.08	4.40
5(β)	5.6	2.0	2.70	5.15	4.20	4.20	5.40	5.40	4.20	4.40
6(β)	5.5	2.0	2.40	5.20	4.25	4.25	5.40	5.20	4.02	4.40

TABLE II

^{1}H–^{1}H COUPLING CONSTANTS (Hz) FOR COMPOUNDS **4, 5,** AND **6**

Compound	$J_{3a,3e}$	$J_{3e,4}$	$J_{3a,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$	$J_{3e,F}$	$J_{3a,F}$
4	−13.8	4.9	10.6	10.7	10.4	2.1	6.8	5.9	2.5	−12.5		
5	−13.8	4.8	10.0	10.6	10.8	0.8	6.7	6.1	2.6	−12.5	4.9	−24.0
6	−13.8	5.5	9.4	9.5	10.7	0.8	0	4.7	2.2	−11.2	8.6	−10.0

TABLE III

BOND ANGLES (DEGREES) ESTIMATED[20] FOR COMPOUNDS **4, 5,** AND **6**

Compound	*Conformation*	$\phi_{3a,4}$	$\phi_{3e,4}$	$\phi_{4,5}$	$\phi_{5,6}$	$\phi_{6,7}$	$\phi_{7,8}$	$\phi_{8,9}$	$\phi_{8,9'}$
4		163	52	180	180	62	154	212	59
5		158	52	180	180	90	154	214	59
6		154	48	180	180	87	90	214	60
Observed[a]	$^2C_5$	160	52	180	180	60	180	180	60

[a]From a Dreiding model.

Karplus equation described by Altona *et al.*[20] in which the orientation and electronegativities of the substituent groups are included in the calculation. The electronegativities were calculated from values given by Huggins[21] as OAc = 1.22, NAc = 0.77, C-9 = 0.15, C-4, C-5, C-6, C-7, C-8 = 0.08, C-2-F = 0.25, and C-2-Cl = 0.1.

The bond angles calculated from the ^{1}H–^{1}H coupling constants are shown in Table III, together with bond angles measured from Dreiding models of compounds **4, 5,** and **6** in the $^2C_5$ conformation. The close agreement between the expected and calculated bond angles indicates that these AcNeu derivatives all have the $^2C_5$ pyranose conformation, as has also been observed by others[7,18,19].

The ^{1}H–^{1}H-coupling constants of the side chain indicate slight conformational differences between compounds **5** and **6**. The small $J_{6,7}$ value indicates a bond angle of ~90° or a gauche relationship between H-6 and H-7. The $J_{7,8}$ value for **4** and **5** is ~6.7 Hz, indicating H-7 and H-8 to be *trans*. In **6** the $J_{7,8}$ value is ~0°, indicating that the H-7–H-8 bond angle is ~90° (*gauche* disposition). The *gauche* disposition should be of higher energy than the *trans* because of interactions between C-9 and either C-6 or the acetoxy group at C-7, and between acetoxy groups at C-8 and C-6 or the acetoxy group at C-7. The strong preference for the *gauche* relationship for H-7–H-8 is hard to explain on the basis of a simple change in configuration at C-2, as there should be no long range interactions and there was no change in the conformation of the pyranose ring-system. The favored orientation of C-9 seems to be one in which H-8 is *trans* to H-9 and *gauche* to H-9, resembling that proposed by Brown *et al.*[19].

Based on the ^{13}C-n.m.r. spectra of **4, 5,** and **6** and published assignments for other AcNeu derivatives[7,22,23], the carbon spectra were totally assigned (see Table IV). C-2 in **4** was readily assigned at δ 96.9, which is in the characteristic region for the anomeric carbon resonance. C-2 of compounds **5** and **6** show a marked downfield shift because of replacement of fluorine by chlorine. Also, C-2 of **6** lies downfield from C-2 of **5**, which is consistent with the observation that equatorial, electronegative groups cause more deshielding than axial ones. This shift of C-2 also confirms the assignment of the β configuration for **5** and α for **6**. The only other changes observed for compounds **5** and **6** occur at C-1 and C-8. The shift at C-1 is expected, because of the change in anomeric configuration. The shift of C-8 was

TABLE IV

^{13}C CHEMICAL SHIFTS (p.p.m.) AND $J_{C\text{-}F}$ COUPLING CONSTANTS (Hz) FOR COMPOUNDS **4**, **5**, AND **6**

Atom	*Chemical shift (p p.m.)*			*$J_{C\text{-}F}$ Coupling (Hz)*	
	4	**5**	**6**	**5**	**6**
C-1	165.9	165.9[a]	165.0[a]	30	29.7
C-2	96.9	106.9[a]	108.0[a]	223.6	231.0
C-3	40.8	35.9[a]	35.5[a]	25.0	28.3
C-4	69.0	68.7[a]	68.5	8.6	0
C-5	48.8	49.0	48.6		
C-6	74.1	73.4	73.2		
C-7	67.2	67.5	67.4		
C-8	70.3	69.9	70.4		
C-9	62.3	62.3	62.5		
O-CH_3	53.8	53.2	53.4		
N-Ac	23.0	23.0	23.0		
O-Ac	20.7	20.8	20.7		
Carbonyl	170–171.1	166.1–171.0	170.1–171.1		

[a]Center of the doublet.

unexpected as C-8 is remote from the anomeric center, but in light of the differences in $J_{7,8}$ values for **5** and **6**, it may be rationalized as being due to a change in conformation of the side chain.

The C–F coupling constants in **5** and **6** proved useful in confirming the assignments of C-1, C-2, C-3, and C-4. The magnitudes of the C–F coupling constants are similar to those reported by Bock and Pedersen[24] for various glycosyl fluorides, the major difference being that $J_{C\text{-}1,F}$ for an axial fluoride was always larger (10 Hz) than $J_{C\text{-}1,F}$ of an equatorial fluoride. They[24] attributed the difference to the effect on the coupling of the lone pair of electrons of the ring oxygen atom. In the case of **5** and **6**, exactly the reverse is observed. The $J_{C\text{-}1,F}$ value of the equatorial fluoride **6** is 10 Hz larger than $J_{C\text{-}1,F}$ of the axial fluoride **5**. It may be that the carbonyl group of C-1 has a larger and opposite effect on the coupling constant as compared with the ring-oxygen atom. Bock and Pedersen[24] also reported that $J_{C\text{-}3,F}$ is 4–10 Hz for compounds in which C-3 and fluorine are *trans* and 0–6 Hz for compounds in which the orientation is *cis*. The opposite situation is observed between C-4 and F in **5** and **6**. The fluorine atom and C-4 in **5** are *cis*-disposed and $J_{C\text{-}4,F}$ is 8.6 Hz, whereas, the C-4–F orientation is *trans* in **6** and $J_{C\text{-}4,F}$ is 0. Again, this reversal may arise from the influence of the carbonyl group at C-1.

Based on our work and previous studies, it appears the derivatives of AcNeu favor the $^2C_5$ conformation in which the substituents at C-4, C-5, and C-6 are equatorial, and that changing the substituents at C-2 has little effect on the conformation of the ring.

EXPERIMENTAL

General methods. — ^{1}H-N.m.r. spectra were recorded with a Bruker 360-MHz spectrometer in the pulse-transform mode, for solutions in chloroform-*d* with tetramethylsilane as the internal standard, or in deuterium oxide. ^{13}C-n.m.r. spectra were recorded with a Varian XL 100-15 spectrometer in pulsed-Fourier, noise-decoupled, transform mode in chloroform-*d* with tetramethylsilane as the internal standard. Chemical shifts are in p.p.m. downfield of the tetramethylsilane signal. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter in jacketed, 1-dm cells at 25°. Solutions were evaporated *in vacuo*. The compounds were purified on a semipreparative liquid chromatograph equipped with a differential refractometer (Waters R401), a u.v. detector, and a Glenco high-performance l.c. pumping system. Ethyl acetate or ethyl acetate–hexanes were used as eluents with a column of silica gel (Whatman, Partisil M9 10/25) at a flow rate of 8 mL/min. A C_{18} reverse-phase column (Partisil M9 10/25 ODS-3, Whatman) was used with water at a flow rate of 8 mL/min for the purification of **7, 8,** and **9**. For large-scale separations, a Waters Associates Model 500 l.c. was used with a silica gel column. Melting points were determined on a Mel-Temp apparatus (Laboratory Devices, Cambridge, Massachusetts) and are uncorrected. The assignments of ^{1}H-n.m.r. spectra were made by use of a Varian spin-simulation program publication No. 87-131-214, Program No. 994029-B, and a modified LAOCN-III program run on a 48K Apple II Plus microcomputer.

Isolation of 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-*2-nonulosonic acid* (**1**). — The procedure of Flashner and coworkers[15] was modified. Edible bird's-nest substance (300 g) was added to boiling water (6 L) and the suspension was boiled under reflux for 5 h. The gluey solids were allowed to settle overnight at room temperature and removed by filtration through MIRA cloth. A small amount of sodium azide was added to the filtrate to prevent bacterial degradation. The filtrate was further purified to remove high-molecular-weight proteins by ultrafiltration at 0° through an Amicon hollow-fiber, cartridge filter (type H1 P10, Amicon Corporation, Lexington, MA 02173) to remove material of mol. wt. >10,000, followed by two washings with water (2 × 200 mL) over a period of ~4 h. The solution was subjected to second filtration through an Amicon hollow-fiber, cartridge filter (type H1 P2) to remove polypeptides and other impurities having molecular weights >1,000, and was washed twice with water (2 × 200 mL) over a total period of ~48 h. The washings and filtrate were combined and lyophilized to yield **1** (7.2 g). The lyophilized **1** was recrystallized from 1:10 water–acetic acid; m.p. 188–189° (dec.), lit.[16] m.p. 187–189° (dec.); ν_{max}^{KBr} 3400–3300 broad (OH, NH), 1690, 1625 (CO, NH-CO), 1400, and 1130 cm^{-1}.

5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-*2-nonulosonic acid methyl ester* (**2**). — Compound **1** (6.0 g, 97 mmol), Dowex 50 X8-400 ion-exchange resin (18 g, washed with 50 mL of methanol and dried in a vacuum overnight at 25°), dry methanol (600 mL) were stirred for 2 h at room temperature. The mixture was

filtered and the filter washed with methanol (25 mL). The filtrate and washings were combined, more methanol (600 mL), was added and the solution was stirred for 1 h more at room temperature, and then evaporated to yield **2** as a colorless solid (4.8 g, 76%), m.p. 179° (lit.[9] m.p. 179–180°); ν_{max}^{KBr} 3400–3300 (broad OH, NH), 1740, 1650 (CO, NH-CO), 1550, 1440, and 1040 cm^{-1}.

*5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-*D-glycero-D-galacto-*2-nonulosonic acid methyl ester* (**3**). — Compound **2** (3.23 g, 10 mmol) was cooled in an ice bath and dry pyridine (40 mL) and acetic anhydride (45 mL) were added with stirring. The mixture was further stirred for 4 h at 0° and by 48 h at room temperature. The resulting solution was evaporated at low temperature. Toluene (4 × 10 mL) was evaporated from the residue to remove traces of pyridine. The resultant colorless, foamy solid was further purified by preparative l.c. (silica gel, ethyl acetate), and dried in a vacuum to give **3** (3.8 g, 71%). Recrystallization from 1:3 ethyl acetate–benzene gave a foamy colorless solid, m.p. 157–158° (lit.[9] m.p. 156–157°), $[\alpha]_D^{24}$ −3.4° (*c* 1, chloroform) (lit.[9] m.p. 156–157°), $[\alpha]_D^{24}$ −3.4° (*c* 1, chloroform) (lit.[9] $[\alpha]_D^{20}$ −3.3° in chloroform); ν_{max}^{KBr} 3500–3380 (NH), 1730 (C=O) 1650 (NHCO), 1530, 1425, and 1360 cm^{-1}; ^{1}H-n.m.r.: δ 3.8 (s, 3 H, Me), 1.9–2.2 (18 H, Ac), 5.5 (d, NH).

*5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-*D-glycero-β-D-galacto-*2-nonulosonic acid methyl ester* (**4**). — Compound **3** (2.66 g, 5 mmol) was dissolved in acetyl chloride (80 mL) and diethyl ether (100 mL) and cooled in ice bath. Hydrogen chloride gas was passed into the solution under nitrogen for 15 min. The mixture was refrigerated for 4 days. The excess of hydrogen chloride was removed by bubbling nitrogen through the solution and the solvents were removed. Subsequent evaporation of carbon tetrachloride (4 × 10 mL) from the residue gave a colorless solid that was further purified by preparative l.c. with ethyl acetate as eluant. Evaporation of the solvent gave **4** as a foamy, colorless solid (1.66 g, 65%), which was crystallized from a mixture of dichloromethane, diethyl ether, and petroleum ether at 0°; m.p. 84–86° (sintered), 105° (dec.), $[\alpha]_D^{25}$ −68.3° (*c* 1, chloroform) (lit.[9] $[\alpha]_D^{25}$ −63° (*c* 1, chloroform); ν_{max}^{KBr} 3460, 3380 (NH), 1750, 1720, 1650 (CO, NHCO), 1540, 1515, 1425, and 1360 cm^{-1}; ^{1}H-n.m.r. data see Tables I and II; ^{13}C-n.m.r. data see Table IV.

*5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-fluoro-*D-glycero-β-D-galacto-*2-nonulosonic acid methyl ester* (**6**). — Compound **4** (1.02 g, 2 mmol) was dissolved in dry and freshly distilled acetonitrile (4 mL) under vacuum and treated with silver fluoride (Alfa Products, recrystallized from acetonitrile, 0.28 g, 2.2 mmol) under vacuum. The mixture was stirred for 1.5 h at room temperature in the dark. The solution was filtered through Celite and the filter washed with acetonitrile (2 × 5 mL). The filtrate and washings were combined and evaporated. The residue was dissolved in dichloromethane (50 mL) and the solution successively washed with saturated sodium thiosulfate (5 mL), distilled water (10 mL), and saturated sodium chloride (10 mL), dried (sodium sulfate, 2 h), and evaporated to give a colorless solid. T.l.c. (ethyl acetate) revealed one major and two minor

products, which were separated by l.c. on a column of silica gel (4:1 ethyl acetate–hexane) to give **6** (0.5 g, 50%); m.p. 162–164°, $[\alpha]_D^{25}$ +13.3° (*c* 1, chloroform); ν_{max}^{KBr} 3370, 3280 (NH), 1740, 1655 (CO, NHCO), 1530, 1430, and 1365 cm^{-1}; ^{1}H-n.m.r. see Tables I and II; ^{13}C-n.m.r. see Table IV.

Anal. Calc. for $C_{20}H_{28}FNO_{12}$: C, 48.65; H, 5.72. Found: C, 48.45; H, 5.85.

5-Acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-2-fluoro-D-glycero-*α*-D-galacto-*2-nonulosonic acid methyl ester* (**5**). — Compound **5** was one of the minor products from the preceding experiment; it was obtained as a colorless solid (0.15 g, 15%); m.p. 137–138°, $[\alpha]_D^{25}$ −17.5° (*c* 1, chloroform); ν_{max}^{KBr} 3430, 3280 (NH), 1750, 1650 (CO, NHCO), 1530, 1430, 1365, and 1220 cm^{-1}; ^{1}H-n.m.r. see Tables I and II; ^{13}C-n.m.r. see Table IV.

Anal. Calc for $C_{20}H_{28}FNO_{12}$: C, 48.65; H, 5.72. Found: C, 48.33; H, 5.62.

Preparation of compound **5** *from* **3**. — Compound **3** (0.53 g, 1 mmol) was stirred for 1 h at 0° with 70% hydrogen fluoride–pyridine solution[17]. The mixture was diluted with ice–cold water (200 mL) and extracted with dichloromethane (2 × 100 mL). The extract was washed with water (100 mL) and dried over anhydrous magnesium sulfate. The remaining, water-soluble portion was made neutralized with sodium hydrogencarbonate solution and extracted with dichloromethane (2 × 50 mL). The extract was washed with water and dried. Evaporation of both solutions gave **5** from which traces of pyridine were removed by evaporation of toluene (2 × 20 mL) from the residual colorless, foamy solid, which was further purified by l.c. (yield 0.39 g, 80%). The i.r., ^{1}H-, and ^{13}C-n.m.r. spectra were identical to those of **5** prepared by the method previously described.

5-Acetamido-2,3,5-trideoxy-2-fluoro-D-glycero-*α*-D-galacto-*2-nonulosonic acid methyl ester* (**7**). — A solution of **5** (0.124 g, 0.25 mmol) in methanol (1.0 mL) and sodium methoxide (0.019 g, 0.35 mmol) was stirred at room temperature. After 2.5 h, t.l.c. (3:2:1 ethyl acetate–1-propanol–water) revealed the absence of **5**. The solution was evaporated at low temperature and diluted with water (1.0 mL) and brought to pH 7.0 with few drops of sodium hydrogenphosphate buffer. The product was further purified by reverse-phase, semi-preparative l.c. with water as eluant; freeze-drying gave **7** (0.30 g, 37%) as a white, fluffy solid, m.p. 143–145° $[\alpha]_D^{25}$ +11.5° (*c* 1, water); ν_{max}^{KBr} 3400 (broad, OH, NH), 1730, 1620 (CO_2Me, NHAc), 1550, 1430, 1370 cm^{-1}; ^{1}H-n.m.r.: δ 2.3 (s, 3 H, NAc), 2.6 (dd, 1 H, $J_{3a,3e}$ 12.8 $J_{3a,4a}$ 12.3, $J_{3a,F}$ 24.7 Hz, H-3a), 2.9 (t, 1 H, $J_{3e,3a}$ −12.8, $J_{3e,4a}$ 4.9 Hz, H-3e).

Anal. Calc. for $C_{12}H_{20}FNO_{12} \cdot 0.5\ H_2O$: C, 43.09; H, 6.33. Found: C, 42.99; H, 6.27.

5-Acetamido-2,3,5-trideoxy-2-fluoro-D-glycero-*α*-D-galacto-*2-nonulosonic acid methyl ester* (**8**). — A solution of **6** (0.124 g, 0.25 mmol) in methanol (1.0 mL) in which sodium (3 mg) had been dissolved was stirred at room temperature. After 2.5 h, t.l.c. (3:2:1 ethyl acetate–1-propanol–water) revealed the absence of starting material. The solution was brought to pH 7.0 with phosphate buffer and the product separated by reverse-phase l.c. with water to give, after freeze drying, 35 mg (43%) of **8**, m.p. 150–151°, $[\alpha]_D^{25}$ −18.3° (*c* 1, water); ν_{max}^{KBr} 3480, 3300 (broad, OH,

NH), 1710, 1620 (CO_2Me, NHAc), 1555, 1430, 1365, 1260 cm^{-1}; ^{1}H-n.m.r.: δ 2.3 (s, 3 H, NAc), 2.6 (m, 1 H, H-3a), 2.8 (m, 1 H, H-3e).

Anal. Calc. for $C_{12}H_{20}FNO_8 \cdot 3\,H_2O$: C, 37.97; H, 6.86. Found: C, 37.53; H, 6.76.

Sodium 5-acetamido-2,3,5-trideoxy-2-fluoro-D-glycero-α-D-galacto-*2-nonulosonate* (**9**). — Compound **6** (0.124 g, 0.25 mmol) in methanol (1.0 mL) and sodium methoxide (0.019 g, 0.34 mmol) was stirred for 2.5 h at room temperature. T.l.c. (3:2:1 ethyl acetate–1-propanol–water) revealed the absence of starting material. The solution was evaporated, the residue diluted with water (1.0 mL), and M sodium hydroxide (015 mL) was added. The mixture was stirred for 1 h at 0° and the pH raised by addition of 3 drops of M sodium hydroxide. Stirring was continued overnight at 0°. The product was purified by reverse-phase, semipreparative l.c. with water as eluant to give, after lyophilizing, **9** (40 mg, 50%), m.p. 162–164°, $[\alpha]_D^{25}$ −12.8° (*c* 1, water); ν_{max}^{KBr} 3400–3300 (OH, NH broad), 1710, 1630 (CO, NHAc), 1550, 1430, and 1140 cm^{-1}; ^{1}H-n.m.r.: δ 2.3 (s, 3 H, NAc), 2.6 (m, 1 H, $J_{3e,3a}$ −12.8, $J_{3e,4}$ 4.8, $J_{3e,F}$ 7.8 Hz, H-3e), 2.5)m, $J_{3a,4}$ 11.0 $J_{3a,F}$ 22.2 Hz, H-3a).

Anal. Calc. for $C_{11}H_{17}FNNaO_8 \cdot 2\,H_2O$: C, 35.75; H, 5.73. Found: C, 36.04; H, 5.95.

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