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Note

Fluorinated carbohydrates as potential plasma-membrane modifiers and inhibitors: synthesis of 2-amino-2,6-dideoxy-6-fluoro-D-mannopyranose hydrochloride*

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As part of our program[2] on design and synthesis of inhibitors and modifiers of cell-surface glycoconjugates, we have developed potential inhibitors of the biosynthesis of cell-surface sialic acid. Sialic acid is primarily responsible for the negative charge on the cell surface, and the inhibition of its synthesis may affect the cancer cell in two ways. Firstly, studies with neuraminidase-treated cells have shown that sialic acid may mask the antigenic sites[3] and that such cells may act as a vaccine[4]. Secondly, the metastatic behavior of cells may be modified, particularly as it has been shown that the "homing" of cells may be influenced with neuraminidase[5], and metastasizing cells have been found to have a higher amount of cell-surface sialic acid than their non-metastasizing counterparts[6].

It has been shown that 2-acetamido-2-deoxy-D-mannose 6-phosphate is the obligatory intermediate in the biosynthetic pathway to sialic acid; it is formed from 2-acetamido-acetyl- or 2-(*N*-glycolyl)amino-D-mannose and ATP in the presence of a specific kinase, which has been partially purified from rat-liver extracts[7]. Therefore, substitution of the 6-OH group by fluorine in 2-acetamido-2-deoxy-D-mannose (**2**) might furnish an effective inhibitor of this enzyme, thus blocking the biosynthesis of sialic acid.

As 2-amino-2-deoxy-D-mannose was found to be tumoristatic[8a] and to exert a selective effect on the ultrastructure of ascites tumor-cells[8b], derivatives of this sugar are of interest as cytostatic agents.

It appeared initially that the target compound **2** could readily be synthesized simply by epimerizing **1**; the latter may be readily obtained from the appropriately protected 2-amino-2-deoxy-D-glucose derivative by application of the (diethylamino)-sulfur trifluoride reagent[9]. Epimerization of **1** under alkaline conditions has been monitored by ^{13}C-n.m.r. spectroscopy as described earlier[10]. A mixture was obtained containing 20% of **2**. Unfortunately, **2** is not readily separable from the mixture, and

*A preliminary report of these studies has appeared; see ref. 1.

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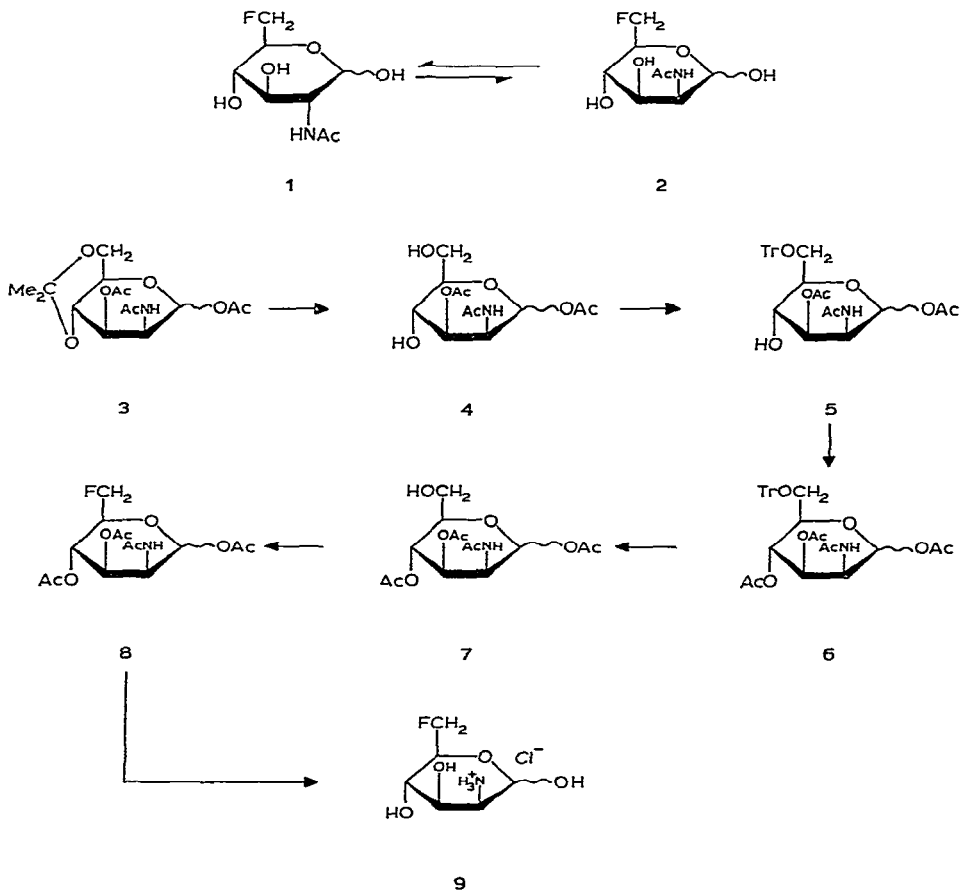
we had to resort to its synthesis from 2-amino-2-deoxy-D-mannose as the precursor. Surprisingly, tritylation of 2-acetamido-2-deoxy-D-mannopyranose with chlorotriphenylmethane in pyridine resulted in a mixture of D-*gluco* and D-*manno* products. When 2-acetamido-2-deoxy-D-mannopyranose was kept in anhydrous pyridine for 48 h at room temperature, it was ~20% epimerized to the D-*gluco* epimer; a small extent of epimerization under similar conditions has been observed earlier[11]. In order to avoid epimerization, the anomeric carbon atom in 2-acetamido-2-deoxy-D-mannopyranose was protected before tritylation. This was accomplished by acetonation followed by acetylation, yielding 2-acetamido-1,3-di-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-D-mannopyranose (**3**) as an anomeric mixture in which the α anomer preponderated[12] (77%). The assignments of anomeric protons in the mixtures were based on studies by Horton *et al.*[13] for 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranose anomers. Hydrolysis of **3** with aqueous acetic acid gave **4**, which was subsequently tritylated to **5** and fully acetylated to **6**. Detritylation of **6** was effected by hydrogenolysis to give **7**, which reacted slowly with an excess of diethylaminosulfur trifluoride in diglyme, giving the acetylated 6-fluoro-6-deoxy analog **8**.



It should be mentioned that the acetylated derivatives generally undergo fluorination with (diethylamino)sulfur trifluoride at a lower rate, giving lower yields than the corresponding derivatives having benzyl or acetal protecting groups. Deacetylation of **8** was accomplished with hydrochloric acid, giving the hydrochloride of the target compound **9**, which was *N*-acetylated to give **2**.

The ^{19}F-n.m.r. spectrum of **9** (D_2O) gave two superposed sextets centered at 159.0 and 158.8 p.p.m. (from external CF_3CO_2H) for the α and β anomers, respectively. As the mutarotation of **9** is very fast, giving rise to an almost equimolar mixture of the anomers, these peaks were assigned by analogy with other 6-deoxy-6-fluorohexoses, for which the ^{19}F chemical shifts of the α anomers were found at a higher field than those of the β anomers[8,14]. The geminal, ^{19}F–^{1}H J constants were 47.0 and 45.9 Hz for the α and β anomers, respectively, and the corresponding vicinal, ^{19}F–^{1}H J constants were 27.6 and 27.9 Hz, respectively. Comparison of the ^{13}C-n.m.r. spectrum of **2** with that of 2-acetamido-2-deoxy-D-mannopyranose[15] shows that introduction of fluorine at C-6 deshields C-6 from 61.70 to 83.0 p.p.m. ($J_{C\text{-}6,F}$ 171.6 Hz), whereas C-1 remains unchanged[15] at 94.1 p.p.m.

Compounds synthesized in this study were tested as inhibitors of growth of murine L-1210 leukemia cells in culture. Whenever the ID_{50} of a compound was greater than 10^{-3}M, the compound was designated as inactive. The fully acetylated (**8**) and the non-acetylated derivative (**9**) had ID_{50} values of 1×10^{-4} and 1×10^{-3}M, respectively, whereas the *N*-acetyl compound **2** was inactive. It was shown earlier that *O*-acetylated sugar derivatives are more inhibitory than their *O*-deacetylated counterparts, and that *O*-acetylation promotes uptake by passive diffusion[16]. The foregoing results are consistent with these observations. Similarly, *N*-acetyl derivatives of amino sugars are only poorly taken up by cells[8a], and this factor may explain the inactivity of **2**, as compared with its fully acetylated (**8**) and deacetylated (**9**) derivatives, which were found to be inhibitory. The corresponding non-fluorinated compound, 2-amino-2-deoxy-D-mannose, inhibited only 16% at 10^{-3}M, and its *N*-acetyl derivative was not inhibitory at all at this concentration.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Evaporations were performed in a rotary evaporator *in vacuo* at a bath temperature below 40°. Organic solutions were dried with sodium sulfate. Column chromatography was performed on silica gel (Bio-Sil A, 100–200 mesh). T.l.c. was effected on Analtech, Inc. "Uniplate" precoated t.l.c. plates (Silica Gel GF, 250 μm) developed with sulfuric acid spray at 100°. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 457 spectrometer. ^{1}H-N.m.r. spectra were recorded (in δ values) with Varian A-60 A and XL-100 instruments. The latter was operated in the Fourier-transform mode, and was also used for ^{13}C-n.m.r. spectra at 25.4 MHz and for ^{19}F-n.m.r. spectra at 94.1 MHz.

For ^{1}H-n.m.r. and ^{13}C-n.m.r. spectra, tetramethylsilane, and for ^{19}F-n.m.r., $CFCl_3$ (1–5%), were used as standards.

2-Acetamido-1,3-di-O-acetyl-2-deoxy-D-mannopyranose (**4**). — A stirred solution of 2-acetamido-2-deoxy-1,3-di-*O*-acetyl-4,6-*O*-isopropylidene-D-mannopyranose[12] (**3**, 7 g) in 50% aqueous acetic acid (100 mL) was heated for 90 min to 65°, and the hydrolysis was monitored by t.l.c. The solution was evaporated and the residue freed from acetic acid by evaporating water and then toluene from it, yielding the product as a waxy solid (6.0 g, 98%), $[\alpha]_D^{22}$ +5° (*c* 1, methanol); ν_{max}^{KBr} 3250–3550 (OH and NH), 1730 (C=O), 1650, and 1540 cm^{-1} (amide).

Anal. Calc. for $C_{12}H_{19}NO_8 \cdot H_2O$: C, 44.58; H, 6.55; N, 4.33. Found: C, 44.88; H, 6.72; N, 4.03.

2-Acetamido-1,3-di-O-acetyl-2-deoxy-6-O-trityl-D-mannopyranose (**5**). — To a solution of **4** (6.0 g) in anhydrous pyridine (75 mL) was added chlorotriphenylmethane (6 g), and the solution was stirred for 48 h at room temperature, and then for 1 h at 60–70°. The mixture was poured into ice–water and extracted with chloroform. The chloroform extracts were successively washed with water, dried, and evaporated. The residue was freed from pyridine by evaporating toluene from it. The thick syrup was chromatographed on a column of silica gel. Ether eluted triphenylmethanol, and **5** was eluted with ethyl acetate. The product solidified on trituration with ether and was crystallized from chloroform–ether; yield 9.0 g (91%), m.p. 130–132°, $[\alpha]_D^{22}$ +4° (*c* 1, chloroform); ν_{max}^{KBr} 3500–3200 (OH and NH), 1740 (C=O), 1650 and 1530 (amide), and 700 cm^{-1} (aromatic); ^{1}H-n.m.r. ($CDCl_3$): δ 1.8–2.2, (9 H, NAc and OAc), 5.65 (d, 1 H, *J* 9.0 Hz, NH), 5.89 (d, *J* 1.65 Hz, H-1β), 6.09 (d, *J* 1.8 Hz, H-1α), and 7.35 (m, 15 H, aromatic).

Anal. Calc. for $C_{31}H_{33}NO_8$: C, 67.99; H, 6.07; N, 2.56. Found: C, 67.76; H, 6.14; N, 2.56.

2-Acetamido-1,3,4-tri-O-acetyl-2-deoxy-6-O-trityl-D-mannopyranose (**6**). — Compound **5** (1.5 g) was acetylated with acetic anhydride (5 mL) and pyridine (20 mL) overnight at room temperature. The mixture was poured into ice–water, the product extracted with chloroform, and the extract washed with water and dried. The solvent was evaporated off and the product freed from pyridine by evaporation of toluene from it, and it was then crystallized from ether–petroleum ether; yield 1.3 g (92%), m.p. 122–125°, $[\alpha]_D^{22}$ +50.2° (*c* 1, chloroform); ν_{max}^{KBr} 3320 (NH), 1740 (C=O), 1660 and 1540 (amide), and 710 cm^{-1} (aromatic); ^{1}H-n.m.r. ($CDCl_3$): δ 1.8–2.2 (12 H, NAc and OAc), 5.87 (d, *J* 1.6 Hz, H-1β), 6.13 (d, *J* 2.0 Hz, H-1α), and 7.4 (m, 15 H, aromatic).

Anal. Calc. for $C_{33}H_{35}NO_9$: C, 67.20; H, 5.98; N, 2.38. Found: C, 67.46; H, 5.96; N, 2.18.

2-Acetamido-1,3,4-tri-O-acetyl-2-deoxy-D-mannopyranose (**7**). — To a solution of **6** (8.5 g) in acetic acid (100 mL) was added 10% palladium-on-carbon (3.5 g), and the suspension was hydrogenated for 48 h. After filtration of the mixture and evaporation of the filtrate, the residue was freed from acetic acid by evaporation of water and then of toluene from it. A solution of the residue in chloroform was

chromatographed on a column of silica gel. Ether eluted triphenylmethane, and ethyl acetate eluted the product, which was crystallized from chloroform–ether; yield 4.2 g (80.5%), m.p. 70–75°, $[\alpha]_D^{22}$ +41° (*c* 1, chloroform); ν_{max}^{KBr} 3400 (OH), 3300 (NH), 1740 (C=O), 1650, and 1525 cm^{-1} (amide); ^{13}C-n.m.r. (D_2O): 175.2, 173.7, 173.6, 172.5, 172.3 (C=O), 93.1 (C-1α, 73%), 91.9 (C-1β, 27%), 75.7, 73.0, 72.7, 71.0, 66.5, 60.5, 50.5, 50.2, 22.9, and 21.3 (CH_3). The C-1 peak in 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose in D_2O was observed at 92.0; C-1 of the α anomer resonated at 92.9 p.p.m.

Anal. Calc. for $C_{14}H_{21}NO_9 \cdot 0.5\ H_2O$: C, 47.19; H, 6.18; N, 3.93. Found: C, 47.21; H, 6.15; N, 3.85.

2-Acetamido-1,3,4-tri-O-acetyl-2,6-dideoxy-6-fluoro-D-mannopyranose (**8**). — To a stirred solution of (diethylamino)sulfur trifluoride (2 mL) in dry diglyme (3 mL), a solution of **7** (2.0 g) in dry diglyme (10 mL) was rapidly added at room temperature. Stirring was continued for 1 h at room temperature and then for 3 h at 40°. The mixture was poured onto ice and the product repeatedly extracted with ethyl acetate. The extract was washed with water, dried, and evaporated at 50° to remove diglyme. The residue was chromatographed on a column of silica gel. After eluting impurities with ether, the product was eluted with ethyl acetate, giving a waxy solid (1.0 g, 62%), $[\alpha]_D^{22}$ +51.9° (*c* 1, chloroform); ν_{max}^{KBr} 3230 (NH), 1750 (C=O), 1640, and 1550 (amide); ^{1}H-n.m.r. ($CDCl_3$): δ 1.8–2.2 (9 H, NAc and Ac), 5.19 (d, *J* 1.6 Hz, H-1β), and 6.07 (d, *J* 1.8 Hz, H-1β); ^{13}C-n.m.r. ($CDCl_3$): 91.9 (C-1α, 77%) and 90.8 (C-1β, 23%). The C-1 peak in 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-mannopyranose in $CDCl_3$ was observed at δ 90.6; C-1 of the α anomer lay at 91.7 p.p.m.; ^{19}F-n.m.r. ($CDCl_3$–$CFCl_3$): sextet, β, δ −233.74 ($J_{F,H-6}$ 47.5, $J_{F,H-5}$ 23.8 Hz), sextet, α, δ −234.28 ($J_{F,H-6}$ 47.2, $J_{F,H-5}$ 25.5 Hz). Continued elution of the column with ethyl acetate yielded the starting material **7** (800 mg).

Anal. Calc. for $C_{14}H_{20}NO_8F$: C, 48.12; H, 5.73; N, 4.00; F, 5.44. Found: C, 48.12; H, 5.88; N, 3.86; F, 5.35.

2-Amino-2,6-dideoxy-6-fluoro-D-mannopyranose hydrochloride (**9**). — A solution of **8** (200 mg) in 3M hydrochloric acid (7 mL) was heated for 3 h at 90–95°. The cooled solution was evaporated to dryness and freed from excess hydrochloric acid by evaporation of water. The light-yellow, semisolid residue was chromatographed on a column of Bio-Rad Ag-50 W × 8 H^+-form resin (200–400 mesh). The column was washed with water and the product eluted with M hydrochloric acid and crystallized from methanol–ether; yield 125 mg (90%), m.p. 185–187°, $[\alpha]_D^{22}$ −8.1→ −7.1° (after 24 h) (*c* 1, water); ^{19}F-n.m.r. [D_2O–CF_3CO_2H (ext.)]: sextet, β, δ −158.8 ($J_{F,H-6}$ 47.00, $J_{F,H-5}$ 27.5 Hz), sextet, α, δ −159.00 ($J_{F,H-6} = J_{F,H-6'}$ = 45.9, $J_{F,H-5}$ 27.9 Hz).

Anal. Calc. for $C_6H_{13}ClNO_4F$: C, 33.10; H, 5.90; N, 6.43; F, 8.73. Found: C, 33.21; H, 5.95; N, 6.47; F, 8.56.

2-Acetamido-2,6-dideoxy-6-fluoro-D-mannopyranose (**2**). — To a solution of **9** (50 mg) in abs. methanol (3 mL) was added anhydrous sodium acetate (10 mg). The mixture was stirred for 30 min at room temperature, cooled to 0°, and acetic

anhydride (0.5 mL) added. After stirring for 3 h at room temperature, ice was added, and the solvent was evaporated. The residue was dried to remove acetic acid. The semisolid mass was taken up in water, and passed through a short column of Bio-Rad Ag-50 W × 8 H^+-form resin (2 mL). The aqueous eluate was evaporated, and the residue crystallized from ethanol–ether; yield 32 mg (64%), m.p. 102–104°, $[\alpha]_D^{22}$ +4.4→+3.2° (after 24 h) (*c* 1, water); ν_{max}^{KBr} 3200–3500 (NH, OH), 1630 and 1520 cm^{-1} (C=O, amide); ^{1}H-n.m.r. (methyl sulfoxide-d_6): 1.90 (s, 3 NAc), 4.95 (broad s, 1 H, H-1), and 7.6 (d, 1 H, *J* 10 Hz, NH); ^{19}F-n.m.r. [D_2O–CF_3CO_2H (ext.)]: complex sextet, δ −156.2.

Anal. Calc. for $C_8H_{14}NO_5F$: C, 43.05; H, 6.28; N, 6.25; F, 8.52. Found: C, 43.16; H, 6.20; N, 5.99; F, 8.40.

L1210 Cell-culture test-system. — An inoculum of 50,000 cells in 1 mL of RPMI 1640 medium containing 10% of heat-inactivated, fetal-calf serum and 20mM HEPES buffer was supplemented with 1 mL of the same medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated either by protein assay or by cell counts using a Coulter counter. The growth in control cultures varied from 3- to 6-fold after 2 days. Each concentration was tested in triplicate. For compounds found inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50%-inhibitory concentration. Results are expressed in terms of ID_{50}, the molar concentration of the sugar analog in the nutrient medium leading to 50% of cell growth, as compared with the drug-free control.

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