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GENERAL METHODS FOR MODIFICATION OF SIALIC ACID AT C-9. SYNTHESIS OF *N*-ACETYL-9-DEOXY-9-FLUORONEURAMINIC ACID*

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ABSTRACT

Methyl 5-acetamido-3,5-dideoxy-2-*O*-methyl-D-*glycero*-D-*galacto*-2-nonulopyranosate was converted into the 9-*O*-trityl derivative and the remaining hydroxyl groups were protected as benzyl ethers. Removal of the trityl group, followed by treatment with diethylaminosulfur trifluoride gave the 9-deoxy-9-fluoro derivative, and deprotection *N*-acetyl-9-deoxy-9-fluoroneuraminic acid (**8**). In another procedure, coupling of 2-acetamido-2,6-dideoxy-6-fluoro-D-glucopyranose with potassium di(*tert*-butyl) oxaloacetate, followed by hydrolysis and decarboxylation gave **8**. Some of the derivatives were active as inhibitors of growth of mouse mammary adenocarcinoma (TA_3) and L1210 cells in culture.

INTRODUCTION

Cell-surface sialic acids have been implicated in several cellular functions involving the plasma membrane. These functions include masking of the cell surface antigens[2], bacterial cell-surface activity, mitogenic-receptor activity to some lectins[3], cell-to-cell recognition[2], and other social behavior[2,4]. There is also increasing evidence that sialic acid is involved in the process of metastasis[5–7]. Sialic acid is also greatly responsible for the negative charge on the cell surface. Among the sialic acids, *N*-acetylneuraminic acid is the one which occurs most commonly and, therefore, it is relevant to investigate its cell-surface molecular interactions.

Alteration of the cell surface sialic acid could be achieved either by inhibition of its incorporation or by incorporation of a modified sialic acid residue. Incorporation of sialic acid into the membrane glycoconjugates involves two steps. First, its activation through formation of a nucleotide, cytidine 5′-monophosphosialic acid (CMP-NeuAc), followed by the tranfer of the sialic acid residue from the nucleotide sugar to the glycoconjugate by a sialyl transferase. The hypothesis that the inhi-

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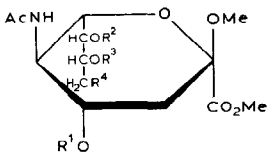
bition of either of these two enzyme-catalyzed processes might be possible by the modified *N*-acetylneuraminic acid enhanced our interest in the synthesis of *N*-acetyl-9-deoxy-9-fluoroneuraminic acid.

RESULTS AND DISCUSSION

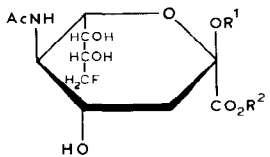
Considerable difficulties have been encountered in the development of methods for the modification of the *N*-acetylneuraminic acid molecule[8]. The only known fluorinated sialic acid, which is a marked inhibitor of the *N*-acetylneuraminic acid aldolase, is *N*-acetyl-3-deoxy-3-fluoroneuraminic acid[9], obtained only in 1% yield. Hence, we are reporting herein a successful application of a protection–deprotection approach to the synthesis on *N*-acetyl-9-deoxy-9-fluoroneuraminic acid.

The starting material, *N*-acetylneuraminic acid, was initially prepared from 2-acetamido-4,6-*O*-benzylidene-D-glucopyranose[10]. Later, it was isolated from edible bird's nest substance (purchased from Chinese groceries)[11]. Initially, the easily accessible and the most recommended route which we investigated involved the preparation of methyl 5-acetamido-2,4,7,8-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosate and then substitution at C-9 with fluorine. The 9-*O*-trityl derivative[12] was prepared and the trityl group removed by various procedures. The product obtained was a mixture of 9-*O*-acetyl compounds, resulting from the acetyl migration from O-7 and O-8, as shown by ^{1}H-n.m.r. spectroscopy. Identical acyl migrations were also observed when the 9-*O*-trityl derivative **2** was acetylated and detritylated. Hence, the acetyl group was found not suitable as a protecting group.

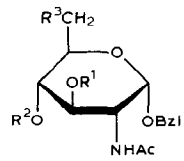
Esterification of the carboxylic acid group and simultaneous formation of the methyl glycoside was accomplished in one step by refluxing *N*-acetylneuraminic acid with absolute methanol in the presence of dry Dowex 50W (H^+) resin to give **1** (ref. 13). During this reaction, a small portion of the product was *N*-deacetylated, and this was recovered from the Dowex 50W (H^+) column by elution with M formic acid. The *N*-deacetylated product was *N*-reacetylated with acetic anhydride in methanol to give **1** and the overall yield of **1** was thus improved. This compound, without further purification, was tritylated in pyridine to give **2** in 72% yield. Protection of OH-4,7, and 8 was achieved as benzyl ethers[14] by treatment of **2** with barium oxide–barium hydroxide in *N,N*-dimethylformamide containing benzyl bromide. During this reaction, the carbomethoxy group of **3** was partially hydrolyzed; hence, the crude product was treated, after benzylation, with an ethereal solution of diazomethane to give **3** in 75% yield. Detritylation was first accomplished by treatment of **3** with hot aqueous acetic acid to give **4** in 78% yield. Some of the product was lost due to decomposition, possibly due to heating under acidic conditions[15]. However, the yield of the key intermediate compound **4** was significantly improved (94%) when **3** was detritylated with boron trifluoride–methanol complex[16] in dichloromethane.



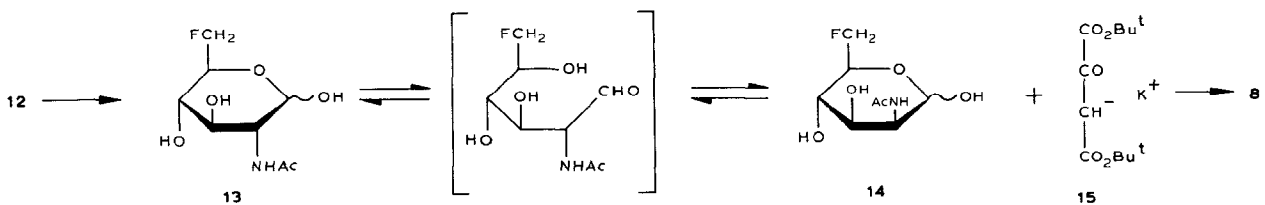
1 $R^1 = R^2 = R^3 = H, R^4 = OH$
2 $R^1 = R^2 = R^3 = H, R^4 = OTr$
3 $R^1 = R^2 = R^3 = Bzl, R^4 = OTr$
4 $R^1 = R^2 = R^3 = Bzl, R^4 = OH$
5 $R^1 = R^2 = R^3 = Bzl, R^4 = F$



6 $R^1 = R^2 = Me$
7 $R^1 = Me, R^2 = H$
8 $R^1 = R^2 = H$



9 $R^1 = R^2 = H, R^3 = OTr$
10 $R^1 = R^2 = Bzl, R^3 = OTr$
11 $R^1 = R^2 = Bzl, R^3 = OH$
12 $R^1 = R^2 = Bzl, R^3 = F$



The protected 9-deoxy-9-fluoroneuraminic acid derivative **5** was prepared by treatment of **4** with diethylaminosulfur trifluoride[17] in dichloromethane. Removal of the benzyl groups by catalytic hydrogenolysis of **5** in acetic acid and in the presence of palladium-on charcoal gave **6** in very good yield (86%). Attempts to remove simultaneously the glycosyl methoxy group and to hydrolyze the ester group of **6** with aqueous acid were not successful. It appears that the ester linkage of methoxy-sialic acid is quite stable[15] to acid hydrolysis, and when the acid concentration was increased only extensive decomposition occurred. Saponification of **6** with dilute sodium hydroxide solution yielded **7** as a crystalline solid, which on careful acid hydrolysis gave *N*-acetyl-9-deoxy-9-fluoroneuraminic acid (**8**) as a colorless crystalline compound.

In another approach for the synthesis of **8**, which was an adaptation of an earlier method[10] for the synthesis of *N*-acetylneuraminic acid, the 6-*O*-trityl derivative[18] of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[19,20] (**9**) was converted into **10** (88%) by a modification of the previous method[14] using barium oxide and barium hydroxide in *N,N*-dimethylformamide containing benzyl bromide. Compound[14] **11** was obtained as a crystalline solid after detritylation of **10** with boron trifluoride–methanol complex[16] in dichloromethane. Fluorination of **11** was accomplished by treatment with diethylaminosulfur trifluoride[17] to afford compound[19] **12** in 92% yield, and subsequently **13** by catalytic hydrogenolysis in acetic acid. In aqueous solution, 2-acetamido-2,6-dideoxy-6-fluoro-D-glucopyranose epimerized, in 48 h at pH 11, to give a mixture of 2-acetamido-2,6-dideoxy-6-fluoro-D-mannopyranose[21] **14** (20%) and the starting material **13** (80%), as observed by ^{13}C-n.m.r. spectroscopy of a solution in deuterium oxide. In the experimental condition of coupling with potassium di(*tert*-butyl) oxaloacetate[10] (**15**), compound **13** epimerized to **14** to undergo condensation. After hydrolysis and decarboxylation, the condensation product gave **8** (21% yield) identical in all respect with the product obtained previously.

The compounds described herein were tested as inhibitors of growth of mouse mammary adenocarcinoma (TA_3) and murine L1210 leukemia cells in culture. Whenever the ID_{50} of a compound was < 1mM, the compound was designated as cytotoxic. *N*-Acetylneuraminic acid and most of its synthetic derivatives demonstrated no growth inhibition at concentration up to 1mM. However, compounds **6** and **7** demonstrated a 40% growth inhibition at 1mM, and **8** inhibited cell growth to the greatest extent, showing an ID_{50} value of 0.16mM. *N*-Acetyl-9-deoxy-9-fluoroneuraminic acid (**8**) also was found to be a good substrate (K_m 6.3mM), as compared to *N*-acetylneuraminic acid (K_m 2.3mM) for CMP sialatesynthase (EC 2.7.7.43). It also demonstrated a marked inhibitory effect on hemagglutination by *Limulus polyphemus* (limulin) lectin[22].

EXPERIMENTAL

General methods. — Melting points (uncorrected) were determined by the

capillary method. Optical rotations were measured in a 10-cm cell with a Perkin-Elmer model 141 polarimeter. I.r. spectra were recorded on a Perkin-Elmer model 457 spectrophotometer and ^{1}H-n.m.r. spectra with Varian 390 and XL100 instruments. The latter instrument operating in the F.T. mode was also used for the determination of ^{13}C- and ^{19}F-n.m.r. spectra. Evaporation was performed in a rotary evaporator *in vacuo* at a bath temperature $<40°$. Column chromatography was performed on silica gel (Bio-sil, 100–200 mesh). T.l.c. was conducted on uniplate Silica Gel GF-250 and the spots were detected with H_2SO_4 at 100°.

*Methyl (methyl 5-acetamido-3,5-dideoxy-9-*O*-trityl-β-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**2**). — A mixture of *N*-acetylneuraminic acid (500 mg, 1.7 mmol) and dry Dowex 50W-X8 (H^+, 1 g) in anhydrous methanol (20 mL) was refluxed for 48 h to give methyl (methyl 5-acetamido-3,5-dideoxy-β-*D*-*glycero*-*D*-*galacto*-2-nonulopyranosid)onate (**1**, 380 mg); ^{13}C-n.m.r. ($CDCl_3$): δ 175.70 (C=O, ester), 171.29 (C=O, NAc), 110.21 (C-2), 71.62 (C-8), 70.93 (C-6), 69.14 (C-7), 67.46 (C-4), 64.46 (C-9), 54-62 (CH_3, ester) 52.83 (C-5), 52.10 (OCH_3), 40.32 (C-3), and 23.24 (CH_3, NAc). Without purification, **1** was stirred with chlorotriphenylmethane (400 mg, 1.4 mmol) in dry pyridine (5 mL) for 48 h at room temperature. The mixture was poured into ice-water, extracted with chloroform, washed with water, dried (Na_2SO_4), and evaporated. The residue was freed from pyridine by distilling with toluene and dried under vacuum. The gummy residue solidified on trituration with ether and recrystallized from chloroform–ether (475 mg, 72%), m.p. 138–40°, $[\alpha]_D^{22}$ −31° (*c* 1, chloroform); ν_{max}^{KBr} 3200–3400 (OH, NH), 1735 (C=O, ester), 1645 and 1540 (C=O, amide), and 705 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 7.45 (m, 15 H, arom.), 3.82 (s, 3 H, CO_2CH_3), 3.25 (s, 3 H, OCH_3), 2.42 (q, 1 H, $J_{3e,4}$ 4.20 Hz, H-3*e*), 1.95 (s, 3 H, NAc), and 1.68 (t, 1 H, $J_{3a,3e}$ 13.5, $J_{3a,4}$ 12.20 Hz, H-3*a*).

Anal. Calc. for $C_{32}H_{37}NO_9 \cdot 0.5\ H_2O$; C, 65.23; H, 6.45; N, 2.38. Found: C, 65.28; H, 6.45; N, 2.52.

*Methyl (methyl 5-acetamido-4,7,8-tri-*O*-benzyl-3,5-dideoxy-9-*O*-trityl-β-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**3**). — To a mixture of BaO (1.8 g) and $Ba(OH)_2$ (470 mg) in dry *N,N'*-dimethylformamide (15 mL) containing benzyl bromide (2.5 mL), cooled to 0°, was added a solution of **2** (2.05 g, 6.08 mmol) under N_2 with stirring. The mixture was stirred for 1 h at 0°, and then overnight at room temperature. After dilution with chloroform, the mixture was made neutral with aqueous 1% formic acid at 0°, and extracted with chloroform. The chloroform solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was taken up in absolute methanol, treated with an ethereal solution of diazomethane (~1 g), and kept overnight. After evaporation, the residue was taken up in benzene and poured into a column of silica gel. After elution of some low boiling impurities with petroleum ether, **3** was eluted with chloroform and crystallized from ether–petroleum ether (265 mg, 88%), m.p. 135–137°, $[\alpha]_D^{22}$ −7.2° (*c* 1, chloroform); ν_{max}^{KBr} 3390 (NH), 1750 (C=O, ester), 1680 (C=O, amide), and 700 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (m, 30 H, arom.), 3.72 (s, 3 H, O_2CH_3), 3.17 (s, 3 H, OCH_3), 2.42 (q, 1 H, $J_{3a,3e}$ 13.00, $J_{3e,4}$ 4.50 Hz, H-3*e*), and 1.89 (t, 4 H, H-3 and

NAc); ^{13}C-n.m.r. ($CDCl_3$): δ 169.69 (C=O, ester), 168.16 ($NHCOCH_3$), δ 143.71 (quater. arom. C of Tr), 138.36 (quater. arom. C of Bzl) 127.5 (m, arom.), 98.99 (C-2), 86.56 (quater. aliph. C of Tr), 78.23 (C-8), 75.2, 74.00, 73.4, 71.87, 70.93, 70.49, 62.35, 62.35 (C-9), 52.32 (O_2CH_3), 51.50 (C-5), 50.54 (OCH_3), 37.01 (C-3), and 23.56 ($NHCOCH_3$).

Anal. Calc. for $C_{53}H_{55}NO_9$: C, 74.91; H, 6.47; N, 1.64. Found: C, 74.73; H, 6.70; N, 1.54.

*Methyl (methyl 5-acetamido-4,7,8-tri-*O*-benzyl-3,5-dideoxy-β-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**4**). — (*a*). To a solution of **3** (850 mg, 1 mmol) in acetic acid (20 mL) at 90–100° (bath temperature) was added water (10 mL) slowly with stirring to avoid precipitation. The clear solution was stirred for 2 h at the same temperature, cooled, evaporated to dryness, and the residue freed from acetic acid by distillation with toluene. The dry residue was chromatographed on a column of silica gel. Triphenylmethanol was eluted with ether, and **4** subsequently with 9:1 chloroform–methanol as a waxy solid. It crystallized from ether–petroleum-ether (550 mg, 78%), m.p. 57°, $[\alpha]_D^{22}$ −29° (*c* 1, chloroform).

(*b*). A solution of **3** (1.7 g, 1.2 mmol) in dry dichloromethane (75 mL) was stirred at room temperature with BF_3–methanol complex [prepared from BF_3 etherate (500 mg) and absolute methanol (1.5 mL)] for 40 min when the reaction was complete. Water (1 mL) was added with stirring and the solution poured onto a column of silica gel. After eluting once with dichloromethane to remove impurities, **4** was eluted with 1:9 methanol–chloroform (1.1 g, 94%), m.p. 58–60°, $[\alpha]_D^{22}$ −28° (*c* 1, chloroform); ν_{max}^{KBr} 3500–3200 (OH,NH), 1740 (C=O, ester), 1655 and 1550 (C=O, amide), and 720 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 7,42 (m, 15 H, arom.), 4.60 (m, 6 H, CH_2Ph), 3.85 (m, 2 H, H_2-9), 3.75 (s, 3 H, O_2CH_3), 3.15 (s, 3 H, OCH_3), 2.55 (q, 1 H, $J_{3a,3e}$ 12.2, $J_{3e,4}$ 4.5 Hz, H-3*e*), and 1.75 (t, 4 H, H-3*a* and NAc); ^{13}C-n.m.r. ($CDCl_3$): δ 170.19 (C=O, ester), 168.10 ($NHCOCH_3$), 138.24 and 138.06 (quater. arom. C of Bzl), 129.00–127.13 (arom.), 98.95 (C-2), 81.83 (C-8), 75.28, 73.08, 72.72, 71.28, 71.46, 71.13, 60.43 (C-9), 53.17 (O_2CH_3), 52.62 (C-5), 51.10 (OCH_3), 37.55 (C-3), and 23.60 ($NHCOCH_3$).

Anal. Calc. for $C_{34}H_{41}NO_9$: C, 67.20; H, 6.80; N, 2.30. Found: C, 67.07; H, 6.75; N, 2.05.

*Methyl (methyl 5-acetamido-4,7,8-tri-*O*-benzyl-3,5,9-trideoxy-9-fluoro-β-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**5**). — To a solution of *N,N*-diethylaminosulfur trifluoride (1 mL) in dry dichloromethane (4 mL) at −20° was added **9** (500 mg, 0.82 mmol) in dichloromethane (2 mL) at a moderate rate with stirring under N_2. The mixture was stirred for 1 h at −10° and then for 2 h at 0°. When t.l.c. indicated the absence of starting material, the mixture was cooled to −20°, and absolute ethanol (1 mL) and then ice-water were added. The dichloromethane extract was washed with water, dried (Na_2SO_4), and evaporated to dryness. The light-colored solid residue was chromatographed on a column of silica gel. After eluting of some impurities with petroleum ether, the column was eluted with 1:1 ether–petroleum ether to give **5** which crystallized from ether–petroleum ether (390

mg, 68%), m.p. 55°, $[\alpha]_D^{22}$ −19° (*c* 1, chloroform); ν_{max}^{KBr} 3260 (NH), 1745 (C=O), 1650 and 1545 (C=O, amide), and 730 cm^{-1} (arom.); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35 (m, 15 H, arom.), 3.75 (s, 3 H, O_2CH_3), 3.15 (s, 3 H, OCH_3), 2.55 (q, 1 H, $J_{3a,3e}$ 12.00, $J_{3e,4}$ 4.5 Hz, H-3*e*), and 1.70 (t, 4 H, NAc and H-3*a*); ^{13}C-n.m.r. ($CDCl_3$): δ 169.95 (C=O, ester), 167.96 ($NHCOCH_3$), 138.17 and 137.94 (quater. arom. C of Bzl), 128.23–127.55 (arom.), 98.91 (C-2), 83.09 (d, $J_{F,H-9a}$ = $J_{F,H-9b}$ 187.8 Hz, C-9), 80.08 (C-8), 74.75, 74.45, 73.42, 73.06, 72.06, 71.27, 70.91, 52.47 (O_2CH_3), 52.29 (C-5), 50.88 (OCH_3), 37.36 (C-3), and 23.53 ($NHCOCH_3$); ^{19}F-n.m.r. ($CDCl_3$): δ −230.48 (sext., $J_{F,H-9a}$ = $J_{F,H-9b}$ 46.50, $J_{F,H-8}$ 26.60 Hz).

Anal. Calc. for $C_{34}H_{40}FNO_8$: C, 67.00; H, 6.61; N, 2.30; F, 3.12. Found: C, 66.90; H, 6.66; N, 2.19; F, 3.24.

*Methyl (methyl 5-acetamido-3,5,9-trideoxy-9-fluoro-β-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**6**). — A solution of **5** (435 mg, 0.71 mmol) in acetic acid (20 mL) was hydrogenolyzed in the presence of Pd–C (500 mg) for 48 h. After the usual workup, the residue was freed from acetic acid by distillation with water *in vacuo* and chromatography on a column of silica gel, and the product was eluted with 1:4 methanol–chloroform. It crystallized from ether, and was recrystallized from methanol–ether (210 mg, 86%), m.p. 155°, $[\alpha]_D^{22}$ −44.5° (*c* 1, methanol); ν_{max}^{KBr} 3500–3150 (NH, OH), 1735 (C=O), 1640 and 1550 (C=O, amide); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 3.72 (s, 1 H, $COCH_3$), 3.20 (s, 3 H, OCH_3), 2.19 (q, 1 H, $J_{3e,4}$ 4.2 Hz, H-3*e*), 1.90 (s, 3 H, NAc), and 1.5 (t, 1 H, $J_{3a,3e}$ 1.35, $J_{3a,4}$ 11.00 Hz, H-3*a*); ^{13}C-n.m.r. (D_2O): δ 175.72 (C=O, ester), 171.35 ($NHCOCH_3$), 100.31 (C-2), 81.54 (d, $J_{C-9,F}$ 164.9 Hz, C-9) 71.45 (C-6), 69.64 ($J_{C-8,F}$, 14.10 Hz, C-8), 68.17 ($J_{C-7,F}$ 6.4 Hz, C-7), 67.55 (C-4), 54.72 (CH_3, ester), 52.80 (C-5), 52.15 (OCH_3), 40.36 (C-3), and 23.29 ($NHCOCH_3$); ^{19}F-n.m.r. (CD_3OD–$CFCl_3$): δ −236.05 (sextet, $J_{F,H-9a}$ = $J_{F,H-9b}$ 47.90, $J_{F,H-8}$ 24.70 Hz).

Anal. Calc. for $C_{13}H_{22}FNO_8$: C, 46.02; H, 6.53; N, 4.13; F, 5.60. Found: C, 46.12; H, 6.62; N, 3.94; F, 5.75.

(*Methyl 5-acetamido-3,5,9-trideoxy-9-fluoro-*D*-glycero-*D*-galacto-2-nonulopyranosyl)onic acid* (**7**). — Compound **6** (240 mg, 0.7 mmol) was dissolved in 0.1M NaOH (20 mL), and kept for 5 h at room temperature. The solution was made neutral with Dowex 50W-X8 (H^+) resin, the suspension filtered, and the filtrate evaporated to dryness. The residue crystallized from methanol–ether (210 mg, 90%), m.p. 174–175°, $[\alpha]_D^{22}$ −45° (methanol); ν_{max}^{KBr} 3400–3200 (OH, NH), 1725 (C=O), 1635 and 1520 cm^{-1} (C=O, amide); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 3.20 (s, 3 H, OCH_3), 2.17 (q, 1 H, $J_{3e,4}$ 4.1 Hz, H-3*e*), 1.97 (s, 3 H, NAc), and 1.48 (t, 1 H, $J_{3a,3e}$ 13, $J_{3a,4}$ 11.2 Hz, H-3*a*); ^{13}C-n.m.r. (D_2O; external Me_4Si): δ 175.99 (CO_2H), 172.5 ($NHCOCH_3$), 99.94 (C-2), 81.19 ($J_{C-9,F}$, 165.6 Hz, C-9), 71.10 (C-6), 69.81 ($J_{C-8,F}$, 17.70 Hz, C-8), 68.00 ($J_{C-7,F}$ 6.00 Hz, C-7), 52.60 (C-5), 51.86 (OCH_3), 40.10 (C-3), and 23.05 ($NHCOCH_3$); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −236.87 (sextet, $J_{F,H-9a}$ = $J_{F,H-9b}$ 47.70, $J_{F,H-8}$ 27.10 Hz).

Anal. Calc. for $C_{12}H_{20}FNO_8$: C, 44.31; H, 6.15; N, 4.31; F, 5.85. Found: C, 44.69; H, 6.19; N, 4.31; F, 5.72.

5-Acetamido-3,5,9-trideoxy-9-fluoro-D-glycero-D-galacto-*2-nonulopyranosonicacid (N-acetyl-9-deoxy-9-fluoroneuraminic acid,* **8**). — *From* **7**. A solution of **7** (500 mg, 1.54 mmol) in aqueous mM HCl (20 mL) was heated at 100–105° (oil bath) with stirring for 2 h, when the reaction was complete. The solution was evaporated to dryness and again co-evaporated with water *in vacuo*. The residue was taken up in water and the solution passed through a column of Dowex 1-X8 (HCO_2^-) resin. After eluting with water to remove the impurities, **8** was eluted with M formic acid. After evaporation, the dry residue crystallized from acetic acid–ether (400 mg, 84%), m.p. 165–167°), $[\alpha]_D^{22}$ −47° (*c* 1, water); ν_{max}^{KBr} 3200–3500 (OH, NH), 1740 (COOH) 1620 and 1550 cm^{-1} (C=O, amide); ^{13}C-n.m.r. (D_2O; external Me_4Si): δ175.5 (CO_2H), 175.1 ($NHCOCH_3$), δ 96.73 (C-2), 86.10 (d, $J_{C-9,F}$ 164.9 Hz, C-9), 71.3 (C-6), 69.89 (d, $J_{C-8,F}$ 17.80 Hz, C-8), 68.25 (d, $J_{C-7,F}$ 7.20 Hz, C-7), 67.88 (C-4), 53.18 (C-5), 40.09 (C-3), and 23.27 ($NHCOCH_3$); ^{19}F-n.m.r. (D_2O; external $CFCl_3$): δ −235.4 (sextet, $J_{F,H-9a}$ = $J_{F,H-9b}$ 46.8, $J_{F,H-8}$ 26.0 Hz).

Anal. Calc. for $C_{11}H_{18}FNO_8$: C, 42.45; H, 5.83; N, 4.50; F, 6.12. Found: C, 42.68; H, 6.04; N, 4.50; F, 6.40.

From 2-acetamido-2,6-dideoxy-6-fluoro-D-*glucopyranose* (**13**). A mixture of **13** (1.32 g, 5.9 mmol) and potassium di(*tert*-butyl) oxaloacetate (**15**; 3.0 g, 10.6 mmol) in absolute methanol (50 mL) was vigorously shaken until all the solid went into solution. The solution was stirred for 10 days at room temperature. After filtration, the methanolic solution was made neutral with Dowex 50W-X8 (H^+) resin and the suspension filtered. After evaporation of the filtrate, the syrupy residue was taken up in water and extracted with ether, and the aqueous solution evaporated to dryness. The residue was taken up in 2-propanol and kept for 2 days to separate the starting material. After filtration, the solution was evaporated to a dry foamy residue, which was dissolved in water (30 mL). The solution was heated over a steam bath in the presence of microporous chips until the gas evolution ceased. The brown solution was cooled rapidly in ice-water, and a solution of 2M NaOH was added dropwise until the pH was 9; it was kept for 24 h at room temperature, the pH being adjusted to 9. The solution was poured into a column of Dowex 1-X8 (HCO_2^-) resin and eluted with water until all the starting material **13** had been removed (500 mg). Finally, the column was eluted with M formic acid and the eluant evaporated to dryness to give a light-colored glassy solid residue (500 mg). After treatment of the residue with activated charcoal in 1:1 water–acetic acid, the solution was evaporated to dryness and the residue cyrstallized from acetic acid–ether (370 mg, 19%), m.p. 161°, dec., $[\alpha]_D^{22}$ −45.5° (*c* 1, water). The product was identical with **8** described above.

*Benzyl 2-acetamido-3,4-*O*-benzyl-2-deoxy-6-*O*-trityl-α-*D*-glucopyranoside*[14] (**10**). — Benzyl 2-acetamido-2-deoxy-6-*O*-trityl-α-D-glucopyranoside[18] (**9**; 5 g, 10.8 mmol) was treated with BaO (4 g) and $Ba(OH)_2$ (1 g) in dry *N,N*-dimethylformamide (15 mL) containing benzyl bromide (2 mL) overnight at room temperature with stirring. The mixture was diluted with chloroform (100 mL), made neutral with M aqueous formic acid at 0°, and extracted with chloroform. The chloroform

solution was washed with water, dried (Na_2SO_4), and evaporated. The residue was passed through a column of silica gel. After a forerun with petroleum ether, **10** was eluted with ether as a thick syrup which crystallized from ether–petroleum ether (5.5 g, 88%), m.p. 160–161°, $[\alpha]_D^{22}$ +84° (*c* 1, chloroform); lit.[14] m.p. 161–162.5°, $[\alpha]_D$ +82° (*c* 1, chloroform).

*Benzyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-*α*-*D*-glucopyranoside* (**11**). — A solution of **10** (5 g, 6.9 mmol) in dry dichloromethane (60 mL) was stirred in the presence of BF_3–methanol complex [prepared from BF_3 etherate (0.5 mL)] and dry methanol (0.7 mL) for 40 min at room temperature. Water (1 mL) was added and the solution passed through a column of silica gel. The column was eluted with dichloromethane to give **11** mixed with triphenylmethanol. After evaporation, the product crystallized from chloroform–ether (3.1 g, 90%), m.p. 202–204°, $[\alpha]_D^{22}$ +125° (*c* 1, chloroform); lit.[14] m.p. 204.5°; $[\alpha]_D^{22}$ +121° (*c* 0.73, chloroform).

*Benzyl 2-acetamido-3,4-di-*O*-benzyl-2,6-dideoxy-6-fluoro-*α*-*D*-glucopyranoside* (**12**). — A solution of **11** (2.5 g, 5.0 mmol) in dichloromethane (2 mL) was added rapidly to a solution of *N,N*-diethylaminosulfur trifluoride (2 mL) in dichloromethane (2 mL) at 0°, under N_2 with stirring. The mixture was stirred for 3 h at room temperature, cooled to −10°, and absolute ethanol (2 mL) added. The mixture was poured into ice–water and after the usual extraction with chloroform and evaporation of the solvent, the residue was chromatographed on a column of silica gel. The product **12** was eluted with ethyl acetate as a colorless solid, and crystallized from chloroform–ether–petroleum ether (2.3 g, 92%), m.p. 202–204°, $[\alpha]_D^{22}$ +106.5° (*c* 1, chloroform); lit.[19] m.p. 204–205°, $[\alpha]_D^{22}$ +108°, (*c* 1, chloroform).

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