Stepwise Synthesis of *N*-Acetylneuraminic Acid and *N*-Acetyl[1-^{13}C]neuraminic Acid

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N-Acetylneuraminic acid (NANA) has been synthesized from 2-acetamido-2-deoxy-D-mannose by the stepwise extension of the carbon chain. Addition of nitromethane to 2-acetamido-2-deoxy-D-mannose gave 3-acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol, which underwent a modified Nef reaction to yield 3-acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptose. The heptose was converted to 3-acetamido-3-deoxy-4,5:6,7-di-*O*-isopropylidene-*aldehydo*-D-*glycero*-D-*galacto*-heptose via its isopropylidenated dimethyl dithioacetal derivative. The *aldehydo*-heptose was converted via a second nitromethane addition, and subsequent standard reactions, to 4-acetamido-1,2,4-trideoxy-1-nitro-D-*glycero*-D-*galacto*-octitol, which underwent a modified Nef reaction to yield 4-acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-octose. Treatment of the octose with aqueous sodium cyanide gave the expected nononic acid derivatives 5-acetamido-3,5-dideoxy-D-*erythro*-L-*manno*-nononic acid and 5-acetamido-3,5-dideoxy-D-*erythro*-L-*gluco*-nononic acid, which were selectively oxidized at C-2 to give 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (NANA). Using Na^{13}CN in the synthesis gave [1-^{13}C]-NANA.

Acylneuraminic acids (sialic acids)[1,2] are important natural glycoses found in glycoproteins, glycolipids,[3,4] and microbial polysaccharides,[5-7] where they influence physicochemical, immunological, and other biological properties. These acids are N-acylated with acetyl or glycoloyl groups, and frequently *O*-acetyl substituents are present.

N-Acetylneuraminic acid (NANA), the most widely occurring member of the sialic acid family, was first obtained crystalline from submaxillary mucin,[8] and subsequent investigations showed NANA to be 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid.[9] Chemical,[10] ^{1}H NMR,[11] and ^{13}C NMR[5,12] studies showed it to exist in aqueous solution as 5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid[13] (Figure 1). The assigned structure of the acid is supported by x-ray analysis data.[14,15]

N-Acetylneuraminic acid has been synthesized in low yield by the reaction of oxalacetic acid with either 2-acetamido-2-deoxy-D-glucose[16,17] or 2-acetamido-2-deoxy-D-mannose,[18,19] and improved yields were reported by the condensation of di-*tert*-butyl oxaloacetate with the acetamidoglycoses or their 4,6-*O*-benzylidene derivatives.[20] Application of the Wittig reaction to 3-acetamido-2,4,5,6,7-penta-*O*-acetyl-3-deoxy-*aldehydo*-D-*glycero*-D-*galacto*-heptose has been used to effect the stereochemically defined synthesis of NANA.[21]

The aim of the present work was to develop a practical general method for the stepwise synthesis of NANA specifically labeled in the carbon chain with either ^{14}C or ^{13}C, which could also be adapted to the synthesis of configurational analogues of NANA.

The stepwise ascent procedure selected was the Fischer–Sowden nitromethane addition method.[22-24] It has been found that nitromethane did not always undergo base-catalyzed addition to glycoses. For example, while 2-acetamido-2-deoxy-D-mannose undergoes nitromethane addition, 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose fail to react with nitromethane under the usual experimental conditions. In other experiments, it was found that the latter glycoses readily underwent base-catalyzed addition of nitromethane to their protected acyclic aldehydo derivatives prepared by demercaptalation of their ketal-substituted dialkyl dithioacetal derivatives.[25] The use of the *aldehydo*-glycose derivatives to effect successful nitromethane additions appears to be of general application, and it was used in the present work when the conversion of the intermediate aldoheptose to the corresponding 1-deoxy-1-nitrooctitol derivatives could not be effected under the normal nitromethane addition conditions.

Discussion

2-Acetamido-2-deoxy-D-mannose (1) underwent base-catalyzed addition of nitromethane to yield 3-acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (**2**), with the formation of less than 1% of the corresponding D-*glycero*-D-*talo*-heptitol. The stereospecificity of this addition follows that found in the base-catalyzed nitromethane addition to D-mannose[26] and is that required for the introduction of the asymmetric center at C-4 of NANA. Heptitol **2** under modified[27] Nef[28] reaction conditions afforded 3-acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptose (**3**), which was identical with an authentic sample prepared by reduction of the nitrile produced by the addition of hydrogen cyanide to 2-acetamido-2-deoxy-D-mannose.[29]

Heptose **3**, on treatment with methanethiol in concentrated hydrochloric acid, was converted in high yield to crystalline 3-acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptose dimethyl dithioacetal (**4**), which on acid-catalyzed isopropylidenation gave crystalline 3-acetamido-3-deoxy-4,5:6,7-di-*O*-isopropylidene-D-*glycero*-D-*galacto*-heptose dimethyl dithioacetal (**5**), the assigned positional substitution of the isopropylidene groups being inferred from stereochemical considerations.[30] Demercaptalation of **5** produced 3-acetamido-3-deoxy-4,5:6,7-di-*O*-isopropylidene-*aldehydo*-D-*glycero*-D-*galacto*-heptose (**6**), which gave IR and NMR data consistent with the existence of the aldehydo function.

The substituted *aldehydo*-heptose **6** readily underwent base-catalyzed addition of nitromethane to give an almost theoretical yield of 4-acetamido-1,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-1-nitro-D-*erythro*-L-*manno*-octitol (**7**), which on mild acid hydrolysis underwent deisopropylidenation to give crystalline 4-acetamido-1,4-dideoxy-1-nitro-D-*erythro*-L-*manno*-octitol (**8**). The nitromethane addition to **6** was stereospecific, giving only the octitol having the D-*erythro*-L-*manno* configuration, the assignment of the configuration of the new asymmetric center at C-7 being based on the observed positive Cotton effect at 340 nm in the ORD spectrum of **8**.[31,32] Acetylation of **8** afforded crystalline 4-acetamido-2,3,5,6,7,8-hexa-*O*-acetyl-1,4-dideoxy-1-nitro-D-*erythro*-L-*manno*-octitol (**9**), which on refluxing in benzene solution in the presence of sodium bicarbonate was converted to crystalline 4-acetamido-3,5,6,7,8-pentaacetoxy-D-*glycero*-D-*galacto*-1-nitro-1-octene (**10**). Octene **10** on reduction with sodium borohydride in ethanol solution afforded crystal-

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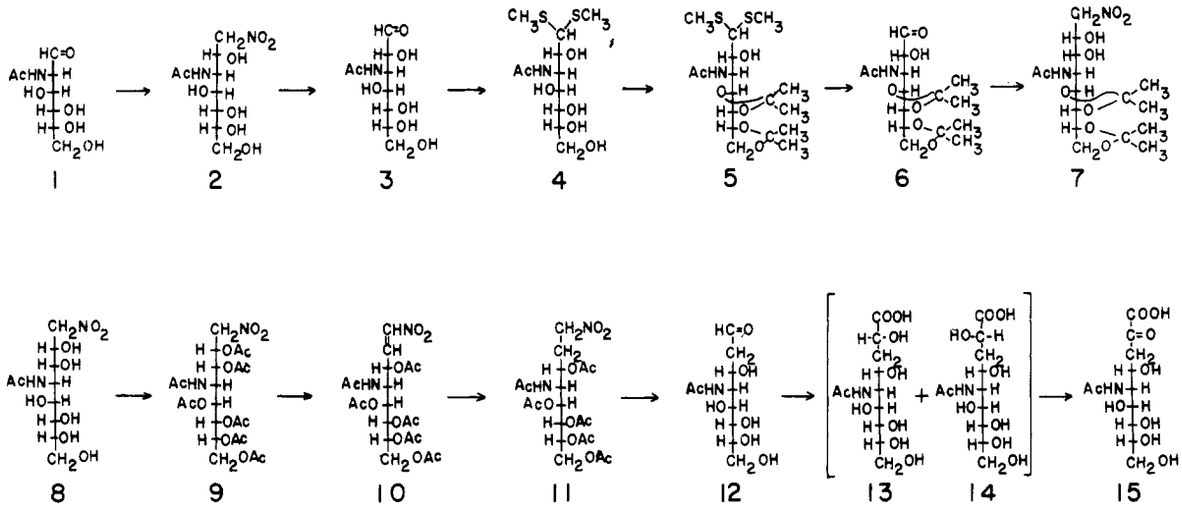

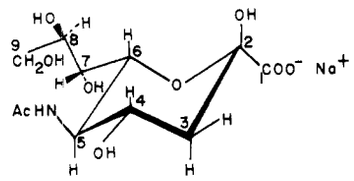

Figure 1. The assigned structure of NANA in aqueous solution.

line 4-acetamido-3,5,6,7,8-penta-*O*-acetyl-1,2,4-trideoxy-1-nitro-D-*glycero*-D-*galacto*-octitol (**11**), which underwent a modified Nef reaction to yield crystalline 4-acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-octose (**12**).

Octose **12**, which had the correct configuration for direct conversion to NANA, was converted to its epimeric nononic acid derivatives, 5-acetamido-3,5-dideoxy-D-*erythro*-L-*manno*-nononic acid (**13**) and 5-acetamido-3,5-dideoxy-D-*erythro*-L-*gluco*-nononic acid (**14**), by treatment with sodium cyanide. Acids **13** and **14** were formed in approximately equal proportions as evidenced by ^{13}C NMR and chromatographic analysis and were indistinguishable from the same acids produced in essentially the same proportion by the sodium borohydride reduction of the keto function of the salt of authentic NANA.

The mixed nononic acids **13** and **14** were oxidized with a vanadium pentoxide–potassium chlorate catalyst,[33-35] which effected the selective oxidation of the hydroxy groups at C-2 to yield 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (NANA, **15**), which had identical physical, chromatographic, and NMR data with authentic NANA.

Starting from 2-acetamido-2-deoxy-D-mannose, the above synthetic route can give NANA in 17% overall yield. While most of the steps can be made in better than 90% yield, a significant loss of materials occurs in the nitromethane addition to 2-acetamido-2-deoxy-D-mannose, where it was observed that the unreacted material was mainly 2-acetamido-2-deoxy-D-glucose, which arises from the epimerization of the starting material in the alkaline medium. A second significant loss occurs in the isopropylidenation step, and it is possible that alternative protection groups would lead to improved yields. Although the synthesis of NANA using nitromethane-^{13}C or -^{14}C would not be practical under the described conditions, preliminary experiments have shown that nitromethane addition to some protected *aldehydo*-glycoses can be effected in excellent yield in *N,N*-dimethylformamide solvent using stoichiometric amounts of nitromethane catalyzed with 50% aqueous sodium hydroxide.[25] If this finding proves to be of general application, the use of nitromethane-^{13}C or -^{14}C in the synthesis of specifically labeled glycoses may be of practical value.

5-Acetamido [1-^{13}C]-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid was synthesized in 51% yield from the nononic acids **13** and **14** using Na^{13}CN and [1-^{13}C]NANA gave a ^{13}C NMR spectrum consistent with that expected. It is probable that the synthetic route could be used for the synthesis of configurational isomers of NANA.

All the compounds synthesized in the work had elemental analyses, IR, ^1H NMR, and ^{13}C NMR data consistent with the assigned structures.

Experimental Section

General Methods. Evaporations were performed under reduced pressure and below 40 °C. Melting points were determined on a Fisher-Johns apparatus and are corrected. Optical rotations were determined at 20 °C in 1-dm tubes using a Perkin-Elmer Model 141 polarimeter. Optical rotary dispersion (ORD) measurements were made using a Jasco Model ORD/UV-5 automatically recording spectrophotometer. Infrared (IR) spectra were obtained by the KBr disk technique using a Perkin-Elmer 237B Infracord.

Paper chromatography was performed by the descending method on Whatman No. 1 filter paper using the solvent systems (A) pyridine–ethyl acetate–water (2:5:5 v/v, top layer) and (B) 1-propanol–acetic acid–water (54:8:18 v/v). Compounds were detected with (a) 2% silver nitrate in acetone followed by 2% sodium hydroxide in ethanol,[36] (b) 2% *p*-anisidine hydrochloride in ethanol,[37] or (c) 0.02 M aqueous sodium metaperiodate followed by ethylene glycol–acetone–sulfuric acid (50:50:0.3 v/v) and 6% sodium 2-thiobarbiturate.[38] The rates of movement of the compounds are quoted relative to 2-acetamido-2-deoxy-D-mannose (R_M).

Thin-layer chromatography (TLC) was done on precoated 0.25-mm silica gel 60 plates (Merck) using (A) benzene–methanol (9:1 v/v) or (B) 1-butanol–acetone–water (4:5:1 v/v), and compounds were located by spraying with 10% sulfuric acid in ethanol and heating at 120 °C. Mobilities are quoted relative to the solvent front (R_f).

Gas chromatography (GC) was performed using a Hewlett-Packard 402 gas chromatograph with a hydrogen flame detector, fitted with glass U-tubes (4 ft × 6 mm × 3 mm i.d.) packed with (A) 3% (w/w) ECNSS-M on 100–120 mesh Gas Chrom Q or (B) 3% (w/w) OV-17 on 100–120 mesh Gas Chrom Q. Development was made with helium, and samples were injected directly onto the column packings without the use of the flash-heating system. Retention times of the compounds are quoted relative to 2-acetamido-2-deoxy-1,3,4,5,6-penta-*O*-acetyl-D-mannitol (T_{MA}).

Proton magnetic resonance (^1H NMR) spectra were recorded using a Varian EM-360 spectrometer at 60 MHz with tetramethylsilane as an internal standard. ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra were measured on a Varian CFT-20 (20 MHz) spectrometer in 10-mm o.d. tubes, and the spectra were recorded with complete proton decoupling, spectra windows of 200 ppm, and digitization into 4K data points. Solvent deuterium resonance was used as a field frequency lock, and chemical shifts are expressed relative to tetramethylsilane contained in a coaxial 5-mm o.d. sample tube.

3-Acetamido-1,3-dideoxy-1-nitro-D-*glycero*-D-*galacto*-heptitol (2). To a stirred suspension of 2-acetamido-2-deoxy-D-mannose (**1**, 20 g) in nitromethane (400 mL) was added dropwise over 20 min a solution of 1.7 M sodium methoxide in methanol (52 mL), followed by methanol (300 mL) to give a clear solution which was stirred at room temperature for 18 h. Following the addition of ether (1 L), the precipitated material was collected by filtration, and the ether-washed solid, dissolved in a minimum amount of cold water, was passed down a column of Rexyn 101 (H^+) ion-exchange resin (800 mL). The eluate and water washings from the column were concentrated under reduced pressure to yield a syrup which, on trituration with warm methanol, afforded crystalline **2** (14.62 g, 57.5%), which on paper chromatography (solvent A) gave a single silver nitrate positive spot with R_M 3.81. A sample of **2** recrystallized from methanol had mp 200–202 °C; $[\alpha]_D$ −59° (*c* 0.75, water); ORD $[\phi]_{350}$ +111° (*c* 0.2, water); IR 1560 cm^{-1} (NO_2); ^{13}C NMR (D_2O) δ 175.5 (C=O), 80.10 (CH_2NO_2), 71.89, 70.23, 68.57, 67.89, 64.37 (CH_2OH), 52.65 (CH-NHCOCH$_3$), 22.99 (CH_3CONH).

Anal. Calcd for $C_9H_{18}N_2O_8$: C, 38.29; H, 6.42; N, 9.92. Found: C, 38.40; H, 6.43; N, 9.97.

3-Acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptose (3). Compound **2** (14.62 g) dissolved in a solution of $Ba(OH)_2{\cdot}8H_2O$ (15.9 g) in water (280 mL) was added dropwise with stirring to a cooled (0 °C) solution of concentrated sulfuric acid (32 mL) in water)170 mL), and the mixture was then stirred at 20 °C for 16 h. The reaction mixture was neutralized in the cold with saturated barium hydroxide solution (to pH 6); following the addition of barium carbonate (1 g), the mixture was filtered and the filtrate and water washings were passed through columns of Rexyn 101 (H^+) (6 mL) and Duolite A4 (OH^-) (5 mL) ion-exchange resins. Concentration of the eluate afforded **3** as a solid (11.8 g, 90%), which on paper chromatography (solvent A) gave a single silver nitrate and *p*-anisidine positive spot with R_M 0.52. A sample of **3** after two recrystallizations from ethanol had mp 215–216 °C and $[\alpha]_D$ +81.7° (4 min) → +116° (equilibrium) (*c* 1.2, water) (lit.[29] mp 223–225 °C, $[\alpha]_D$ +149.5 → +110.5° (water); the difference in melting point and mutarotation values is probably due to crystallization of **3** in different anomeric forms).

Anal. Calcd for $C_9H_{17}O_7N$: C, 43.02; H, 6.82; N, 5.58. Found: C, 43.31; H, 6.80; N, 5.51.

GC analysis (column A, 225 °C) of reduced and acetylated **3**[39] gave a single peak with T_{MA} 1.90, having the same retention time as authentic 3-acetamido-1,2,4,5,6,7-hexa-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-heptitol. Selective periodate oxidation and subsequent reduction of the methyl glycosides of **3** afforded 3-amino-3-deoxy-D-galactose hydrochloride with mp 180 °C and $[\alpha]_D$ +90° (*c* 0.4, water), which was chromatographically identical with an authentic sample of the aminoglycose.

3-Acetamido-3-deoxy-D-*glycero*-D-*galacto*-heptose Dimethyl Dithioacetal (4). Compound **3** (10.1 g) was dissolved in concentrated hydrochloric acid (20 mL) and cooled in an ice bath, and following the addition of methanethiol (20 mL) the stirred mixture was allowed to warm to 20 °C over 20 min. The reaction mixture was poured into ice water (300 mL) containing a few drops of 2-octanol, and the stirred solution was quickly neutralized by the addition of lead carbonate. Following filtration through a bed of Celite, the filtrate and washings were concentrated to dryness, and the residue, after extraction with boiling ethanol (400 mL), was filtered while hot (to remove $PbCl_2$). On cooling the ethanol solution to 0 °C, it gave crystalline **4** (10.5 g, 80%), which had mp 168–169 °C and $[\alpha]_D$ −34° (*c* 1.7, water), and on paper chromatography (solvent A) **4** gave a single silver nitrate positive spot with R_M 2.80.

Anal. Calcd for $C_{11}H_{23}NO_6S_2$: C, 40.11; H, 7.04; N, 4.25; S, 19.43. Found: C, 40.18; H, 7.07; N, 4.19; S, 19.31.

3-Acetamido-3-deoxy-4,5:6,7-di-*O*-isopropylidene-D-*glycero*-D-*galacto*-heptose Dimethyl Dithioacetal (5). Compound **4** (5 g) was added to a stirred solution prepared by the addition of 2,2-dimethoxypropane (20 mL) to acetone (7.5 mL) containing concentrated sulfuric acid (0.12 mL). After stirring at 20 °C for 90 min, the reaction mixture was neutralized by the addition of saturated barium hydroxide followed by barium carbonate (0.5 g). The neutralized mixture was filtered through Celite, and the filtrate, after the addition of three drops of pyridine, was concentrated to a syrup. The syrup was extracted with boiling hexane (3 × 150 mL), which on concentration and cooling gave crystalline **5** (2.37 g). Chromatographic separation of the residual hexane extract on a column of silica gel (3 × 50 cm) using a benzene–methanol mixture (10:1 v/v) as the mobile phase afforded further pure **5** (1.46 g, total yield 3.83 g, 62%).

Crystalline **5** gave a single spot on TLC (solvent A, R_f 0.29) and had mp 125–126 °C and $[\alpha]_D$ +61° (*c* 2.6, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.98 (d, 1 H, NH), 4.8 (t, 1 H), 2.22 and 2.16 (2 s, 6 H, $2SCH_3$), 2.04 (s, 3 H, $COCH_3$), 1.42 (m, 12 H, CH_3 of isopropyl); D_2O exchange caused the signal at δ 5.98 to disappear and the triplet at δ 4.80 to collapse to a doublet.

Anal. Calcd for $C_{17}H_{30}NO_6S_2$: C, 49.86; H, 7.63; N, 3.42; S, 15.63. Found: C, 49.75; H, 7.61; N, 3.29; S, 15.51.

4-Acetamido-1,4-dideoxy-1-nitro-D-*erythro*-L-*manno*-octitol (8). A stirred solution of **5** (5.45 g) in an acetone–water mixture (10:1 v/v, 115 mL) was treated at 20 °C with mercuric oxide (9.5 g), followed by the addition of a solution of mercuric chloride (9.5 g) in an acetone–water mixture (10:1 v/v, 20 mL), and the mixture was stirred for 24 h. The reaction mixture was filtered through Celite into a flask containing a little mercuric oxide, and the filtrate was concentrated to a syrup. The syrup was extracted with chloroform (2 × 150 mL), and the extract, after filtration through Celite, was washed with 17% aqueous potassium iodide (150 mL), followed by water (2 × 50 mL). The dried (sodium sulfate) chloroform extract was concentrated to a syrup (2.75 g) and was combined with the syrup (0.83 g) obtained by back-extraction of the potassium iodide wash solution with chloroform to give 3-acetamido-3-deoxy-4,5:6,7-di-*O*-isopropylidene-*aldehydo*-D-*glycero*-D-*galacto*-heptose (**6**, total yield 3.58 g, 81%). The *aldehydo*-heptose **6** gave a single spot on TLC (solvent A, R_f 0.13); IR 1740 cm^{-1} (CHO); partial ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1 H, CHO), 6.1 (m, 1 H, NH), 1.82 (s, 3 H, $COCH_3$), 1.32 (m, 12 H, CH_3 of isopropyl). The syrup **6** was used in the next step without further purification.

To a stirred solution of **6** (3.28 g) in nitromethane (145 mL) was added dropwise 1.7 M sodium methoxide in methanol (11.9 mL), followed by the addition of methanol (70 mL) to dissolve the precipitated material, and the mixture was stirred at 20 °C for 48 h. The reaction mixture was saturated with carbon dioxide and was concentrated to a syrup. The syrup was extracted at 35 °C with chloroform (150 mL), and concentration of the filtered chloroform extract afforded essentially pure 4-acetamido-1,4-dideoxy-5,6:7,8-di-*O*-isopropylidene-1-nitro-D-*erythro*-L-*manno*-octitol (**7**, 3.64 g, 94%), which gave a single spot on TLC (solvent A, R_f 0.17) and was used in the next step without further purification.

A solution of **7** (3.5 g) in 0.02 N sulfuric acid (175 mL) was heated on a boiling water bath for 17 min, and the cooled solution was neutralized with barium carbonate and filtered. The filtrate was passed through a column of Rexyn 101 (H^+) (5 mL) and Duolite A4 (OH^-) (5 mL) ion-exchange resins, and the eluate was concentrated to yield **8** as a slightly yellow solid (2.57 g, 92%), which gave a single spot on TLC (solvent B, R_f 0.6) and on paper chromatography (solvent A, R_M 2.06). Recrystallization of the product (2.5 g) from methanol (20 mL) afforded pure crystalline **8** which had mp 173–174 °C; $[\delta]_D$ −22° (*c* 1.15, water); ORD $[\phi]_{340}$ +316° (*c* 0.19, water); IR 1545 cm^{-1} (NO_2); ^{13}C NMR (D_2O) δ 175.89 (CO), 80.19 (CH_2NO_2), 71.89, 70.52, 70.33, 69.85, 68.57, 64.45 (CH_2OH), 51.46 (CHNHCOCH$_3$), 23.06 (CH_3CO).

Anal. Calcd for $C_{10}H_{20}N_2O_9$: C, 38.45; H, 6.45; N, 8.97. Found: C, 38.29; H, 6.60; N, 8.76.

Reduced and acetylated **8**[39] on GLC (column A, 225 °C) gave a single peak with T_{MA} 3.87.

4-Acetamido-2,3,5,6,7,8-hexa-*O*-acetyl-1,4-dideoxy-1-nitro-D-*erythro*-L-*manno*-octitol (9). A solution of **8** (1.4 g) in acetic anhydride (60 mL) was treated with three drops of concentrated sulfuric acid, and the mixture was heated on a boiling water bath for 75 min. The cooled mixture was poured into ice water (600 mL) and, following extraction with chloroform (1 L), the chloroform solution was washed successively with saturated sodium bicarbonate solution (500 mL) and water (500 mL). Concentration of the dried (sodium sulfate) extract gave **9** (2.4 g, 95%), which was pure by TLC (solvent A, R_f 0.20) and was used directly in the next synthetic step.

An analytical sample of **9** obtained as rod-shaped crystals from a water–hexane–ethyl acetate mixture had mp 80–81 °C and $[\alpha]_D$ 0° (*c* 1.15, chloroform); IR 1560 cm^{-1} (NO_2); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3 H, $NHCOCH_3$), 2.12 (m, 18 H, CO_2CH_3).

Anal. Calcd for $C_{22}H_{32}N_2O_{15}$: C, 46.80; H, 5.71; N, 4.96. Found: C, 46.65; H, 5.89; N, 4.81.

4-Acetamido-3,5,6,7,8-pentaacetoxy-D-*glycero*-D-*galacto*-1-nitro-1-octene (10). A solution of **9** (2.4 g) in benzene (75 mL) was boiled under reflux for 3 h with sodium bicarbonate (2.7 g). The cooled reaction mixture was filtered, and the filtrate was concentrated to yield **10** as a solid (1.92 g, 90%), which gave a single spot on TLC (solvent A, R_f 0.19) and was used without further purification in the next synthetic step. An analytical sample of **10** obtained crystalline from an ether–petroleum ether (bp 65–110 °C) mixture had mp 149–150 °C and $[\alpha]_D$ +6.8° (*c* 0.80, chloroform); IR 1530 cm^{-1} (NO_2); ^{1}H NMR ($CDCl_3$) δ 6.89 (m, 2 H, CH=CH), 2.18 (m, 15 H, CO_2CH_3), 1.90 (s, 3 H, $NHCOCH_3$); ^{13}C NMR ($CDCl_3$) δ 141.40 and 136.14 (C-1

and C-2).

Anal. Calcd for $C_{20}H_{28}N_2O_{13}$: C, 47.61; H, 5.59; N, 5.55. Found: C, 47.67; H, 5.60; N, 5.59.

4-Acetamido-3,5,6,7,8-penta-*O*-acetyl-1,2,4-trideoxy-1-nitro-D-*glycero*-D-*galacto*-octitol (11). A solution of **10** (2.4 g) in ethanol (100 mL) was treated with sodium borohydride (2 g), and after 8 min at 20 °C the cooled mixture was acidified with acetic acid. When no more hydrogen was evolved, the reaction mixture was concentrated, and the residue was evaporated with methanol (5 × 60 mL). The residue was extracted with chloroform (150 mL). Concentration of the filtered chloroform extract afforded **11** (1.05 g, 99%) as a glass, which gave a single spot on TLC (solvent A, R_f 0.16) and was used directly in the next step.

An analytical sample of **11** obtained crystalline from a benzene–petroleum ether (bp 60–110 °C) mixture had mp 137–138 °C and $[\alpha]_D$ +5.2° (*c* 0.59, chloroform); IR 1560 cm^{-1} (NO_2); ^{13}C NMR ($CDCl_3$) δ 29.20 (CH_2), and no signals at δ 141.40 or 136.14 (found in the spectrum of **10**).

Anal. Calcd for $C_{20}H_{30}N_2O_{13}$: C, 47.43; H, 5.97; N, 5.53. Found: C, 47.27; H, 5.87; N, 5.41.

4-Acetamido-2,4-dideoxy-D-*glycero*-D-*galacto*-octose (12). Compound **11** (0.81 g) was dissolved in 1 N sodium hydroxide (10.4 mL), and the solution was kept at 36 °C for 1 h. The cooled solution was added dropwise with stirring to an ice-cold solution of concentrated sulfuric acid (1.52 mL) in water (4 mL), and the reaction was allowed to proceed for 2 h at 20 °C. The reaction mixture was neutralized by the addition of saturated barium hydroxide solution, followed by barium carbonate (0.2 g), and the insoluble material was removed by filtration. The filtrate and water washings were passed down a column of Rexyn 101 (H^+) (14 mL) and Duolite A4 (OH^-) (6 mL) ion-exchange resins, and the eluate on concentration afforded **12** (0.40 g, 86%) as a glass, giving a single *p*-anisidine and silver nitrate positive spot on paper chromatography (solvent A, R_M 0.75). An analytical sample of crystalline **12** obtained from ethanol solution had mp 182–183 °C and $[\alpha]_D$ −26.6° (*C* 1.37, water); ^{13}C NMR (D_2O) δ 94.72 (C-1 β), 92.59 (C-1 α), 53.20 and 53.80 (CHNHAc), 41.00 and 38.60 (CH_2), 23.20 (CH_3CONH). GC (column A, 225 °C) of reduced and acetylated **12**[39] gave a single peak with T_{MA} 2.59.

Anal. Calcd for $C_{10}H_{19}NO_7$: C, 45.28; H, 7.22; N, 5.28. Found: C, 45.21; H, 7.16; N, 5.26.

5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic Acid (NANA, 15). Compound **12** (0.30 g) in water (2.5 mL) was treated with a fresh solution of sodium cyanide (0.15 g) in water (1.7 mL), and the mixture was kept at 4 °C for 7 days. The mixture was heated to 70 °C for 3 h while a slow stream of nitrogen was blown over the surface, and the cooled solution was then passed down a column of Rexyn 101 (H^+) ion-exchange resin (5 mL). The column eluate on concentration afforded almost equal amounts of 5-acetamido-3,5-dideoxy-D-*erythro*-L-*manno*-nononic acid (**13**) and 5-acetamido-3,5-dideoxy-D-*erythro*-L-*gluco*-nononic acid (**14**, 0.26 g, 79%), which gave a ^{13}C NMR spectrum indistinguishable from that of the mixed acids **13** and **14** obtained by the sodium borohydride reduction of a solution of the authentic potassium salt of NANA, and the product was used directly in the next synthetic step; ^{13}C NMR (D_2O) δ 22.60 and 22.98 ($NHCOCH_3$), 33.45 and 33.84 (CH_2), 52.19 and 53.47 (CHNHAc), 76.76 and 79.29 (C-2), 175.55 and 175.88 (CO_2H), 180.25 ($NHCOCH_3$), 64.33 (CH_2OH).

The mixture of nononic acids **13** and **14** (0.25 g) in water (6 mL) was adjusted to pH 8.5 with 0.1 N potassium hydroxide and was kept at 20 °C for 2 h while maintaining a pH of 8.5. The solution was treated with potassium chlorate (0.12 g), the oxidizing catalyst (5.4 mL), prepared by stirring vanadium pentoxide (75 mg) in concentrated hydrochloric acid (4.5 mL) at 0 °C and immediately adding pyridine (4.5 mL), was added, and the mixture was stirred at 20 °C for 20 h. The reaction mixture was diluted with water (15 mL) and was extracted with chloroform (15 mL). The water layer was then passed down a column of Dowex 50 (H^+) ion-exchange resin (14 mL), followed by Dowex 1-X8 (formate) ion-exchange resin (18 mL). The latter column was eluted with 5% (v/v) formic acid (50 mL), and the total eluate from the column was concentrated to a syrup (200 mg). The syrup, dissolved in water (1 mL), was chromatographed on a column of Dowex 1-X8 (formate) ion-exchange resin (1 × 24 cm) which was eluted with 300 mL of a 0–10% formic acid gradient. Fractions of the eluate found by paper chromatography to contain NANA were combined and concentrated under reduced pressure to yield crystalline NANA (**15**; 125 mg, 50%).

Crystalline NANA, mp 182–190 °C and $[\alpha]_D$ −32° (*c* 0.8, water) (lit.[20] mp 181–183 °C, $[\alpha]_D$ −32.1°), gave a single periodate–thiobarbiturate positive spot on paper chromatography (R_M 0.79, solvent B) and on TLC (R_f 0.44, 2-propanol–acetic acid–water, 54:8:18 v/v) having the same mobility as authentic NANA. Trimethylsilylated[40] (Me_3Si) **15** on GC (column B, 210 °C) gave a single peak (T_{MA} 0.49) having the same retention time as authentic Me_3Si–NANA. Compound **15** gave the following data: ^{13}C NMR (D_2O) δ 174.6 (C-1), 96.4 (C-2), 40.0 (C-3), 67.9 (C-4), 53.2 (C-5), 71.4 (C-6), 69.4 (C-7), 71.4 (C-8), 64.3 (C-9), 23.2 ($NHCOCH_3$), 176.0 ($NHCOCH_3$); the spectrum is indistinguishable from that of an authentic sample of NANA.

5-Acetamido[1-^{13}C]-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic Acid. The reaction of **12** (0.30 g) with $Na^{13}CN$ (0.15 g, 90 atom % ^{13}C) and catalytic oxidation of the resulting nononic acids **13** and **14** as described above in the preparation of **15** gave crystalline [1-^{13}C]NANA (128 mg, 51%) having the same physical properties as **15**. The ^{13}C NMR spectrum (limited number of transients) showed a single signal at δ 174.6, and the full spectrum of the sample showed the same signals for C-(1–8) as those recorded for **15**.

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Registry No.—**1**, 3615-17-6; **2**, 38191-81-0; **3**, 28434-33-5; **4**, 64130-80-9; **5**, 64130-81-0; **6**, 64130-82-1; **7**, 64130-83-2; **8**, 64130-84-3; **9**, 64130-85-4; **10**, 64130-86-5; **11**, 64130-87-6; **12**, 64130-88-7; **13**, 64130-89-8; **14**, 64130-90-1; **15**, 131-48-6; [1-^{13}C]-**15**, 64162-77-2; 3-amino-3-deoxy-D-galactose hydrochloride, 64162-10-3.

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