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Synthesis of the deculopyranosonic acid analog of *N*-acetylneuraminic acid, its 5-epimer and 6-epimer, and of 5-acetamido-1,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranose *,**

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ABSTRACT

Condensation of 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-mannofuranose with disodium acetonedicarboxylate and subsequent esterification with diphenyldiazomethane yielded diphenylmethyl 6-acetamido-2,4,6-trideoxy-D-*glycero*-β-D-*galacto*-dec-3-ulopyranosonate (**3**), and its 5- and 6-epimer. Hydrogenation gave the corresponding free acids. Hydrogenation of ester **3** and working-up under alkaline conditions afforded stable ammonium 6-acetamido-2,4,6-trideoxy-D-*glycero*-β-D-*galacto*-dec-3-ulopyranosonate which, on heating, led to an anomeric mixture of 5-acetamido-1,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranose.

INTRODUCTION

N-Acetylneuraminic acid (NeuAc) is synthesized in nature by enzymatic aldol condensation of 2-acetamido-2-deoxy-D-mannose 6-phosphate and phosphoenolpyruvate. The studies of Kuhn and Baschang[1], who condensed 2-acetamido-2-deoxy-D-mannose with di-*tert*-butyl oxaloacetate to yield NeuAc, represents an analogy to the natural process. An enhancement of the reactivity of the amino

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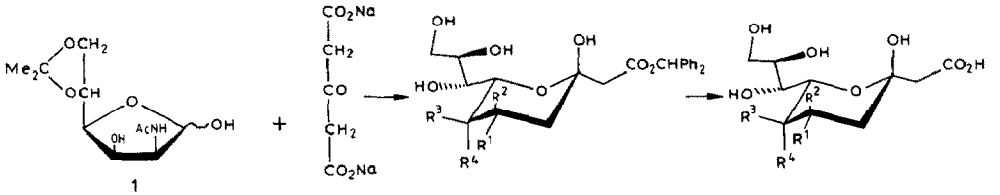
sugar was recently achieved [2] by use of the 2-acetamido-2-deoxy-D-mannofuranose derivative **1**. In addition, we have also obtained several NeuAc derivatives including *N*-acetyl-3*a*-hydroxyneuraminic acid[3] and *N*-acetyl-3*e*-methylneuraminic acid[4] by modifying the methylene component involved in the condensation. The use of this reaction with a more complex methylene component, acetonedicarboxylic acid, would afford deculopyranosonic acid derivatives of NeuAc. Such NeuAc analogs are potential probes for the study of substrate specificities of enzymes involved in sialic acid metabolism and for examining the interaction with viruses of the influenza group.

RESULTS AND DISCUSSION

Since aldol condensation affords analogs of *N*-acetylneuraminic acid with biological potential, optimal conditions for this reaction had to be found. According to previous investigations[4], the reaction is performed near neutrality to prevent the epimerization of acetamidodeoxymannofuranose. Further, some catalytic additives enhance the reactivity of the methylene component. In the case of oxaloacetic acid, metal cations shift the keto–enol equilibrium towards the enol form that reacts. Using Ni^{2+} ions, Charon and Szabo[5] were able to improve the yield of 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) from 6 to 50–60%. However, catalysts like Ni^{2+} have undesirable properties, for example, the velocity of decarboxylation of the methylene component, which is a β-ketocarboxylic acid, is enhanced. This leads to NeuAc derivatives that lack the carboxylic group. For oxaloacetic acid in the presence of various metal ions, a linear relationship was observed between the rate constants for the decarboxylation of the keto acid–metal complexes and the keto–enol ratios[6]. Therefore, the selection of the metal ion is of importance, and the following series of ions was found[7] to increasingly affect the rate of decarboxylation of dimethyl oxaloacetic acid, $Mn^{2+} < Ni^{2+} < Al^{3+} < Cu^{2+}$. Ni^{2+} seemed to be a good compromise between the undesired decarboxylation and the desired shift in the keto–enol equilibrium. In aqueous 1,4-dioxane or ethanol, the enol form is favored, but a limited hydration of metal ions takes place in these two solvents resulting in an increased rate of decarboxylation[8], and pure water was selected. The relationship between the concentration of metal ion and methylene component is also important, because a higher concentration of metal ions prevent the condensation reaction. Ni^{2+} concentrations of 10–20 mol% relative to the methylene component turned out to be the best[4]. Whereas in the presence or absence of Ni^{2+} catalyst additional reaction products were obtained at pH 9, at pH 7 the formation of byproducts was negligible, and **1** and **2** were condensed at this pH. After removal of the isopropylidene protecting group, the byproducts of the decarboxylation were separated by anion-exchange chromatography. The mixture of acids was converted to the diphenylmethyl esters **3**, **4**, and **5**, which were separated by silica gel chromatography.



1 2

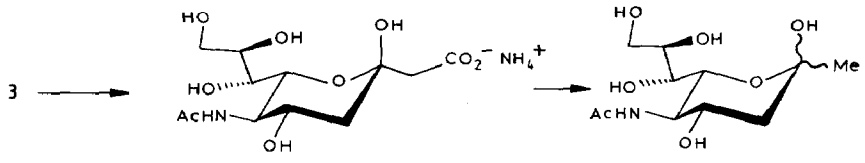
3 R^1 = OH, R^2 = R^4 = H, R^3 = NHAc 6 R^1 = OH, R^2 = R^4 = H, R^3 = NHAc
4 R^1 = R^4 = H, R^2 = OH, R^3 = NHAc 7 R^1 = R^4 = H, R^2 = OH, R^3 = NHAc
5 R^1 = OH, R^2 = R^3 = H, R^4 = NHAc 8 R^1 = OH, R^2 = R^3 = H, R^4 = NHAc

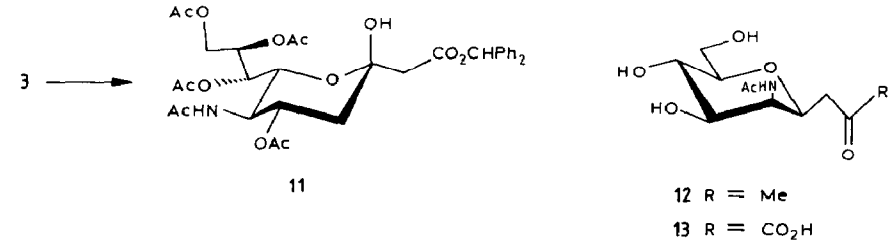
The ^{1}H NMR spectrum proved the structure of ester **3** to be chain extended. Interestingly, the spectrum showed a lower integration of the signals of CH_2 protons at C-2 (δ 2.85–2.83, m, 0.6 H) than expected. This suggests an H–D exchange at C-2. In spite of the lack of vinylic H-signals, the immeasurably fast "passing through" the vinyl ether structure between C-2 and C-3 in the open-chain form seems plausible. In contrast, the protons H-4*a* and H-4*e* would only be exchanged under alkaline conditions[9]. Except both 6-epimer derivatives **5** and **8**, all chain-extended NeuAc derivatives described here tend to undergo, to varying degrees, the H–D exchange of CH_2 protons at C-2. They appear, in the ^{1}H NMR spectra, as singlet and AB-system.

The structure of **4**, the epimer of **3** at C-5, was established by the coupling pattern of an equatorial H-5 signal ($J_{4a,5}$ 2.7, $J_{4e,5}$ 3.5, $J_{5,6}$ 3.1 Hz) in good agreement with the coupling pattern of the 4-epimer of *N*-acetylneuraminic acid[10].

The H-6 signal with the shape of a paramagnetic-shifted doublet and a small coupling constant of 3 Hz is a characteristic feature of the NMR spectra of **5** and **8**, the epimers of compounds **3** and **6** at C-6 with an axial acetamido group. While the H-5 signal of **5** is overlapped with other signals, the H-5 signal of **8** appears as a dd-signal and couples only with both H-4 protons; therefore, $J_{5,6}$ equals 0 Hz. Yet, the latter coupling constant is only attainable by distortion of the sugar ring, because of a repulsion between the *cis* *N*-acetyl group and the side chain. This reasoning, though plausible, has not yet been supplemented by additional NMR data.

Acetylation of the diphenylmethyl ester **3** led to a major product having one nonacetylated OH group which, based on spin-decoupling experiments, was assigned to the anomeric position. The NMR data agree well with the values obtained for methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulosonate[11]. The free acids **6**, **7**, and **8** were obtained from the corresponding esters by hydrogenation in the presence of palladium(II) oxide. The instability of acid **6** is evident by the appearance of signals of both anomers of the decarboxylated derivative **10** in the ^{1}H NMR spectrum. These signals were found to be present for **6**:**10**β:**10**α in the ratio 40:50:1. In contrast to **6**, acids **7** and **8**, epimeric at C-5 and C-6, respectively, did not decarboxylate. The ammonium salt **9**, obtained from **3** by hydrogenation, also proved to be stable. However, on





heating **9** decarboxylated to give both anomers of **10** in the ratio $\alpha:\beta$ of 1:6. The anomers were identified by NMR spectra.

The spontaneously decarboxylated products were separated from the mixture formed by aldol condensation by anion-exchange chromatography and isolated by silica gel chromatography. In addition to the major component **10**, very hygroscopic **12** was obtained. Recently, Pozsgay et al.[12] desdribed 5-acetamido-4,8-anhydro-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulosonic acid (**13**) which was formed as a side product, in addition to NeuAc, by mild acid hydrolysis of edible birds' nests. ^{1}H NMR spectra of **12** and **13** in D_2O were very similar. It is noteworthy that the keto–enol equilibrium, described[12] for **13** in D_2O, was not observed with **12**, which exists entirely in the keto form. The β configuration was assigned[12] to **13**. The thermodynamic preference for the β anomer reflects the tendency of the alkyl substituent to occupy the equatorial position of the sugar ring. Thus, the assignment of the β configuration to **12** is in order. The small coupling constants $J_{4,5}$ of 1.3 (**12**), 0.5 (**13**), and 1.2 Hz (peracetyl derivative of **13**[12]) cannot unequivocally establish the relative steric position of H-4. However, these values are in good agreement with the anomeric configuration found for 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-mannopyranosyl cyanide[13], $J_{a,e}$ 1.4 Hz (β-D-*manno* configuration) and $J_{e,e}$ 2.0 Hz (α-D-*manno* configuration).

EXPERIMENTAL

Methods.—Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241 polarimeter at the D-line. ^{1}H NMR spectra were recorded with a Bruker WM-300 (300 MHz) spectrometer. Analytical TLC was

performed on Merck Silica Gel 60 F-254, and column chromatography on Merck Silica Gel 60 (63–200 mesh). For anion-exchange chromatography, a peristaltic pump P-1 (Pharmacia, Freiburg, Germany) was used. HPLC equipment consisted of a Knauer pump 64 (Berlin, Germany) a LiChrosorb Si 60, 7-μ column (250 × 8 mm) and a RI detector ERC-7520 (ERC, Alteglofsheim, Germany).

*Condensation of 2-acetamido-2-deoxy-5,6-O-isopropylidene-*D*-mannofuranose* (**1**) *with disodium 1,3-acetonedicarboxylate* (**2**).—A solution of 1,3-acetonedicarboxylic acid (16.9 g, 115.7 mmol, 3 equiv) in water (67 mL; pH 2) was slowly added at 0° to a solution of NaOH (9.23 g, 230.8 mmol, 6 equiv) in water (50 mL; pH 14). The pH was adjusted to 7.0 by the addition of 1,3-acetonedicarboxylic acid or NaOH, and **1** (10.0 g, 38.3 mmol, 1 equiv) and nickel acetate tetrahydrate (3.83 g, 15.4 mmol, 0.4 equiv) were added, and the solution was stirred at room temperature overnight. TLC (5:3 propanol–water) indicated complete conversion of **1**. The pH of the green solution was adjusted to 2 by the addition of Amberlite IR-120 (H^+) ion-exchange resin and the mixture was stirred for 2–3 h at room temperature. The resin was then filtered off, washed thoroughly with water, and the resulting solution was lyophilized. The residue was dissolved in a small volume of water and the pH adjusted to 7 by addition of Bio-Rad AG MP-1 (OH^-) anion-exchange resin. The suspension was deposited on a column (250 mL) of Bio-Rad AG MP-1 (HCO_3^-) anion-exchange resin, which was developed with water (500 mL), and subsequently with an aq $(NH_4)HCO_3$ gradient (0–0.5 M; 3 L). The first fraction (water phase) contained a mixture of decarboxylated products (4.0 g) (*vide infra*). The second (750 mL) and third (1200 mL) fractions (HCO_3^- phase) (6.8 and 0.55 g, respectively) were combined and their pH adjusted to 2 by addition of Amberlite IR-120 (H^+) cation-exchange resin to remove NH_4^+. After filtration and lyophilization, the solid (7.3 g) was dissolved in MeOH (100 mL) and treated with an etheral solution of diphenyldiazomethane until the red color of the solution persisted. The solvent was evaporated and the residue was chromatographed on a 60 × 6 cm column of silica gel with 5:1 $CHCl_3$–MeOH. The resulting three compounds were eluted in the order, **4**, **5**, and **3**.

*Diphenylmethyl 6-acetamido-2,4,6-trideoxy-*D*-glycero-β-*D*-talo-dec-3-ulopyranosonate* (**4**) *(C-5 epimer of* **3**).—Yield 1.1 g (6%); mp 146–147° (MeOH–diethyl ether); $[\alpha]_D^{20}$ −41° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): δ 7.38–7.24 (m, 10 H, aryl-H), 6.85 (s, 1 H, C*H*Ph_2), 4.24 (dd, 1 H, H-7), 4.05 (ddd, 1 H, H-5), 4.01 (dd, 1 H, H-6), 3.74 (dd, 1 H, H-10b), 3.70 (ddd, 1 H, H-9), 3.61 (dd, 1 H, H-10a), 3.48 (dd, 1 H, H-8), 2.81 (s, 1.7 H, CH_2-2), 2.21 (dd, 1 H, H-4*e*), 2.00 (s, 3 H, NAc), and 1.94 (dd, 1 H, H-4*a*); $J_{4a,4e}$ 14.2, $J_{4a,5}$ 2.7, $J_{4e,5}$ 3.5, $J_{5,6}$ 3.1, $J_{6,7}$ 10.6, $J_{7,8}$ 1.3, $J_{8,9}$ 8.8, $J_{9,10a}$ 5.3, $J_{9,10b}$ 3.1, and $J_{10a,10b}$ 11.1 Hz.

Anal. Calcd for $C_{25}H_{31}NO_9$ (489.51): C, 61.34; H, 6.38; N, 2.86. Found: C, 60.82; H, 6.28; N, 2.93.

*Diphenylmethyl 6-acetamido-2,4,6-trideoxy-*D*-glycero-β-*D*-gulo-dec-3-ulopyranosonate* (**5**) *(C-6 epimer of* **3**).—Yield 1.8 g (10%); mp 108–110° (water–MeOH); $[\alpha]_D^{20}$ −31.5° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): δ 7.38–7.23 (m, 10 H, aryl-H),

6.83 (s, 1 H, C*H*Ph_2), 4.49 (d, 1 H, H-6), 3.91–3.80 (m, 3 H, H-5,7,8), 3.57 (dd, 1 H, H-10b), 3.46 (dd, 1 H, H-10a), 3.23 (ddd, 1 H, H-9), 2.90 (s, 2 H, CH_2-2), 2.39 (dd, 1 H, H-4*e*), 1.97 (s, 3 H, NAc), and 1.71 (dd, 1 H, H-4*a*); $J_{4a,4e}$ 13.3, $J_{4a,5}$ 9.3, $J_{4e,5}$ 5.8, $J_{9,10a}$ 4.9, $J_{9,10b}$ 3.1, and $J_{10a,10b}$ 11.5 Hz.

Anal. Calcd for $C_{25}H_{31}NO_9 \cdot H_2O$: C, 59.16; H, 6.55; N, 2.76. Found: C, 59.15; H, 6.31; N, 2.53.

Diphenylmethyl 6-acetamido-2,4,6-trideoxy-D-glycero-*β*-D-galacto-*dec-3-ulopyranosonate* (**3**).—Yield 2.0 g (11%); mp 153–154° (MeOH–diethyl ether–hexane); $[\alpha]_D^{20}$ −17.5° (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD): *δ* 7.42–7.24 (m, 10H, aryl-H), 6.86 (s, 1 H, C*H*Ph_2), 4.01 (ddd, 1 H, H-5), 3.92 (dd, 1 H, H-7), 3.74 (dd, 1 H, H-6), 3.75–3.58 (m, 3 H, H-9,10a,10b), 3.47 (dd, 1 H, H-8), 2.85–2.83 (m, 0.6 H, CH_2-2), 2.31 (dd, 1 H, H-4*e*), 2.01 (s, 3 H, NAc), and 1.65 (dd, 1 H, H-4*a*); $J_{4a,4e}$ 12.9, $J_{4a,5}$ 11.2, $J_{4e,5}$ 4.7, $J_{5,6}$ 10.0, $J_{6,7}$ 10.6, and $J_{7,8}$ 1.5 Hz.

Anal. Calcd for $C_{25}H_{31}NO_9$ (489.51): C, 61.34; H, 6.38; N, 2.86. Found: C, 60.91; H, 6.40; N, 2.66.

Diphenylmethyl 6-acetamido-5,8,9,10-tetra-O-*acetyl-2,4,6-trideoxy*-D-glycero-*β*-D-galacto-*dec-3-ulopyranosonate* (**11**).—Acetic anhydride (1 mL) was added to an ice-cold solution of **3** (150 mg, 0.31 mmol) in dry pyridine (2 mL). After several h at room temperature and storage overnight in a refrigerator, the solution was concentrated to dryness and the residual solvent codistilled twice with toluene (5 mL). The major component was isolated by HPLC (EtOAc) and lyophilized (yield 90 mg, 45%); $[\alpha]_D^{19}$ −11.0° (*c* 0.5, MeOH); ^{1}H NMR [$(CD_3)_2SO$]: *δ* 7.73 (d, 1 H, NH), 7.46–7.24 (m, 10 H, aryl-H), 6.76 (s, 1 H, C*H*Ph_2), 6.45 (d, 1 H, *J* 1.2 Hz, OH), 5.23 (dd, 1 H, H-8), 5.10 (ddd, 1 H, H-9), 5.01 (ddd, 1 H, H-5), 4.35 (dd, 1 H, H-10b), 4.15 (dd, 1 H, H-7), 4.00 (dd, 1 H, H-10a), 3.87 (ddd, 1 H, H-6), 2.87 (AB system, 0.76 H, CH_2-A), 2.71 (AB system, 0.76 H, CH_2-B), 2.14 (dd, 1 H, H-4*e*), 1.98, 1.96, 1.90, 1.87, 1.66 (5 s, 15 H, NAc 4 OAc), and 1.64 (1 H, H-4*a*, partially covered by Ac signal); $J_{A,B}$ 14.2, $J_{4a,4e}$ 12.4, $J_{4a,5}$ 11.1, $J_{4e,5}$ 5.1, $J_{5,6}$ 10.6, $J_{6,NH}$ 9.7, $J_{6,7}$ 10.6, $J_{7,8}$ 2.2, $J_{8,9}$ 5.5, $J_{9,10a}$ 7.1, $J_{9,10b}$ 2.7, and $J_{10a,10b}$ 11.9 Hz.

Anal. Calcd for $C_{33}H_{39}NO_{13} \cdot 0.5H_2O$: C, 59.45; H, 6.05; N, 2.10. Found: C, 59.40; H, 5.63; N, 1.75.

6-Acetamido-2,4,6-trideoxy-D-glycero-*β*-D-galacto-*dec-3-ulopyranosonic acid* (**6**).—A solution of **3** (500 mg, 1.02 mmol) in absolute MeOH (20 mL) was hydrogenated in the presence of PdO (50 mg) for 3 h at room temperature under a H_2 pressure of 0.1 MPa. TLC (4:1 $CHCl_3$–MeOH) revealed complete conversion. The catalyst was filtered off, and the filtrate was evaporated to give a syrup which was dissolved in water (20 mL). Three extractions with diethyl ether removed diphenylmethane. The aqueous layer was freeze-dried (yield 300 mg, 91%); $[\alpha]_D^{20}$ −22.5° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): *δ* 4.10 (ddd, 1 H, H-5), 4.05 (dd, 1 H, H-7), 3.91 (dd, 1 H, H-6), 3.90 (dd, 1 H, H-10b), 3.80 (ddd, 1 H, H-9), 3.68 (dd, 1 H, H-10a), 3.61 (dd, 1 H, H-8), 2.88–2.86 (m, 0.4 H, CH_2-2), 2.41 (dd, 1 H, H-4*e*), 2.12 (s, 3 H, NAc), and 1.79 (dd, 1 H, H-4*a*); $J_{4a,4e}$ 12.8, $J_{4a,5}$ 11.7, $J_{4e,5}$ 4.9, $J_{5,6}$ 10, $J_{6,7}$ 9.5, $J_{7,8}$ 1, $J_{8,9}$ 8.8, $J_{9,10a}$ 6.2, $J_{9,10b}$ 2.7, and $J_{10a,10b}$ 11.5 Hz.

Anal. Calcd for $C_{12}H_{21}NO_9 \cdot H_2O$: C, 42.22; H, 6.79; N, 4.10. Found: C, 42.11; H, 6.91; N, 4.00.

Ammonium 6-acetamido-2,4,6-trideoxy-D-glycero-β-D-galacto-*dec-3-ulopyranosonate* (**9**).—Compound **3** (500 mg) was hydrogenated as described for **6**. After separation of the water layer, the pH was adjusted to 8–9 by addition of 25% NH_4OH (~ 5 drops) and freeze-dried (yield 230 mg, 66%), $[\alpha]_D^{20}$ −30.5° (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 4.03 (ddd, 1 H, H-5), 3.97 (dd, 1 H, H-7), 3.83 (dd, 1 H, H-10b), 3.83 (dd, 1 H, H-6), 3.74 (ddd, 1 H, H-9), 3.60 (dd, 1 H, H-10a), 3.52 (dd, 1 H, H-8), 2.61 (AB system, 0.8 H, C*H*$_2$-A), 2.53 (AB system, 0.8 H, C*H*$_2$-B), 2.23 (dd, 1 H, H-4*e*), 2.06 (s, 3 H, NAc), and 1.65 (dd, 1 H, H-4*a*); $J_{A,B}$ 14.8, $J_{4a,4e}$ 12.8, $J_{4a,5}$ 11.5, $J_{4e,5}$ 4.9, $J_{5,6} = J_{6,7} = 10.2$, $J_{7,8}$ 1.1, $J_{8,9}$ 8.8, $J_{9,10a}$ 6.6, $J_{9,10b}$ 2.7, and $J_{10a,10b}$ 11.5 Hz.

Anal. Calcd for $C_{12}H_{24}N_2O_9 \cdot H_2O$: C, 40.22; H, 7.31; N, 7.82. Found; C, 40.89; H, 7.30; N, 7.35.

6-Acetamido-2,4,6-trideoxy-D-glycero-β-D-talo-*dec-3-ulopyranosonic acid* (**7**) *(C-5 epimer of* **6***).*—Compound **4** (500 mg) was hydrogenated as described for **6** (yield 300 mg, 91%); $[\alpha]_D^{19}$ −69° (1 day; *c* 0.5, MeOH); ^1H NMR (D_2O): δ 8.02 (d, 0.4 H, NH), 4.28 (d, 1 H, H-7), 4.18 (ddd, 1 H, H-5), 4.08 (ddd, 1 H, H-6), 3.86 (dd, 1 H, H-10b), 3.80 (ddd, 1 H, H-9), 3.63 (dd, 1 H, H-10a), 3.56 (d, 1 H, H-8), 2.75–2.72 (m, 0.43 H, C*H*$_2$-2), 2.22 (dd, 1 H, H-4*e*), 2.05 (s, 3 H, NAc), and 2.00 (dd, 1 H, H-4*a*); $J_{4a,4e}$ 14.6, $J_{4a,5} = J_{4e,5} = J_{5,6} = 3.1$, $J_{6,NH}$ 9.3, $J_{6,7}$ 10.6, $J_{8,9}$ 8.8, $J_{9,10a}$ 6.2, $J_{9,10b}$ 2.7, and $J_{10a,10b}$ 11.5 Hz.

Anal. Calcd for $C_{12}H_{21}NO_9 \cdot 0.5H_2O$: C, 43.37; H, 6.67; N, 4.22. Found: C, 43.52; H, 6.86; N, 4.09.

6-Acetamido-2,4,6-trideoxy-D-glycero-β-D-gulo-*dec-3-ulopyranosonic acid* (**8**) *(C-6 epimer of* **6***).*—Compound **5** (200 mg) was hydrogenated as described for **6** (yield 90 mg, 68%), $[\alpha]_D^{20}$ −35.0° (*c* 0.5, MeOH); ^1H NMR (D_2O): δ 4.64 (d, 1 H, H-6), 4.08 (dd, 1 H, H-5), 4.06–3.98 (m, 2 H, H-7,8), 3.79 (dd, 1 H, H-10b), 3.64 (dd, 1 H, H-10a), 3.53 (ddd, 1 H, H-9), 3.02 (AB system, 1 H, C*H*$_2$-A), 2.98 (AB system, 1 H, C*H*$_2$-B), 2.50 (dd, 1 H, H-4*e*), 2.12 (s, 3 H, NAc), and 1.89 (dd, 1 H, H-4*a*); $J_{A,B}$ 14.7, $J_{4a,4e}$ 13.2, $J_{4a,5}$ 9.6, $J_{4e,5}$ 5.9, $J_{9,10a}$ 5.9, $J_{9,10b}$ 2.6, and $J_{10a,10b}$ 11.8 Hz.

Anal. Calcd for $C_{12}H_{21}NO_9$ (323.30): C, 44.58; H, 6.55; N, 4.33. Found: C, 44.73; H, 6.64; N, 4.34.

5-Acetamido-1,3,5-trideoxy-D-glycero-α,β-D-galacto-*non-2-ulopyranose* (**10**).—A solution of **9** (150 mg) in water was heated at 70° for 1 day. After cooling to room temperature the solution was passed through a 6 × 1.5 cm column of DEAE-Sephadex A-25 (CO_3^{2-}) to remove unreacted product. Further purification was achieved by HPLC (5:3 $CHCl_3$–MeOH), yield 80 mg (65%), mp (α,β mixture) 91–93° (MeOH–diethyl ether), $[\alpha]_D^{20}$ −28.5° (10 min) → −51° (1 h) → −64° (2.5 days; *c* 0.5, MeOH); ^1H NMR (D_2O): δ 3.99 (ddd, 0.86 H, H-4β), 3.95 (dd, 0.86 H, H-6β), 3.85 (dd, 0.86 H, H-9bβ), 3.80 (dd, 0.86 H, H-5β), 3.77 (ddd, 0.86 H, H-8β), 3.62 (dd, 0.86 H, H-9bβ), 3.53 (dd, 0.86 H, H-7β), 3.48 (d, 0.14 H, H-7α), 2.75–2.71 (m, 0.28 H, H-3*e*α,*a*α), 2.23 (s, 0.42 H, CH_3α), 2.22 (dd, 0.86 H, H-3*e*β), 2.07 (s,

0.42 H, NAcα), 2.06 (s, 2.58 H, Nacβ), 1.64 (dd, 0.86 H, H-3$a\beta$), and 1.48 (s, 2.58 H, $CH_3\beta$); α anomer: $J_{7,8}$ 8.8 Hz; β anomer: $J_{3a,3e}$ 13.3, $J_{3a,4}$ 11.5, $J_{3e,4}$ 4.9, $J_{4,5}$ 9.7, $J_{5,6}$ 10.2, $J_{6,7}$ 0.9, $J_{7,8}$ 8.8, $J_{8,9a}$ 6.2, $J_{8,9b}$ 2.7, and $J_{9a,9b}$ 11.5 Hz.

Anal. Calcd for $C_{12}H_{21}NO_7 \cdot H_2O$: C, 44.44; H, 7.80; N, 4.71. Found: C, 44.47; H, 7.56; N, 4.44.

5-Acetamido-4,8-anhydro-1,3,5-trideoxy-D-glycero-β-D-galacto-*non-2-ulose* (**12**). —The product mixture (1 g) obtained as the first fraction (water phase) of the Bio-Rad AG MP-1 separation (in the condensation of **1** with **2**) was resolved in a 30×3 cm column of silica gel (5:3 $CHCl_3$–MeOH). In addition to **10** (710 mg), a not identified unstable product (60 mg), and the very hygroscopic **12** (144 mg) were isolated and freeze-dried; $[\alpha]_D^{20}$ −28.5° (3 days, *c* 0.45, H_2O); ^1H NMR (D_2O): δ 4.33 (dd, 1 H, H-5), 4.17 (ddd, 1 H, H-4), 3.87 (dd, 1 H, H-6), 3.86 (dd, 1 H, H-9b), 3.77 (dd, 1 H, H-9a), 3.54 (dd, 1 H, H-7), 3.41 (ddd, 1 H, H-8), 2.82 (dd, 1 H, H-3a), 2.68 (dd, 1 H, H-3b), 2.22 (s, 3 H, CH_3), and 2.10 (s, 3 H, NAc); $J_{3a,3b}$ 17.2, $J_{3a,4}$ 8.0, $J_{3b,4}$ 5.1, $J_{4,5}$ 1.3, $J_{5,6}$ 4.6, $J_{6,7} = J_{7,8} = 9.7$, $J_{8,9a}$ 4.9, $J_{8,9b}$ 2.4, and $J_{9a,9b}$ 12.4 Hz.

Anal. Calcd for $C_{11}H_{19}NO_6 \cdot 0.25H_2O$: C, 49.71; H, 7.39; N, 5.27. Found: C, 49.50; H, 7.21; N, 4.98.

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