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Synthesis of methyl 4-acetamido-*N*-acetyl-4-deoxy-α- and β-4-*epi*-neuraminic acids ☆

B.P. Bandgar [a,*], S.V. Patil [a], Erich Zbiral [b,1]

[a] *Department of Chemistry, Postgraduate and Research Centre, R.B.N.B. College, Shrirampur 413 709, Maharashtra, India*

[b] *Institute for Organic Chemistry, University of Vienna, A-1090, Vienna, Austria*

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Abstract

Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-2,3-didehydro-2,3,4,5-tetradeoxy-D-*glycero*-D-*talo*-nonulopyranosonate with *N*-bromosuccinimide in methanol gave methyl (methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3-bromo-3,4,5-trideoxy-α-D-*glycero*-D-*talo*-nonulopyranosid)onate and methyl (methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3-bromo-3,4,5-trideoxy-β-D-*glycero*-D-*talo*-nonulopyranosid)onate, which on reductive acetylation with H_2/Pd–C in acetic anhydride–ethyl acetate yielded the corresponding 4-acetamido analogues. Debromination with Bu_3SnH/AIBN in toluene, followed by hydrolysis, gave methyl 4-acetamido-*N*-acetyl-4-deoxy-α-and β-4-*epi*-neuraminic acids.

Keywords: Neuraminic acid, *N*-acetyl-; 4-*epi*-Neuraminic acid, 4-acetamido-*N*-acetyl-4-deoxy-

1. Introduction

N-Acetylneuraminic acid (Neu5Ac) and various related derivatives, the sialic acids, play an important role in a series of biochemical and biological processes. During the last few years considerable work has been done to gain more insight into the structure–

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* Corresponding author.

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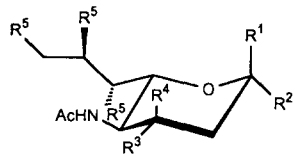
activity relationships of these most interesting biomolecules, including some artificial sialic acid analogues. Studies have been conducted concerning their behavior toward the most important enzymes of sialic acid metabolism, e.g. CMP-sialate synthase [1–5], acylneuraminate lyase [6–9], and sialidases [10]. The structure–activity relationship with sialidases [11], as well as the hemagglutinin [12] of the influenza virus illustrates another aspect of the interaction between protein structures and substrates containing sialic acids. X-Ray analysis of this viral hemagglutinin complexed with 2-α-sialyllactose provides detailed information about the positions and distances of the particular functional groups of the sialic acid moiety relative to the protein [13]. Recently developed 4-amino- and 4-guanidino-substituted Neu5Ac2en are found to be high-affinity inhibitors for influenza virus sialidase [14].

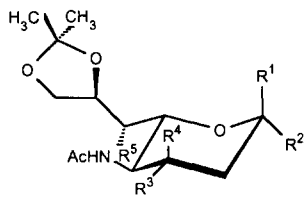
Functional groups of sialic acid that are important for the binding of this sugar to the hemagglutinin of influenza A virus were found in the glycerol side chain, the *N*-acetyl group and the carboxyl function. The model deduced from X-ray difference electron density maps of crystallographic studies demonstrates the interaction involved in the binding of sialic acids to the binding pocket of the influenza A hemagglutinin. Furthermore, it was determined that for biological activity the carboxyl group must exhibit an axial orientation corresponding to a natural α glycoside. The model deduced from X-ray crystallographic studies showed that the hydroxyl group of Ser-136 and the amido group of Asn-137 in the peptide backbone of the hemagglutinin are close enough to the axially oriented carboxyl group of sialic acid to form hydrogen bonds [13,15]. Recently it was found that the glycosidic oxygen can be substituted by an equatorially orientated hydrogen (H_{eq}), whereby the recognition was only diminished by 25% [15,16]. This recent information is helpful in developing novel compounds that may enhance or alter sialic acid–hemagglutinin interactions, leading to sialic acid analogues that can bind more widely and more specifically to the influenza virus hemagglutinin and possibly contribute to the development of new anti-influenza drugs. Therefore, αMe 4d4*epi*Neu4NHAc5Ac was chosen a suitable candidate for obtaining information about the details of the sialic acid–hemagglutinin interaction [11,12]. We wish to report here the synthesis of some related 4-acetamido-4-deoxy analogues of *N*-acetylneuraminic acid.

2. Results and discussion

A series of attempts were made to synthesize sialic acid analogues such as **1**, **2**, **3** and **4**. Recently described compound **5** [17] was transformed into the corresponding per-acetylated 2-chloro compound **6** by reaction with acetyl chloride [16]. When catalytic hydrogenation of **6** was carried out according to the previously reported sequence [2b], a non-separable mixture of H-2_{eq} and H-2_{ax} derivatives of Neu5Ac was formed. The glycosidation of **6** with MeOH under Koenigs–Knorr conditions [18] in the presence of Ag_2CO_3 exclusively gave the corresponding β-methyl glycoside. The classical substitution reaction of substrates **7**, **8**, **9** and **10** via their corresponding 4-*O*-methylsulfonyl (mesyl), 4-*O*-trifluoromethylsulfonyl or 4-*O*-(*p*-tolylsulfonyl) (tosyl) derivatives with the N_3^- nucleophile to obtain 4-azido derivatives of Neu5Ac was unsuccessful.



	R^1	R^2	R^3	R^4	R^5
1	COOH	H	NHAc	H	OH
2	COOH	OCH_3	NHAc	H	OH
3	COOH	H	H	NHAc	OH
4	COOH	OCH_3	H	NHAc	OH
5	OH	$COOCH_3$	NHAc	H	OAc
6	Cl	$COOH_3$	NHAc	H	OAc



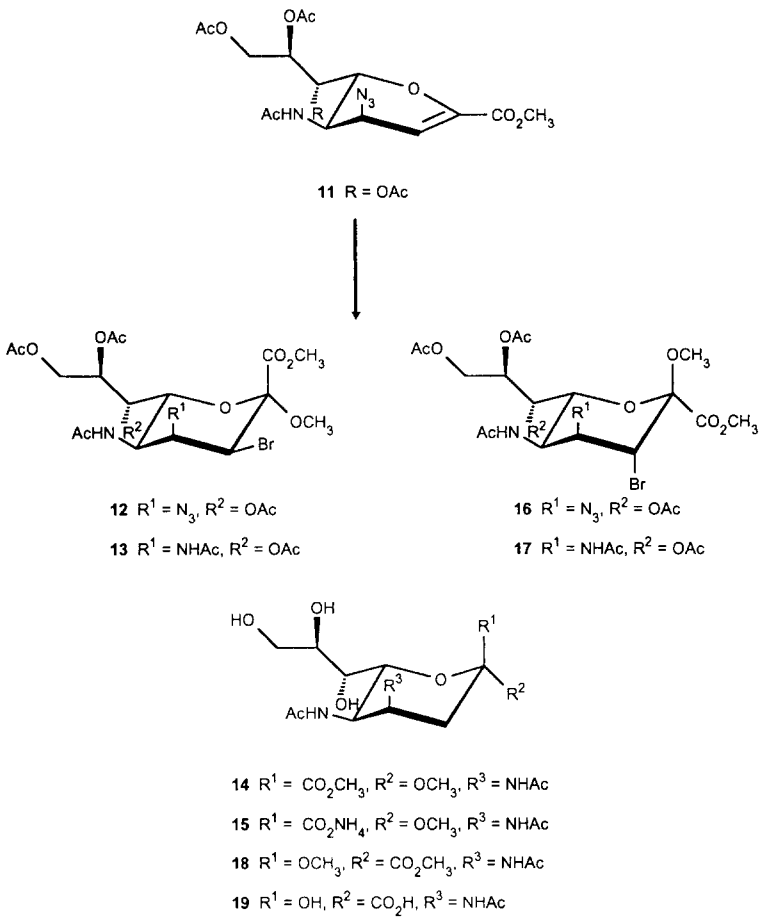
	R^1	R^2	R^3	R^4	R^5
7	$COOCH_3$	H	OH	H	OH
8	$COOCH_3$	OCH_3	OH	H	OH
9	$COOCH_3$	H	H	OH	OH
10	$COOCH_3$	OCH_3	H	OH	OH

As all the above trials failed to prepare the desired sialic acid analogues, the long-route strategy in Scheme 1 was used.

The preparation of **11** was performed according to the reported procedure [19]. The transformation of **11** into the mixture of corresponding diequatorial and diaxial isomers **12** and **16** was performed with *N*-bromosuccinimide in MeOH at 20°C under an argon atmosphere [20], and the products could be separated by flash chromatography (2:1 petroleum ether–ethyl acetate). The fast-migrating zone was the diequatorial isomer **12**, whereas the slower migrating zone was the diaxial isomer **16** (Scheme 1). The yields thus obtained were 20% and 40% for **12** and **16**, respectively.

Due to the failure of obtaining the expected simultaneous debromination and reduction of the 4-N_3 of **12** and **16**, a recently developed strategy [17] for the reduction of the azido group was used to obtain **13** and **17**; however, debromination of **13** and **17** was not observed (^{1}H NMR spectroscopy). Debromination [21] of **13** and **17** was achieved by Bu_3SnH/AIBN in toluene at 70 to 80°C to give **14** and **18**. The removal of the ester groups was carried out by aq NaOH to get the corresponding α-methyl glycoside **15**.

Compound **18** was heated with aq NaOH, followed by aq HCl and Amberlyst-15 [H^+], to get the β-anomeric acid **19**.



Scheme 1.

The structural assignments by ^{1}H NMR spectroscopy for the α and β anomers are in accord with the general feature [20,22,23] that the chemical shifts of H-3_{eq} and H-6 are shifted further downfield in the α anomers than in the β anomers, whereas the chemical shift of H-4 is shifted upfield in the α anomers relative to that of the β anomers. Therefore, the chemical shifts of H-3_{eq}, H-4 and H-6 showed that **12**, **13**, **14** and **15** are α anomers and **16**, **17**, **18** and **19** are β anomers. Another interesting argument is based on the conformation of the side chain. The trihydroxypropyl side chain at C-6 adopts a different conformation in **12–15** than that of **16–19** as deduced from the quite different coupling constants: $J_{7,8}$ 7.9–8.7 Hz in **12–15** and $J_{7,8}$ 5.2–5.5 Hz in **16–19**. This

difference in conformation may be a result of steric interaction with the anomeric substituents with a large NHAc at C-4 and C-5 and a Br group at C-3. Thiem and co-workers had also observed similar results [22]. The coupling constants, $J_{3ax,4}$ for **14**, **15**, **18** and **19** are 2.50, 2.90, 3.1 and 2.6 Hz, respectively, which indicate that these compounds are 4-epimers of Neu5Ac.

3. Experimental

General procedures.—Solvents were freshly distilled before use. All reactions, with exception of those in water, were conducted in oven-dried (140°C) or flame-dried two- and three-necked flasks closed by rubber septa. Addition of reagents, and consequently control of the progress of the reactions, was achieved by use of syringes and monitoring by thin-layer chromatography (TLC). Analytical TLC was carried out using Silica Gel 60 F254 plates, thickness 0.2 mm (E. Merck). Compounds were visualized by spraying with a solution of $Ce(NO_3)_4$ in 2 N H_2SO_4, followed by heating at 200°C. Flash chromatography was carried out using Silica Gel-60, 0.040–0.063 mm (E. Merck). Bruker WM 250 and AM 400 WB instruments were used for NMR spectroscopy. ^1H NMR (at 250 and 400 MHz) used $CDCl_3$ with Me_4Si as an internal standard. For solutions in D_2O, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in D_2O was used as an internal reference, or the spectra were correlated with HDO ($\delta = 4.80$). Coupling constants (J) are reported as first order. ^{13}C NMR spectra were determined at 62.9 and 100.6 MHz. For solutions in D_2O at 303 K, either an external reference of tetramethylsilane or the signal of 1,4-dioxane (67.40 ppm downfield from the external Me_4Si signal in D_2O) was used. *N*-Acetylneuraminic acid (Neu5Ac) was prepared from edible bird's nest glycoprotein [24]. The starting material **11** was prepared according to ref. 19.

General procedure for acetylation.—Approximately 100 mg of the compound to be acetylated was dissolved in 1 mL of dry pyridine, and 1 mL of acetic anhydride and 5 mg of 4-(dimethylamino)pyridine were added. The reaction mixture was allowed to stand at room temperature for 14 h, at the end of which time the solvents were evaporated under reduced pressure (0.001 torr), and the crude product was purified as given in each protocol.

*Methyl (methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3-bromo-3,4,5-trideoxy-α-*D*-glycero-*D*-talo-nonulopyranosid)onate (**12**).*—To a stirred solution of **11** (400 mg, 0.88 mmol) in abs methanol (20 mL) was added *N*-bromosuccinimide (180 mg, 1 mmol) at 20°C under an argon atmosphere. The mixture was stirred for 4 h and evaporated under reduced pressure to give a residue. The residue was dissolved in ethyl acetate, and the solution was washed with water and brine, and the organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo to a syrup that was purified by flash chromatography on silica gel (2:1 ethyl acetate–petroleum ether). The fast-migrating zone was the 2,3-diequatorial isomer **12**, whereas the slower migrating zone was the 2,3-diaxial isomer **16**. The yields of **12** and **16** were 60 mg (0.12 mmol, 20%) and 240 mg (0.52 mmol, 80%), respectively, before purification. IR (CH_2Cl_2): 2130 cm^{-1} (N_3); ^1H NMR data for **12** (400 MHz, $CDCl_3$): δ 1.99, 2.04, 2.11, 2.15 (4s, 4×3 H,

4 × $COCH_3$), 3.31 (s, 3 H, OCH_3), 3.88 (s, 3 H, $COOCH_3$), 4.02 (dd, 1 H, $J_{8,9a}$ 6.00 Hz, $J_{9a,9b}$ 12.30 Hz, H-9a), 4.30 (dd, 1 H, $J_{3ax,4}$ and $J_{4,5}$ 2.90 Hz, H-4), 4.09 (d, 1 H, H-3_{ax}), 4.30–4.50 (m, 2 H, H-5, H-9b), 4.70 (dd, 1 H, $J_{5,6}$ 11.00 Hz and $J_{6,7}$ 2.50 Hz, H-6), 5.13 (dd, 1 H, $J_{7,8}$ 8.70 Hz, H-7), 5.42 (ddd, 1 H, $J_{8,9b}$ 2.90 Hz, H-8), 6.02 (d, 1 H, $J_{5,NH}$ 9.30 Hz, NH). Anal. Calcd for $C_{19}H_{27}BrO_{11}N_4$ (567.349): C, 40.22; H, 4.80; N, 9.88. Found: C, 40.68; H, 4.48; N, 9.65.

*Methyl (methyl 4,5-diacetamido-7,8,9-tri-*O*-acetyl-3-bromo-3,4,5-trideoxy-α-*D-glycero-D-talo-*nonulopyranosid)onate* (**13**).—Compound **12** (60 mg, 0.12 mmol) was dissolved in ethyl acetate (10 mL). To this solution 5 mL of acetic anhydride and 10% Pd–C (80 mg) were added, and hydrogenation was carried out at 50 psi for 14 h. The solution was filtered and the catalyst was washed several times with ethyl acetate. (Caution: fire hazard!) Evaporation of the solvent under reduced pressure, followed by flash chromatography on silica gel with ethyl acetate as the eluant, yielded 52 mg (0.12 mmol, 84%) of **13**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97, 2.04, 2.12, 2.15, 2.16 (5s, 5 × 3 H, 5 × $COCH_3$), 3.32 (s, 3 H, OCH_3), 3.85 (s, 3 H, $COOCH_3$), 4.11 (dd, 1 H, $J_{8,9a}$ 5.20 Hz, $J_{9a,9b}$ 12.00 Hz, H-9a), 4.22 (ddd, 1 H, $J_{3ax,4}$ 2.85 Hz, $J_{4,5}$2.87 Hz, $J_{4,NH}$ 8.30 Hz, H-4), 4.07 (d, 1 H, H-3_{ax}), 4.32–4.8 (m, 2 H, H-5, H-9b), 4.60 (dd, 1 H, $J_{5,6}$ 11.00 Hz, $J_{6,7}$ 2.50 Hz, H-6), 5.13 (dd, 1 H, $J_{7,8}$ 8.70 Hz, H-7), 5.41 (ddd, 1 H, $J_{8,9b}$ 2.82 Hz, H-8), 5.87 (d,1 H, NH), 6.12 (d, 1 H, $J_{5,NH}$ 9.30 Hz, NH). Anal. Calcd for $C_{21}H_{31}BrO_{12}N_2$ (583.389): C, 43.24; H, 5.36; N, 4.80. Found: C, 43.31; H, 5.38; N, 4.79.

*Methyl (methyl 4,5-diacetamido-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-α-*D-glycero-D-talo-*nonulopyranosid)onate* (**14**).—To **13** (45 mg, 0.077 mmol) in abs toluene (10 mL), Bu_3SnH (49.5 mg, 0.17 mmol) and AIBN (5 mg) was added, and the reaction mixture was stirred at 70–80°C for 4 h. The progress of the reaction was monitored by TLC (ethyl acetate). The solvent was removed under reduced pressure, and the product was purified by flash chromatography. Yield of **14**: 23 mg (0.046 mmol, 59%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87 (dd, 1 H, $J_{3ax,3eq}$ 14.10 Hz, $J_{3ax,4}$ 2.50 Hz, H-3_{ax}), 1.96, 2.04, 2.07, 2.16, 2.18 (5s, 5 × 3 H, 5 × $COCH_3$), 2.62 (dd, 1 H, $J_{3eq,4}$ 3.75 Hz, H-3_{eq}), 3.30 (s, 3 H, OCH_3), 3.81 (s, 3 H, $COOCH_3$), 4.07 (dd, 1 H, $J_{8,9a}$ 6.10 Hz, $J_{9a,9b}$ 11.80 Hz, H-9a), 4.09 (m, 1 H, H-4), 4.13 (dd, 1 H, $J_{8,9b}$ 2.40 Hz, H-9b), 4.37 (ddd, 1 H, $J_{4,5}$ 2.75 Hz, $J_{5,NH}$ 9.50 Hz, $J_{5,6}$ 11.10 Hz, H-5), 4.48 (dd, 1 H, $J_{6,7}$ 1.70 Hz, H-6), 4.89 (dd, 1 H, $J_{7,8}$ 8.30 Hz, H-7), 5.33 (dd, 1 H, H-8), 5.78 (d, 1 H, $J_{4,NH}$ 8.70 Hz, NH), 6.09 (d, 1 H, NH). Anal. Calcd for $C_{21}H_{32}N_2O_{12}$ (504.491): C, 50.00; H, 6.39; N, 5.55. Found: C, 50.11; H, 6.28; N, 5.48.

*Ammonium (Methyl 4,5-diacetamido-3,4,5-trideoxy-α-*D-glycero-D-talo-*nonulopyranosid)onate* (**15**).—Compound **14** (20 mg, 0.04 mmol) in 1 N NaOH (3 mL) was stirred at room temperature for 5 h, and the progress of the reaction was monitored by TLC (15:4:0.5, 1-propanol–water–acetic acid). The reaction mixture was neutralized by solid CO_2 and then passed over a column of Dowex 50 NH_4^+ (5 g), which was thoroughly eluted with water. The removal of water under vacuum, followed by lyophilization, yielded 12 mg (0.03 mmol, 80%) of **15** in pure form: ^{1}H NMR (400 MHz, D_2O): δ 1.89 (dd, 1 H, $J_{3ax,4}$ 2.90 Hz, $J_{3ax,3eq}$ 14.50 Hz, H-2_{ax}), 2.10, 2.21, (2s, 2 × 3 H, 2 × $NCOCH_3$), 2.68 (dd, 1 H, $J_{3eq,4}$ 3.52 Hz, H-3_{eq}), 3.31 (s, 3 H, OCH_3), 3.67 (dd, 1 H, $J_{8,9a}$ 6.30 Hz, $J_{9a,9b}$ 11.30 Hz, H-9a), 3.74 (ddd, 1 H, $J_{7,8}$ 7.90 Hz, $J_{8,9b}$

2.50 Hz, H-8), 3.94 (dd, 1 H, $J_{6,7}$ 2.25 Hz, H-7), 4.11 (dd, 1 H, H-9b), 4.15 (dd, 1 H, $J_{4,5}$ 2.90 Hz, $J_{5,6}$ 10.75 Hz, H-5), 4.23 (ddd, 1 H, H-4), 4.43 (dd, 1 H, H-6); ^{13}C NMR (100.6 MHz, D_2O): δ 24.60, 25.87 (2 × $COCH_3$), 41.58 (C-3), 51.25 (OCH_3), 53.65 (C-5), 65.42 (C-9), 68.81, 71.23, 72.15, 74.43 (C-4, C-6, C-7, C-8), 101.98 (C-2), 177.42, 178.11, 179.20 (3 C, C-1,2 × N*C*OCH$_3$.

*Methyl (methyl 5-acetamido-7,8,9-tri-*O*-acetyl-4-azido-3-bromo-3,4,5-tri-deoxy-β-*D-glycero-D-talo-*nonulopyranosid)onate* (**16**).—The preparation of **16** was carried out in a manner similar to that for **12**. IR (CH_2Cl_2): 2125 cm^{-1} (N_3); ^{1}H NMR data for **16** (250 MHz, $CDCl_3$): δ 1.95, 2.04, 2.08, 2.16 (4s, 4 × 3 H, 4 × $COCH_3$), 3.30 (s, 3 H, OCH_3), 3.85 (s, 3 H, $COOCH_{3eq}$, 4.10–4.25 (m, 3 H, H-5, H-7, H-9a), 4.50 (d, 1 H, $J_{3Heq,4}$ 2.20 Hz, H-3$_{eq}$), 4.83 (dd, 1 H, $J_{5,6}$ 10.00 Hz, $J_{6,7}$ 3.50 Hz, H-6), 4.92 (dd, 1 H, $J_{8,9b}$ 2.40 Hz, $J_{9a,9b}$ 12.90 Hz, H-9b), 5.25 (ddd, 1 H, $J_{8,9a}$ 5.90 Hz, $J_{7,8}$ 5.50 Hz, H-8), 5.40 (dd, 1 H, $J_{4,5}$ 4.00 Hz, H-4), 5.60 (d, 1 H, $J_{5,NH}$ 10.80 Hz, NH). Anal. Calcd for $C_{19}H_{27}BrNO_{11}$ (567.349): C, 40.22; H, 4.80; N, 9.88. Found: C, 40.34; H, 4.63; N, 9.92.

*Methyl (methyl 4,5-diacetamido-7,8,9-tri-*O*-acetyl-3-bromo-3,4,5-trideoxy-β-*D-glycero-D-talo-*nonulopyranosid)onate* (**17**).—The preparation of **17** was similar to that of **13**. Compound **16** (50 mg, 0.11 mmol) yielded **17** (45 mg, 0.1 mmol, 84.5%): ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.95, 2.03, 2.08, 2.09, 2.15 (5s, 5 × 3 H, 5 × $COCH_3$), 3.30 (s, 3 H, OCH_3), 3.85 (s, 3 H, $COOCH_3$), 4.15 (dd, 1 H, $J_{8,9a}$ 2.5 Hz, $J_{9a,9b}$ 12.00 Hz, H-9a), 4.20 (ddd, 1 H, $J_{4,5}$ 3.60 Hz, $J_{5,NH}$ 10.40 Hz, $J_{5,6}$ 10.10 Hz, H-5), 4.48 (d, 1 H, $J_{3eq,4}$ 2.50 Hz, H-3$_{eq}$), 4.83 (dd, 1 H, $J_{6,7}$ 3.60 Hz, H-6), 4.86 (dd, 1 H, $J_{7,8}$ 5.50 Hz, H-7), 4.92 (dd, 1 H, $J_{8,9b}$ 3.00 Hz, H-9b), 4.25 (m, 2 H, NH, H-8), 5.39 (ddd, 1 H, $J_{4,NH}$ 3.50 Hz, H-4), 5.67 (d, 1 H, NH). Anal. Calcd for $C_{12}H_{31}BrN_2O_{12}$ (583.387): C, 43.24; H, 5.36; N, 4.80. Found: C, 43.17; H, 5.41; N, 4.69.

*Methyl (methyl 4,5-diacetamido-7,8,9-tri-*O*-acetyl-3,4,5-trideoxy-β-*D-glycero-D-talo-*nonulopyranosid)onate* (**18**).—Compound **17** (45 mg, 0.08 mmol) was dissolved in abs toluene (10 mL), Bu_3SnH (49.5 mg, 0.17 mmol) and AIBN (5 mg) were added, and the mixture was stirred at 70–80°C for 4 h. The progress of the reaction was monitored by TLC (ethyl acetate). Evaporation of the solvent under reduced pressure, followed by flash chromatography, yielded pure **18** (25 mg, 65%): ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.96 (dd, 1 H, $J_{3ax,3eq}$ 15.10 Hz, $J_{3ax,4}$ 3.10 Hz, H-3$_{ax}$), 1.97, 2.03, 2.07, 2.09, 2.15 (5s, 5 × 3 H, 5 × $COCH_3$), 2.38 (dd, 1 H, J_{3eq} 4 2.10 Hz, H-3$_{eq}$), 3.27 (s, 3 H, O-CH_3), 3.79 (s, 3 H, $COOCH_3$), 3.97 (dd, 1 H, $J_{8,9a}$ 7.40 Hz, $J_{8,9b}$ 9.70 Hz, H-9a), 4.07 (dd, 1 H, $J_{8,9b}$ 5.70 Hz, H-9b), 4.27 (dd, 1 H, $J_{5,6}$ 10.15 Hz, $J_{6,7}$ 2.10 Hz, H-6), 4.35 (ddd, 1 H, $J_{4,5}$ 3.06 Hz, $J_{5,NH}$ 9.20 Hz, H-5), 4.41 (m, 1 H, $J_{4,NH}$ 8.90 Hz, H-4), 4.67 (dd, 1 H, $J_{7,8}$ 5.25 Hz, H-7), 5.15 (m, 2 H, NH, H-8), 5.61 (d, 1 H, NH). Anal. Calcd for $C_{21}H_{32}N_2O_2$ (504.491): C, 50.00; H, 6.39; N, 5.55. Found: C, 49.97; H, 6.32; N, 5.47.

*4,5-Diacetamido-3,4,5-trideoxy-β-*D-glycero-D-talo-*nonulosonic acid* (**19**).—Compound **18** (25 mg, 0.05 mmol) was dissolved in aq NaOH (1 M, 5 mL) and heated for 3 h at 40°C. At the end of this time, the mixture was neutralized with Amberlyst-15 [H^+], the resin was filtered off and washed with water, and the combined washings were evaporated under reduced pressure. The residue was dissolved in HCl (0.025 M, 5 mL), and 1 g of Amberlyst-15 [H^+] was added. The mixture was then heated at 80°C for 2.5 h. The reaction mixture was cooled and filtered. Separation of the products was carried

out on a column of 5 g of Dowex-1 × 8 [$HCOO^-$] using a gradient of 0.1 N CH_3COOH (100 mL). Removal of the solvent at 30°C under reduced pressure, followed by lyophilization, gave 10 mg (0.029 mmol, 57%) of **19**. ^1H NMR of **19** (250 MHz, D_2O): δ 1.84 (dd, 1 H, $J_{3ax,3eq}$ 13.10 Hz, $J_{3ax,4}$ 2.60 Hz, H-3_{ax}), 2.25 (dd, 1 H, $J_{3eq,4}$ 1.90 Hz, H-3_{eq}), 3.71 (dd, 1 H, $J_{8,9a}$ 7.60 Hz, $J_{9a,9b}$ 10.10 Hz, H-9a), 3.92 (dd, 1 H, $J_{8,9b}$ 5.40 Hz, H-9b), 4.11 (dd, 1 H, $J_{5,6}$ 9.70 Hz, $J_{6,7}$1.80 Hz, H-6), 4.19 (dd, 1 H, $J_{7,8}$ 2.80 Hz, H-5), 4.27 (ddd, 1 H, H-4), 4.55 (dd, 1 H, $J_{7,8}$ 5.20 Hz, H-7), 4.72 (ddd, 1 H, H-8). Anal. Calcd for $C_{13}H_{22}N_2O_9$ (350.331): C, 44.57; H, 6.33; N, 7.99. Found: C, 44.63; H, 6.24; N, 7.87.

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