225. Synthesis and Biological Properties of *N*-Acetyl-4-deoxy-D-neuraminic Acid

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N-Acetylneuraminic acid (**1**) can be transformed into the methyl α-D-ketoside **2** which, by reaction with methanesulfonyl chloride, yields the corresponding 4-*O*-mesylate **3** and the 4,7-di-*O*-mesylate **4** as a by-product. Compound **3** reacts with NaI giving the 4-deoxy-4-iodo compound **5** with equatorial orientation of the I-atom. As second product, the dihydrooxazole **6** is produced. Catalytic hydrogenation of **5** is followed by ester cleavage and removal of the isopropylidene group yielding the methyl α-D-ketoside **8** which affords the title compound, *N*-acetyl-4-deoxyneuraminic acid (**9**), by reaction with *fowl plague virus* sialidase. Further biochemical activities of **8** and **9** are reported.

Introduction. – Sialic acids as constituents of glycoproteins and glycolipids are of considerable importance for a multitude of biological phenomena: cell-cell recognition, cell adhesion, interactions within the immune system and between virus and host cell, to mention only a few examples. For some years, this laboratory is carrying out a program aiming at the relationship between chemical structure and biological activity of sialic acids. This work has resulted in the synthesis of sialic-acid analogues which are modified at the particular regions of the parent molecule.

Position C(4) in *N*-acetylneuraminic acid (Neu5Ac; **1**) appears to be particularly sensitive to the action of some enzymes involved in the metabolism of **1**. Thus, α-ketosides of *N*,4-*O*-diacetylneuraminic acid (Neu4,5Ac$_2$) are not attacked by bacterial sialidases, but are slowly cleaved by viral sialidases [1] [2]. Ketosides of *N*-acetyl-4-*O*-methylneuraminic acid (Neu4Me5Ac) are completely resistent towards bacterial sialidases, but are cleaved rapidly by viral sialidases [1]. Neu4Me5Ac was activated to the corresponding CMP-glycoside and transferred onto asialo glycoprotein [1]. In contrast, Neu4,5Ac$_2$ apparently was not activated [3]. Neu4,5Ac$_2$ is poorly cleaved, and Neu4Me5Ac is not cleaved at all by the action of aldolase [1]. The biological properties of some further C(4) analogues have been reported [4–6]. In our laboratory, synthesis and biological properties of two additional substrates, 5-acetamido-3,5-dideoxy-D-*glycero*-D-*talo*-2-nonulosonic acid (4-epi-Neu5Ac) [7] and 5-acetamido-3,5-dideoxy-D-*manno*-2-non-4-ulosonic acid (4-oxo-Neu5Ac) [8] have been studied.

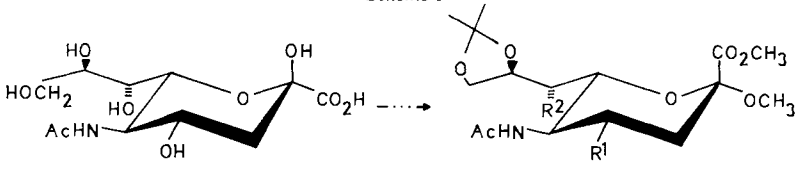
Here, we report on the synthesis of novel O-devoided C(4) analogues of **1** [9] by some appropriate steps starting with the natural compound itself.

Results and Discussion. – A simple and approved standard reaction for modifying OH groups is to prepare sulfonate esters which are prone to nucleophilic substitution at the same C-atom. The more it is surprising that this common method never has been used to modify the substitution pattern at C(4) of *N*-acetylneuraminic acid (**1**). To achieve this, **1**

had to be protected in a convenient way. According to the method of *Kuhn et al.* [10], **1** was transformed into the per-*O*-acetylated methyl β-D-ester followed by replacement of the anomeric acetyl group with a Cl-atom. Reaction with MeOH catalyzed by polymeric silver salt gave the corresponding methyl α-D-ketoside [11] which was de-*O*-acetylated by *Zemplén* saponification affording methyl *N*-acetyl-2-*O*-methyl-α-D-neuraminate [12] [13]. Finally, OH–C(8) and OH–C(9) were protected by an isopropylidene group to give **2** [14] *(Scheme 1)*. This made sure that only OH–C(7) and OH–C(4) were left for the following reactions.

According to our experience, OH–C(7) of derivatives like **2** proves to be less reactive than OH–C(4), whereupon a selective transformation at C(4) should be possible. In fact, mesylation of **2** yielded the crystalline 4-*O*-mesylate **3** (50%), and the 4,7-di-*O*-mesylate **4** was found as a by-product (15%), as expected *(Scheme 1)*.

Scheme 1



1

2 R^1 = OH, R^2 = OH
3 R^1 = MsO, R^2 = OH
4 R^1 = MsO, R^2 = MsO

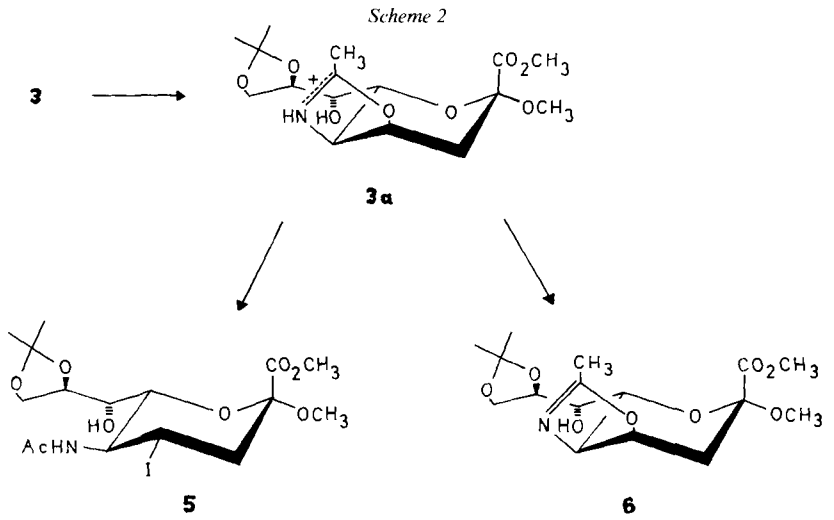
According to *Yamasaki et al.* [15], methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-tosyl-β-L-glucopyranoside readily reacts with NaI in 74% yield to give the 3-deoxy-3-iodo derivative as the only product. Nevertheless, the authors did not report any spectroscopic data in detail. Hence, the configuration at C(3) of this compound remains uncertain.

A comparable *Finkelstein* reaction could be applied to the mesylate **3**. However, the corresponding 4-iodo compound **5** was isolated, after chromatographic separation and recrystallization from CH_2Cl_2/light petroleum, only in 20% yield *(Scheme 2)*. From *Beilstein*'s test and combustion analysis, the presence of an I-atom was deduced. Surprisingly, the I-atom of **5** has exclusively equatorial and not axial orientation at C(4) as shown by the ^{1}H-NMR spectrum (J(3a,4) = J(4,5) = 12.8 Hz). In comparison with **3**, H–C(4) is shifted clearly upfield ($\Delta\delta$ = 0.66 ppm). Furthermore, the structure of **5** was confirmed by X-ray analysis [16]. With these results, it became clear that the substitution does not follow a simple S_N2 mechanism accompanied by *Walden* inversion. This assumption was further substantiated by detection of dihydrooxazole **6** which was isolated as a crystalline compound (25%), after chromatographic separation.

The ^{1}H-NMR spectrum of **6** shows at 1.97 ppm the *d* of the CH_3 group at the oxazole moiety with $^5J(5,CH_3)$ = 0.9 Hz. Other, noteworthy coupling constants are J(3a,4) = 9.7 and J(3e,4) = 6.6 Hz. All results are in accordance with the postulated structure of **6**.

Brandstetter and *Zbiral* [17] prepared the methyl β-D-ketoside analogous to **6** in a different way. But it could not be characterized sufficiently because of lacking stability. Only when OH–C(7) was acetylated, this dihydrooxazole became stable and could be analyzed by ^{1}H-NMR spectroscopy.

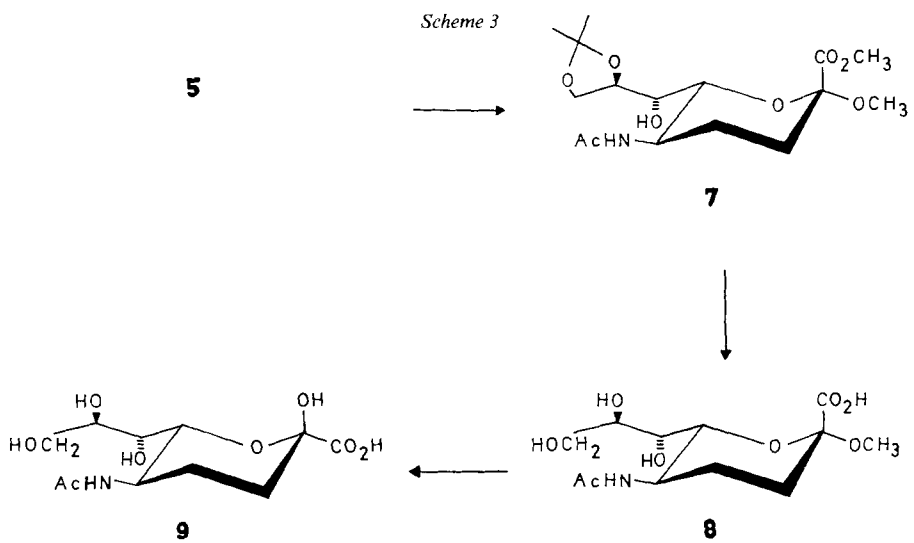
Scheme 2



Obviously, mesylate **3**, before reacting with NaI, is attacked at C(4) by the *N*-acetyl group (neighbouring-group participation) which represents an intramolecular S_N2 mechanism *(Scheme 2)*. The resulting dihydrooxazolium ion **3a** is then converted into the dihydrooxazole **6** by loss of a proton or, alternatively, is attacked at C(4) by I^- thereby regenerating the acetylamino group at C(5) *(Scheme 2)*.

Catalytic hydrogenation of the iodo compound **5** with H_2/Pd readily gave the crystalline 4-deoxy compound **7** in a yield of 84% *(Scheme 3)*. The structure of **7** has been confirmed by ^{1}H-NMR spectroscopy (H_{ax}–C(4) (1.45 ppm) and H_{eq}–C(4) (1.96 ppm) appear as *dddd*'s with J(4ax,4eq) = 13.3 Hz).

Scheme 3



Subsequently, the methyl ester at C(1) and the 8,9-*O*-isopropylidene group of **7** were cleaved in a one-pot reaction. First, **7** was treated with 1N NaOH, and after complete saponification of the ester, an excess of *Dowex 50 WX 4* (H^+) was added. Finally, methyl ketoside **8** was purified by ion-exchange chromatography on *DEAE Sephadex A-25* (HCO_3^-). The results of the deblocking reactions have been proven by the ^{1}H-NMR spectrum of **8** (the characteristic *s* of the isopropylidene group and of the ester of **7** are absent).

In order to obtain free *N*-acetyl-4-deoxyneuraminic acid (**9**), the ketoside had to be cleaved. Although there are approved chemical procedures [18], the particularly careful enzymatic cleavage by a suitable sialidase was recommended as **9** is rather sensitive to acid. At the same time, the biological activity of **8** could be studied. The reaction of **8** with fowl plaque virus sialidase proved to be promising and was complete after 20 h at 37°. After purification by ion-exchange chromatography on *DEAE Sephadex A-25* (HCO_3^-) and freeze-drying, **9** was isolated in a yield of 92%.

In the ^{1}H-NMR spectrum of **9**, CH_2(3), CH_2(4), and CH_3CONH are found as a *m* at 2.09–1.95 ppm which could not be analyzed. H–C(5) and H–C(6) give also a *m* at 4.12–4.07 ppm, whereas the signals of the protons at C(7), C(8), and C(9) are coming up clearly.

We found that ketoside **8** is not a substrate for bacterial sialidases from *Vibrio cholerae, Clostridium perfringens,* and *Arthrobacter ureafaciens.* However, **8** was also cleaved by sialidase from bovine testis [19].

Furthermore, the biological activity of **9** was studied. As expected, **9** was not cleaved by *N*-acetylneuraminate pyruvate-lyase (EC 4.1.3.3), but proved to be an inhibitor for the cleavage of the natural compound **1** (inhibition rate 80%, **1**/**9** ratio 1:2) [19].

Interestingly, **9** was activated to the corresponding CMP-glycoside by CMP-sialic-acid synthase and was transferred onto asialo-α_1-acid glycoprotein by α-2,6-sialyltransferase from rat liver [20].

In this study, we were able to prepare novel C(4) analogues of *N*-acetylneuraminic acid which show interesting properties and thus require further investigations of the biological relevance of the C(4) OH group.

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Experimental Part

General. All chemicals were obtained from commercial sources. Fowl plague virus sialidase was a generous gift from Prof. *R. Rott,* Universität Giessen. Solvents were distilled before use. *N*-Acetylneuraminic acid (**1**) was prepared from edible-bird's nests [21]. Solns. were evaporated in a rotary evaporator below 40°. TLC: *Merck* precoated plates, silica gel *60* F_{254}, layer thickness 0.2 mm; detection by spraying with 2N H_2SO_4 followed by heating at 150°. Column chromatography: *Merck* silica gel *60*, 0.063–0.200 mm. M.p.: *Tottoli-Büchi* apparatus; uncorrected. Optical rotations: *Perkin-Elmer-141* polarimeter. ^{1}H-NMR spectra: if no mention, on *Bruker WM-300* (^{1}H(300 MHz)), otherwise on *Bruker AM-500* (^{1}H(500 MHz)) using tetramethylsilane (TMS) as the internal standard; δ in ppm relative to TMS; coupling constants were determined first order. Elemental analysis was performed in the *Max-Planck-Institut für Medizinische Forschung,* Heidelberg.

*Methyl (Methyl 5-Acetamido-3,5-dideoxy-8,9,*O*-isopropylidene-*α*-*D*-glycero-*D*-galacto-2-nonulopyranosid)-onate* (**2**). To a soln. of methyl (methyl 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate [12] (6.0 g, 17.8 mmol) in anh. acetone (100 ml), $TsOH \cdot H_2O$ (100 mg, 0.53 mmol) was added and stirred at r.t. for

4 h. Then, *Dowex 1 × 4* (AcO^-, 1.0 g) was added, and vigorous stirring was continued for 15 min. Finally, the resin was filtered off and washed with acetone (3 × 10 ml). After removing of the solvent, the residue was crystallized from MeOH/Et_2O affording 5.0 g (75%) of **2**, m.p. 181°, R_f 0.55 ($CHCl_3$/MeOH 4:1), $[\alpha]_D^{20} = -9.0°$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3/D_2O$): 4.30 (*ddd*, $J = 6.3, 6.3, 6.3$, H–C(8)); 4.12 (*dd*, $J = 8.6, 6.3$, H–C(9)); 4.06 (*dd*, $J = 8.6, 6.3$, H–C(9)); 3.85 (*m*, H–C(5)); 3.81 (*s*, CO_2CH_3); 3.72 (*m*, H–C(4)); 3.57 (*dd*, $J = 6.3, 1.3$, H–C(7)); 3.51 (*dd*, $J = 10.4, 1.3$, H–C(6)); 3.39 (*s*, CH_3O); 2.69 (*dd*, $J = 12.9, 4.8$, H–C(3)); 2.06 (*s*, AcN); 1.83 (*dd*, $J = 12.9, 11.0$, H–C(3)); 1.40, 1.37 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{16}H_{27}NO_9$ (377.39): C 50.92, H 7.21, N 3.71; found: C 50.99, H 7.24, N 3.61.

*Methyl (Methyl 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4-*O*-methanesulfonyl-α-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**3**) *and Methyl (Methyl 5-Acetamido-3,5-dideoxy-8,9-*O*-isopropylidene-4,7-di-*O*-methanesulfonyl-α-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**4**). To a soln. of **2** (950 mg, 2.52 mmol) in anh. pyridine (10 ml) cooled in ice, methanesulfonyl chloride (200 µl, 2.58 mmol) was added dropwise during 40 min and the mixture stored in the refrigerator at −10° overnight. The soln. was poured on ice/H_2O (20 ml), extracted with CH_2Cl_2 (3 × 20 ml), dried ($MgSO_4$), and evaporated. The residue was co-distilled with toluene (10 ml) and gave, after chromatography (toluene/AcOEt/EtOH 5:1:1), 580 mg (51%) of **3** (recrystallized from AcOEt/light petroleum) and 200 mg (15%) of **4**. *Data of* **3**: M.p. 133–134°, R_f 0.33, $[\alpha]_D^{20} = -19.2°$ ($c = 1.0$, $CHCl_3$). ^{1}H-NMR ($CDCl_3/D_2O$): 4.87 (*ddd*, $J = 7.5, 6.2, 5.7$, H–C(8)); 4.86 (*dd*, $J = 11.5, 11.5, 5.3$, H–C(4)); 4.12 (*dd*, $J = 8.4, 6.2$, H–C(9)); 4.05 (*dd*, $J = 8.4, 5.7$, H–C(9)); 4.01 (*dd*, $J = 11.5, 10.4$, H–C(5)); 3.84 (*s*, CO_2CH_3); 3.52 (*d*, $J = 7.5$, H–C(7)); 3.45 (*s*, CH_3O); 3.39 (*d*, $J = 10.4$, H–C(6)); 3.10 (*s*, MsO); 2.78 (*dd*, $J = 12.4, 5.3$, H–C(3)); 2.07 (*dd*, $J = 12.4, 11.5$, H–C(3)); 2.06 (*s*, AcN); 1.38, 1.36 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{17}H_{29}NO_{11}S$ (455.48): C 44.83, H 6.42, N 3.07; found: C 45.00, H 6.42, N 3.01.

Data of **4**: R_f 0.41, $[\alpha]_D^{20} = +5.25°$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3/D_2O$): 5.45 (*ddd*, $J = 11.5, 9.7, 5.3$, H–C(4)); 5.11 (*dd*, $J = 2.4, 1.1$, H–C(7)); 4.62 (*dd*, $J = 10.6, 1.1$, H–C(6)); 4.38 (*ddd*, $J = 8.0, 6.6, 2.4$, H–C(8)); 4.18 (*dd*, $J = 8.4, 6.6$, H–C(9)); 4.04 (*dd*, $J = 8.4, 8.0$, H–C(9)); 3.88 (*s*, CO_2CH_3); 3.34 (*s*, MsO); 3.30 (*dd*, $J = 10.6, 9.7$, H–C(5)); 3.22 (*s*, MsO); 3.01 (*s*, CH_3O); 2.92 (*dd*, $J = 12.8, 5.3$, H–C(3)); 1.99 (*s*, AcN); 1.90 (*dd*, $J = 12.8, 11.5$, H–C(3)); 1.44, 1.37 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{18}H_{31}NO_{13}S_2$ (533.57): C 40.52, H 5.86, N 2.62; found: C 40.29, H 5.58, N 2.67.

*Methyl (Methyl 5-Acetamido-3,4,5-trideoxy-4-iodo-8,9-*O*-isopropylidene-α-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**5**) *and Methyl (Methyl 3,4,5-Trideoxy-4,5-dihydro-8,9-*O*-isopropylidene-2'-methyloxazolo[5,4-*d*]-α-*D*-glycero-*D*-talo-2-nonulopyranosid)onate* (**6**). A soln. of **3** (500 mg, 1.1 mmol) and anh. NaI (5.00 g, 39.4 mmol) in anh. acetone (40 ml) was heated in a steel bomb for 3 h at 100°. After cooling, the suspension was evaporated, and the solid residue was extracted with a mixture of sat. sodium thiosulfate soln. (20 ml) and CH_2Cl_2 (2 × 20 ml). The combined org. layers were dried ($MgSO_4$) and evaporated. The yellow-brown syrup left yielded, after chromatography (AcOEt), 105 mg (20%) of **5** (recrystallized from CH_2Cl_2/light petroleum) and 100 mg (25%) of **6** (recrystallized from AcOEt/light petroleum). *Data of* **5**: M.p. 140–141°, R_f 0.43, $[\alpha]_D^{20} = +18.0°$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3/D_2O$): 4.27 (*ddd*, $J = 12.4, 6.2, 6.2$, H–C(8)); 4.20 (*ddd*, $J = 12.8, 12.8, 4.4$, H–C(4)); 4.14–4.05 (*m*, 2 H–C(9)); 4.05 (*dd*, $J = 12.8, 10.2$, H–C(5)); 3.82 (*s*, CO_2CH_3); 3.60 (*d*, $J = 6.2$, H–C(7)); 3.52 (*d*, $J = 10.2$, H–C(6)); 3.40 (*s*, CH_3O); 3.08 (*dd*, $J = 13.4, 4.4$, H–C(3)); 2.53 (*dd*, $J = 13.4, 12.8$, H–C(3)); 2.09 (*s*, AcN); 1.39, 1.36 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{16}H_{26}INO_8$ (487.29): C 39.44, H 5.38, I 26.04, N 2.87; found: C 39.21, H 5.26, I 26.11, N 2.83.

Data of **6**: M.p. 115°, R_f 0.15, $[\alpha]_D^{20} = -5.3°$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR (CD_3OD): 4.88 (*m*, H–C(4)); 4.46 (*m*, H–C(5)); 4.27 (*ddd*, $J = 6.6, 6.2, 6.2$, H–C(8)); 4.12 (*dd*, $J = 8.4, 6.2$, H–C(9)); 4.00 (*dd*, $J = 8.4, 6.6$, H–C(9)); 3.85 (*dd*, $J = 6.2, 1.1$, H–C(7)); 3.77 (*s*, CO_2CH_3); 3.68 (*dd*, $J = 10.2, 1.1$, H–C(6)); 3.35 (*s*, CH_3O); 2.51 (*dd*, $J = 13.9, 6.6$, H–C(3)); 2.12 (*dd*, $J = 13.9, 9.7$, H–C(3)); 1.97 (*d*, $J = 0.9$, CH_3–C(2')); 1.38, 1.34 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{16}H_{24}NO_8$ (358.37): C 53.62, H 6.75, N 3.91; found: C 53.50, H 7.02, N 3.82.

*Methyl (Methyl 5-Acetamido-3,4,5-trideoxy-8,9-*O*-isopropylidene-α-*D*-manno-2-nonulopyranosid)onate* (**7**). A soln. of **5** (50 mg, 0.1 mmol) in anh. MeOH (8 ml) and anh. pyridine (20 µl) was hydrogenated over H_2/Pd during 2 d at r.t. After evaporation of the pale yellow soln., the residue was dissolved in $CHCl_3$ (15 ml) and shaken with a soln. of sat. sodium thiosulfate (8 ml). The aq. layer was re-extracted with $CHCl_3$ (10 ml). The combined org. layers were dried ($MgSO_4$) and evaporated affording a syrup which slowly crystallized. Recrystallization from Et_2O/light petroleum yielded 31 mg (84%) of **7**, m.p. 120–121°, R_f 0.47 (AcOEt), $[\alpha]_D^{20} = +2.8°$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR (CD_3OD): 4.27 (*m*, H–C(8)); 4.08 (*dd*, $J = 8.4, 6.2$, H–C(9)); 4.00 (*dd*, $J = 8.4, 6.6$, H–C(9)); 3.95 (*ddd*, $J = 11.5, 11.5, 4.0$, H–C(5)); 3.80 (*s*, CO_2CH_3); 3.64–3.60 (*m*, H–C(6), H–C(7)); 3.33 (*s*, CH_3O); 2.30 (*ddd*, $J = 13.7, 4.0, 4.0$, H–C(3)); 1.96 (*dddd*, $J = 13.3, 4.0, 4.0, 4.0$, H–C(4)); 1.94 (*s*, AcN); 1.72 (*ddd*, $J = 13.7, 13.3, 4.0$, H–C(3)); 1.45 (*dddd*, $J = 13.3, 13.3, 11.5, 4.0$, H–C(4)); 1.37, 1.34 (2*s*, $(CH_3)_2C$). Anal. calc. for $C_{16}H_{27}NO_8$ (361.39): C 53.18, H 7.53, N 3.88; found: C 53.03, H 7.72, N 3.96.

*Methyl 5-Acetamido-3,4,5-trideoxy-α-*D*-manno-2-nonulopyranosidonic Acid* (**8**). To a stirred soln. of **7** (310 mg, 0.86 mmol) in MeOH (1 ml) cooled in ice, 1N NaOH (1.5 ml) was added dropwise and then, the stirring was continued for 2 h at r.t. After addition of H_2O (2 ml), the soln. was acidified with *Dowex WX 4* (H^+) to pH 2.5 and vigorously stirred for 2.5 h. The resin was filtered off, intensively washed with H_2O (30 ml), and the combined filtrate was freeze-dried. For purification, the solid residue was transferred onto a column filled with *DEAE Sephadex A-25* (HCO_3^-), washed with H_2O (70 ml), and eluted with 0.075M NH_4HCO_3. The fractions containing **8** were collected and evaporated (bath temp. max. 20°). A soln. of the residue in H_2O (20 ml) was rapidly acidified with *IR 120* (H^+) to pH 2.5, then filtered, and the resin was washed intensively with H_2O (30 ml). The combined filtrates were freeze-dried giving 200 mg (72%) of **8**, R_f 0.55 (EtOH/AcOH 5:1), $[\alpha]_D^{20} = -7.8°$ ($c = 0.5$, H_2O). ^{1}H-NMR (500 MHz, D_2O): 3.93–3.87 (*m*, H–C(5), H–C(8)); 3.87 (*dd*, $J = 11.8, 2.6$, H–C(9)); 3.81 (*dd*, $J = 10.4, 1.7$, H–C(6)); 3.64 (*dd*, $J = 11.8, 6.3$, H–C(9)); 3.57 (*dd*, $J = 8.9, 1.7$, H–C(7)); 3.35 (*s*, CH_3O); 2.36 (*ddd*, $J = 13.5, 3.7, 3.7$, H–C(3)); 2.05 (*dddd*, $J = 13.5, 12.0, 4.3, 3.7$, H–C(4)); 1.98 (*s*, AcN); 1.71 (*ddd*, $J = 13.5, 13.5, 4.3$, H–C(3)); 1.50 (*dddd*, $J = 13.5, 13.5, 12.0, 3.7$, H–C(4)). Anal. calc. for $C_{12}H_{21}NO_8 \cdot 1\,H_2O$ (325.31): C 44.31, H 7.13, N 4.31; found: C 44.78, H 7.17, N 4.43.

*5-Acetamido-3,4,5-trideoxy-*D*-manno-2-nonulosonic Acid* (**9**). Acid **8** (36.1 mg, 0.12 mmol) was dissolved in 0.1M NaOAc buffer (1.8 ml), and the pH was focused to 6.0 by addition of 1N NaOH. After addition of fowl plague virus sialidase (0.5 U), the soln. was incubated 20 h at 37°. When TLC (EtOH/AcOH 5:1) indicated complete cleavage of **8**, the soln. was dialyzed 6 times each 0.5 h *vs.* H_2O (100 ml). The dialysates were transferred onto a column filled with *DEAE Sephadex A-25* (HCO_3^-), washed with H_2O (100 ml), and eluted with 0.08M NH_4HCO_3 soln. The fractions containing **9** were collected, evaporated (bath temp. max. 25°) and dissolved in H_2O (15 ml). The soln. was acidified with *IR 120* (H^+) to pH 2.5 and freeze-dried yielding 31.8 mg (92%) of **9**, R_f 0.26 (EtOH/AcOH 5:1), $[\alpha]_D^{20} = -38.0°$ ($c = 0.4$, H_2O, $pK_a = 3.30$ (H_2O, 22°). ^{1}H-NMR (D_2O): 4.12–4.07 (*m*, H–C(5), H–C(6)); 3.92 (*dd*, $J = 11.5, 2.6$, H–C(9)); 3.85 (*ddd*, $J = 8.8, 6.2, 2.6$, H–C(8)); 3.70 (*dd*, $J = 11.5, 6.2$, H–C(9)); 3.66 (*d*, $J = 8.8$, H–C(7)); 2.09–1.95 (*m*, 2 H–C(3), 2 H–C(4), AcN). Anal. calc. for $C_{11}H_{19}NO_8 \cdot 1\,H_2O$ (311.29): C 42.44, H 6.80, N 4.50; found: C 42.20, H 6.46, N 4.57.

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