Monatshefte für Chemie 120, 899—906 (1989)

Monatshefte für Chemie
Chemical Monthly

Structural Variations of N-Acetylneuraminic Acid, 12 [1]: A New Useful Approach to 4-epi- and 8-epi-N-Acetylneuraminic Acid

Michael Hartmann and **Erich Zbiral***

Institut für Organische Chemie, Universität Wien, A-1090 Wien, Austria

Summary. Readily available Neu5*Ac* derivatives **1** and **7** are oxidized by RuO_4 to the ketones **2** and **8** which are reduced diastereoselectively by the borane-ammonia complex to yield the 4- and 8-epimers **4a** and **10**. Subsequent deprotection leads to the title compounds **5** and **12**. This few step procedure is also applicable on gram scale.

Keywords. Borane-ammonia complex; Ruthenium tetroxide; Sialic acids.

Strukturelle Abwandlungen an N-Acetylneuraminsäure, 12: Eine neue, nützliche Synthese von 4-epi- und 8-epi-N-Acetylneuraminsäure

Zusammenfassung. Die leicht zugänglichen Neuraminsäurederivate **1** und **7** werden mittels RuO_4 in die Ketone **2** und **8** umgewandelt, welche mit dem Ammoniak-Boran-Komplex diastereoselektiv zu den entsprechenden 4- und 8-Epimeren **4a** und **10** reduziert werden. Anschließende Deblockierungsschritte führen zu den Titelverbindungen **5** und **12**. Diese Synthesewege sind für den Grammaßstab ausgearbeitet worden.

Introduction

Sialic acids are widespread in living systems as terminal units of oligosaccharide chains of numerous glycoproteins and glycolipids. Therefore they are involved in various biologically important processes [2, 3].

This is the reason why many structural variations at C-4 [4], C-7 [5], C-8 [5] and C-9 [6] were performed during the last few years. Earlier procedures [4] leading to suitable derivatives of **5** made use of neighbouring participation of the acetamido group forming an oxazoline with inverted configuration at C-4. Opening of the oxazoline ring led to a 4-*epi*-Neu5*Ac* derivative. A multistep-synthesis of **5** was published by Vasella and Baumberger [7] applying a different strategy.

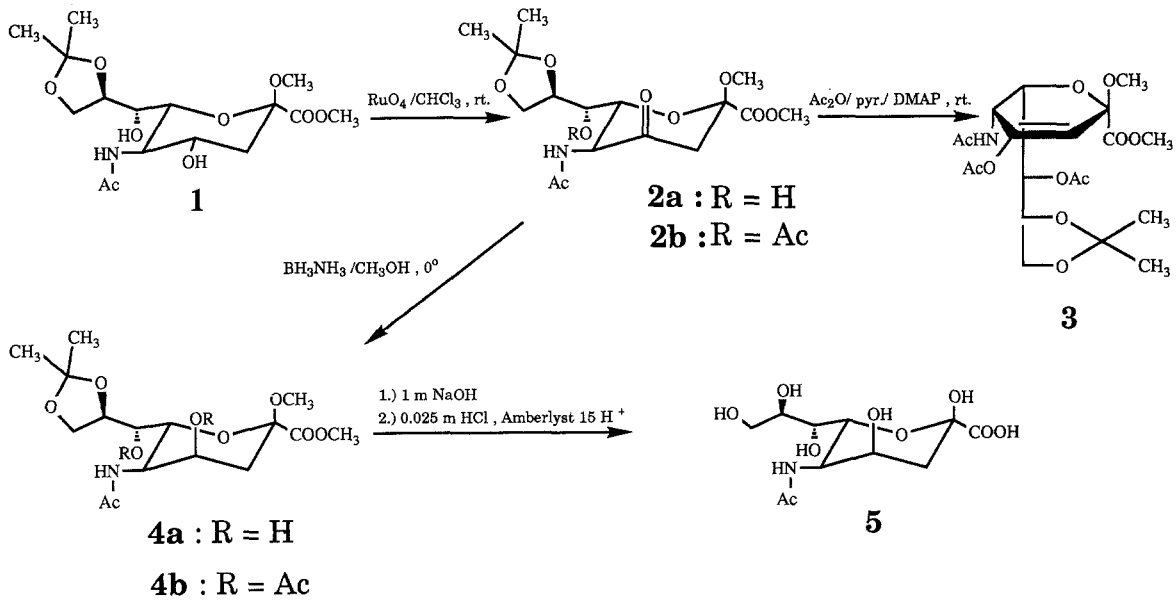
In this contribution we wish to report on a new synthesis feasible for gram scale preparation of 5-acetamido-3,5-dideoxy-*D*-glycero-*D*-talo-2-nonulosonic acid (4-*epi*-Neu5*Ac*) **5** and 5-acetamido-3,5-dideoxy-*L*-glycero-*D*-galacto-2-nonulosonic acid (8-*epi*-Neu5*Ac*) **12**.

For further investigations and chemical variations we explored an easy access not only to derivatives **4a** and **10** but also to free acids **5** and **12**.

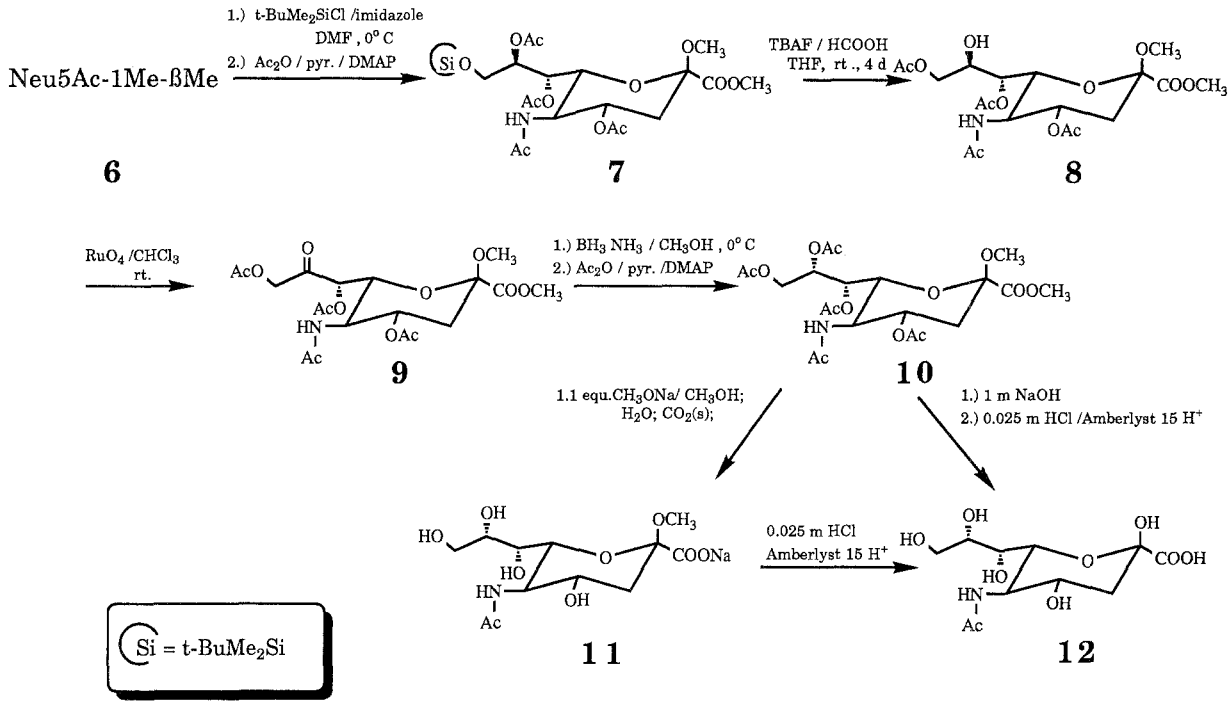
Results and Discussion

The first step (Scheme 1) in our synthesis of 4-*epi*-Neu5*Ac* **5** is the selective oxidation of the 8,9-isopropylidene derivative **1** [4] to the ketone **2** by means of RuO_4. We compared the "single-phase" (stoichiometric) oxidation with the "two-phase" (catalytic method [8] and found that the first was superior in terms of shorter reaction time and higher yields. Lower yields were also observed in the case of PCC-oxidation, probably due to coprecipitation of the relatively polar product during work-up. Encouraged by our experience on the diastereoselectivity of the reduction of a 7-oxo-Neu5*Ac* derivative to the corresponding 7-epi-compound [9] with borane-ammonia-complex we applied the same reagent for the transformation of **2** into the alcohol **4a**. In this case the selectivity was good (**4a** : **1** = 10 : 1). In this connection it is interesting to note that the borane-trimethylamine-complex was also successfully applied in diastereoselective reduction of carbonyl groups of other sugar derivatives [10]. When we acetylated **2a** in the presence of 4-dimethylamino-pyridine we observed enolization yielding the enolacetate **3**, whereas in the absence of the catalyst only **2b** was formed. The removal of protecting groups and purification on an ion-exchange resin column led to desired 4-*epi*-Neu5*Ac* **5**.

We applied an analogous way to synthesize the 8-*epi*-Neu5*Ac* and derivatives (Scheme 2). Preparation of the fully protected derivative **7** was an improvement of earlier procedures [11]. Removing the 9-O-*t*-butyldimethylsilyl group under acidic conditions (*TBAF*/HCOOH) caused a simultaneous migration of the acetyl group from C-8 to C-9. By this procedure leaving only C-8 functionality accessible for further transformations the useful derivative **8** is obtained. Subsequent oxidation of alcohol **8** with RuO_4 gave the corresponding ketone **9** in excellent yields. The key step reduction of **9** with borane-ammonia-complex led with good diastereoselectivity to the desired 8-*epi*-Neu5*Ac* derivative which is easily separated



Scheme 1



Scheme 2

from the byproduct **8** after acetylation (**10**:**8** = 20:1). Removing the protective groups gave derivative **11**, which could be transformed into **12** by cleavage of *β*-methylketoside under acidic conditions. Deprotection of **10** to **12** was performed in analogy to a published method by Schauer et al. [12].

In comparison to this procedure the earlier reported synthesis of **12** consisted of much more steps [5].

Acknowledgments

We are grateful to Miss S. Kotzinger and Mister W. Schneider for technical assistance. This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (project number 6805).

Experimental Part

Solvents were distilled before use. N-acetylneuraminic acid (Neu5*Ac*) was prepared either from meconium [13], or from edible bird's nest glycoprotein [14]. Starting materials **1** and **6** were prepared according to Refs. [15] and [4].

Solutions were evaporated in a rotatory evaporator below 40°C. TLC: Merck plates, silica gel 60 F_{254}, layer thickness 0.2 mm; detection by spraying with a solution of $Ce(NO_3)_4$ in 2*n* H_2SO_4, followed by heating at 200°C. Deprotected Neu5*Ac* compounds were detected by spraying with Bial's reagent [2] and heating at 130°C for 10 minutes. Column chromatography: Merck silica gel 60, 0.040–0.063 mm. Flash-chromatography [16] Merck silica gel 60, 0.040–0.063 mm.

^{1}H- and ^{13}C-nmr spectra and 2-D-experiments were recorded with a Bruker WM 250 instrument. In the case of ^{1}H-nmr (250 MHz) using tetramethylsilane (*TMS*) in $CDCl_3$-solutions as internal

standard and in D_2O as external standard. ^{13}C-nmr (62.9 MHz) were calibrated on *TMS* in $CDCl_3$ and with 1,4-dioxane external in D_2O. Chemical shifts are given in δ-values, coupling constants were determined by first-order evaluation. ^{13}C-nmr were recorded *J*-modulated and the assignment was established by ^{1}H—^{13}C-correlation experiments. 3 Å molecular sieves were used commonly after drying 4 h in vacuo (0.001 Torr; 130°C). All reactions were carried out in two- or three-necked flasks under argon, closed by rubber septa. Addition of reagents and control of reactions was achieved by using syringes. Imidazole was sublimed under reduced pressure at 80°C bath temperature in vacuo (0.001 Torr). $CHCl_3$ used for RuO_4-oxidations was liberated from alcohol and impurities by treatment on a column filled with Merck Al_2O_3 90 active neutral 0.063–0.200 mm. For characterization of **5** and **12** 20 mg of each were passed over 1 g of Dowex 50 Na^+ and lyophilized. The ^{1}H NMR data were identical with those given in Ref. [17].

Methyl-5-acetamido-8,9-O-(1'-methyl-ethylidene)-3,5-dideoxy-β-D-manno-2,4-nonodiulopyranosidonic Acid Methyl Ester (**2a**)

2.14 g KIO_4 (9.3 mmol), 0.12 K_2CO_3 (0.83 mmol) and 0.64 g $RuO_2 \cdot H_2O$ (4.81 mmol) were dissolved in 50 ml of water and stirred for 10 min. This solution was extracted 5 times with 10 ml of $CHCl_3$. The combined organic layers were added to a vigorously stirred solution of 1.4 g (3.71 mmol) of compound **1** in 10 ml or $CHCl_3$. The reaction was monitored by TLC: ethylacetate; R_f(**1**) = 0.10, R_f(**2a**) = 0.34. After starting material had completly disappeared (0.5 h) the reaction was stopped by the addition of 0.5 ml of 2-propanol and stirred further ten minutes. The precipitate was removed by filtration over celite, washed three times with 20 ml of $CHCl_3$ and the crude product was liberated from solvent under reduced pressure. Flash-chromatography with ethyl acetate on 50 g of silica yielded 1.08 g of **2a** (2.88 mmol, 78%) as a colorless foam. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.30, 1.38 [2 s, 2 × 3 H, $C(CH_3)_2$], 2.14 (s, 3 H, CH_3CO), 2.88 (d, 1 H, 3-H'), 2.97 (d, 1 H, 3-H), 3.33 (s, 3 H, OCH_3), 3.46 (dd, 1 H, 7-H), 3.88 (dd, 1 H, 6-H), 3.85 (s, 3 H, $COOCH_3$), 4.05 (dd, 1 H, 9-H_a), 4.19 (dd, 1 H, 9-H_b), 4.41 (ddd, 1 H, 8-H), 4.74 (dd, 1 H, 5-H), 6.70 (d, 1 H, N—H); $J(3_a, 3_b)$ = − 15.5, J(5, N—H) = 6.6, J(5, 6) = 10.4, J (6, 7) = 1.7, J (7, 8) = 8.7, J (8, 9_a) = 5.3, J (8, 9_b) = 6.2, J (9_a, 9_b) = − 9.2. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 200.48 (C=O, C-4), 172.95 (C=O, *Ac*), 167.02 (C=O, C-1), 109.13 [**C**$(CH_3)_2$], 100.70 (C-2), 74.75, 74.27, 70.71 (C-6, C-7, C-8), 67.99 (C-9), 56.67 (C-5), 52.81, 51.50 (OCH_3, $COOCH_3$), 48.34 (C-3), 27.06, 25.77 [C(**C**$H_3)_2$], 22.93 (**C**H_3CO). MS (70 eV, 140°C): *m/z* (%) = 360 (3.48) = [M^+-CH_3], 316 (6.59) = [M^+-$C_2H_3O_2$]. $C_{16}H_{25}NO_9$ (375.3). Calcd. C 51.20, H 6.71, N 3.73; found C 51.47, H 6.81, N 3.53.

Methyl-5-acetamido-7-O-acetyl-8,9-O-(1'-methyl-ethylidene)-3,5-dideoxy-β-D-manno-2,4-nonodiulopyranosidonic Acid Methyl Ester (**2b**)

149 mg (0.40 mmol) **2a** were dissolved in 3 ml pyridine, 3 ml acetic anhydride were added and the resulting solution was allowed to stand overnight at room temperature. Evaporation of volatile components yielded a brown oil, which was purifid by flash-chromatography. 131 mg (0.31 mmol, 77.5%) of **2b** were obtained as a colorless foam. TLC: ethyl acetate; R_f (**2b**) = 0.38. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.28, 1.34 [2 s, 2 × 3 H, $C(CH_3)$], 1.99, 2.14 (2 s, 2 × 3 H, CH_3CO), 2.81 (d, 1 H, 3-H_a), 2.91 (s, 3 H, OCH_3), 3.83 (s, 3 H, $COOCH_3$), 3.92 (dd, 1 H, 9-H_a), 4.01 (dd, 1 H, 6-H), 4.02 (dd, 1 H, 9-H_b), 4.35 (ddd, 1 H, 5-H), 5.06 (dd, 1 H, 7-H), 5.88 (d, 1 H, N—H); $J(3_a, 3_b)$ = − 14.0, J(5, N—H), J(5, 6) = 10.7, J(6, 7) = 2.0, J(8, 9_a) = 6.7, J(8, 9_b) = 6.8, J (9_a, 9_b) = − 8.8. ^{13}C NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 200.21 (C=O, C-4), 170.72, 170.16 (2 C=O, CH_3CO), 166.61 (C=O, C-1), 108.79 [$C(CH_3)_2$], 100.09 (C-2), 73.86, 72.67, 69.71 (C-6, C-7, C-8), 66.52 (C-9), 55.15 (C-5), 52.64 ($COOCH_3$), 51.34 (OCH), 48.11 (C-3), 26.42, 25.30 (2 CH_3CO). MS (70 eV, 140°C): *m/z* (%) = 402 (5.7%) = [M^+-CH_3].

Methyl-5-acetamido-4,7-O-diacetyl-8,9-O-(1'-methyl-ethylidene)-3,5-dideoxy-3,4-didehydro-β-D-manno-2-nonulopyranosidonic Acid Methyl Ester (**3**)

163 mg (0.43 mmol) **2a** were dissolved in 3 ml pyridine, 3 ml acetic anhydride were added and the solution was allowed to stand overnight at room temperature. Removal of pyridine (0.01 Torr, 40°C) and subsequent flash-chromatography (10 g silica, ethylacetate: *n*-hexane/9 : 1) yielded 108 mg (0.24 mmol, 54.7%) of enolacetate **3**. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.28, 1.35 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.92, 2.09, 2.12 (3 s, 3 × 3 H, 2 CH_3CO), 3.31 (s, 3 H, OCH_3), 3.79 (s, 3 H, $COOCH_3$), 3.90 (dd, 1 H, 9-H_a), 4.02 (dd, 1 H, 9-H_b), 4.30 (dd, 1 H, 6-H), 4.37 (ddd, 1 H, 8-H), 4.77 (ddd, 1 H, 5-H), 5.13 (dd, 1 H, 7-H), 5.49 (d, 1 H, N—H), 5.75 (d, 1 H, 3-H); J (3, 5) = 2.2, J (5, N—H) = 9.8, J (5, 6) = 10.4, J (6, 7) = 2.2, J (7, 8) = 7.4, J (8, 9_a) = 6.5, J (8, 9_b) = 6.1, J (9_a, 9_b) = − 8.7. MS (70 eV, 140°C): m/z = 444 (2.62) = [M^+-CH_3], 400 (10.89) = [M^+-$C_2H_3O_2$]. $C_{20}H_{29}NO_{11}$ (459.4). Calcd. C 52.29, H 6.36, N 3.05; found C 51.94, H 6.47, N 2.98.

Methyl-5-acetamido-8,9-O-(1'-methyl-ethylidene)-3,5-dideoxy-β-D-glycero-D-talo-nonulopyranosidonic Acid Methyl Ester (**4a**)

A solution of 1.08 g (2.88 mmol) of compound **2a** in 42 ml of methanol was cooled in an ice-bath to 0°C for 15 min. Then 184 mg $BH_3 \cdot NH_3$ (5.9 mmol) were added and the reaction was monitored by TLC: ethyl acetate; R_f (**4a**) = 0.14, R_f (**1**) = 0.10, R_f (**2a**) = 0.34. After 20 min the reaction was finished and methanol was evaporated at 0°C under reduced pressure. Subsequent flash-chromatography (ethyl acetate, 40 g silica) yielded 780 mg (2.07 mmol, 71.6%) of **4a** and 60 mg (0.15 mmol, 7.6%) of **1** both ascolorless foams. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.32, 1.40 [2 s, 2 × 3 H, $C(CH_3)_2$], 2.03 (dd, 1 H, 3-H_{ax}), 2.08 (s, 3 H, CH_3CO), 2.40 (dd, 1 H, 3-H_{equ}), 3.38 (s, 3 H, OCH_3), 3.46 (d, 1 H, 7-H), 3.76–4.09 (m, 4 H, 4-H, 5-H, 6-H, 9-H_a), 3.79 (s, 3 H, $COOCH_3$), 4.17 (dd, 1 H, 9-H_b), 4.39 (ddd, 1 H, 8-H), 6.37 (d, 1 H, N—H); J (3_{ax}, 3_{equ}) = − 14.5, J (3_{ax}, 4) = 2.7, J (3_{equ}, 4) = 2.5, J (4, 5) = n.d., J (5, N—H) = 8.1, J (5, 6) = n.d., J (6, 7) = 1.5, J (7, 8) = 8.4, J (8, 9_a) = 7.9, J (8, 9_b) = 5.6, J (9_a, 9_b) = − 8.7. ^{13}C NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 172.16, 172.02 (2 C=O, CH_3CO), 167.54 (C=O, C-1), 108.94 [$C(CH_3)_2$], 99.13 (C-2), 74.04 (C-8), 70.61, 68.24 (C-6, C-7), 68.08 (C-9), 67.00 (C-4), 52.61 ($COOCH_3$), 51.54 (OCH_3), 47.98 (C-5), 37.25 (C-3), 27.08, 25.45 [$C(CH_3)_2$], 23.06 (CH_3CO). MS (70 eV, 150°C): m/z (%) = 362 (13.25) = [M^+-CH_3]. $C_{16}H_{27}NO_9$ (377.4). Calcd. C 50.92, H 7.21, N 3.71; found C 50.62, H 7.24, N 3.66.

Methyl-5-acetamido-4,7-di-O-acetyl-8,9-O-(1'-methyl-ethylidene)-3,5-dideoxy-β-D-glycero-D-talo-nonulopyranosidonic Acid Methyl Ester (**4b**)

To a solution of 25 mg (0.066 mmol) of compound **4a** in 1 ml of pyridine 1 ml of acetic anhydride and 4 mg of *DMAP* were added and stirred overnight at room temperature. The solvents were removed in vacuum (0.01 Torr). The yellow residue was purified by flash-chromastography (ethyl acetate, 2 g silica) yielding 26 mg (0.056 mmol, 84.8%) of **4b**. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.32, 1.38 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.96 (ddd, 1 H, 3-H_{ax}), 2.13 (s, 3 H, CH_3CO), 2.58 (dd, 1 H, 3-H_{equ}), 3.27 (s, 3 H, OCH_3), 3.80 (s, 3 H, $COOCH_3$), 3.93 (dd, 1 H, 9-H_a), 4.06 (dd, 1 H, 9-H_b), 4.23 (dd, 1 H, 6-H), 4.34–4.46 (m, 2 H, 5-H, 8-H), 5.36 (dd, 1 H, 7-H), 5.66 (d, 1 H, N—H); J (3_{ax}, 3_{equ}) = − 15.4, J (3_{ax}, 4) = 3.1, J (3_{equ}, 4) = 2.2, J (4, 5) = 3.0, J (5, N—H) = 9.3, J (5, 6) = 10.1, J (6, 7) = 2.0, J (7, 8) = 6.8, J (8, 9_a) = 7.5, J (8, 9_b) = 5.8, J (9_a, 9_b) = − 8.3. MS (70 eV, 140°C): m/z (%) = 446 (3.71) = [M^+-CH_3]. $C_{20}H_{31}NO_{11}$ (461.5). Calcd. C 52.06, H 6.77, N 3.04; found C 51.84, H 6.84, N 2.89.

5-Acetamido-3,5-dideoxy-D-glycero-D-talo-nonulosonic Acid (**5**)

800 mg (2.08 mmol) of **4a** were dissolved in 15 ml 1 *m* NaOH and heated for 120 min on 40°C, neutralized with Amberlyst 15 H^+ to pH = 3. The ion exchange resin was filtered off and water was

evaporated under reduced pressure. The residue was dissolved in 30 ml of 0.025 m HCl and 4 g of Amberlyst 15 H^+ were added. Heating for 100 min on 80°C, let cool down, filtration and separation on 30 g of Dowex 1 × 8 $HCOO^-$ by a gradient of 0–1 *N* HCOOH (1 000 ml), removal of solvent at 30°C under reduced pressure and lyophilization led to 502 mg (1.62 mmol, 78%) of **5**. $C_{11}H_{19}NO_9$ (309.3). Calcd. C 42.72, H 6.19, N 4.52; found C 41.82, H 6.44, N 4.22.

Methyl-5-acetamido-4,7,8-tri-O-acetyl-9-O-t-butyldimethylsilyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (**7**)

1.5 g **6** (4.45 mmol), 757 mg imidazole (11.1 mmol), 1.5 g 3 Å molecular sieve were dried together (3 h, 0.001 Torr), dissolved in 17 ml *DMF* abs. and cooled to 0°C. After 10 minutes 805 mg (5.34 mmol) *t*-butyldimethylsilylchloride was added and let come to room temperature. Molecular sieve was filtered off and *DMF* removed at reduced pressure (40°C, 0.01 Torr). The yellow residue was dissolved in a mixture of 25 ml of diethyl ether and 20 ml of water. The water-phase was extracted 5 times more with 25 ml portions of ether. The combined organic layers were dried with Na_2SO_4. Removal of ether under reduced pressure yielded 1.88 g of a yellow foam, which was acetylated by means of 5 ml pyridine, 5 ml acetic anhydride and 20 mg of *DMAP* overnight. Distilling pyridine off (0.01 Torr 30°C) gave a brown residue, which was purified by flash-chromatography ethyl acetate: *n*-hexane (4 : 1), 80 g silica. In this way 2.0 g (3.47 mmol, 78%) of **7** were obtained as a white foam. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): $\delta = -0.01$, 0.01 (2 s, 2 × 3 H, 2 × CH_3Si), 0.85 [s, 9 H, $(CH_3)_3CSi$], 1.84 (dd, 1 H, 3-H_{ax}), 1.85, 1.98, 2.04, 2.10 (4 s, 4 × 3 H, CH_3CO), 2.40 (dd, 1 H, 3-H_{equ}), 3.22 (s, 3 H, OCH_3), 3.67 (dd, 1 H, 9-H_a), 3.78 (s, 3 H, $COOCH_3$), 3.92 (dd, 1 H, 6-H), 4.03 (ddd, 1 H, 5-H), 4.08 (dd, 1 H, 9-H_b), 5.07 (ddd, 1 H, 4-H), 5.25 (ddd, 1 H, 8-H), 5.34 (dd, 1 H, 7-H), 5.41 (d, 1 H, N—H); $J(3_{ax}, 3_{equ}) = -13.7$, $J(3_{ax}, 4) = 11.4$, $J(3_{equ}, 4) = 5.1$, $J(4, 5) = 10.2$, $J(5, \text{N—H}) = 10.0$, $J(5, 6) = 10.5$, $J(6, 7) = 1.5$, $J(7, 8) = 4.9$, $J(8, 9_a) = 7.0$, $J(8, 9_b) = 2.6$, $J(9_a, 9_b) = -11.4$. ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 170.98$ (2 C = O, 2 × *Ac*), 170.58, 170.21 (2 C = O, 2 × *Ac*), 167.56 (C = O, C-1), 99.03 (C-2), 74.90, 71.66, 69.18, 68.85 (C-4, C-6, C-7, C-8), 61.57 (C-9), 52.58 ($COOCH_3$), 51.22 (OCH_3), 49.50 (C-5), 37.47 (C-3), 25.77 [3 C, $C(CH_3)_3$], 23.01, 21.06, 20.85, 20.80 (4 C, 4 × CH_3CO), 18.21 [$C(CH_3)_3$], −5.39, −5.47 (2 $SiCH_3$). MS (70 eV, 150°C): *m/z* (%) = 520 (9.91) = [M^+-C_4H_9]. $C_{25}H_{43}NO_{12}Si$ (577.7). Calcd. C 51.98, H 7.50, N 2.42; found C 51.40, H 7.74, N 2.31.

Methyl-5-acetamido-4,7,9-tri-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (**8**)

A solution of 1.8 g (3.12 mmol) **7** in 10 ml of *THF* was stirred 1 min with 57 ml 0.1 *m* HCOOH in *THF*. 15.5 ml of 0.5 m *TBAF* in *THF* (7.75 mmol) were added and stirred at 25°C. The reaction was monitored by TLC: ethyl acetate; R_f(**7**) = 0.38, R_f(**8**) = 0.16. After 72 h and removal of *THF* a yellow oil was obtained. Flash-chromatography (60 g silica, ethylacetate) yielded 1.3 g **8** (2.805 mmol, 90%) as a colorless foam. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): $\delta = 1.90$ (dd, 1 H, 3-H_{ax}), 1.91, 2.04, 2.11, 2.17 (4 s, 4 × 3 H, CH_3CO), 2.45 (dd, 1 H, 3 H_{equ}), 3.33 (s, 3 H, OCH_3), 3.83 (s, 3 H, $COOCH_3$), 4.06–4.30 (m, 5 H, 5-H, 6-H, 8-H, 9-H_a, 9-H_b), 5.07 (dd, 1 H, 7-H), 5.27 (ddd, 1 H, 4-H), 5.66 (d, 1 H, N—H); $J(3_{ax}, 3_{equ}) = -13.0$, $J(3_{ax}, 4) = 11.6$, $J(3_{equ}, 4) = 5.2$, $J(4, 5) = 11.9$, $J(5, \text{N—H}) = 9.0$, $J(5, 6)$ = n.d., $J(6, 7) = 1.2$, $J(7, 8) = 7.3$, $J(8, 9_a) = J(8, 9_b) = J(9_a, 9_b)$ = n.d. ^{13}C NMR (62.9 MHz, $CDCl_3$/*TMS*): $\delta = 171.26$, 171.00, 170.25, 170.32 (4 C = O, 4 × *Ac*), 167.57 (C = O, C-1), 98.72 (C-2), 70.61, 69.63, 69.18, 68.45 (C-4, C-6, C-7, C-8), 65.95 (C-9), 52.59 ($COOCH_3$), 51.37 (OCH_3), 49.23 (C-5), 37.36 (C-3), 22.96, 20.83, 20.80, 20.73 (4 CH_3CO). MS (70 eV, 150°C): *m/z* (%) = 404 (9.91) = [M^+-$C_2H_3O_2$]. $C_{19}H_{29}NO_{12}$ (463.4). Calcd. C 49.24, H 6.31, N 3.02; found C 47.69, H 6.30, N 2.78.

Methyl-5-acetamido-4,7,9-tri-O-acetyl-3,5-dideoxy-β-D-galacto-2,8-nonodiulopyranosidonic Acid Methyl Ester (**9**)

1.55 g KIO_4 (6.74 mmol) and 448 mg $RuO_2 \cdot H_2O$ (3.37 mmol) were dissolved in 60 ml water and stirred for 10 min. This solution was extracted 5 times with 10 ml of $CHCl_3$. The combined organic

layers were added to a vigorous stirred solution of 1.2 g (2.58 mmol) of compound **8** dissolved in 10 ml $CHCl_3$. The reaction was monitored by TLC: ethyl acetate; R_f(**8**) = 0.16, R_f(**9**) = 0.32. After 30 min the reaction has been completed and excess RuO_4 was destroyed by addition of 0.5 ml 2-propanol and stirred further 10 min. Filtration over celite, washing three times with 10 ml of $CHCl_3$ and evaporation of solvent gave a colorless residue, which was purified by flash-chromatography (30 g silica, ethyl acetate: *n*-hexane = 9 : 1). Yield: 1.08 g **9** (2.34 mmol, 91%) colorless foam. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.89 (dd, 1 H, 3-H_{ax}), 1.90, 2.05, 2.16, 2.31 (4 s, 4 × 3 H, CH_3CO), 2.44 (dd, 1 H, 3-H_{equ}), 3.10 (s, 3 H, OCH_3), 3.81 (s, 3 H, $COOCH_3$), 4.00 (dd, 1 H, 6-H), 4.23 (ddd, 1 H, 5-H), 4.78 (d, 1 H, 9-H_a), 5.09 (d, 1 H, 9-H_b), 5.26 (d, 1 H, 7-H), 5.27 (ddd, 1 H, 4-H), 5.66 (d, 1 H, N—H); $J(3_{ax}, 3_{equ})$ = − 13.4, $J(3_{ax}, 4)$ = 10.7, $J(3_{equ}, 4)$ = 5.2, $J(4, 5)$ = 10.7, $J(5, N—H)$ = 9.5, $J(5, 6)$ = 10.5, $J(6, 7)$ = 2.2, $J(9_a, 9_b)$ = − 17.9. ^{13}C NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 201.40 (C=O, C-8), 170.54, 170.51, 170.46, 169.63 (4 C=O, CH_3CO), 167.03 (C=O, C-1), 98.38 (C-2), 75.08 (C-7), 72.68 (C-6), 68.32 (C-4), 66.92 (C-9), 52.35 ($COOCH_3$), 50.89 (OCH_3), 48.09 (C-5), 37.05 (C-3), 22.48, 20.48, 20.38, 19.97 (4 CH_3CO). MS (70 eV, 150°C): m/z (%) = 402 (17.0) = [M^+-$C_2H_3O_2$]. $C_{19}H_{27}NO_{12}$ (461.4). Calcd. C 49.46, H 5.90, N 3.04; found C 48.69, H 6.02, N 2.71.

Methyl-5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-L-glycero-D-galacto-2-nonulopyranosidonic Acid Methyl Ester (**10**)

To a cooled solution (0°C) of 1 g (2.17 mmol) **9** 80 mg $BH_3 \cdot NH_3$ were added and the reaction mixture was stirred for 1 h at 0°C. The solvent was removed under reduced pressure yielding a colorless residue. After drying the solid 1 h (0.01 Torr, rt.) 5 ml pyridine, 5 ml acetic anhydride and 20 mg *DMAP* were added. The resulting yellow solution was allowed to stand overnight at room temperature. A yellow oil was obtained after distilling the volatile components off (0.01 Torr, 40°C). Purification was achieved by flash-chromatography (50 g silica, ethyl acetate) yielding 960 mg (1.90 mmol, 87.5%) of compound **10**. TLC: ethyl acetate; R_f(**10**) = 0.25. ^{1}H NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.90 (s, 3 H, CH_3CO), 1.91 (dd, 1 H, 3-H_{ax}), 2.05, 2.06, 2.11 (3 s, 3 × 3 H, CH_3CO), 2.64 (dd, 1 H, 3-H_{equ}), 3.35 (s, 3 H, OCH_3), 3.84 (s, 3 H, $COOCH_3$), 3.91 (dd, 1 H, 6-H), 4.20 (ddd, 1 H, 5-H), 4.46 (ABX-system, 2 H, 2 × 9-H), 5.26 (dd, 1 H, 7-H), 5.27 (ddd, 1 H, 4-H), 5.37 (d, 1 H, N—H), 5.47 (ddd, 1 H, 8-H); $J(3_{ax}, 3_{equ})$ = − 13.4, $J(3_{ax}$, 4) = 10.5, $J(3_{equ}, 4)$ = 5.0, $J(4, 5)$ = 10.5, $J(5, N—H)$ = 9.5, $J(5, 6)$ = 10.0, $J(6, 7)$ = 2.0, $J(7, 8)$ = 6.7, $J(8, 9_a)$ = 4.2, $J(8, 9_b)$ = 6.5, $J(9_a, 9_b)$ = n.d. ^{13}C NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 170.80, 170.53, 170.10, 169.98, 169.42 (5 C=O, CH_3CO), 167.05 (C=O, C-1), 98.75 (C-2), 70.83 (C-6), 69.91 (C-8), 68.74 (C-7), 63.30 (C-9), 52.42 ($COOCH_3$), 51.36 (OCH_3), 49.08 (C-5), 37.10 (C-3), 22.78 (CH_3CO), 20.57 (4 C, CH_3CO). MS (70 eV, 140°C); m/z (%) = 446 = [M^+-$C_2H_3O_2$]. $C_{21}H_{31}NO_{13}$ (505.5). Calcd. C 49.90, H 6.18, N 2.77; found C 49.52, H 6.24, N 2.83.

Sodium Methyl-5-acetamido-3,5-dideoxy-β-L-glycero-D-galacto-2-nonulopyranosidonat (**11**)

269 mg (0.53 mmol) **10** dissolved in 1 ml of CH_3OH abs. was reacted with 6 ml of 0.1 *M* CH_3O Na in CH_3OH overnight at 4°C. Solvent was removed and the residue was dissolved in 2 ml of methanol and evaporated twice. After stirring 10 min with 2 ml of water the solution was neutralized by CO_2 (s). Treatment with charcoal, filtration over celite and lyophilization yielded 155 mg (0.43 mmol, 84.6%) of methylketoside **11**. ^{1}H NMR (250 MHz, D_2O/HDO = 4.80, 298 K): δ = 1.63 (dd, 1 H, 3-H_{ax}), 2.02 (s, 3 H, CH_3CO), 2.31 (dd, 1 H, 3-H_{equ}), 3.18 (s, 3 H, $COOCH_3$), 3.48–3.66 (m, 3 H, 5-H, 7-H, 9-H_a), 3.70 (dd, 1 H, 9-H_b), 3.76–4.03 (m, 3 H, 4-H, 6-H, 8-H); $J(3_{ax}, 3_{equ})$ = − 13.5, $J(3_{ax}, 4)$ = 11.2, $J(3_{equ}, 4)$ = 4.9, $J(8, 9_b)$ = 3.2, $J(9_a, 9_b)$ = − 12.0, $J(4, 5)$ = $J(5, 6)$ = $J(6, 7)$ = $J(7, 8)$ = $J(8, 9_b)$ = n.d. ^{13}C NMR (62.9 MHz, D_2O/1,4-dioxane): δ = 175.66, 175.55 (2 C=O, C-1, CH_3CO), 101.53 (C-2), 73.34, 73.26 (C-6, C-8), 69.17 (C-7), 67.51 (C-4), 63.40 (C-9), 53.24 (C-5), 51.72 (OCH_3), 40.57 (C-3), 23.07 (CH_3CO). $C_{12}H_{20}NNaO_9$ (345.3). Calcd. C 41.74, H 5.84, N 4.06; found C 40.14, H 6.10, N 3.84.

5-Acetamido-3,5-dideoxy-L-glycero-D-galacto-2-nonulosonic Acid (**12**)

Method A: 400 mg (1.06 mmol) **10** were treated analogously to **4a** yielding 229 mg (0.74 mmol).

Method B: 60 mg (0.17 mmol) of **11** dissolved in 0.025 *M* HCl were heated 150 min on 80°C in the presence of 1 g Amberlyst 15 H^+. The ion-exchange resin was filtered off and the solution was treated with charcoal. Further filtration over celite yielded 40 mg (0.13 mmol, 76%) of the free acid **12**. $C_{11}H_{19}NO_9$ (309.3). Calcd. C 42.72, H 6.19, N 4.52; found C 41.50, H 6.52, N 4.38.

References

[1] Previous commun. (no. 11): Zbiral E., Schreiner E., Salunkhe M. M., Schulz G., Kleineidam R. G., Schauer R. (1989) Liebigs Ann. Chem.: 589
[2] Schauer R. (1982) Cell Biology Monographs, Vol. 10. Springer, Wien New York
[3] Schauer R. (1982) Adv. Carbohydr. Chem. Biochem. **40**: 132
[4] Brandstetter H. H., Zbiral E. (1983) Liebigs Ann. Chem.: 2055; Gross H. J., Kovac A., Rose U., Watzlawik H., Brossmer R. (1988) Biochemistry **27**: 4279
[5] Zbiral E., Brandstetter H. H. (1985) Monatsh. Chem. **116**: 87
[6] Petrik C. R., Korytnyk W. (1981) Anal. Biochem. **131**: 153; Brossmer R., Rose U., Kasper D., Smith T. L., Grasmuk H., Unger F. M. (1980) Biochem. Biophys. Res. Commun. **96**: 1282; Gross H. J., Bunsch A., Paulsen J. C., Brossmer R. (1987) Eur. J. Biochem. **168**: 595
[7] Baumberger F., Vasella A. (1986) Helv. Chim. Acta **69**: 1205
[8] Courtney J. L. (1986) Ruthenium Tetroxide Oxidations. In: Mijs W. J., de Jonge C. R. H. I. (ed.) Organic Synthesis by Oxidation with Metal Compounds. Plenum Press, New York, p. 464
[9] Salunkhe M. M., Hartmann M., Schmid W., Zbiral E. (1988) Liebigs Ann. Chem.: 187
[10] Babirad S. A., Wong Y., Kishi Y. (1987) J. Org. Chem. **52**: 1370
[11] Brandstetter H. H., Zbiral E., Schulz G. (1982) Liebigs Ann. Chem.: 1
[12] Reuter G., Schauer R. (1988) Glycoconjugate J. **5**: 123; Zbiral E., Schreiner E., Salunkhe M. M., Schulz G., Kleineidam R. G., Schauer R. (1989) Liebigs Ann. Chem.: 159
[13] Zilliken F., O'Brien P. J. (1960) Biochem. Prep. **7**: 1; Martensson E., Raal A., Svennerholm L. S. (1958) Biochim. Biophys. Acta **30**: 124
[14] Czarniecki M. F., Thornton E. R. (1977) J. Am. Chem. Soc. **99**: 8273
[15] Kuhn R., Lutz P., MacDonald D. L. (1966) Chem. Ber. **99**: 611
[16] Still W. C., Kahn M., Mitra A. (1978) J. Org. Chem. **43**: 2923
[17] Christian R., Schulz G., Brandstetter H. H., Zbiral E. (1987) Carbohydr. Res. **162**: 1

Received April 7, 1989. Accepted April 21, 1989