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Structural Variations of N-Acetylneuraminic Acid, Part 19 [1]: Synthesis of Both Epimeric Pairs of the 4-C-Methyl- and 4-Deoxy-4-C-methyl- as Well as of the β-Methylketoside of 4-Deoxy-4-C-methylene-N-acetylneuraminic Acid

Behaviour Towards CMP-Sialate Synthase

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Summary. While the reaction of the 4-oxo-Neu 5 Ac derivative **2 a** with tributoxy methyl zirconate led exclusively to equatorial 4-C-methyl derivative **3 a**, the analogous reaction with tetramethyl zirconate yielded a 3 : 2 mixture of both diastereoisomeres **3 a** and **4 a**. After removal of protecting groups the 5-acetamido-3,4-dideoxy-4-C-methyl-*D*-glycero-*D*-galacto-2-nonulosonic acid **5 a** and 5-acetamido-3,4-dideoxy-4-C-methyl-*D*-glycero-*D*-talo-2-nonulosonic acid **6 a** were obtained. The 4-C-methylene derivative was prepared by treatment of the same 4-oxo-derivative with $CH_2I_2/Zn/Cp_2ZrCl_2$. Subsequent hydrogenation led to both epimeric 4-deoxy-4-C-methyl derivatives **8 a** and **9 a**. Final removal of protecting groups gave the 5-acetamido-3,4,5-trideoxy-4-C-methyl-*D*-glycero-*D*-galacto-2-nonulosonic acid **10 a** respectively the 5-acetamido-2,7-anhydro-4-C-methyl-3,4,5-trideoxy-*D*-glycero-*D*-talo-2-nonulosonic acid **11 a**. The β-methylketosides of the 4-deoxy-4-C-methyl- (**16**) and 4-C-methylene-Neu 5 Ac (**15**) were prepared via the peracetylated derivatives to obtain modell substrates for enzymatic studies. Thus all free acids were tested for inhibition of CMP-sialate synthase. Only the 4-C-methylene compound **15** showed most unexpectedly a strong competitive inhibition of this enzyme.

Keywords. Sialic acids; Methyl-branched sugars; Zirconium organyls.

Strukturelle Abwandlungen an N-Acetylneuraminsäure, 19. Mitt.: Synthese der beiden Epimerenpaare der 4-C-Methyl- und 4-Deoxy-4-C-methyl- sowie des β-Methylketosids der 4-Deoxy-4-C-methylen-N-acetylneuraminsäure. Verhalten gegenüber CMP-Sialat-Synthase

Zusammenfassung. Während die Umsetzung des 4-Oxoderivates **2 a** mit $(BuO)_3MeZr$ ausschließlich zur equatorialen 4-C-Methylverbindung **3 a** führt, wurde bei der Reaktion mit Me_4Zr ein 3 : 2-Gemisch der beiden Diastereomeren **3 a** und **4 a** erhalten. Das 4-C-Methylenderivat **7 a** wurde durch Reaktion derselben 4-Oxoverbindung mit $CH_2I_2/Zn/Cp_2ZrCl_2$ erhalten. Eine anschließende Hydrierung (H_2-Pd/C) führte zu einem trennbaren Gemisch der beiden 4-Deoxy-4-C-methylderivative **8 a** und **9 a**. Diese Verbindungen konnten durch das Entfernen der Schutzgruppen einerseits in die 5-Acetamido-3,4,5-trideoxy-4-C-methyl-*D*-glycero-*D*-galacto-2-nonulosonsäure **10 a** und 5-Acetamido-2,7-anhydro-4-C-methyl-3,4,5-tridoxy-*D*-glycero-*D*-talo-2-nonulosonsäure **11 a** umgewandelt werden. Die

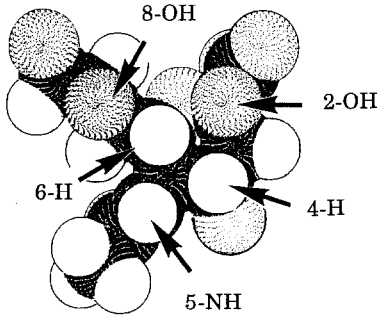
Verbindungen Methyl-5-acetamido-4-C-methylen-3,4,5-trideoxy-β-*D*-manno-2-nonulopyranosidonat (**15**) und Methyl-5-acetemido-4-C-methyl-3,4,5-tridoxy-β-*D*-glycero-*D*-talo-2-nonulopyranosidonat (**16**) wurden als Modellverbindungen für enzymatische Untersuchungen über peracetylierte Zwischenstufen hergestellt. Überraschenderweise zeigte nur die 4-C-Methylenverbindung **15** eine starke kompetitive Hemmung gegenüber CMP-Sialat-Synthase.

Introduction

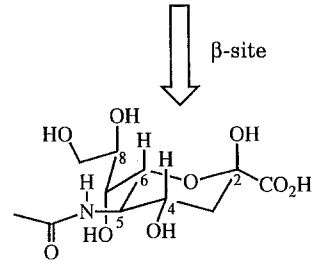
N-Acetylneuraminic acid **1 b** and its various derivatives – the sialic acids – are widespread in the animal kingdom [2, 3]. As terminal units of glycoproteins and glycolipids they are involved in a variety of biochemical and biophysical processes. This is the obvious reason for the synthesis of sialic acid analogues and the study of their behaviour towards the enzymes of the sialic acid metabolism.

In a series of publications we were able to evaluate the essential parameters for the recognition of sialic acids by CMP-sialate synthase [EC 2.7.7.43] [4–9], sialidases [EC 3.2.1.18] [10–12], and acylneuraminat lyase [EC 4.1.3.3.] [13, 14].

Especially the structural conditions for the activation by CMP-sialate synthase could be understood very clearly [4–9]. Thus, if we look at the β-face (see Fig. 1), the positions 2,5, and 8 require nearly coaxially orientated hydrophilic functional groups (the β-oriented anomeric 2-OH, 5-NH respectively 5-OH, and 8-OH), whereas the positions 4 and 6 are occupied by the hydrophobic hydrogens. All structural variations leading to a change of this relation – three hydrophilic to two hydrophobic centers – are not accepted by this enzyme. According to this rule the 4-OH_{eq}-group is not necessary for the recognition. It can be exchanged by hydrogen without influence on the activation [14]. In this connection an additional question arose: What happens if the equatorial 4-OH group is substituted by the more bulky as well as most hydrophobic methyl group? Furthermore it was interesting to investigate the behaviour of a sialic acid analogue bearing an axial 4-methyl group instead of the aforementioned necessary hydrophobic axial 4-H towards CMP-sialate synthase. Though there is one report [15] about a 4-C-methyl branched natural occurring sialic acid **1 a** (Scheme 1), no synthetic route was published until now. Therefore we undertook the syntheses of a series of 4-C-methyl branched sialic acids in order to investigate the influence of the 4-position on the activation of sialic acids by CMP-sialate synthase.

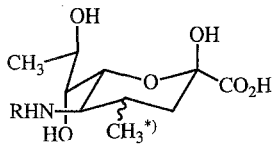


CPK-Model of
N-Acetylneuraminic Acid
(look from the β-site)



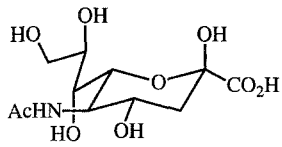
N-Acetylneuraminic Acid

Fig. 1



1a

R = $CH_3CO_2CH_2CO$
*) stereochemistry not assigned



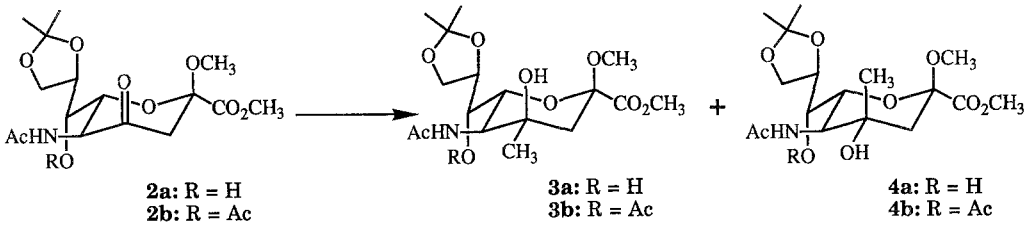
1b

N-Acetylneuraminic Acid

Scheme 1

Results and Discussion

Our first synthetic target was a 4-C-methyl branched derivative with an hydroxyl function at the branching point. A lot of such transformations has been carried out in the field of carbohydrate chemistry by reacting glycosiduloses with carbon nucleophiles [16]. Especially diazomethane [17, 18], Grignard- [19–22] and organo lithium compounds [23, 24] were employed. For our problem we selected the 4-oxo derivative **2b** as carbonyl compound. As mentioned earlier [25] we observed an easy enolization of this ketone. Therefore it was not unexpected that first experiments with usual methyl nucleophiles were unsuccessful. Thus we applied the cerium variants [26–30] of the Grignard and lithium organyls which are recommended to suppress the enolization. But also in this case only starting material was recovered after work up.



2a: R = H
2b: R = Ac

3a: R = H
3b: R = Ac

4a: R = H
4b: R = Ac

2a (2b)	3a (3b) : 4a (4b)	
$ZrMe_4$	60	40
$(BuO)_3ZrMe$	100	0

Scheme 2

Thus we decided to apply methyl zirconium compounds [31], which should even react with highly enolizable ketones. One method recently described [32] applied tetramethyl zirconium in a mixture of toluene and ether [33]. Using this procedure we obtained a separable mixture of **3b** and **4b**, but also small amounts of **3a** and **4a** were isolated (Scheme 2). After reacetylation of the mixture we isolated only small total amounts of **3b** and **4b**. An in situ preparation of tetramethyl

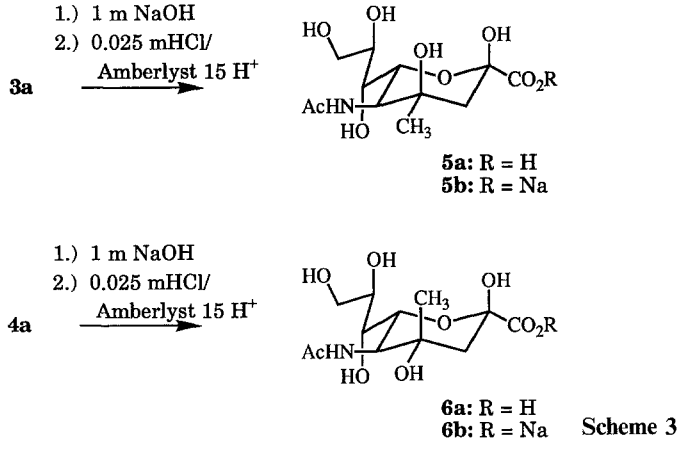
zirconate in *THF* and subsequent reaction increased the yields markedly. Furthermore we observed that it was not necessary to protect the 7-OH-group since the reaction of **2a** in *THF* gave also a 3:2 mixture of **3a** and **4a** in comparable yield. In order to increase the diastereoselectivity of this reaction we choose the more suitable tributoxy methyl zirconium [34, 35]. In the case of **2a** this transformation yielded only **3a**, whereas the same conditions applied on **2b** led to a mixture of **3a** and **3b**. Nevertheless this mixture was acetylated to yield **3b** as a single product.

The unambiguous assignment of the configuration at the 4-C of **3b** (methyl equatorial, i.e.: *D*-gylcero-*D*-talo) and **4b** (methyl axial, i.e.: *D*-glycero-*D*-galacto) could be achieved as follows:

(a) All relevant coupling constants gave clear evidence that both diastereoisomers existed in the $^2C_5$-conformation. A NOE-experiment on compound **3b** with irradiation of the 4-methyl group gave a response at the signals of the 5-H and both 3-H's corresponding to a gauche interaction. This findings are only compatible with an equatorially oriented methyl group. Furthermore irradiation of the methyl group of **4b** led to a NOE effect on the 6-H, N-H, and somewhat less intensive on 3-H_{ax}. This confirms an axial orientation of this methyl group.

(2) ^{13}C-nmr spectra confirmed this assignment, as for the 4-C-methyl group a downfield shift of approximately 3.2 ppm to 26.05 ppm was found for compound **3b** in contrast to 22.83 ppm for **4b** [36]. Furthermore the branching carbon of the *D*-glycero-*D*-talo compound **3b** expectedly absorbed at higher field (70.22 ppm) as its diastereomer **4b** (71.22 ppm) [37].

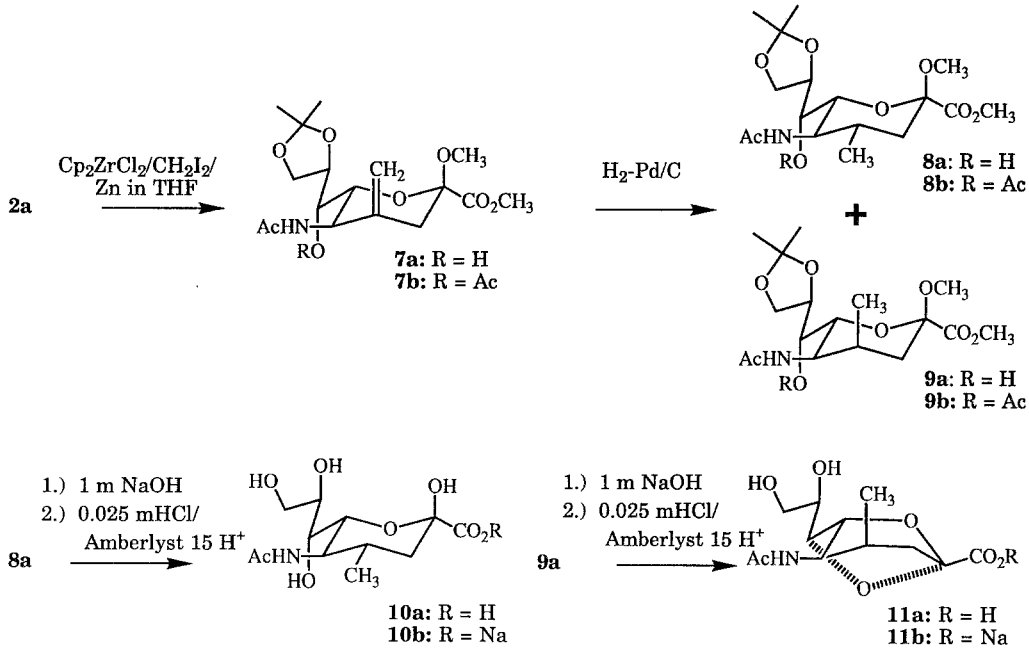
(3) According to an old rule in carbohydrate chemistry [38] we found that compounds with axial hydroxyl and equatorial methyl group showed a higher R_f-value on silica gel (TLC) than the other epimer.



Scheme 3

Finally the 5-acetamido-3,5-dideoxy-4-C-methyl-*D*-glycero-*D*-talo-2-nonulosonate (**5b**) and 5-acetamido-3,5-dideoxy-4-C-methyl-*D*-glycero-*D*-galacto-2-nonulosonate (**6b**) could be isolated as their sodium salts after treatment of **3b** respectively **4b** with 1*M* NaOH followed by Amberlyst 15 H^+ and 0.025 *M* HCl followed by passing the free acids **5a** and **6a**, respectively, through a column filled with Dowex 50 Na^+ (Scheme 3).

A direct transformation of **3b** as well as **4b** into the corresponding 4-deoxy-4-C-methyl neuraminates via the envisaged *p*-tolylthionocarbonyl derivatives succeeded by tributyltin hydride could not be achieved, obviously due to steric hindrance. Therefore we changed the strategy and planned the synthesis of the 4-C-methylene derivative **7a** as a versatile intermediate for further transformations [39] starting with ketones **2a** or **2b** [25, 40]. Remarkably, the usual olefination conditions according to Wittig [41] and Peterson [42, 43] (as well as the modified form with cerium(III) chloride [44]) were unsuccessful, obviously due to the aforementioned tendency of enolization. The application of Tebbe's reagent [45] was to be renounced due to the present ester group.



Scheme 4

Other comparable problems were described in 1978 when methylenation of enolizable ketones was performed by systems like CH_2I_2-Zn-Me_3Al and CH_2Br_2-Zn-$TiCl_4$ [46–48]. Recently these methods were improved by the application of Cp_2ZrCl_2 on similar problems [49]. Therefore we treated ketone **2a** with CH_2I_2-Zn-Cp_2ZrCl_2 in *THF* and were successfully isolating 75% of the desired 4-C-methylene derivative **7a** [39]. Subsequent hydrogenation by means of H_2-Pd/C in *i*-propanol/acetone led to a 3:2 mixture of **8a** and **9a** which could be easily separated by flash chromatography (Scheme 4). The hydrogenation of the peracetylated derivative **12** too showed no increase in diastereoselectivity. In addition a separation of the diastereoisomers was not possible.

The removal of protecting groups from derivative **8a** led exclusively to the 5-acetamido-3,4,5-trideoxy-4-C-methyl-*D*-glycero-*D*-galacto-2-nonulosonic acid **10a** which was again transformed into its sodium salt **10b**.

Analogous reaction conditions applied to **9a** yielded exclusively the 2,7-anhydro product **11b**. This result was not unexpected since we had observed an ether

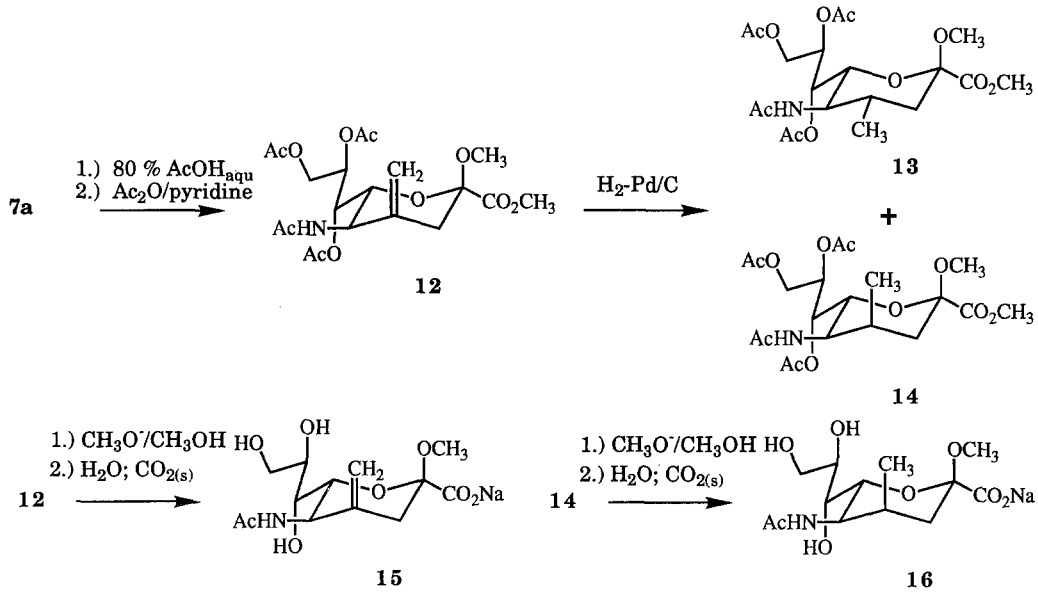
formation under conditions suitable for the cleavage of the β-methylketosides either quantitatively in the case of the 4-deoxy or up to 20% for the 4-*epi*-Neu 5 Ac [50].

(1) The assignment of the structue **11 b** is illustrated by the down field shift of 0.45 ppm of the 6-H (4.50 ppm) in contrast to the corresponding signal in the ^{1}H-nmr of Neu 5 Ac (4.05 ppm) and a severe change in the pattern of the ^{1}H-^{1}H-coupling constants ($J_{4,5}$ = 4.0 Hz and $J_{5,6}$ = 1.0 Hz).

(2) The 2-C resonance is shifted downfield from 95–98 ppm – typical for semiketals in the pyranose ring – to 107.68 ppm, indicating a 2,7-anhydro structure [51].

All experiments to remove the protecting groups from derivative **7 a** to furnish the corresponding free 4-C-methylene sialic acid led to a mixture of unidentified products.

Therefore we decided to prepare the more stable β-methylketosides of the free 4-C-methylene sialic acid and of the 4-d-4-C-methyl$_{ax}$-Neu 5 Ac, because we have learned from earlier results [5, 7] that β-methylketosides are suitable substrates to study the recognition by CMP-sialate synthase measuring the inhibition constants.



Scheme 5

In continuation of this program we started with the peracetylated derivative **12** and **14** and hydrolyzed it to the methyl-5-acetamido-4-C-methylene-3,4,5-trideoxy-β-*D*-manno-2-nonulopyranosidonate (**15**) and the methyl-5-acetamido-4-C-methyl-3,4,5-trideoxy-β-*D*-glycero-*D*-talo-2-nonulopyranosidonate (**16**), respectively (Scheme 5).

Experiments with CMP-Sialate Synthase

The compounds **5 b**, **6 b**, **10 b**, **15**, and **16** were tested as inhibitors of the activation of Neu 5 Ac to its CMP-derivative. Surprisingly the derivative **15** (K_i = 4.4 m*M*) was similar to the inhibition of the corresponding β-methylketoside of Neu 5 Ac

($K_i = 2.5$) [5]. In addition the significant lower inhibition of the 4-Oxo-Neu 5 Ac-β-methylketoside ($K_i = 19$ m*M*) may be recalled [7]. The compounds **5 b**, **6 b**, **10 b** [52], and **16** showed no inhibition within experimental error of the test.

Acknowledgement

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Experimental Part

Commercially available compounds were dried and used without further purification. Solvents were dried and distilled before use. All reactions, with exception of those in water, were conducted in oven dried (140 °C) or flame dried two- or three-necked flasks under an argon atmosphere. Addition of reagents and control of the progress of reactions (TLC) was achieved using syringes. Analytical thinlayer chromatography (TLC): Merck™ plates, silica gel 60 F_{254}, layer thickness 0.2 mm. 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: Merck™ silica gel 0.040–0.063 mm. ^1H NMR (250): Bruker WM 250, tetramethylsilane (*TMS*) as internal standard; the determined coupling constants are of first order. In the case of solutions in D_2O sodium 4,4-dimethyl-4-silapentanesulfate (*DSS*) in D_2O was used as internal reference, or spectra were correlated to HDO (4.80 ppm). ^{13}C NMR spectra (62.9 MHz); Bruker WM 250 instrument equipped with a 5 mm probe head. For solutions in D_2O (303 K) an external reference of tetramethylsilane (67.40 ppm upfield from the signal of 1,4-dioxane in D_2O) was used. N-Acetylneuraminic acid Neu5Ac **1 b** was prepared from edible birds nest glycoproteine [53]. Starting materials **2 a** and **2 b** were prepared as reported earlier [25].

General Procedure for Acetylation

Approximately 100 mg of the compound to be acetylated were dissolved in 1 ml of pyridine and 1 ml of Ac_2O and 5 mg of 4-(dimethylamino)pyridine was added. The reaction mixture was allowed to stand c. 12 h, then the solvents were removed under reduced pressure (0.01 Torr), and the crude material was purified as given in each protocol.

Activation Experiments of CMP-Sialate Synthase

The enzyme kinetics were studied as already described [4] using N-acetylneuraminic acid concentrations from 0.15 to 3.5 m*M*, β-methylketoside from 0–7 m*M*, 10 m*M* CTP and 10 mU per ml rat liver CMP-Sialate Synthase [EC 2.7.7.43]. Product formation was monitored using TBA test [3].

Methyl (Methyl 5-acetamido-3,5-dideoxy-4-C-methyl-8,9-O-(methyl-ethylidene)-β-D-glycero-D-talo-nonulopyranosid)onate (**3 a**)

Method A. 2.52 ml of a 1.23 *M* solution of tributoxymethyl zirconate in ether and 6 ml of anhydr. *THF* were stirred in an ice-bath at −10 °C. After addition of 1.8 ml of methyllithium solution (1.6 *M* in ether) the mixture was allowed to come to 0 °C within 1 h and 375 mg **2 a** (1 mmol) in 5 ml of *THF* was added. The solution was allowed to come to room temperature overnight. TLC (ethyl acetate): R_f(**2 a**) = 0.40, r_f(**3 a**) = 0.22. As no more starting material could be detected the reaction was quenched by addition of 40 ml of a NH_4Cl solution (1 : 1 $NH_4Cl_{sat.} - H_2O$) and diluted with 30 ml of ethyl acetate. The resulting mixture ways extracted three times with ethyl acetate and the combined organic layers were dried over Na_2SO_4 sicc. After removal of the solvents the resulting residue was purified

by flash-chromatography (20 g silica gel, ethyl acetate). Yield: 368 mg of compound **3a** (0.85 mmol, 84.9%).

Method B. In a 100 ml two-necked flask equipped with a condenser 593 mg $ZrCl_4$ (2.54 mmol) were carefully dried (0.01 Torr, 40 °C). After short cooling to 0 °C 28 ml of dry *THF* was added and the resulting mixture was heated at 50 °C until the whole precipitate was dissolved (approx. 10 min). Then the solution was cooled to −50 °C and 6 ml of a 1.6 *M* methyllithium solution in ether was added. This mixture was allowed to come to 0 °C within 30 min. The reaction was accompanied by the formation of a pale yellow color. Subsequent the solution was cooled to −78 °C and 480 mg (1.28 mmol) of **2a** in 8 ml of *THF* was added. The reaction was monitored by TLC (ethyl acetate): R_f (**3a**) = 0.22, R_f(**4a**) = 0.06. After 1 h all starting material was consumed and the reaction was quenched by addition of 50 ml of a semisaturated NH_4Cl-solution and diluted with 30 ml of ethyl acetate. The aqueous phase was extracted three times more with ethyl acetate and the combined organic layers were dried over Na_2SO_4 sicc. After removal of solvents the resulting residue was purified by flash chromatography (20 g silica gel, ethyl acetate). Yield: 340 mg (0.87 mmol, 68%) of **3a** and 120 mg (0.32 mmol, 25%) of **4a**. ^{1}H NMR (250 MHz, CDCl: δ = 1.18 (s, 3 H, CH), 1.27, 135 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.89 (d, 1 H, 3-H_{ax}), 2.04 (s, 3 H, CH_3CO), 2.24 (d, 1 H, 3-H_{equ}), 3.34 (s, 3 H, OCH_3), 3.42 (dd, 1 H, 7-H), 3.64 (dd, 1 H, 6-H), 3.76 (s, 3 H, CO_2CH_3), 3.84 (dd, 1 H, 5-H), 3.97 (dd, 1 H, 5-H), 3.97 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.33 (ddd, 1 H, 8-H), 6.09 (d, 1 H, N-H); $J(3_{ax}, 3_{eq}) = -14.5$ Hz, J (5, N-H) = 8.5, J (5, 6) = 10.8, J (6, 7) = 1.6, J(7, 8) = 8.4, J(8, 9_a) = 5.5, J(8, 9_b) = 6.1, $J(9_a, 9_b) = -8.6$. MS (70 eV, 140 °C): m/z (%) = 376 (2.4) [$M^+ - CH_3$]. $C_{17}H_{29}NO_9$ (391.41). Calcd. C 52.17, H 7.46, N 3.57; found C 52.13, H 7.52, N 3.48.

Methyl (Methyl 5-acetamido-7-O-acetyl-3,5-dideoxy-4-C-methyl-8,9-O-(methyl-ethylidene)-β-D-glycero-D-talo-nonulopyranosid)onate (**3b**)

Method A. 417 mg (1 mmol) of **2b** was treated as described for **3a** (method A). TLC (ethyl acetate): R_f(**2b**) = 0.40, R_f(**3b**) = 0.16. Yield: 320 mg of **3b** (0.75 mmol. 74.9%).

Method B. 417 mg (1 mmol) of **2b** was treated as described for **3a** (method B). TLC (ethyl acetate): R_f(**3b**) = 0.22, R_f(**4b**) = 0.06. After work up the crude mixture was reacetylated by means of 5 ml pyridine and 5 ml acetic anhydride. Subsequent removal of solvents and purification of the resulting residue by flash chromatography (20 g silica gel, ethyl acetate) yielded 265 mg **3b** (0.61 mmol, 68%) and 113 mg **4b** (0.26 mmol, 26%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.10 (s, 3 H, CH_3), 1.26, 1.33 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.88 (d, 1 H, 3-H_{ax}), 1.92, 2.09 (2 s, 2 × 3 H, CH_3CO), 2.22 (d, 1 H, 3-H_{equ}), 3.32 (s, 3 H, OCH_3), 3.77 (s, 3 H, CO_2CH_3), 3.84−4.00 (m, 3 H, 6-, 9_a-, 9_b-H), 4.01 (dd, 1 H, 5-H), 4.32 (ddd, 1 H, 5-H), 5.15 (dd, 1 H, 7-H), 5.64 (d, 1 H, N-H); $J(3_{ax}, 3_{eq}) = -14.4$ Hz, J(5, NH) = 9.6, J(5, 6) = 11.2, J(6, 7) = 2.1, J(7, 8) = 7.3, J(8, 9_a) = 6.3, J(8, 9_b) = 7.0, J (9_a, 9_b) not determined. ^{13}C NMR (62.9 MHz, $CDCl_3$: δ = 170.45, 170.17 (2 C, CH_3CO), 167.17 (1-C), 108.78 [1 C, **C**$(CH_3)_2$], 99.23 (2-C), 73.94 (7-C), 70.22 (4-C), 69.96, 68.90 (2 C, 6-, 8-C), 52.58, 51.49, 50.30 (3 C, 5-C, OCH_3, CO_2CH_3), 42.58 (3-C), 26.56 [1 C, C$(CH_3)_2$], 26.05 (10-C), 25.50 [1 C, C$(CH_3)_2$], 23.00, 20.96 (2 C, CH_3CO). MS (70 eV, 130 °C): m/z (%) = 418 (5.3) [$M^+ - CH_3$]. $C_{19}H_{31}NO_{10}$ (433.45). Calcd. C 52.65, H 7.21, N 3.23; found C 52.73, H 7.23, N 3.12

Methyl (Methyl 5-acetamido-3,5-dideoxy-4-C-methyl-8,9-0-(methylethylidene)-β-D-glycero-D-galacto-2-nonulopyranosid)onate (**4a**)

For the preparation see **3a**. There are no nmr-data as this compound was not soluble in $CDCl_3$. For further characterization the compound was transformed − according to the general acetylation procedure − into **4b**. $C_{17}H_{29}NO_9$ (391.41). Calcd. C 52.17, H 7.46, N 3.57; found C 52.25, H 7.41, N 3.52.

Methyl (Methyl 5-acetamido-7-O-acetyl-3,5-dideoxy-4-C-methyl-8,9-0-(methylethylidene)-β-D-glycero-D-galacto-2-nonulopyranosid)onate (**4b**)

For the preparation see **3b** respectively acetylation of **4a** led to **4b**. ^{1}H NMR (250 MHz, $CDCl_3$: δ = 1.28 [s, 3 H, $C(CH_3)_2$], 1.31 (s, 3 H, CH_3), 1.35 [s, 3 H, $C(CH_3)_2$]. 1.92 (d, 1 H, 3-H_{ax}), 1.98, 2.09 (2 s, 2 × 3 H, CH_3CO), 2.30 (d, 1 H, 3-H_{ax}), 3.21 (s, 3 H, OCH_3), 3.78 (dd, 1 H, 6-H), 3.79 (s, 3 H, CO_2CH_3), 3.87 (dd. 1 H, 9-H_a), 4.00 − 4.08 (m, 2 H, 5-H, 9-H_b), 4.36 (ddd, 1 H, 7-H), 5.51 (d, 1 H, N-H); $J(3_{ax}, 3_{eq})$ = − 13.9 Hz, J(5, NH) = 9.5, J(5, 6) = 10.6, J(6, 7) = 2.3, J(7, 8) = 6.6, $J(8, 9_a)$ = 7.1, $J(8, 9_b)$ = 6.3, $J(9_a, 9_b)$ = − 8.7. ^{13}C NMR (62.9 MHz, $CDCl_3$: δ = 172.11, 172.49 (2 C, CH_3CO), 168.18 (1-C, 108.96 [1 C, $C(HC_3)_2$], 99.11 (2-C), 74.11 (7-C), 71.22 (4-C), 70.05, 69.90 (2 C, 6-, 8-C), 66.47 (9-C), 53.97, 52.56, 51.07 (3 C, 5-C, OCH_3, CO_2CH_3), 26.56, 25.35 [2 C, $C(CH_3)_2$], 23.22 (CH_3CO), 22.83 (10-C), 20.83 (CH_3CO). MS (70 eV, 130 °C): m/z (%) = 418 (1.3) [$M^+ - CH_3$]. $C_{19}H_{31}NO_{10}$ (433.45). Calcd. C 52.65, H 7.21, N 3.23; found C 52.51, H 7.36, N 3.11.

5-Acetamido-3,5-dideoxy-4-C-methyl-D-glycero-D-talo-nonulosonic Acid (**5a**)

65 mg **3b** (0.17 mmol) were dissolved in 5 ml of a 1 *M* NaOH and stirred 90 min at 40 °C. Subsequent the solution was neutralized with Amberlyst 15 H^+. After filtration of the ion-exchange resin water was removed under reduced pressure. The resulting residue was dissolved in 15 ml 0.025 *M* HCl, 2 g Amberlyst 15 H^+ were added and the resulting mixture was stirred for 2 h at 80 °C. TLC (*n*-propanol : acetic acid : water [15 : 0.5 : 4]): R_f(**5a**) = 0.35. After cooling to room temperature the mixture was filtered through cotton and treated with char coal. Further filtration through celite yielded a clear, colorless solution that was concentrated in vacuum and lyophilized. Yield: 37 mg of **5a** (0.114 mmol, 67%). For further characterizatioan see **5b**.

Sodium 5-Acetamido-3,5-dideoxy-4-C-methyl-D-glycero-D-talo-nonulosonoate (**5b**)

37 mg (0.114 mmol) of **5a** was dissolved in 1 ml of H_2O and passed over a column filled with 2 g of Dowex 50 Na^+. Yield after lyophilization: 35 mg (0.101 mmol, 89%) of compound **5b**. ^{1}H NMR (250 MHz, D_2O/HDO): δ = 1.19 (s, 3 H, CH_3), 1.99 (d, 1 H, 3-H_a), 2.06 (d, 1 H, 3-H_b), 2.07 (s, 3 H, CH_3CO), 3.51 (dd, 1 H, 7-H), 3.61 (dd, 1 H, 9-H_a), 3.76–3.88 (m, 2 H, 8-, 9-H_b), 4.00 (d, 1 H, 5-H), 4.19 (dd, 1 H, 6-H); $J(3_a, 3_b)$ = − 14.6, J(5, 6) = 10.8, J(6, 7) = 1.0, J(7, 8) = 9.0, $J(8, 9_a)$ = 6.0, $J(9_a, 9_b)$ = − 11.3, $J(8, 9_b)$ not determined. ^{13}C NMR (62.9 MHz, D_2O/dioxane): δ = 176.71 (1-C), 175.45 (CH_3CO), 97.05 (2-C), 71.94 (4-C), 71.18, 69.74 68.10 (3 C, 6-, 7-, 8-C), 64.10 (9-C), 52.36 (5-C), 42.39 (3-C), 26.56 (10-C), 22.64 (CH_3CO).

5-Acetamido-3,5-dideoxy-4-C-methyl-D-glycero-D-galacto-2-nonulosonic Acid (**6a**)

Treatment of 50 mg of compound **4b** (0.13 mmol) in analogy to the procedure described for 5a yielded 35 mg (0.11 mmol, 85%). For further characterization see **6b**.

Sodium 5-Acetamido-3,5-dideoxy-4-C-methyl-D-glycero-D-galacto-2-nonulosonate (**6b**)

35 mg (0.11 mmol) of **6a** was dissolved in 1 ml of H_2O and passed over a column filled with 2 g of Dowex 50 Na^+. Yield after lyophilization: 36 mg (0.10 mmol, 91%) of compound **6b**. ^{1}H NMR (250 MHz, D_2O/HDO): δ = 1.43 (s, 3 H, CH_3). 198 (d, 1 H, 3-H_a), 2.06 (d, 1 H, 3-H_b), 2.06 (s, 3 H, CH_3CO), 3.53 (dd, 1 H, 7-H), 3.61 (dd, 1 H, 9-H_a), 3.73–3.88 (m, 2 H, 8-, 9-H_b), 4.05 (dd, 1 H, 6-H), 4.17 (d, 1 H, 5-H), $J(3_a, 3_b)$ = − 14.2 Hz, J(5, 6) = 10.6, J(6, 7) = 1.2, J(7, 8) = 8.7, $J(8, 9_a)$ = 6.1, $J(9_a, 9_b)$ = − 10.6, $J(8, 9_b)$ = not determined. ^{13}C NMR (62.9 MHz, D_2O/dioxane): δ = 176.43 (1-C), 175.68 (CH_3CO), 96.4 (2-C), 72.39 (4-C), 71.34, 70.24, 69.74 (3 C, 6-, 7-, 8-C), 64.12 (9-C), 54.86 (5-C), 45.31 (3-C), 22.88, 22.80 (2 C, 10-C, CH_3CO).

Methyl (Methyl 5-acetamido-3,5-dideoxy-4-C-methylene-8,9-O-(methyl-ethylidene)-β-D-manno-2-nonulopyranosid)onate (**7a**)

In a 50 ml two necked flask 1.33 g Zn powder and 750 mg zirkonocendichloride were dried carefully for 1 h (0.01 Torr, 50 °C). This mixture was cooled at 0 °C and 5 ml of *THF* was added. After stirring this mixture 15 min in an ice bath 563 mg of ketone **2a** (1.5 mmol) in 5 ml *THF* were added. After further 15 min the ice bath was removed and 413 µl of CH_2I_2 was added within 1 min. Approximately 30 s later an exothermic reaction was observed. After 8 min the reaction mixture was cooled again in an ice bath and the reaction was quenched by quick addition of 15 ml of a 1 : 1 mixture of $NH_4Cl_{(sat)}$ and water. Subsequent extraction with three times 30 ml of ethyl acetate, drying of the combined organic layers over Na_2SO_4 and removal of the solvent led to a crude mixture which was purified by flash chromatography (silica gel, ethyl acetate. Yield: 395 mg (1.05 mmol, 70.5%) of compound **7a**. ^{1}NMR (250 MHz, $CDCl_3$: $\delta = 1.28$, 1.35 [2 s, 2 × 3 H, $C(CH_3)_2$, 2.08 (s, 3 H, CH_3CO), 2.56 (ddd, 1 H, 3-H_a), 2.76 (d, 1 H, 3-H_b) 3.25 (s, 3 H, OCH_3), 3.47 (dd, 1 H, 7-H), 3.53 (dd, 1 H, 6-H), 3.78 (s, 3 H, CO_2CH_3), 4.00 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.29 (ddd, 1 H, 8-H), 4.67 (dddd, 1 H, 8-H), 4.96 (dd, 1 H, 10-H_a), 5.00 (dd, 1 H, 10-H_b), 5.84 (d, 1 H, N-H); J (3_a, 3_b) = −14.1 Hz, J (3_a, 10_a) = 1.9, J (3_a, 10_b) = 1.9, J (5, NH) = 9.3, J(5, 6) = 10.6, J(5, 10_a) = 1.9, J(5, 10_b) = 1.9, J(6, 7) = 1.3, J(7, 8) = 8.2, J(8, 9_a) = 5.4, J(8, 9_b) = 6.1, J(9_a, 9_b) = −8.1. ^{13}C NMR (62.9 MHz, $CDCl_3$: $\delta = 171.50$ (CH_3CO), 168.66 (1-C), 134.53 (4-C), 109.60 (10-C), 108.88 [$C(CH_3)_2$], 98.56 (2-C), 74.33, 73.23, 70.46 (6-, 7-, 8-C), 67.33 (9-C), 52.58, 51.20 (2 C, OCH_3, $COOCH_3$), 48.57 (5-C), 41.47 (3-C) 27.00, 25.31 [2 C, $C(CH_3)_2$], 23.14 (CH_3CO). MS (70 eV, 160 °C): m/z /%) = 598 (0.7) [$M^+ - CH_3$], 402 (48.4) [$M^+ - C_{14}H_{11}O_2$]. $C_{17}H_{27}NO_8$ (373.41). Calcd. C 54.68, H 7.83, N 3.75; found C 54.81, H 7.89, N 3.66.

Methyl (Methyl 5-acetamido-7-O-acetyl-3,5-dideoxy-4-C-methylene-8,9-O-(methylethylidene)-β-D-manno-2-nonulopyranosid)onate (**7b**)

Application of the general acetylation procedure on 20 mg (0.054 mmol) of compound **7a** and subsequent flash chromatography (5 g silica gel, ethyl acetate) yielded 20 mg (0.048 mmol, 88.9%) of compound **7b**. ^{1}H NMR (250 MHz, $CDCl_3$: $\delta = 1.28$, 1.33 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.99, 2.10 (2 s, 2 × 3 H, CH_3CO), 2.55 (ddd, 1 H, 3-H_a), 2.76 (d, 1 H, 3-H_b), 3.23 (s, 3 H, OCH_3), 3.76 (dd, 1 H, 6-H), 3.80 (s, 3 H, CO_2CH_3), 3.91 (dd, 1 H, 9-H_a), 4.03 (dd, 1 H, 9-H_b), 4.33 (ddd, 1 H, 8-H), 4.64 (m, 1 H, 5-H), 4.92 (m, 2 H, 10-H's), 5.28 (dd, 1 H, 7-H), 5.42 (d, 1 H, N-H)M; J(3_{ax}, 3_{eq}) = −14.4 Hz, J(5, NH) = 10.2, J(5, 6) = 10.6, J(6, 7) = 2.3, J(7, 8) = 6.4, J(8, 9_a) = 6.9, J(8, 9_b) = 6.1, J(9_a, 9_b) = −8.8. MS (70 eV, 150 °C): m/z(%) = 460 (1.7) [$M^+ - CH_3$]. $C_{19}H_{29}NO_9$ (415.4). Calcd. C 54.93, H 7.04, N 3.37; found C 54.98, H 6.99, N 3.25.

Methyl (Methyl 5-acetamido-3,4,5-trideoxy-4-C-methyl-8,9-0-(methylethylidene)-β-D-glycero-D-galacto-2-nonulopyranosid)onate (**8a**)

A solution of 200 mg of **7a** (0.53 mmol) in 20 ml of acetone was hydrogenated by addition of 50 mg Pd/C in a H_2-atmosphere (50 psi). TLC (ethyl acetate): R_f (**8a**) = 0.18, R_f (**9a**) = 0.09. Subsequent filtration of the catalyst and removal of the solvent under reduced pressure led to a colorless residue. The two diastereoisomers were separated by flash chromatography. Yield: 110 mg **8a** (0.29 mmol, 55%), 73 mg **9a** (0.19 mmol, 36%). ^{1}H NMR (250 MHz, $CDCl_3$: $\delta = 1.21$ (d, 3 H, CH_3), 1.27, 1.35 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.98 (m, 2 H, 3-H's), 2.04 (s, 3 H, CH_3CO), 2.21 (dddd, 1 H, 4-H), 3.24 (s, 3 H, OCH_3), 3.42 (dd, 1 H, 7-H), 3.70 (dd, 1 H, 6-H), 3.75 (s, 3 H, CO_2CH_3), 3.96 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.21 (ddd, 1 H, 5-H), 4.30 (ddd, 1 H, 8-H), 5.54 (d, 1 H, N-H); J(3_{ax}, 4) = 12.1 Hz, J(4, 5) = 10.5, J (4, 10) = 7.2, J(5, NH) = 9.3, J(5, 6) = 10.5, J(6, 7) = 1.2, J(7, 8) = 8.2, J(8, 9_a) = 6.0, J(8, 9_b) = 6.2, J(9_a, 9_b) = −8.6, J(3_{eq}, 4) not determined. ^{13}C NMR (62.9 MHz, $CDCl_3$: $\delta = 171.51$ (CH_3CO), 168.73 (1-C), 108.87 [$C(CH_3)_2$], 98.0.1 (2-C), 74.22, 70.99, 67.86 (3 C, 6-, 6-, 8-C), 67.96 (9-C), 52.42, 50.93 (2-C), OCH_3, CO_2CH_3), 47.02 (5-C), 37.73 (3-C), 28.92 (4-C), 26.95, 25.35 [2 C, $C(CH_3)_2$], 23.37 (CH_3CO), 14.86 (10-C). MS (60 eV, 150 °C): m/z(%) = 360 (1.76) [$M^+ - CH_3$]. $C_{17}H_{29}NO_8$ (375.4). Calcd. C 54.39, H 7.79, N 3.73; found C 54.32, H 7.81, N 3.65.

Methyl (Methyl 5-acetamido-7-O-acetyl-3,4,5-trideoxy-4-C-methyl-8,9,0-(methylethyliden)-β-D-glycero-D-galacto-2-nonulopyranosid)onate (**8b**)

Application of the general acetylation procedure on 32 mg (0.085 mmol) of compound **8a** and subsequent flash chromatography (12 g silica gel, ethyl acetate) yielded 26 mg (0.062 mmol, 72.9%) of compound **8b**. TLC (ethyl acetate): R_f (**8b**) = 0.27. ^{1}H NMR (250 MHz, $CDCl_3$: δ = 0.90 (d, 3 H, CH_3), 1.28, 1.33 [2 s, 2 × 3 H, C)$CH_3)_2$], 1.50 (dd, 1 H, 3-H_{ax}), 1.92 (s, 3 H, CH_3CO), 1.92–2.08 (m, 2 H, 3-H_{eq}, 4-H), 2.10 (s, 3 H, CH_3CO), 3.24 (s, 3 H, OCH_3), 3.68 (ddd, 1 H, 5-H), 3.77 (s, 3 H, CO_2CH_3), 3.79 (dd, 1 H, 6-H), 3.91 (dd, 1 H, 9-H_a), 4.03 (dd, 1 H, 9-H_b), 4.32 (ddd, 1 H, 8-H), 5.16 (d, 1 H, N-H), 5.27 (dd, 1 H, 7-H); $J(3_{ax}, 3_{eq}) = -13.4$ Hz, $J(3_{ax}, 4) = 12.1$, $J(4, 5) = 11.5$, $J(4, 10) = 6.5$, $J(5, N\text{-}H) = 9.9$, $J(5, 6) = 10.2$, $J(6, 7) = 2.2$, $J(7, 8) = 6.2$, $J(8, 9_a) = 7.2$, $J(8, 9_b) = 6.1$, $J(9_a, 9_b) = -8.8$, $J(3_{eq}, 4)$ not determined. MS (70 eV, 170 °C): m/z (%) = 402 (11.34) [$M^+ - CH_3$]. $C_{19}H_{31}NO_9$ (417.4). Calcd. C 54.67, H 7.48, N 3.36; found C 54.52, H 7.61, N 3.32.

Methyl (Methyl 5-acetamido-3,4,5-trideoxy-4-C-methyl-8,9-O(methyl-ethylidene)-β-D-glycero-D-talo-nonulopyranosid)onate (**9a**)

^{1}H NMR (250 MHz, $CDCl_3$: δ = 0.97 (d, 3 H, CH_3), 1.28, 1.35 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.53 (dd, 1 H, 3-H_{ax}), 1.96–2.18 (m, 2 H, 3-H_{equ}, 4-H), 2.04 (s, 3 H, CH_3CO), 3.27 (s, 3 H, OCH_3), 3.45 (dd, 1 H, 7-H), 3.50 (dd, 1 H, 6-H), 3.65 (ddd, 1 H, 5-H), 3.76 (s, 3 H, CO_2CH_3), 3.99 (dd, 1 H, 9-H_a), 4.12 (dd, 1 H, 9-H_b), 4.30 (ddd, 1 H, 8-H), 5.35 (d, ^{1}H, N-H); $J(3_{ax}, 3_{eq}) = -13.6$ Hz, $J(3_{ax}, 4) = 12.1$, $J(4, 5) = 10.5$, $J(4, 10) = 6.4$, $J(5, NH) = 8.7$, $J(5, 6) = 10.2$, $J(6, 7) = 1.4$, $J(7, 8) = 8.0$, $J(8, 9_a) = 5.7$, $J(8, 9_b) = 6.2$, $J(9_a, 9_b) = -8.6$, $J(3_{eq}, 4)$ not determined. ^{13}C NMR (62.9 MHz, $CDCl_3$: δ = 172.04 (CH_3CO), 168.54 (1-C), 108.75 [$C(CH_3)_2$], 98.10 (2-C), 74.34, 72.62, 70.43 (3 C, 6-, 7-, 8-C), 67.81 (9-C), 52.42, 51.24, 50.93 (3-C, 5-C, OCH_3, CO_2CH_3), 39.93 (3-C), 30.12 (4-CD), 26.98, 25.39 [2 C, $C(CH_3)_2$], 23.06 (CH_3CO), 18.21 (10-C). MS (70 eV, 135 °C): m/z (%) = 360 (6.8) [$M^+ - CH_3$]. $C_{17}H_{29}NO_8$ (375.4). Calcd. C 54.39, H 7.79, N 3.73; found C 54.28, H 7.74, N 3.64.

Methyl (Methyl 5-acetamido-7-O-acetyl-3,4,5-trideoxy-4-C-methyl-8,9-O-(methylethylidene)-β-D-glycero-D-talo-nonulopyranosid)onate (**9b**)

Application of the general acetylation procedure on 50 mg (0.133 mmol) of compound **9a** and subsequent flash chromatography (10 g silica gel, ethyl acetate) yielded 54 mg (0.129 mmol, 96.9%) of compound **9b**. ^{1}H NMR (250 MHz, $CDCl_3$: δ = 1.12 (d, 3 H, CH_3), 1.28, 1.34 [2 s, 2 × 3 H, $C(CH_3)_2$], 1.92 (s, 3 H, CH_3CO), 1.95 (mc, 2 H, 3-H's), 2.09 (s, 3 H, CH_3CO), 2.19 (m, 1 H, 4-H), 3.22 (s, 3 H, OCH_3), 3.77 (s, 3 H, CO_2CH_3), 3.89 (dd, 1 H, 9-H a), 3.91 (dd, 1 H, 6-H), 4.02 (dd, 1 H, 9-H_b), 4.27 (ddd, 1 H, 8-H), 5.24 (dd, 1 H, 7-H), 5.35 (d, 1 H, N-H); $J(4, 5) = 4.9$ Hz, $J(4, 10) = 7.3$, $J(5, N\text{-}H) = 10.2$, $J(5, 6) = 11.2$, $J(6, 7) = 2.4$, $J(7, 8) = 6.5$, $J(8, 9_a) = 6.9$, $J(8, 9_b) = 6.2$, $J(9_a, 9_b) = -8.8$, $J(3, 4)$ not determined. MS (70 eV, 150 °C): m/z (%) = 402 (4.68) [$M^+ - CH_3$], 358 (10.23) [$M^+ - C_2H_3O_2$]. $C_{19}H_{31}NO_9$ (417.4). Calcd. C 54.67, H 7.48, N 3.36; found C 54.61, H 7.44, N 3.28.

5-Acetamido-3,4,5-trideoxy-4-C-methyl-D-glycero-D-galacto-2-nonulonic Acid (**10a**)

30 mg of compound **8a** was dissolved (0.08 mmol) in a mixture of 2 ml *M* NaOH and 1 ml methanol and heated for 120 min at 40 °C. After cooling to room temperature the solution was neutralized by addition of Amberlyst 15 H^+. Then the resin was filtered off and the filtrate was concentrated (approx. 1 ml) in vacuum. The residue was diluted with 15 ml of 0.025 *M* HCl and 2 g of Amberlyst 15 H^+ was added. After heating this mixture 1 h at 80 °C TLC showed only one spot corresponding to the product (*n*-propanol : acetic acid : water [15 : 0.5 : 4]): R_f (**10a**) = 0.38. After filtration of the resin the filtrate was lyophilized. Yield: 20 mg **10a** (0.065 mmol, 81%).

Sodium 5-Acetamido-3,4,5-trideoxy-4-C-methyl-D-glycero-D-galacto-2-nonulosonate (**10 b**)

20 mg (0.065 mmol) of **10 a** was dissolved in 1 ml H_2O and passed over a column filled with 2 g of Dowex 50 Na^+. Yield after lyophilization: 21 mg (0.064 mmol, 98%) of compound **10 b**. ^1H NMR (250 MHz, D_2O/HDO: $\delta = 0.95$ (d, 3 H, CH_3), 1.65 (dd, 1 H, 3-H_{ax}), 1.99 (dd, 1 H, 3-H_{equ}), 2.03 (s, 3 H, CH_3CO), 2.06 (ddd, 1 H, 4-H), 3.53 (dd, 1 H, 7-H), 3.59 (dd, 1 H, 9-H_a), 3.67 (dd, 1 H, 5-H), 3.73 (ddd, 1 H, 8-H), 3.81 (dd, 1 H, 9-H), 3.98 (dd, 1 H, 6-H); $J(3_{ax}, 3_{eq}) = -13.3$ Hz, $J(3_{ax}, 4) = 12.0$, $J(3_{eq}, 4) = 4.14$, $J(4, 5) = 11.2$, $J(5, 6) = 9.0$, $J(6, 7) = 1.1$, $J(7, 8) = 9.1$, $J(8, 9_a) = 5.3$, $J(8, 9_b) = 2.6$, $J(9_a, 9_b) = -11.5$ ^{13}C NMR (62.9 MHz, D_2O/dioxane): $\delta = 175.6$ (CH_3CO), 95.49 (2-C 71.90, 71.00, 69.29 (3 C, 6-, 7-, 8-C), 64.00 (9-C), 51.54 (5-C), 39.25 (3-C), 30.72 (4-C), 22.61 (CH_3CO), 18.02 (10.C).

5-Acetamido-2,7-anhydro-4-C-methyl-3,4,5-trideoxy-D-glycero-D-talo-2-nonulosonic Acid (**11 a**)

35 mg (0.093 mmol) of compound **9 a** were treated as described for **10 a** (0.03 mmol). TLC (*n*-propanol : acetic acid : water [15 : 0.5 : 4]): R_f (**11 a**) = 0.42. Yield: 24 mg **11 a** (0.083 mmol, 89%).

Sodium 5-Acetamido-2,7-anhydro-4-C-methyl-3,4,5-trideoxy-D-glycero-D-talo-2-nonulosonate (**11 b**)

Compound **11 a** was transformed into its sodium salt by passing 10 mg (0.035 mmol) through a column filled with 2 g of Dowex 50 Na^+ and subsequent lyophilization of the filtrate yielded 10 mg (0.032 mmol) of **11 b**. ^1H NMR (250 MHz, D_2O/dioxane): $\delta = 0.87$ (d, 3 H, CH_3), 1.56 (dd, 1 H, 3-H_a), 1.91 (dd, 1 H, 3-H_b), 2.08 (s, 3 H, CH_3CO), 2.39 (dddd, 1 H, 4-H), 3.54–3.63 (m, 2 H, 8-H, 9-H_a), 3.75 (dd, 1 H, 9-H_b), 3.96 (dd, 1 H, 5-H), 4.50 (dd, 1 H, 6-H); $J(3_{ax}, 3_{ax}, 3_{eq}) = -14.2$ Hz, $J(3_{ax}, 4) = 12.7$, $J(3_{eq}, 4) = 5.1$, $J(4, 5) = 4.0$, $J(5, 6) = 1.0$, $J(6, 7) = 1.2$, $J(7, 8) = 6.8$, $J(8, 9_b) = 6.0$, $J(9_a, 9_b) = -8.5$, $J(8, 9_a)$ not determined. ^{13}C NMR (62.9 MHz, $CDCl_3$: $\delta = 174.95$ (CH_3CO), 107.68 (2-C), 79.88, 78.45 (2 C, 6-, 7-C), 72.59 (C-8), 62.96 (9-C), 50.66 (5-C), 37.36 (3-C), 26.96 (4-C), 22.57 (CH_3CO), 16.34 (10-C).

Methyl (Methyl 4-acetamido-7,8,9-tri-O-acetyl-4-C-methylene-3,4,5-trideoxy-β-D-manno-2-nonulopyranosid)onate (**12**)

A solution of 145 mg (0.39 mmol) of compound **7 a** in 5 ml of aqueous acetic acid (80%) was heated for 3 h at 60 °C. After cooling to room temperature the solvents were removed under reduced pressure. The resulting residue was coevaporated three times with methanol and dried in vacuum (0.01 Torr, 40 °C). Following acetylation was carried out by addition of 3 ml of pyridine and 3 ml of acetic anhydride. After 14 h the solvents were removed and the resulting residue was purified by flash chromatography (20 g silica gel, ethyl acetate). Yield: 130 mg (0.28 mmol, 72.6%) of cerivative **12**. TLC (ethyl acetate): R_f (**12**) = 0.31. ^1H NMR (250 MHz, $CDCl_3$: $\delta = 1.97$, 2.00, 2.03, 2.11 (4 s, 4 × 3 H, CH_3CO), 2.54 (mc, 1 H, 3-H_a), 2.77 (d, 1 H, 3-H_b), 3.22 (s, 3 H, OCH_3), 3.78 (dd, 1 H, 6-H), 3.79 (s, 3 H, CO_2CH_3), 4.11 (dd, 1 H, 9-H_a), 4.61 (m, 1 H, 5-H), 4.83 (dd, 1 H, 6-H), 4.92 (m, 2, 10 H's), 5.19 (dd, 1 H, 8-h), 5.43 (dd, 1 H, 7-H), 5.50 (d, 1 H, N-H); $J(3_a, 3_b) = -14.1$ Hz, $J(5, \text{N-H}) = 10.0$, $J(5, 6) = 10.5$, , $J(6, 7) = 2.5$, $J(7, 8) = 3.8$, $J(8, 9_a) = 7.7$, $J(8, 9_b) = 2.5$, $J(9_a, 9_b) - 12.4$, $J(3, 4)$, $J(4, 5)$ not determined. MS (70 eV, 150 °C): m/z (%) = 459 (0.78) [M^+], 400 (13.89) [$M^+ - C_2H_3O_2$]. $C_{20}H_{29}NO_{11}$ (459.4). Calcd. C 52.29, H 636, N 3.05; found C 52.28, H 6.28, N 2.95.

Methyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-C-methyl-3,4,5-trideoxy-β-D-glycero-D-galacto-2-nonulopyranosid)onate (**13**)

A solution of 250 mg of **12** (0.55 mmol) in 20 ml of *i*-propanol was hydrogenated by addition of 50 mg Pd/C in a H_2-atmosphere (50 psi). TLC (ethyl acetate): R_f (**13**, **14**) = 0.37. Subsequent filtration of the catalyst and removal of the solvent under reduced pressure led to a colorless residue. The mixture

of the two diastereoisomers was not separable by flash chromatography. Yield: 160 mg of a 2:3 mixture of **13** and **14** (0.35 mmol, 64%).

Methyl (Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-C-methyl-3,4,5-trideoxy-β-D-glycero-D-talo-nonulopyranosid)onate (**14**)

45 mg (0.12 mmol) of **9a** was treated as described for **12**. TLC (ethyl acetate): R_f (**14**) = 0.16. Yield: 41 mg of **14** (0.089 mmol, 74.2%). ^{1}H NMR (250 MHz, $CDCl_3$: $\delta = 1.10$ (d, 3 H, CH_3), 1.90 (s, 3 H, CH_3CO),1.95 (mc, 2 H, 3-H's), 2.01, 2.06, 2.11 (3 s, 3 × 3 H, CH_3CO), 2.11–2.22 (m, 1 H, 4-H), 3.21 (s, 3 H, OCH_3), 3.77 (s, 3 H, CO_2CH_3), 3.95 (dd, 1 H, 9-H_a), 4.26 (ddd, 1 H, 5-H), 4.83 (dd, 1 H, 8-H), 5.38 (d, 1 H, N-H), 5.40 (dd, 1 H, 7-H); $J(3_a, 4) = 2.2$ Hz, $J(3_b, 4) = 3.2$, $J(4, 5) = 5.2$, $J(5, \text{N-H}) = 10.4$, $J(5, 6) = 10.2$, $J(6, 7) = 2.6$, $J(7, 8) = 3.4$, $J(8, 9_a) = 7.7$, $J(8, 9_b) = 2.4$, $J(9_a, 9_b) = -12.5$. MS (70 eV, 160 °C): m/z (%) = 461 (0.65) [M^+], 446 (12.89) [$M^+ - CH_3$]. $C_{20}H_{31}NO_{11}$ (461.4). Calcd. C 52.06, H 6.77, N 3.04; found C 52.13, H 6.76, N 3.00.

Sodium (Methyl 5-acetamido-4-C-methylene-3,4,5-trideoxy-β-D-manno-2-nonulopyranosid)onate (**15**)

Sodium methoxide (3 ml, 0.1 *M* in methanol) was added to a solution of **12** (130 mg, 0.28 mmol) in 2.2 ml anhydrous methanol and stirred for ca. 12 h. The solvent was removed under reduced pressure, and the residue coevaporated twice with 5 ml of anhydrous methanol. The resulting residue was dissolved in 10 ml of water and this solution was neutralized after 20 minutes by addition of $CO_{2(s)}$. Subsequent treatment with charcoal, filtration by suction through Celite and lyophilization yielded **15** (90 mg, 0.26 mmol, 93%). ^{1}H NMR (250 MHz, D_2O/HDO): $\delta = 2.10$ (s, 3 H, CH_3CO), 2.50 (d, 1 H, 3-H_a), 2.70 (d, 1 H, 3-H_b), 3.20 (s, 3 H, OCH_3), 3.61 (dd, 1 H, 7-H), 3.66 (dd, 1 H, 9-H_b), 3.77 (dd, 1 H, 6-H), 3.86 (dd, 1 H, 9-H_b), 3.90 (ddd, 1 H, 8-H), 4.59 (d, 1 H, 5-H), 4.98 (mc, 2 H, 10-H's); $J(3_a, 3_b) = -14.2$, $J(5, 6) = 10.5$, $J(6, 7) = 1.1$, $J(7, 8) = 9.7$, $J(8, 9_a) = 6.2$, $J(8, 9_b) = 2.6$, $J(9_a, 9x) = -12.3$. ^{13}C NMR (62.9 MHz, D_2O/1,4-dioxane): $\delta = 176.38$, 175.21 (2 C, 1-C, CH_3CO), 140.57 (10-C), 110.97 (4-C), 100.97 (2-C), 72.60, 70.87, 69.59 (3 C, 6-, 7-, 8-C), 64.37 (9-C), 51.36, 49.99 (2, 5-C, OCH_3), 4.75 (3-C), 22.58 (CH_3CO).

Sodium (Methyl 5-acetamido-4-C-methyl-3,4,5-trideoxy-β-D-glycero-D-talo-nonulopyranosid)onate (**16**)

The analogous procedure as described for **15** applied on 41 mg (0.089 mmol) of **14** yielded 27 mg (0.078 mmol, 88%) of **16**. ^{1}H NMR (250 MHz, D_2O/HDO): $\delta = 1.02$ (d, 3 H, CH_3), 1.77 (dd, 1 H, 3-H_a), 1.93 (dd, 1 H, 3-H_b), 2.01 (s, 3 H, CH_3CO), 2.09 (m, 1 H, 4-H), 3.19 (s, 3 H, OCH_3), 3.53 (dd, 1 H, 7-H), 3.66 (dd, 1 H, 9-H_a), 3.84 (dd, 1 H, 9-H_b), 3.88 (ddd, 1 H, 8-H), 3.94 (dd, 1 H, 6-H), 4.25 (dd, 1 H, 5-H); $J(3_a, 3_b) = -14.3$ Hz, $J(3_a, 4) = 6.7$, $J(3_b, 4) = 4.6$, $J(4, 5) = 6.1$, $J(4, 10) = 7.2$, $J(5, 6) = 10.1$, $J(6, 7) = 1.5$, $J(7, 8) = 8.9$, $J(8, 9_a) = 6.0$, $J(8, 9_b) = 2.7$, $J(9_a, 9_b) = -12.3$. ^{13}C NMR (62.9 MHz, D_2O/dioxane): $\delta = 174.98$, 174.90 (2 C, 1-C, CH_3CO), 102.03 (2-C), 71.07, 70.35, 68.31 (3 C, 6-, 7-, 8-C), 64.31 (9-C), 51.22, 48.41 (2 C, 5-C, OCH_3), 37.44 (3-C), 29.15 (4-C), 22.61 (CH_3CO), 15.12 (10-C).

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