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# Structural Variations of N-Acetylneuraminic Acid, 24 [1]: Synthesis of the α-Methylketoside of 8-Oxo-N-Acetylneuraminic Acid and Related Derivatives

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**Abstract.** The synthesis of the sodium 5-acetamido-2,3,5-trideoxy-2,3 didehydro-*D*-galacto-2,8-nondiulopyranosidonate 8,8-dimethyl acetal (**8**) and of the methyl-5-acetamido-3,5-dideoxy-α-*D*-galacto-2,8-nondiulopyranosidonate 8,8 dimethyl acetal (**11**) as well as of the methyl-5-acetamido-3,5-dideoxy-α-*D*-galacto-2,8-nondiulopyranosidonic acid (**12**) is reported involving the easily accessible 8-oxo-derivative **5**. Compounds **11** and **12**, respectively, showed to have glycosidic bond with remarkable stability to acidic conditions.

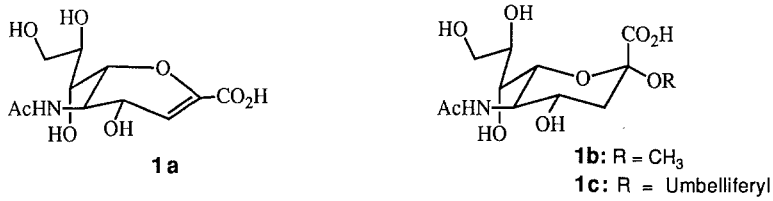
**Keywords.** Sialic acids; Keto-sugars; Synthesis of a methyl glycoside.

**Strukturelle Variationen an N-Acetylneuraminsäure, 24. Mitt.: Synthese des α-Methylketosids von 8-Oxo-N-acetylneuraminsäure und verwandten Derivaten**

**Zusammenfassung.** Es wird ein Zugang zu Natrium-(5-acetamido-2,3,5-trideoxy-2,3-didehydro-*D*-galacto-2,8-nondiulopyranosid)onat-8,8-dimethylacetal (**8**), Methyl-5-acetamido-3,5-dideoxy-α-*D*-galacto-2,8-nondiulopyranosidonat-8,8-dimethyl acetal (**11**) und Methyl-5-acetamido-3,5-dideoxy-α-*D*-galacto-2,8-nondiulopyranosidonsäure (**12**) über ein gemeinsames, leicht herzustellendes 8-Oxo-Sialinsäurederivat **5** beschrieben. Die glycosidische Bindung der Verbindungen **11** bzw. **12** zeigt eine bemerkenswerte Stabilität gegenüber sauren Reaktionbedingungen, welche im Gegensatz zu Beobachtungen an anderen Neuraminsäuremethylketosiden steht.

## Introduction

The importance of sialic acids in animal tissues is already well documented [2, 3], though parts of their biochemistry and chemistry are still unknown. In most cases these molecules are the terminal residues of glycolipids and glycoproteins attached α-glycosidically to the 3-, respectively 6-O of a subterminal galactose. Therefore, the sialidases – important for their catabolic activity – recognize only sialic acids in this α-configuration. Tuppy and Meindl synthesized first the 2,3-didehydro-N-acetylneuraminic acid **1a** [4], which was shown to be an excellent inhibitor of sialidases [5]. Later, this fact was correlated, using the analogy to the corresponding transition state during the cleavage of N-acetlyneuraminic acids [6] (Scheme 1).



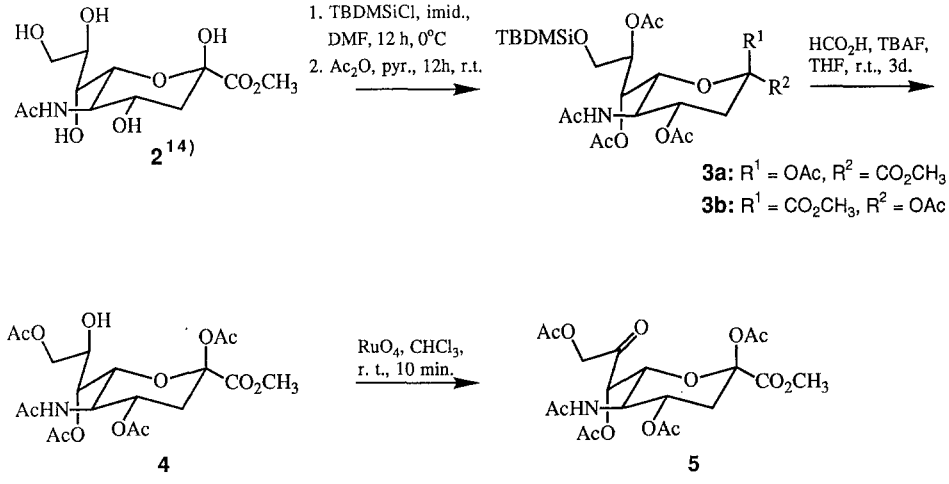

**Scheme 1**

Also the recognition of cell surfaces by the influenza viruses is regulated by the interaction of α-glycosidically bond sialic acids with viral haemagglutinins [7].

As stated earlier, sialic acids with different stereochemistry and functional groups might be correlated biosynthetically to N-acetylneuraminic acid via corresponding carbonyl compounds [8]. We studied the influence of the side chain stereochemistry as well as further chemical variations of N-acetylneuraminic acid on the recognition of sialidases using α-umbeliferyl and α-methyl glycosides (**1b** and **1c**), respectively 2,3-didehydro derivatives as model substrates [9]. Therefore, we were interested in new congeners of neuraminic acid and we wish now to report a synthesis of the α-methylglycosides of the 8-oxo- and 8,8-dimethoxy-N-acetylneuraminic acids and the 8,8-dimethylacetal of the 2,3-didehydro-N-acetylneuraminic acid.

## Results and Discussion

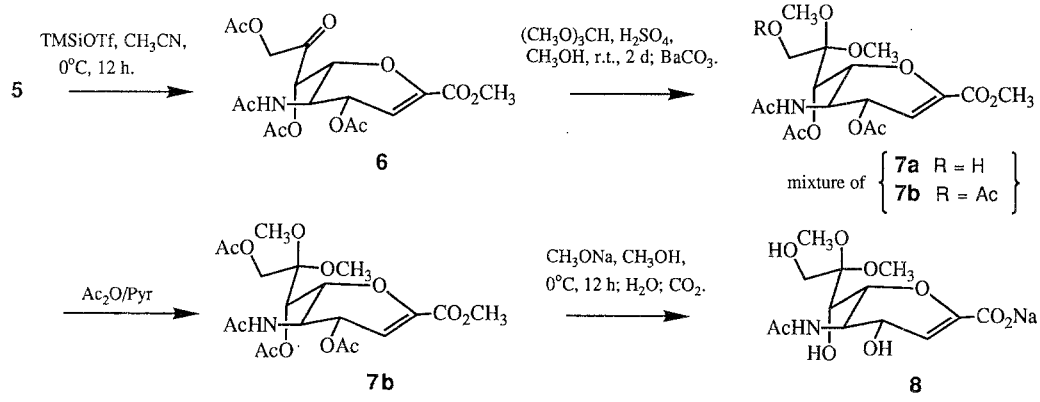
At first we should consider a strategy reported recently by us [10]. Thus derivatives of Neu5Ac only unprotected in 8-position were obtained by controlled migration of one acetyl group from 8- to 9-position. Consequently we prepared the peracetylated 9-O-*t*.butyldimethylsilyl derivative **3a** starting with the methylester of N-acetylneuraminic acid **2**. Thus, monosilylation of the primary alcohol followed by peracetylation yielded the mixture of both anomers **3a** (62%) and **3b** (5.6%) (β : α = 11 : 1), which could be easily separated by flash-chromatography. To have




**Scheme 2**

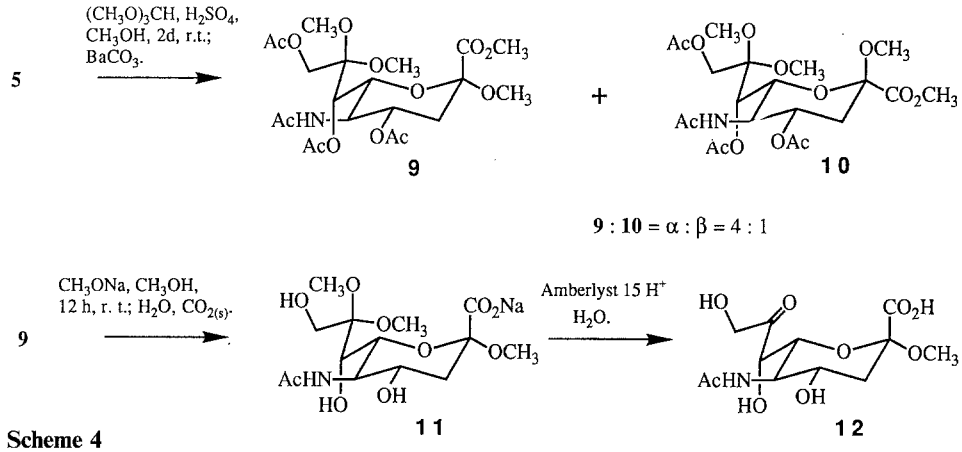
clean analytical conditions, only the β-anomer **3a** was used for further transformations. Removal of the silyl group with *TBAF* and formic acid in *THF* was accompanied – as expected (see above) – by a slow acetyl migration to yield **4** within 3 days as the only product (92%). Subsequent oxidation by means of $RuO_4$ in $CHCl_3$ gave the ketone **5** in excellent yields (96%) (Scheme 2).

Starting with this 8-oxo-compound **5** we prepared the 2,3-didehydro-derivative **6** by treatment with trimethylsilyltrifluoromethanesulfonate in analogy to an improvement [8, 11] of an older procedure by using two equivalents. All trials to remove the acetyl groups by saponification led only to decomposition of **5** into a series of unidentified products. As we were interested in the 2,3-didehydro-8-oxo-N-acetylneuraminic as well as its 8,8-dimethyl acetal, we prepared dimethylacetal **7b** by treatment with trimethyl orthoformate and sulfuric acid in methanol. As we observed a remarkable amount of **7a**, we combined the acetalization with an subsequent acetylation procedure to obtain **7b** as the main product. Now Zemplen saponification and aqueous work up yielded the desired sodium 5-acetamido-2,3,5-trideoxy-2,3-didehydro-*D*-galacto-2,8-nondiulopyranosidonate 8,8-dimethyl acetal **8** (Scheme 3). When we tried to remove the dimethyl acetyl under various acidic condition we observed again severe decomposition. Therefore, we were not able to isolate any 2,3 didehydro-8-oxo-N-acetylneuraminic acid.




**Scheme 3**

When we applied the reagent mixture trimethyl orthoformate $H_2SO_4$/methanol on compound **5** to prepare the corresponding 8,8-dimethylacetal we observed an interesting glycosylation reaction. Thus a 4 : 1 mixture of the α- and β-methylketosides **9** (66%) and **10** (16%) had been formed, although – under stereoelectronic control – the β-glycoside should be favoured (Scheme 4). The assignment of the stereochemistry at C-2 could be achieved by comparison of the β-methylketoside recently prepared by us [8]. The 3-$H_{eq}$ of the α-anomer showed a signal at 2.63 ppm, typical values for α-glycosides [12]. The both anomers could be separated by HPLC. Subsequent removal of O-acetyl groups by Zemplen saponification and aqueous workup under basic conditions yielded the sodium methyl-5-acetamido-3,5-dideoxy-α-*D*-galacto-2,8-nondiulopyranosidonate 8,8-dimethyl acetal **11** (94%). When we treated this material with Amberlyst 15 $H^+$ in $H_2O$ for 2 h at room temperature, we obtained the α-methyl-glycoside **12** as the only product (94%)




**Scheme 4**

(Scheme 4). Even when we heated the material for several hours we only isolated **12** in a reduced yield, but without any indication for the cleavage of the α-glycosidic bond. This result is almost contradictory to what is known about α-glycosides of other sialic acids, which are usually very labile to acidic cleavage.

## Experimental Part

Commercially available compounds were dried and used without further purification. Solvents were dried and distilled before use. Amberlyst 15 $H^+$ was washed with diluted HCl and methanol and dried under vacuum (0.01 Torr). 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 thin-layer 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 charring at 200°C or in the case of unprotected sialic acids by spraying with Bial's reagent [3] and heating for 15 min at 140°C. Flash chromatography: Merck™ silica gel 0.040–0.063 mm. $^1$H-NMR (250): Bruker WM 250, *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 *TMS* (67.40 ppm upfield from the signal of 1,4-dioxane in $D_2O$) was used.

N-acetylneuraminic acid Neu5Ac was prepared from edible birds nest glycoprotein [13]. Starting material **2** was prepared as reported earlier [14].

*Methyl 5-Acetamido-2,4,7,8-tetra-O-acetyl-9-O-t.butyldimethylsilyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosidonate* (**3 a**)

3 g of compound **2** (9.2 mmol), 1.51 g imidazole, and 2 g 3 Å molecular sieve were stirred together with dry *DMF* (30 ml) for 15 min in an ice-bath at 0°C. At this temperature 1.68 g of *t.*butyldimethylsilyl chloride was added and the ice-bath was allowed to reach room temperature within 14 h. After filtration of the molecular sieves the solvent was removed under reduced pressure. The resulting residue was suspended in 20 ml of water and extracted ten times with 50 ml of ether and the combined organic layers were dried over $Na_2SO_4$ sicc. After removal of the solvent and careful drying under reduced pressure, the residue was treated with acetic anhydride (10 ml) and pyridine (10 ml) and the resulting solution was kept at room temperature for 2 days. The solvents were removed under reduced

pressure (0.01 Torr, 40°C) to yield a yellow oil which was purified by flash-chromatography (ethyl acetate : *n*-hexane, 2 : 1). TLC (ethyl acetate: *n*-hexane, 2 : 1): $R_f$(**3a**) = 0.22, $R_f$(**3b**) = 0.18. Yield: 315 mg **3a** (0.52 mmol, 5.7%) and 3.45 g **3b** (5.7 mmol, 62%).

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = −0.01 [s, 6H, $Si(CH_3)_2$], 0.83 [s, 9H, $SiC(CH_3)_3$], 1.86 (s, 3H, $CH_3CO$), 1.90–2.10 (m, 1-H, 3-$H_{ax}$), 2.01, 2.04, 2.07, 2.09 (4s, 4 × 3H, $CH_3CO$), 2.62 (dd, 1H, 3-$H_{eq}$), 3.54 (dd, 1H, 9-$H_a$), 3.72 (s, 3H, $CO_2CH_3$), 3.84 (dd, 1H, 9-$H_b$), 4.02 (ddd, 1H, 5-H), 4.51 (dd, 1H, 6-H), 5.09 (ddd, 1H, 5-H), 5.35 (dd, 1H, 7-H), 5.37 (dd, 1H, N-H); $J$ ($3_{ax}$, $3_{eq}$) = −13.6 Hz, $J$ ($3_{ax}$, 4) = 12.0, $J$ ($3_{eq}$, 4) = 4.9, $J$ (4, 5) = 10.8, $J$ (5, NH) = 9.9, $J$ (5, 6) = 11.1, $J$ (6,7) = 2.0, $J$ (7, 8) = 6.9, $J$ (8, $9_a$) = 3.4, $J$ (8, $9_b$) = 5.6, $J$ ($9_a$, $9_b$) = −11.5. $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 170.67 (1C, $CH_3CO$), 170.31 (2C, $CH_3CO$, 169.75 (1C, $CH_3CO$), 168.13 (1C, 1-C), 96.24 (2-C), 73.57 (8-C), 72.59 (6-C), 69.67, 67.80 (2C, 4-C, 7-C), 61.14 (9-C), 52.69 ($CO_2CH_3$), 49.22 (5-C), 36.55 (3-C), 25.65 [3C, $SiC(CH_3)_3$], 22.95 ($CH_3CO$), 20.70 (3C, $CH_3CO$), 19.64 ($CH_3CO$), 17.90 [$SiC(CH_3)_3$], −5.66 (2C, $SiCH_3$). MS (70 eV, 160°C): *m/z* (%) = 548 (2.3) [$M^+ - C_4H_9$]. $C_{26}H_{43}NO_{13}Si$ (605.71). Calcd. C 51.36, H 7.15, N 2.31; found C 51.47, H 7.23, N 2.12.

*Methyl (5-Acetamido-2,4,7,8-tetra-O-acetyl-9-O-t.butyldimethylsilyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid)onate* (**3b**)

For synthesis see **3a**. $^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = −0.01 (s, 6H, 2 × $SiCH_3$), 0.83 [s, 9H, $SiC(CH_3)_3$], 1.86 (s, 3H, $CH_3CO$), 1.89–2.06 (m, 1H, 3-$H_{ax}$), 2.00, 2.02, 2.09, 2.10 (4s, 4 × 3H, $CH_3CO$), 2.54 (dd, 1H, 3-$H_{eq}$), 3.59 (dd, 1H, 9-$H_a$), 3.75 (s, 3H, $COOCH_3$), 3.84–4.00 (m, 2-H, 5-H, 9-H), 4.17 (dd, 1H, 6-H), 4.92 (ddd, 1H, 8-H), 5.30 (ddd, 1-H, 4-H), 5.34 (dd, 1H, 7-H), 5.51 (d, 1H, N-H); $J$ ($3_{ax}$, $3_{eq}$) = −13.0 Hz, $J$ ($3_{ax}$, 4) = 11, $J$ ($3_{eq}$, 4) = 4.9, $J$ (4, 5) = 11.0, $J$ (5, NH) = 9.2, $J$ (5, 6) = 10.4, $J$ (6, 7) = 1.8, $J$ (7, 8) = 5.8, $J$ (8, $9_a$) = 2.9, $J$ (8, $9_b$) = 6.1, $J$ ($9_a$, $9_b$) = −11.8 $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 170.32, 170.12, 170.08, 169.70, 167.92 (5C, $CH_3CO$), 166.11 (1-C), 97.32 (2-C), 73.50 (8-C), 72.37 (6-C), 68.13 (4-C), 67.64 (7-C), 60.63 (9-C), 52.69 ($CO_2CH_3$), 48.93 (5-C), 35.54 (3-C), 25.39 [3C, $SiC(CH_3)_3$, 22.64, 20.55 (2C, $CH_3CO$, 20.46 (2C, $CH_3CO$), 20.29 ($CH_3CO$), 17.84 [$SiC(CH_3)_3$], −5.92 (2C, Si-$CH_3$), ). MS (70 eV, 150°C): *m/z* (%) = 605 (6.15) [$M^+ - C_4H_9$]. $C_{26}H_{43}NO_{13}Si$ (605.71). Calcd. C 51.56, H 7.15, N 2.31; found C 51.68, H 7.08, N 2.23.

*Methyl (5-Acetamido-2,4,7,9-tetra-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosid)onate* (**4**)

To **3a** (1.43 g, 2.4 mmol) in 0.2 *M* $HCO_2H$ in *THF* (36 ml) tetrabutylammonium fluoride was added and the mixture was stirred for 3d at room temperature. TLC (ethyl acetate): $R_f$(**3a**) = 0.48, $R_f$(**4**) = 0.16. Subsequent removal of solvents and flash-chromatography (60 g silica gel, ethyl acetate) yielded 1.07 g of **4** (2.2 mmol, 92%).

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.92 (s, 3H, $CH_3CO$), 1.99 (dd, 1H, 3-$H_{ax}$), 2.06, 2.10, 2.15, 2.19 (4s, 4 × 3H, $CH_3CO$), 2.49 (dd, 1H, 3-$H_{eq}$), 3.83 (s, 3H, $CO_2CH_3$), 3.92–4.34 (m, 5H, 5-H, 6-H, 8-H, 9-$H_a$, 9-$H_b$), 5.28 (ddd, 1H, 4-H), 5.61 (d, 1H, N-H); $J$ ($3_{ax}$, $3_{eq}$) = −13 Hz, $J$ ($3_{ax}$, 4) = 11.3, $J$ ($3_{eq}$, 4) = 4.9, $J$ (4, 5) = 12.0, $J$ (5, NH) = 8.3, $J$ (6, 7) = 1.6, $J$ (7, 8) = 8.3, $J$ (5, 6), $J$ (8, $9_a$), $J$ (8, $9_b$), $J$ ($9_a$, $9_b$) not determined. $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 171.11, 170.93, 170.56, 170.33, 169.87 (5C, $CH_3CO$), 166.78 (1-C), 97.66 (2-C), 72.22 (6-C), 68.82, 68.75, 68.25 (3C, 4-C, 7-C, 8-C), 65.40 (9-C), 53.27 ($CO_2CH_3$), 49.10 (5-C), 36.87 (3-C), 23.13, 20.95, 20.88 (3C, $CH_3CO$), 20.83 (2C, $CH_3CO$). MS (70 eV, 180°C): *m/z* (%) = 432 (1.8) [$M^+ - C_2H_3O_2$]. $C_{20}H_{29}NO_{13}$ (491.4). Calcd. C 48.88, H 5.95, N 2.85; found C 48.93, H 6.01, N 2.78.

*Methyl (5-Acetamido-2,4,7,9-tetra-O-acetyl-3,5-dideoxy-b-D-galacto-2,8-nondiulopyranosid)onate* (**5**)

$RuO_2 \cdot H_2O$ (244 mg), $K_2CO_3$ (59 mg), and $KIO_4$ (833 mg) were dissolved in $H_2O$ (20 ml). This mixture was stirred till all $RuO_2$ had reacted (10 min) and the resulting yellow solution was extracted 5 times

with $CHCl_3$ (5 × 10 ml), which was freshly distilled and passed through a column filled with alox neutral. The combined organic layer was added all at once to a vigorously stirred solution of alcohol **4** (433 mg, 0.88 mmol) in $CHCl_3$ (2 ml). The reaction was monitored by TLC (ethyl acetate): $R_f$(**5**) = 0.23. After 25 min all starting material had reacted and the reaction was quenched by addition of iso-propanol (0.5 ml). After stirring for further 5 min the mixture was filtered by suction through celite and the precipitate was washed three times with $CH_2Cl_2$ (50 ml). The combined organic layer was concentrated under vacuum and the residue was subjected to flash-chromatography (40 g silica gel, ethyl acetate). Yield 417 mg (0.85 mmol, 96%) of ketone **5**.

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.91 (s, 3 H, $CH_3CO$), 1.97 (dd, 1 H, 3-$H_{ax}$), 2.06, 2.15, 2.18, 2.30 (4 s, 4 × 3 H, $CH_3CO$), 2.54 (dd, 1 H, 3-$H_{eq}$), 3.80 (s, 3 H, $CO_2CH_3$), 4.20 (dd, 1 H, 6-H), 4.31 (dd, 1 H, 5-H), 4.74 (d, 1 H, 9-$H_a$), 4.92 (d, 2 H, 9-$H_b$), 5.25 (ddd, 1 H, 4-H), 5.32 (d, 1 H, 7-H), 5.87 (d, 1 H, N-H); $J$ ($3_{ax}$, $3_{eq}$) = −13.6 Hz, $J$ ($3_{ax}$, 4) = 12.2, $J$ ($3_{eq}$, 4) = 5.0, $J$ (4, 5) = 10.0, $J$ (5, NH) = 8.8, $J$ (5, 6) = 10.5, $J$ (6, 7) = 2.0, $J$ ($9_a$, $9_b$) = −17.5 $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 200.62 (8-C), 170.90 ($CH_3CO$), 170.59 (2 C, $CH_3CO$), 170.36, 168.05 (2 C, $CH_3CO$), 166.50 (1-C), 96.86 (2-C), 75.15, 74.29 (6-C, 7-C), 68.09 (4-C), 66.92 (9-C), 53.11 ($CO_2CH_3$), 48.94 (5-C), 36.49 (3-C), 22.97, 20.82, 20.68, 20.49, 20.49, 20.36 (5 C, $CH_3CO$). MS (70 eV, 150°C): $m/z$ (%) = 429 (3.65) [$M^+ - C_2H_4O_2$]. $C_{20}H_{27}NO_{13}$ (489.4). Calcd. C 49.08, H 5.56, N 2.86; found C 49.01, H 5.62, N 2.74.

*Methyl (5-Acetamido-4,7,9-tri-O-acetyl-2,3,5-trideoxy-2,3-didehydro-β-D-galacto-2,8-nondiulopyranosid)onate* (**6**)

To a chilled (0°C) solution of ketone **5** (300 mg, 0.61 mmol) in acetonitrile anhydr. (12 ml) trimethylsilyltrifluoromethane sulfonate (222 µl) was added all at once and the mixture was kept at 0°C overnight. After the quick addition of $K_2CO_3$ (324 mg) the resulting mixture was stirred for further 5 min and filtered through Celite (pretreated with dry $CH_3CN$) and the precipitate was washed twice with acetonitrile (10 ml). The combined filtrates were concentrated under reduced pressure and subsequent flash-chromatography (20 g silica gel, ethyl acetate) yielded 231 mg of **6** (0.54 mmol, 88%). TLC (ethyl acetate): $R_f$(**6**) = 0.28.

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.93, 2.09, 2.18, 2.26 (5 s, 5 × 3 H, $CH_3CO$), 3.80 (s, 3 H, $CO_2CH_3$), 4.50–4.63 (m, 2 H, 5-H, 6-H), 4.88 (d, 1 H, 9-$H_a$), 4.99 (d, 1 H, 9-$H_a$), 5.47 (dd, 1 H, 4-H, 5.93 (d, 1 H, 3-H), 6.15 (d, 1 H, N-H); $J$ (3, 4) = 2.3 Hz, $J$ (4, 5) = 8.0, $J$ (5, NH) = 8.2, $J$ (6, 7) = 1.2, $J$ ($9_a$, $9_b$) = −17.3, $J$ (5, 6) not determined. $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 200.66 (8-C), 170.98 (1 C, $CH_3CO$), 170.48 (2 C, $CH_3CO$), 170.21 (1 C, $CH_3CO$), 161.43 (1-C), 145.13 (2-C), 108.51 (3-C), 77.86, 74.47 (2 C, 6-C, 7-C), 68.58 (4-C), 66.97 (9-C), 52.55 (5-C), 46.34 $CO_2CH_3$, 23.05, 20.87, 20.64, 20.36 (4 C, $CH_3CO$). MS (70 eV, 160°C): $m/z$ (%) = 429 (0.32) [$M^+$], 370 (0.46) [$M^+ - C_2H_3O_2$]. $C_{18}H_{23}NO_{11}$ (429.4). Calcd. C 50.35, H 5.40, N 3.26; found C 50.28, H 5.32, N 3.12.

*Methyl (5-Acetamido-4,7-di-O-acetyl-2,3,5-trideoxy-2,3-didehydro-β-D-galacto-2,8-nondiulopyranosid)onate 8,8-Dimethylacetal* (**7**)

To a solution of **6** (420 mg, 0.98 mmol) in trimethyl orthoformate (3 ml) 0.036 *M* $H_2SO_4$ in dry methanol (1 ml) was added. After this mixture was stirred for 2 d at room temperature $BaCO_3$ (500 mg) was added all at once and the resulting slurry was stirred for further 2 h. Finally, the suspension was diluted with methanol (5 ml) and filtered through Celite by suction. Subsequent removal of solvents under reduced pressure and flash-chromatography (20 g florisil, ethyl acetate) yielded crude **7** (340 mg). This material was purified by HPLC (Silicapore, ethyl acetate) to yield 160 mg (0.37 mmol, 38%) of **7**. TLC (ethyl acetate: $R_f$(**7**) = 0.14.

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.91, 2.03, 2.07 (3 s, 3 × 3 H, $CH_3CO$), 3.17, 3.33 (2 s, 2 × 3 H, $OCH_3$), 3.55 (d, 1 H, 9-$H_a$), 3.76 (s, 3 H, $CO_2CH_3$), 3.94 (d, 1 H, 9-$H_b$), 4.25 (ddd, 1 H, 5-H), 4.69 (dd, 1 H, 6-H), 5.32 (dd, 1 H, 7-H), 5.58 (dd, 1 H, 4-H), 5.94 (d, 1 H, 3-H), 5.96 (d, 1 H, N-

H); $J(3, 4) = 3.0$ Hz, $J(4, 5) = 7.9$, $J(5, NH) = 8.5$, $J(5, 6) = 9.5$, $J(6, 7) = 2.6$, $J((9_a, 9_b) = -12.5$. $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): $\delta = 170.54$, 170.28, 169.62 (3 c, $CH_3CO$), 162.06 (1 C), 144.62 (2-C), 108.51 (3-C), 100.71 (8-C), 76.29 (6-C), 67.98, 66.74 (2 C, 4-, 7-C), 60.96 (9-C), 52.66, 49.25, 48.25, 47.39 (4 C, 5-C, $CO_2CH_3$, $2 \times OCH_3$), 23.04, 20.76, 20.70 (3 c, $CH_3CO$). MS (70 eV, 160°C): $m/z$ (%) = 598 (3.4) [$M^+ - CH_3$], 402 (48.4) [$M^+ - C_{14}H_{11}O_2$]. $C_{18}H_{27}NO_{11}$ (433.4). C 49.84, H 6.28, N 3.23; found C 49.96, H 6.15, N 3.07.

*Sodium 5-Acetamido-2,3,5-trideoxy-2,3-didehydro-D-galacto-2,8-nondiulopyranosidonate 8,8-Dimethylacetal* (**8**)

0.5 ml of a 0.1 *M* sodium methoxide solution in methanol was added to a chilled solution (0°C) of **6a** (27 mg, 0.062 mmol) in methanol (2 ml) and was kept at 0°C for 12 h. After removal of solvents under reduced pressure the residue was dissolved twice in methanol (5 ml) and again concentrated to dryness. Finally the residue was dissolved in $H_2O$ (10 ml) and kept at room temp. for 2 h till the $CO_{2(s)}$ was added and a neutral solution was obtained. After treatment with char-coal and filtration through Celite a lyophilization led to 16 mg (0.045 mmol, 72%) of compound **8**.

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): $\delta = 2.08$, (s, 3 H, $CH_3CO$), 3.25, 3.30 (2 s, $2 \times 3$ H, $OCH_3$), 3.56 (d, 1 H, 9-$H_a$), 3.82 (d, 1 H, 7-H), 3.96 (d, 1 H, 9-H), 4.05–4.17 (m, 2 H, 5-, 6-H), 4.46 (dd, 1 H, 4-H), 5.73 (d, 1 H, 3-H); $J(3, 4) = 2.5$ Hz, $J(4, 5) = 8.2$, $J(6, 7) = 1.5$, $J(9_a, 9_b) = -13.4$, $J(5, 6)$ not determined. $^{13}$C-NMR (62.9 MHz, $D_2O$/dioxane): $\delta = 175.65$ ($CH_3CO$), 148.30 (2-C), 108.76 (3-C), 102.00 (8-C), 76.28 (4-C), 68.32, 67.56 (2 C, 6-, 7-C), 59.50 (9-C), 51.32, 49.69, 48.44 (3 C, 5-C, $2OCH_3$), 22.89 ($CH_3CO$).

*Methyl (Methyl-5-acetamido-4,7,9-tri-O-acetyl-3,5-dideoxy-α-D-galacto-2,8-nondiulopyranosid)onate 8,8-Dimethylacetal* (**9**)

A solution of **5** (174 mg, 0.38 mmol) in trimethyl orthoformate (2 ml) and 0.018 *M* $H_2SO_4$ in methanol (2 ml) was kept at room temperature for 48 h. After addition of $BaCO_3$ (0.5 g) the slurry was stirred for 2 h, diluted with methanol (10 ml) and filtered through celite by suction. The filtrate was concentrated under reduced pressure followed by coevaporation with dry methanol ($2 \times 5$ ml). The residue was dissolved in pyridine (3 ml) and was kept 12 h at room temperature. After removal of solvents at reduced pressure (0.01 Torr, 40°C), a flash-chromatography (15 g florisil, ethyl acetate) of the resulting residue yielded a mixture of **9** and **10**. This two compounds were separated by HPLC (silica-phase, ethyl acetate). Yield: 125 mg of **9** (0.25 mmol, 66%) and 30 mg of **10** (0.060 mmol, 16%). TLC (ethyl acetate): $\mathbf{R}_f$(**9**) = 0.17, $R_f$(**10**) = 0.15.

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): $\delta = 1.86$ (s, 3 H, $CH_3CO$), 1.89 (dd, 1 H, 3-$H_{ax}$), 2.00, 2.06, 2.12 (3 s, $3 \times 3$ H, $CH_3CO$), 2.59 (dd, 1 H, 3-$H_{eq}$), 3.25, 3.36, 3.40 (3 s, 3 H, $OCH_3$), 3.82 (s, 3 H, $CO_2CH_3$), 3.83 (ddd, 1 H, 5-H), 4.12 (d, 1 H, 6-H), 4.52 (d, 1 H, 9-$H_b$), 4.97 (ddd, 1 H, 4-H), 5.22 (d, 1 H, N-H), 5.23 (d, 1 H, 7-H); $J(3_{ax}, 3_{eq}) = -12.8$ Hz, $J(3_{ax}, 4) = 11.8$, $J(3_{eq}, 4) = 4.9$, $J(4, 5) = 10.2$, $J(5, NH) = 10.5$, $J(5, 6) = 10.8$, $J(6, 7) = 1.4$, $J(9_a, 9_b) = -12.8$. MS (70 eV, 150°C): $m/z$ (%) = 448 (13.7) [$M^+ - C_2H_3O_2$]. $C_{21}H_{33}NO_{13}$ (507.48). Calcd. C 49.70, H 6.55, N 2.76; found C 49.96, H 6.68, N 2.58.

*Methyl (Methyl-5-acetamido-4,7,9-tri-O-acetyl-3,5-dideoxy-β-D-galacto-2,7-nondiulopyranosid)onate 8,8-Dimethyl acetal* (**10**)

See **9**. Analytical data were identical with those previously reported by us [8].

*Sodium (Methyl-5-acetamido-3,5-dideoxy-α-D-galacto-2,8-nondiulopyranosid)onate 8,8-Dimethylacetal* (**11**)

A 0.1 *M* sodium methoxide solution in methanol (0.45 ml) was added to a solution of **9** in methanol (1 ml) and the resulting mixture was kept at room temperature for 12 h. Then the solvent was removed under reduced pressure and the residue was coevaporated twice with dry methanol (3 ml). The resulting residue was dissolved in $H_2O$ (2 ml) and stirred for 1 h till the solution was neutralized by addition of $CO_{2(s)}$. Lyophilization of this solution resulted in 15 mg of **11** (0.039 mmol, 94%).

$^1$H-NMR (250 MHz, $D_2O$/HDO): δ = 1.59 (dd, 1 H, 3-$H_{ax}$), 2.02 (s, 3 H, $CH_3CO$), 2.63 (dd, 1 H, 3-$H_{eq}$), 3.23, 3.29, 3.33 (3 s, 3 × 3 H, $OCH_3$), 3.55 (ddd, 1 H, 4-H), 3.56 (d, 1 H, 9-$H_a$), 3.62 (d, 1 H, 7 H), 3.80 (dd, 1 H, 5-H), 3.92 (dd, 1 H, 6-H), 3.94 (d, 1 H, 9-$H_b$); $J$ ($3_{ax}$, $3_{eq}$) = −12.2 Hz, $J$ ($3_{ax}$, 4) = 12.0, $J$ ($3_{eq}$, 4) = 4.2, $J$ (4, 5) = 9.8, $J$ (5, 6) = 10.2, $J$ (6, 7) < 1 Hz, $J$ ($9_a$, $9_b$) = −12.8 $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 175.81 ($CH_3CO$), 102.36, 101.43 (2 C, 1-C, 8-C), 72.86, 68.29, 68.15 (3 C, 4-, 6-, 7-C), 60.13 (9-C), 53.50, 52.55 (2 C, 5-C, $OCH_3$), 4.92, 48.57 (2 C, $OCH_3$), 40.76 (3-C), 22.96 ($CH_3CO$).

*Methyl-5-acetamido-3,5-dideoxy-α-D-galacto-2,8-nondiulopyranosidonic Acid* (**12**)

To a solution of **9** (24 mg, 0.047 mmol) in methanol (1 ml) 0.1 *M* sodium methoxide in methanol (2 ml) was added and the mixture was kept at room temperature for 12 h. After removal of solvents at reduced pressure, the residue was coevaporated twice with dry methanol (3 ml) and dissolved in $H_2O$ (5 ml). After 1 h the solution was diluted with $H_2O$ (5 ml) and Amberlyst 15H$^+$ was added till the *pH* was below 3. Then the ion-exchange resin was filtered off and washed with $H_2O$ (5 ml). To the resulting clear solution 1.2 g Amberlyst 15 H$^+$ was added and the mixture was stirred for 2 h at room temperature. The reaction was monitored by TLC (*n*-propanol : $H_2O$ : acetic acid = 15 : 4 : 0.5): $R_f$(**11**) = 0.30, $R_f$(**12**) = 0.26. After filtration of the resin the resulting solution was treated with charcoal (0.5 h) and filtered through celite by suction. Lyophilization yielded 14 mg of **12** (0.044 mmol, 94%).

$^1$H-NMR (250 MHz, $CDCl_3$/*TMS*): δ = 1.69 (dd, 1 H, 3-$H_{ax}$), 2.01 (s, 3 H, $CH_3CO$), 2.60 (dd, 1 H, 3-$H_{eq}$), 3.25 (s, 3 H, $OCH_3$), 3.72 (ddd, 1 H, 4-H), 3.90 (dd, 1 H, 5-H), 4.19 (dd, 1 H, 6-H), 4.41 (d, 1 H, 7-H), 4.50 (d, 1 H, 9-$H_a$), 4.78 (d, 1 H, 9-$H_b$); $J$ ($3_{ax}$, $3_{eq}$) = −12.2 Hz, $J$ ($3_{ax}$, 4) = 11.9, $J$ ($3_{eq}$, 4) = 4.3, $J$ (4, 5) = 10.2, $J$ (5, 6) = 10.3, $J$ (6, 7) = 1.6, $J$ ($9_a$, $9_b$) = −19.3. $^{13}$C-NMR (62.9 MHz, $CDCl_3$/*TMS*): δ = 212.64 (8-C), 175.72 ($CH_3CO$), 100.76 (2-C), 75.63, 74.37 (2 C, 6-, 7-C), 67.87 (4-C), 66.61 (9-C), 52.25 (3-C), 22.91 ($CH_3CO$).

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