

128. Deoxy-nitrosugars

15th Communication¹⁾

Synthesis of *N*-Acetylneuraminic Acid and *N*-Acetyl-4-epineuraminic Acid

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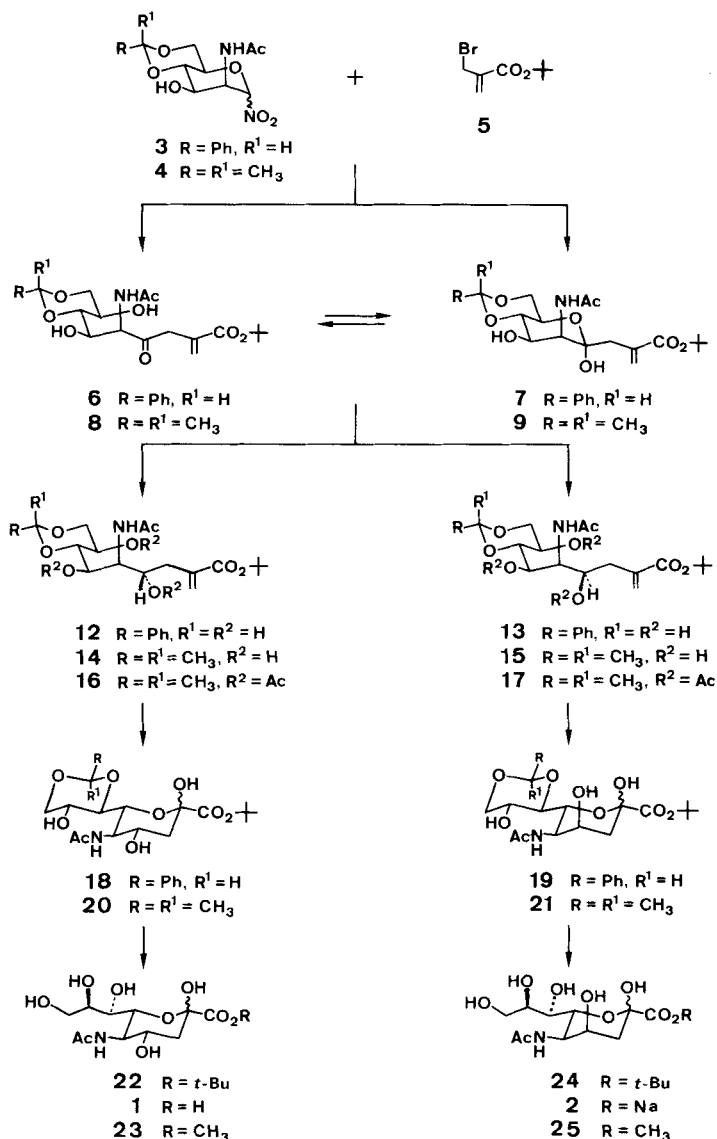
A synthesis of *N*-acetylneuraminic acid (**1**) and of *N*-acetyl-4-epineuraminic acid (**2**, R = H) from 2-acetamido-4,6-*O*-benzylidene-1,2-dideoxy-1-nitro-D-mannopyranose (**3**) and 2-acetamido-1,2-dideoxy-4,6-*O*-isopropylidene-1-nitro-D-mannopyranose (**4**), respectively, is described. *Michael* addition of **3** and **4** to *tert*-butyl 2-(bromomethyl)prop-2-enoate (**5**) and subsequent hydrolytic removal of the NO₂ group gave the 4-nonulosonate tautomers **6/7** and **8/9**, respectively (*Scheme*). Stereoselective reduction of **6/7** and **8/9** with NaBH₄/AcOH in dioxane/H₂O yielded **12/13** (94:6) and **14/15** (92:8), respectively. Reduction of **6/7** and **8/9** in the absence of AcOH or in EtOH gave **12/13** (15:85) and **14/15** (15:85), respectively. Ozonolysis of **12** and **13** followed by hydrolysis gave *tert*-butyl neuraminate **22** and *tert*-butyl 4-epineuraminate **24**, respectively. Ozonolysis of **14/15**, separation of the products **20** and **21**, and hydrolytic removal of the isopropylidene groups gave **22** and **24**, respectively. The *tert*-butyl ester **22** was saponified to give **1**, which was further characterized as the methyl ester **23**. Saponification of **24** gave the crude 4-epimer of **1**, which was converted into the stable Na salt **2** and also into the methyl ester **25**.

Introduction. – Sialic acids [2] [3] and particularly *N*-acetylneuraminic acid (**1**; Neu5Ac) frequently occur at the nonreducing ends of oligosaccharide chains of glycoproteins and glycolipids. Many hypotheses link their presence to biological functions of glycoconjugates (*cf.* [4] [5]). Subsequent to its isolation from gangliosides and from submaxillary mucin by Klenk and coworkers [6] [7], **1** has been synthesized in several ways. The first synthesis by Cornforth *et al.*, based on the reaction of oxaloacetic acid with *N*-acetylglucosamine or *N*-acetylmannosamine [8] [9], has been improved by Kuhn and Baschang [10], who used *N*-acetyl-4,6-*O*-benzylideneglucosamine and potassium di(*tert*-butyl) oxaloacetate. A synthesis of **1** allowing modifications at C(1) to C(3) has been reported by Benzing-Nguyen and Perry [11]. David and coworkers [12] have described an enzymatic synthesis of **1** from pyruvate and an equilibrating mixture of *N*-acetylglucosamine and *N*-acetylmannosamine. We wished to work out a synthesis of *N*-acetylneuraminic acid (**1**), which should allow the preparation of its analogues modified at C(1) to C(5), aiming first at a modification at C(4). Analogues of **1** modified at C(4) are of interest in the context of the mechanism of neuraminidases. The *N*-acetyl-2-deoxy-4-epineuraminic acid [13], the *N*-acetyl-2,3-didehydro-4-epineuraminic acid, its methyl ester [14] [15], and *N*-acetyl-2,3-didehydro-4-oxoneuraminic acid [14] were all competitive inhibitors of *Arthrobacter sialophilus* neuraminidase [15] and of the influenza viral neuraminidase [15]. Bacterial and mammalian sialidases were completely inactive to the

¹⁾ 14th Communication [1].

naturally occurring *N*,4-*O*-diacetylneuraminic acid [16] [17], which was also hardly cleaved by acylneuraminate pyruvate-lyase [18]. Furthermore, the synthetic *N*-acetyl-4-*O*-methylneuraminic acid was resistant to acylneuraminate pyruvate-lyase and did not inhibit the aldol cleavage of **1** by this enzyme [19]. (*N*-acetyl-4-*O*-methylneuraminic acid) fetuin (prepared from *N*-acetyl-4-*O*-methylneuraminic acid and asialo-fetuin with the help of sialyl transferase [20]) releases *N*-acetyl-4-*O*-methylneuraminic acid upon treatment with fowl-plague neuraminidase [19], but is strongly resistant to *Vibrio cholerae* neuraminidase [19].

Scheme



We now report on a synthesis of **1** and of its 4-epimer **2** (*Scheme*), based upon the *Michael* addition of a partially protected 2-acetamido-1,2-dideoxy-1-nitro-D-mannose to a 2-(bromomethyl)acrylate, followed by a β -elimination as the key transformation.

Results. – Two derivatives of 1,2-dideoxy-1-nitro-D-mannopyranose, *viz.* the 4,6-*O*-benzylidene acetal **3** and the 4,6-*O*-isopropylidene acetal **4**²⁾ [**1**] [**21**] were subjected to base-catalyzed addition to *tert*-butyl 2-(bromomethyl)prop-2-enoate³⁾ (**5**) [**22**] [**23**]. *Michael* addition of **3** to **5** (1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), THF, 0°) followed by carefully controlled hydrolytic removal of the NO₂ group [**24**] gave a 65:35 mixture of the benzylidene-protected 4-nonulosonate tautomers **6** and **7** (64%). Similarly, **4** reacted with **5** to give, after hydrolysis, a 65:35 mixture of the isopropylidene-protected 4-nonulosonate tautomers **8** and **9** (83%)⁴⁾.

In the ¹³C-NMR spectra ((D₆)DMSO), the major (hydroxy ketone) isomers **6** and **8** show C(4)=O signals at 206.07 and 206.17 ppm, respectively. The C(4) signals of the hemiacetal isomers **7** and **9** appear at 99.44 and 99.39 ppm, respectively. These are typical values for α -D-anomers [**26**]. Signals of the β -D-anomers were not observed. The ratios of **6**/**7** and **8**/**9** were determined by integration of the NH signals in the ¹H-NMR spectra ((D₆)DMSO) and confirmed by the integrals in the ¹³C-NMR spectra. The same ratio **6**/**7** was observed for a (D₈)dioxane/D₂O 4:1 solution even after addition of 2 equiv. of AcOD. A 65:35 ratio of **8**/**9** was also observed for a CDCl₃ solution.

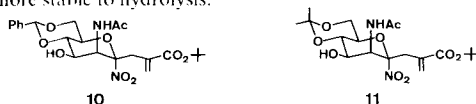
Reduction of **6**/**7** with NaBH₄ in dioxane/H₂O 4:1 in the presence of 2 mol-equiv. of AcOH⁵⁾ gave a 94:6 mixture of the epimeric triols **12** and **13**, which were separated by medium-pressure liquid chromatography (MPLC) [**30**] (92% combined yield). Similarly, reduction of **8**/**9** gave a 92:8 mixture of **14** and **15** (83%). Their tri-*O*-acetates **16**/**17** were separated by MPLC and characterized. The configurations of the epimeric triols **12**/**13** and **14**/**15** were determined by transformation of **12** and **14** into *N*-acetylneuraminic acid (**1**) and by transformation of **13** and **15** into *N*-acetyl-4-epineuraminic acid (**2**) as described below.

Reduction of the benzylidene acetals **6**/**7** and the isopropylidene acetals **8**/**9** with NaBH₄ in the absence of AcOH always gave the D-glycero-D-talo-isomer **13** and **15**, respectively, as the main products (**12**/**13** and **14**/**15** = 15:85). The same ratio of the epimeric triols **12** and **13** was obtained by the reduction of **6**/**7** with NaBH₄ in EtOH (95%) or with Zn(BH₄)₂ [**31**] in THF (95% combined yield). The diastereoselectivity of

²⁾ The overall yields of **4** from D-mannose (35%) [**1**] [**21**] were not affected by upscaling; most intermediates crystallized easily. The overall yield of **3** from D-glucose were 28% for batches up to 10 g [**1**] [**21**]; batches of 50 to 80 g gave a slightly lower yield (21%), mainly due to the formation of by-products during ozonolysis and the chromatographic purification of 1-deoxy-1-nitro-D-glucose.

³⁾ We thank Dr. H. Braunschweiger, Sandoz AG, Basel for a generous gift of this compound.

⁴⁾ Both the reactions of **3** and **4** with **5** gave a main product and a by-product. The IR spectra of the crude products from **3** and **4** showed $\tilde{\nu}(\text{NO}_2)$ at 1555 cm⁻¹ and 1553 cm⁻¹, respectively. The position of these bands indicates the major products to be **10** and **11**, respectively [**25**], implying equatorial attack on the *Michael* acceptor. While **10** was partially transformed into **6** and **7** during aqueous workup, the isopropylidene derivative **11** proved more stable to hydrolysis.



⁵⁾ The diastereoselectivity in the reduction of ketones depends upon the proportions of hydroxylic and non-hydroxylic components of the solvent [**27**]. For the use of sodium acyloxyborohydrides in diastereoselective reductions of cyclic ketones, see [**28**] [**29**].

⁶⁾ Initial experiments were performed by Martin Hugener (undergraduate research project).

these reductions may be rationalized by assuming a H-bond between the acetamido substituent and the C(4)=O group [32]. In the absence of such an H-bond, the *Anh-Felkin* model [33] predicts the formation of **12** and **14**.

Ozonolysis of the epimeric benzylidene acetals **12** and **13** gave the crystalline hemiacetals **18** and **19**, respectively. Both compounds showed no mutarotation in MeOH solution after 5 min. The $^1\text{H-NMR}$ ((D_6) DMSO) and $^{13}\text{C-NMR}$ spectra (CD_3OD) of the neuraminic-acid derivative **18** indicated the presence of only the β -D-anomer. In the $^1\text{H-NMR}$ spectrum ((D_6) DMSO) of the 4-epineuraminic-acid derivate **19** also, only the β -D-anomer was detected. The $^{13}\text{C-NMR}$ (CD_3OD) and the $^1\text{H-NMR}$ spectrum (CD_3OD) of freshly dissolved **19** showed, however, signals for both anomers ($\alpha/\beta = 1:3$). Thus, compound **19** anomerized slowly in DMSO, but rapidly in MeOH (*cf.* [34]).

Ozonolysis of the 92:8 mixture of the epimeric isopropylidene acetals **14** and **15** and flash chromatography of the crude product gave the neuraminic-acid derivative **20** (85%) and its 4-epi analogue **21** (7%). Although **20** and **21** did not crystallize, their $^1\text{H-NMR}$ ((D_6) DMSO) and their $^{13}\text{C-NMR}$ spectra (CD_3OD) showed only traces of the α -D-anomers. No signals of open-chain tautomers of **18**, **19**, **20**, or **21** were observed.

The $^1\text{H-NMR}$ spectra of the neuraminic-acid derivatives **18** and **20** showed two large coupling constants (**18**: $J(4, 5) = 9.5$ and $J(5, 6) = 9.1$ Hz; **20**: $J(4, 5) = 9.8$ and $J(5, 6) = 9.0$ Hz), characteristic for a 1,2-diaxial orientation of H–C(4)/H–C(5) and H–C(5)/H–C(6). The corresponding coupling constant of the 4-epi derivatives **19** and **21** were $J(4, 5) = 3.0$ (**19** and **21**) and $J(5, 6) = 10.5$ (**19**) and 9.0 (**21**) [35] [36], clearly indicating a 1,2-diaxial relation of H–C(5)/H–C(6) and a 1,2-synclinal relation of H–C(4)/H–C(5).

The benzylidene acetal **18** was transformed into **1** by hydrogenolysis of the benzylidene group (Pd/C, aq. MeOH, AcOH; 94%) and saponification of the resulting crystalline *tert*-butyl ester **22** (K_2CO_3 , aq. MeOH; > 95% of **1**, 51% after crystallization [8] [10]). This product could not be distinguished from an authentic sample of *N*-acetylneuraminic acid (*Fluka*; m.p., mixed m.p., $[\alpha]_D$, and $^1\text{H-NMR}$ spectrum [10] [37]). The structure of **1** was confirmed by preparation of the known methyl ester **23** [37] [38]. The *tert*-butyl ester **22** was also obtained from the isopropylidene acetal **20** by hydrolysis with CF_3COOH in CH_2Cl_2 (85%). It did not mutarotate in H_2O solution. According to the $^1\text{H-NMR}$ spectrum it is a 9:1 mixture of the β -D- and α -D-anomers.

Similarly, sodium *N*-acetyl-4-epineuramate (**2**) was obtained from the benzylidene acetal **19**, which gave the crystalline *tert*-butyl ester **24** (95%) upon hydrogenolysis. Saponification of **24** and sequential chromatography on *Dowex 1* (HCOO^-) and *Dowex 50* (Na^+) gave the sodium *N*-acetyl-4-epineuramate **2** (46%)⁷⁾ as a 1:9 mixture of the α - and β -D-anomers ($^1\text{H-NMR}$).

Comparison of the $^{13}\text{C-NMR}$ spectrum of **2** with the one of sodium *N*-acetyl- β -neuramate (**1**; R = Na; pD 7) [39] [40] shows an upfield shift of 3.9 and 5.4 ppm for C(3) and C(5 and 6) of **2**, respectively. The upfield shift of 5.4 ppm for C(6) is a typical value [41] for the difference of the γ -effects exerted by the equatorial and the axial OH group at C(4) of **1** and **2**, respectively.

The *tert*-butyl ester **24** was also obtained from the isopropylidene acetal **21** by hydrolysis with CF_3COOH in CH_2Cl_2 (65%). It did not mutarotate in H_2O solution after 5 min. Its $^1\text{H-NMR}$ spectrum showed the presence of a 7:3 mixture of the β - and

⁷⁾ The free acid has not been described. It did not crystallize in our hands, but decomposed slowly, forming a red oil.

α -D-anomers. The methyl ester **25** was obtained in high yield by treating the *tert*-butyl ester **24** first with CF_3COOH in chlorobutane and then with CF_3COOH in MeOH.

We thank the Swiss National Science Foundation and Sandoz AG, Basel, for generous support.

Experimental Part

General. See [42]. All solvents were distilled before use. THF was distilled from NaH, dioxane from Na, anhydrous MeOH from Mg. Solvents were removed in a rotary evaporator at or below 40° . *tert*-Butyl 2-(bromomethyl)prop-2-enoate (**5**) was distilled before use [23]. TLC: detection by spraying the plates with 0.025M I_2 in 10% aq. H_2SO_4 followed by heating at *ca.* 200° . Column chromatography: silica gel Merck 60, $60\text{--}230\ \mu\text{m}$; flash chromatography (FC): $40\text{--}63\ \mu\text{m}$; MPLC [30]: $15\text{--}40\ \mu\text{m}$. HPLC: Kontron apparatus (LC pump 410) with UV detector. IR: Perkin-Elmer 298 spectrometer (3% , CHCl_3 , unless otherwise specified). ^1H - and ^{13}C -NMR: Varian-HA-100 (^{13}C (25.2 MHz)), Varian-XL-200 (1 H (200 MHz), ^{13}C (50 MHz)) or Bruker-AM-400 spectrometer (1 H (400 MHz), ^{13}C (100.6 MHz)); CDCl_3 solns. unless otherwise specified; δ 's in ppm relative to TMS. MS: Varian-112S (EI: 70 eV; Cl: isobutan) and Varian 711 spectrometer (FAB, bombardement with 8-keV Xe-atoms, glycerol matrix).

tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-manno-4-nonulosonate (**6**) and *tert*-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-manno-4-nonulopyranosonate (**7**). To an ice-cold soln. of 2-Acetamido-4,6-O-benzylidene-1,2-dideoxy-1-nitro-D-mannopyranose (**3**) [1] [21] (8.40 g, 24.8 mmol) and *tert*-butyl 2-(bromomethyl)prop-2-enoate (**5**; 8.40 g, 37.2 mmol) in THF (75 ml) was added dropwise a soln. of DBU (8.40 g, 49.6 mmol) in THF (25 ml) within 4 h. Stirring of the mixture was continued at 0° for 20 h. The precipitate was filtered off, and the filtrate was diluted with AcOEt (200 ml). Extractive workup (H_2O , brine) gave the crude product (13.1 g; TLC: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 92:8), which was redissolved in THF (80 ml) and phosphate buffer of pH 6.6 (20 ml) and treated with urea (1.95 g, 27.3 mmol). The mixture was stirred at r.t. for 3 d and then diluted with AcOEt (100 ml). Extractive workup (5% NaHCO_3 , H_2O , brine) gave a yellow oil, which was redissolved in AcOEt/ Et_2O 1:1. Addition of hexane afforded a precipitate, which was filtered off and crystallized from AcOEt/ Et_2O /hexane yielding slightly yellow crystals of **6/7** (2.2 g). FC of the combined mother liquors (500 g of SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 97:3) gave further **6/7** (5.0 g, total yield 64%). M.p. $143\text{--}144^\circ$; $[\alpha]_D^{25} = +20.5^\circ$ ($c = 1.0$, DMSO). IR: 3435m, 3320m (br.), 3000m, 2985m, 2935w, 2870w, 1715 (sh), 1680s, 1626m, 1502m, 1392m, 1370s, 1356m, 1152s, 1090s, 1030m. ^1H -NMR (400 MHz, $(\text{D}_6)\text{DMSO}$): 8.24 (d, $J = 9.0$, 0.65 NH); 7.60 (d, $J = 10.0$, 0.35 NH); 7.53–7.27 (m, 5 arom. H); 6.04 (d, $J = 1.2$, 0.65 olef. H); 6.00 (d, $J = 1.5$, 0.35 olef. H); 5.63 (d, $J = 1.5$, 0.35 olef. H); 5.51 (s, 0.35 ArCH); 5.50 (d, $J = 1.2$, 0.65 olef. H); 5.40 (s, 0.65 ArCH); 5.19 (d, $J = 6.0$, 0.65 OH); 4.95 (d, $J = 8.0$, 0.65 OH); 4.73 (dd, $J = 9.0$, 9.0, 0.65 H–C(5)); 4.16 (ddd, $J = 10.0$, 10.0, 5.0, 0.65 H–C(8)); 4.21–3.44 (m, 7.35 H); 1.88 (s, 0.35 CH_3); 1.84 (s, 0.65 CH_3); 1.42 (s, 0.35 *t*-Bu); 1.37 (s, 0.65 *t*-Bu). ^{13}C -NMR (50 MHz, $(\text{D}_6)\text{DMSO}$): **6**: 206.07 (s); 168.81 (s); 165.00 (s); 138.15 (s); 135.98 (s); 128.32 (d); 127.70 (d); 126.99 (t); 126.11 (d); 99.77 (d); 80.34 (d); 80.00 (s); 70.88 (t); 67.26 (d); 59.47 (d); 57.12 (d); 44.84 (t); 27.49 (q); 22.36 (q); **7**: 169.76 (s); 166.31 (s); 137.87 (s); 136.10 (s); 128.70 (d); 127.89 (d); 126.64 (t); 126.27 (d); 101.20 (d); 99.44 (s); 79.62 (s); 78.66 (d); 68.31 (t); 66.09 (d); 63.89 (d); 54.64 (d); 37.78 (t); 27.63 (q); 22.77 (q). CI-MS: 450 ($M^{++} + 1$), 432 ($(M^{++} + 1) - 18$), 390 ($(M^{++} + 1) - 60$), 376, 239, 209, 91. Anal. calc. for $\text{C}_{23}\text{H}_{31}\text{NO}_8$ (449.52): C 61.46, H 6.95, N 3.12; found: C 61.46, H 6.80, N 3.33.

tert-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-manno-4-nonulosonate (**8**) and *tert*-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-manno-4-nonulopyranosonate (**9**). To an ice-cold soln. of **4** [1] [21] (2.0 g, 6.90 mmol) and DBU (2.1 g, 13.80 mmol) in THF (25 ml), **5** (2.3 g, 10.40 mmol) in THF (5 ml) was added dropwise. After 2 h, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) indicated the disappearance of **4**. Phosphate buffer of pH 6.6 (10 ml) and urea (0.455 g, 7.60 mmol) were added, and the mixture was stirred at r.t. for 3 d, when TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) showed only traces of the intermediate nitro compound, the mixture was diluted with AcOEt (100 ml) and extracted with brine (50 ml). FC (250 g of SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 20:1) afforded **8/9** (2.3 g, 83%) as a colourless foam. $[\alpha]_D^{25} = -11.4^\circ$ ($c = 1.0$, CHCl_3). IR: 3420m (br.), 2980m, 2930w, 2870w, 1720 (sh), 1680s, 1632 (sh), 1502m, 1370s, 1318m, 1150s, 1064s, 955w, 876m, 856m. ^1H -NMR (200 MHz): 6.88 (d, $J = 8.0$, 0.65 NH); 6.31 (d, $J = 1.0$, 0.65 olef. H); 6.23 (d, $J = 1.2$, 0.35 olef. H); 5.84 (d, $J = 10.0$, 0.35 NH); 5.66 (d, $J = 1.0$, 0.65 olef. H); 5.57 (br. s, 0.35 olef. H); 5.36 (s, 0.35 OH); 5.00 (dd, $J = 8.0$, 5.6, 0.65 H–C(5)); 4.47 (dd, $J = 10.0$, 4.5, 0.35 H–C(5)); 4.40–3.54 (m, 0.65 2 H–C(3), H–C(6), H–C(7), H–C(8), 2 H–C(9), 0.65 OH); 3.43 (d, $J = 8.0$, 0.65 OH); 2.80 (d, $J = 3.0$, 0.35 OH); 2.79, 2.57 (2d, $J = 14.0$, 0.35 2 H–C(3)); 2.14 (s, 0.35 CH_3); 2.05 (s, 0.65 CH_3); 1.52–1.40 (5 CH_3). ^{13}C -NMR (50 MHz, $(\text{D}_6)\text{DMSO}$): **8**: 206.17 (s); 168.45 (s); 165.08 (s); 136.13 (s);

127.07 (*t*); 97.92 (*s*); 80.05 (*d*); 72.45 (*d*); 67.31 (*d*); 64.55 (*t*); 60.81 (*d*); 57.12 (*d*); 44.79 (*t*); 28.45 (*q*); 27.55 (*q*); 22.38 (*q*); 18.85 (*q*); **9**: 169.70 (*s*); 166.37 (*s*); 136.13 (*s*); 126.72 (*t*); 99.39 (*s*); 99.22 (*s*); 79.38 (*s*); 71.10 (*d*); 66.32 (*d*); 64.95 (*d*); 61.92 (*t*); 54.65 (*d*); 37.81 (*t*); 29.27 (*q*); 27.69 (*q*); 22.92 (*q*); 19.68 (*q*). Anal. calc. for $C_{19}H_{31}NO_8$ (401.47): C 56.84, H 7.78, N 3.49; found: C 56.57, H 7.56, N 3.33.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-glycero-D-galacto-nononate (**12**) and *tert*-Butyl 5-Acetamido-7,9-O-benzylidene-2,3,5-trideoxy-2-methylidene-D-glycero-D-talo-nononate (**13**). To an ice-cold soln. of **6/7** (3.0 g, 6.67 mmol) in dioxane/H₂O 4:1 (200 ml) was added a soln. of AcOH (0.80 g, 13.34 mmol) in dioxane/H₂O 4:1 (10 ml). To this mixture, 150 ml of a 0.13M soln. of NaBH₄⁸ in dioxane/H₂O 4:1 were added dropwise during 12 h (= 1 drop per 8 s). According to TLC (CHCl₃/MeOH 9:1), **6/7** had disappeared. The mixture was diluted with AcOEt (200 ml), excess NaBH₄ was destroyed with phosphate buffer (100 ml, pH 6.6). Extractive workup (half-sat. aq. NaCl, brine) gave a 94:6 mixture **12/13** (3.015 g; HPLC; *Zorbax-Sil*, CH₂Cl₂/MeOH 97:3, 254 nm), which were separated by MPLC (500 g of SiO₂, CH₂Cl₂/MeOH 95:5) affording **12** (2.604 g, 86.5%) and **13** (0.166 g, 5.5%) as colourless foams.

Data of 12: $[\alpha]_D^{25} = -37.3^\circ$ (*c* = 1.1, CHCl₃). IR: 3430m, 3005m, 2985m, 2935w, 2870w, 1703m, 1676s, 1630m, 1508m, 1370s, 1152s, 1086s, 1075s, 1030m, 1016 (sh). ¹H-NMR (400 MHz, (D₆)DMSO): 7.58–7.29 (*m*, NH, 5 arom. H); 6.00 (*d*, *J* = 1.2, 1 olef. H); 5.60 (*d*, *J* = 1.2, 1 olef. H); 5.35 (*s*, ArCH); 5.06 (*d*, *J* = 5.8, OH); 4.47 (*d*, *J* = 6.7, OH); 4.43 (*d*, *J* = 7.9, OH); 4.15 (*dd*, *J* = 10.5, 5.0, H–C(9)); 4.08–3.99 (*m*, H–C(4), H–C(5)); 3.85 (*br. d*, *J* = 10.0, H–C(6)); 3.74 (*ddd*, *J* = 10.0, 9.0, 5.0, H–C(8)); 3.49 (*dd*, *J* = 10.5, 10.0, H–C(9)); 4.43 (*br. d*, *J* = 9.0, H–C(7)); 2.23 (*d*, *J* = 6.0, 2 H–C(3)); 1.90 (*s*, CH₃); 1.42 (*s*, *t*-Bu). ¹³C-NMR (50 MHz): 170.90 (*s*); 167.50 (*s*); 138.58 (*s*); 137.50 (*s*); 128.80 (*d*); 128.10 (*d*); 127.30 (*t*); 126.12 (*d*); 100.67 (*d*); 81.38 (*s*); 80.46 (*d*); 71.08 (*t*); 69.59 (*d*); 69.38 (*d*); 61.12 (*d*); 53.51 (*d*); 37.44 (*t*); 27.99 (*q*); 23.18 (*q*). Anal. calc. for C₂₃H₃₃NO₈ (451.53): C 61.18, H 7.37, N 3.10; found: C 61.41, H 7.25, N 3.30.

Data of 13: $[\alpha]_D^{25} = -18.1^\circ$ (*c* = 1.1, CHCl₃). IR: 3420s (*br.*), 3004m, 2985m, 2935m, 2870m, 1704 (sh), 1670s, 1632m, 1520m, 1370s, 1150s, 1086s, 1075s, 1015m. ¹H-NMR (400 MHz, (D₆)DMSO): 7.55–7.30 (*m*, NH, 5 arom. H); 6.00 (*d*, *J* = 2.0, 1 olef. H); 5.58 (*d*, *J* = 2.0, 1 olef. H); 5.41 (*s*, ArCH); 5.15 (*d*, *J* = 5.5, OH); 4.72 (*d*, *J* = 7.8, OH); 4.63 (*d*, *J* = 6.5, OH); 4.15 (*dd*, *J* = 10.5, 5.5, H–C(9)); 4.12 (*dd*, *J* = 9.0, 6.0, H–C(5), after H/D exchange); 3.96 (*br. d*, *J* = 9.0, H–C(6), after H/D exchange); 3.82–3.74 (*m*, H–C(4), H–C(8)); 3.61 (*br. d*, *J* = 10.0, H–C(7)); 3.51 (*dd*, *J* = 10.5, 10.5, H–C(9)); 2.50 (*br. d*, *J* = 15.0, H–C(3)); 2.20 (*dd*, *J* = 15.0, 10.0, H–C(3)); 1.88 (*s*, CH₃); 1.40 (*s*, *t*-Bu). ¹³C-NMR (50 MHz): 171.71 (*s*); 167.09 (*s*); 138.30 (*s*); 137.55 (*s*); 129.11 (*d*); 128.28 (*d*); 127.39 (*t*); 126.08 (*d*); 100.89 (*d*); 81.98 (*d*); 81.07 (*s*); 71.64 (*d*); 71.16 (*t*); 67.46 (*d*); 60.53 (*d*); 56.78 (*d*); 37.73 (*t*); 27.96 (*q*); 23.38 (*q*). Anal. calc. for C₂₃H₃₃NO₈ (451.53): C 61.18, H 7.37, N 3.10; found: C 61.40, H 7.30, N 3.15.

tert-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-galacto-nononate (**14**) and *tert*-Butyl 5-Acetamido-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-talo-nononate (**15**). Similarly to **6/7**, **8/9** (1.50 g, 3.74 mmol) were reduced with NaBH₄/AcOH. After 10 h, TLC (CH₂Cl₂/MeOH 9:1) indicated the disappearance of **8/9**. The mixture was diluted with AcOEt (150 ml). Extractive workup with half-sat. aq. NaCl (100 ml) and brine (100 ml), and FC (130 g SiO₂, CH₂Cl₂/MeOH 19:1) gave a 92:8 mixture **14/15** (1.25 g, 83%) (anal. HPLC (*Zorbax-Sil*), CH₂Cl₂/MeOH 193:7, λ_{obs} 228 nm). ¹H- and ¹³C-NMR showed only signals of **14**. ¹H-NMR (200 MHz): 6.54 (*d*, *J* = 9.7, NH); 6.19 (*d*, *J* = 1.5, 1 H); 5.67 (*br. s*, 1 H); 4.45–3.58 (*m*, 9 H, 2 H exchanged by addn. of D₂O); 3.32–3.18 (*m*, OH); 2.52–2.38 (*m*, 2 H–C(3)); 2.04 (*s*, CH₃); 1.49 (*s*, *t*-Bu); 1.45 (*s*, CH₃); 1.39 (*s*, CH₃). ¹³C-NMR (50 MHz): 170.47 (*s*); 167.41 (*s*); 138.65 (*s*); 127.20 (*t*); 99.01 (*s*); 81.27 (*s*); 72.55 (*d*); 69.27 (*d*); 64.61 (*t*); 62.26 (*d*); 53.30 (*d*); 37.36 (*t*); 28.45 (*q*); 28.01 (*q*); 23.17 (*q*); 19.13 (*q*). Anal. calc. for C₁₉H₃₃NO₈ (403.48): C 56.56, H 8.24, N 3.47; found: C 56.28, H 8.12, N 3.69.

Reduction of 6/7 and 8/9 in the Absence of AcOH. An ice-cold soln. of **6/7** (30 mg, 0.067 mmol) in dioxane/H₂O 4:1 (1.5 ml) was treated with NaBH₄ (6 × 1 mg, 2.5 h). Extractive workup (as described above) gave a 15:85 mixture **12/13** (29 mg, 96%) as indicated by HPLC.

Similarly, treatment of **8/9** (22 mg, 0.055 mmol) with NaBH₄ gave after extractive workup a 15:85 mixture **14/15** (16 mg, 73%) as determined by ¹H-NMR. ¹H-NMR (200 MHz) of **15**: 6.73 (*d*, *J* = 8.3, NH); 6.15 (*d*, *J* = 1.6, 1 olef. H); 5.66 (*d*, *J* = 1.0, 1 olef. H); 4.34–3.54 (*m*, 10 H); 2.68 (*dd*, *J* = 14.0, 1.0, H–C(3)); 2.35 (*dd*, *J* = 14.0, 9.0, H–C(3)); 2.02 (*s*, CH₃); 1.55–1.36 (*t*-Bu, 2 CH₃).

Reduction of 6/7 with NaBH₄ in EtOH. To an ice-cold soln. of **6/7** (100 mg, 0.223 mmol) in EtOH (95%) (2 ml) was added NaBH₄ (50 mg). After 15 min, TLC indicated the disappearance of **6/7**. Extractive workup with AcOEt and half-sat. aq. NaCl soln. gave a 15:85 mixture **12/13** (100 mg, > 95%).

Reduction of 6/7 with Zn(BH₄)₂ in THF. An ice-cold soln. of **6/7** (100 mg, 0.233 mmol) in THF (2 ml) was treated with a 0.15M soln. of Zn(BH₄)₂ [31] in THF (2 ml). After 10 h, TLC indicated the disappearance of **6/7**.

⁸) Addition of solid NaBH₄ to the mixture diminished the diastereoselectivity of the reduction.

Excess of $\text{Zn}(\text{BH}_4)_2$ was destroyed by adding phosphate buffer of pH 6.6 (5 ml). Extractive workup (AcOEt) gave a 15:85 mixture **12/13** (96 mg, 95%).

tert-Butyl 5-Acetamido-4,6,8-tri-O-acetyl-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-galacto-nononate (**16**) and tert-Butyl 5-Acetamido-4,6,8-tri-O-acetyl-2,3,5-trideoxy-7,9-O-isopropylidene-2-methylidene-D-glycero-D-talo-nononate (**17**). A soln. of **14/15** (300 mg, 0.74 mmol) in Et_2O (3 ml) was acetylated at 0° with Ac_2O (1 ml) and pyridine (2 ml). After 3 h, TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1) indicated the disappearance of the starting material. The mixture was concentrated and dried by evaporation with 2×10 ml toluene. The isomeres were separated by MPLC (100 g of SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 40:1) yielding first **17** (220 mg, 56%) as a colourless foam and then **16** (133 mg, 34%) as colourless crystals.

Data of **16**: m.p. $104\text{--}106^\circ$. $[\alpha]_D^{25} = -52.9^\circ$ ($c = 1.2$, CHCl_3). IR: 3435m, 2985m, 2935w, 2880w, 1740s, 1702 (sh), 1687s, 1630w, 1502m, 1370s, 1316w, 1220s (br.), 1150s, 1068m, 1045s, 1030s, 850m. $^1\text{H-NMR}$ (200 MHz): 6.08 (d, $J = 2.0$, 1 olef. H); 5.85 (d, $J = 10.0$, NH); 5.50 (br. s, 1 olef. H); 5.29 (ddd, $J = 9.5$, 4.0, 3.0, H-C(4)); 5.01 (dd, $J = 8.5$, 2.0, H-C(6)); 4.78–4.56 (m, H-C(5), H-C(8)); 4.13 (dd, $J = 10.0$, 2.0, H-C(7)); 3.99 (dd, $J = 11.5$, 5.5, H-C(9)); 3.63 (dd, $J = 11.5$, 8.5, H-C(9)); 2.61 (dd, $J = 14.0$, 3.0, H-C(3)); 2.23 (dd, $J = 14.0$, 9.5, H-C(3)); 2.12–1.90 (4 CH_3); 1.67–1.37 (5 CH_2). $^{13}\text{C-NMR}$ (25.2 MHz): 170.06 (s); 169.68 (s); 169.64 (s); 169.30 (s); 165.27 (s); 137.35 (s); 126.81 (t); 99.88 (s); 80.79 (s); 70.25 (d); 69.42 (d); 67.87 (d); 64.64 (d); 61.78 (t); 50.39 (d); 35.32 (t); 28.02 (q); 27.71 (q); 23.42 (q); 20.82 (q); 20.66 (q); 19.30 (q). EI-MS: 514 (3, $M^{+} - 15$); 458 (7), 414 (2), 398 (5), 356 (8), 316 (8), 296 (4), 270 (6), 228 (36), 198 (15), 186 (16), 168 (50), 115 (51), 57 (23), 43 (100). Anal. calc. for $\text{C}_{25}\text{H}_{39}\text{NO}_{11}$ (529.60): C 56.70, H 7.42, N 2.65; found: C 56.44, H 7.55, N 2.49.

Data of **17**: $[\alpha]_D^{25} = +20.3^\circ$ ($c = 1.2$, CHCl_3). IR: 3425m, 2995m, 2985m, 2935w, 2880w, 1743s, 1705s, 1680s, 1632w, 1510m, 1370s, 1343w, 1310w, 1238s, 1153s, 1052s. $^1\text{H-NMR}$ (200 MHz): 6.57 (d, $J = 10.0$, NH); 6.08 (d, $J = 2.0$, 1 olef. H); 5.56 (s, 1 olef. H); 5.24–5.06 (m, H-C(4), H-C(6)); 4.79 (ddd, $J = 10.0$, 8.5, 5.5, H-C(8)); 4.60 (ddd, $J = 10.0$, 10.0, 5.5, H-C(5)); 4.17 (dd, $J = 10.0$, 2.0, H-C(7)); 3.99 (dd, $J = 11.5$, 5.5, H-C(9)); 3.65 (dd, $J = 11.5$, 8.5, H-C(9)); 2.89 (dd, $J = 14.0$, 1.5, H-C(3)); 2.26 (dd, $J = 14.0$, 10.0, H-C(3)); 2.16–1.96 (4 CH_3); 1.66–1.42 (5 CH_2). $^{13}\text{C-NMR}$ (25.2 MHz): 169.91 (s); 169.83 (s); 169.45 (s); 169.15 (s); 164.97 (s); 137.16 (s); 126.55 (t); 99.58 (s); 80.30 (s); 71.07 (d); 70.71 (d); 66.39 (d); 63.62 (d); 61.44 (t); 51.78 (d); 35.53 (t); 27.80 (q); 23.25 (q); 20.63 (q); 20.40 (q); 19.34 (q). EI-MS: 514 (1, $M^{+} - 15$), 471 (3). Anal. calc. for $\text{C}_{25}\text{H}_{39}\text{NO}_{11}$ (529.60): C 56.70, H 7.42, N 2.65; found: C 56.44, H 7.27, N 2.52.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (**18**). At -78° , a soln. of **12** (100 mg, 0.221 mmol) in CH_2Cl_2 (7 ml) was ozonized until the soln. turned blue⁹). It was purged with N_2 (5 min), treated with a soln. of Ph_3P (87 mg, 0.332 mmol) in CH_2Cl_2 (1 ml) and warmed to r.t. Evaporation of the solvent and FC of the residue (10 g of SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 93:7) gave **18** (89 mg, 89%) as a colourless foam. An anal. sample was obtained by crystallization from anh. EtOH/hexane. M.p. $167\text{--}168^\circ$ (dec.). $[\alpha]_D^{25} = -62.8^\circ$ (20 h, $c = 1.0$, CHCl_3). IR (KBr): 3400s (br.), 2975m, 2905w, 2855w, 1740s, 1632s, 1567m, 1384s, 1370s, 1328m, 1142s, 1124s, 1088s, 1036s, 1026s. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.65 (d, $J = 8.6$, NH); 7.56–7.30 (m, 5 arom. H); 6.15 (d, $J = 2.0$, OH); 5.31 (s, ArCH); 4.94 (d, $J = 5.5$, OH); 4.71 (d, $J = 6.0$, OH); 4.16 (dd, $J = 10.4$, 5.3, H-C(9)); 4.00 (dd, $J = 9.1$, 0.5, H-C(6)); 3.93–3.83 (m, H-C(4), H-C(5), H-C(8)); 3.48 (dd, $J = 10.0$, 0.5, H-C(7)); 3.46 (dd, $J = 10.4$, 10.0, H-C(9)); 1.97 (dd, $J = 13.0$, 4.6, H-C(3)); 1.85 (s, CH_3); 1.58 (dd, $J = 13.0$, 11.0, H-C(3)); 1.41 (s, *t*-Bu); addn. of $\text{D}_2\text{O} \rightarrow 3.81$ (ddd, $J = 9.5$, 9.1, 8.6, H-C(5)); 3.75 (ddd, $J = 11.0$, 9.5, 4.6, H-C(4)); 3.70 (ddd, $J = 10.0$, 10.0, 5.3, H-C(8)). $^{13}\text{C-NMR}$ (50 MHz, CD_3OD): 173.67 (s); 170.22 (s); 139.46 (s); 129.42 (d); 128.73 (d); 127.45 (d); 102.17 (d); 96.51 (s); 83.29 (s); 80.64 (d); 72.11 (t); 70.01 (d); 68.76 (d); 61.41 (d); 52.84 (d); 40.16 (t); 28.06 (q); 22.96 (q). Anal. calc. for $\text{C}_{22}\text{H}_{31}\text{NO}_9$ (453.50): C 58.27, H 6.89, N 3.09; found: C 57.99, H 7.11, N 2.95.

tert-Butyl 5-Acetamido-7,9-O-benzylidene-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (**19**). A sample of **13** (250 mg, 0.554 mmol) was ozonized as described for **12**. The crude product was purified by FC (25 g of SiO_2). $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 97:3 eluted Ph_3P and Ph_3PO ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5 gave **19** (225 mg, 90%) as a colourless foam. Crystallization from anh. MeOH (0.5 ml)/ Et_2O yielded **19** (195 mg). M.p. $164\text{--}165^\circ$ (dec.). $[\alpha]_D^{25} = -80.9^\circ$ ($c = 1.0$, CHCl_3). IR (KBr): 3500s, 3415s, 2985w, 1738s, 1640s, 1536m, 1385m, 1370m, 1320m, 1310m, 1150s, 1095s, 1080s, 1060m, 1020m, 850w, 770m. $^1\text{H-NMR}$ (400 MHz, $(\text{D}_6)\text{DMSO}$): 7.70 (d, $J = 9.4$, NH); 7.50–7.28 (m, 5 arom. H); 6.48 (s, OH-C(2)); 5.34 (s, ArCH); 5.10 (d, $J = 6.0$, OH-C(4)); 5.05 (d, $J = 5.6$, OH-C(8)); 4.36 (dd, $J = 10.5$, 1.0, H-C(6)); 4.16 (dd, $J = 10.5$, 5.0, H-C(9)); 4.11 (ddd, $J = 10.5$, 9.4, 3.0, H-C(5)); 3.93 (br. dd, $J = 6.0$, 3.0, H-C(4); addn. of $\text{D}_2\text{O} \rightarrow$ br. d); 3.78 (dddd, $J = 10.5$, 9.5, 5.6, 5.0, H-C(8); addn. of $\text{D}_2\text{O} \rightarrow$ ddd); 3.57 (dd, $J = 9.5$,

⁹⁾ Continuing the ozonolysis led to by-products, which are likely formed by oxidation of the benzylidene group [43].

1.0, H-C(7)); 3.48 (*dd*, $J = 10.5, 10.5$, H-C(9)); 1.98–1.90 (*m*, 2 H-C(3)); 1.87 (*s*, CH₃); 1.40 (*s*, *t*-Bu). ¹H-NMR (200 MHz, CD₃OD): β -D/ α -D = 3:1; β = D-anomer: 7.60–7.26 (*m*, 5 arom. H); 5.44 (*s*, ArCH); 4.46 (*dd*, $J = 10.5, 1.8$, H-C(6)); 4.33 (*dd*, $J = 10.5, 3.0$, H-C(5)); 4.27 (*dd*, $J = 10.5, 5.5$, H-C(9)); 4.10 (*ddd*, $J = 3.4, 3.0, 3.0$, H-C(4)); 3.98 (*ddd*, $J = 10.5, 9.0, 5.5$, H-C(8)); 3.64 (*dd*, $J = 9.0, 1.8$, H-C(7)); 3.58 (*dd*, $J = 10.5, 10.5$, H-C(9)); 2.17 (*dd*, $J = 14.0, 3.0$, H-C(3)); 2.03 (*dd*, $J = 14.0, 3.4$, H-C(3)); 2.01 (*s*, CH₃); 1.47 (*s*, *t*-Bu); α -D-anomer: 4.55 (*dd*, $J = 10.5, 1.8$, H-C(6)); 2.60 (*dd*, $J = 14.0, 3.0$, H-C(3)); 1.98 (*s*, CH₃); 1.76 (*dd*, $J = 14.0, 2.5$, H-C(3)); 1.51 (*s*, *t*-Bu); the other signals were overlapped by the signals of the β -D-anomer. ¹³C-NMR (50 MHz, CD₃OD): β -D-anomer: 172.94 (*s*); 169.62 (*s*); 139.45 (*s*); 129.52 (*d*); 128.84 (*d*); 127.41 (*d*); 102.22 (*d*); 96.67 (*s*); 83.45 (*s*); 80.96 (*d*); 72.18 (*r*); 67.88 (*d*); 65.98 (*d*); 61.00 (*d*); 48.15 (*d*); 37.04 (*t*); 28.05 (*q*); 22.73 (*q*); α -D-anomer: 172.94 (*s*); 171.74 (*s*); 139.45 (*s*); 129.52 (*d*); 128.84 (*d*); 127.41 (*d*); 102.22 (*d*); 95.73 (*s*); 83.21 (*s*); 81.27 (*d*); 72.26 (*t*); 69.49 (*d*); 67.30 (*d*); 61.51 (*d*); 41.67 (*r*); 28.05 (*q*); 22.73 (*q*). Anal. calc. for C₂₂H₃₁NO₉ (453.50): C 58.27, H 6.89, N 3.09; found: C 58.02, H 6.99, N 2.90.

tert-Butyl 5-Acetamido-3,5-dideoxy-7,9-O-isopropylidene-D-glycero-D-galacto-2-nomulosonate (**20**) and tert-Butyl 5-Acetamido-3,5-dideoxy-7,9-O-isopropylidene-D-glycero-D-taio-nonulosonate (**21**). A mixture **14/15** (1.012 g, 2.50 mmol) was ozonized as described for **12**. The crude products were purified by FC (150 g of SiO₂, CH₂Cl₂/MeOH 15:1 eluted **21** (70 mg, 7%) as a colourless foam and CH₂Cl₂/MeOH 12:1 **20** (861 mg, 85%) as an oil, which solidified after a few hours.

Data of **20**: $[\alpha]_D^{25} = -44.8^\circ$ ($c = 1.0$, MeOH). IR (KBr): 3410s (br.), 2985w, 2940w, 1734m, 1643s, 1553w, 1370s, 1315w, 1227w, 1150s, 1125s, 1070s, 1030m. ¹H-NMR (400 MHz, (D₆)DMSO): 7.58 (*d*, $J = 8.3$, NH); 6.11 (*s*, OH-C(2)); 4.78 (*d*, $J = 5.2$, OH); 4.66 (*d*, $J = 5.5$, OH); 4.06 (*dd*, $J = 9.0, 1.5$, H-C(6)); 3.85–3.64 (*m*, H-C(4), H-C(5), H-C(8), H-C(9)); addn. of D₂O → 3.82 (*ddd*, $J = 11.0, 9.8, 4.5$, H-C(4)); 3.62 (*dd*, $J = 9.2, 1.5$, H-C(7)); 3.48 (*dd*, $J = 10.0, 9.5$, H-C(9)); 2.03 (*dd*, $J = 12.6, 4.5$, H-C(3)); 1.83 (*s*, CH₃); 1.71 (*dd*, $J = 12.6, 11.0$, H-C(3)); 1.44 (*s*, *t*-Bu); 1.29 (*s*, CH₃); 1.25 (*s*, CH₃). ¹³C-NMR (50 MHz, CD₃OD): 173.17 (*s*); 170.24 (*s*); 100.20 (*s*); 96.51 (*s*); 83.26 (*s*); 72.63 (*d*); 70.02 (*d*); 69.06 (*d*); 65.66 (*r*); 62.78 (*d*); 52.72 (*d*); 40.22 (*r*); 28.62 (*q*); 28.05 (*q*); 22.98 (*q*); 19.06 (*q*). Anal. calc. for C₁₈H₃₁NO₉ (405.46): C 53.32, H 7.71, N 3.46; found: C 53.06, H 7.90, N 3.54.

Data of **21**: $[\alpha]_D^{25} = -63.7^\circ$ ($c = 1.0$, CHCl₃). IR: 3420m (br.), 2985s, 2930m, 2870w, 1730s, 1670s, 1505m, 1370s, 1306m, 1136s, 1090s, 1064s, 1024m. ¹H-NMR (400 MHz, (D₆)DMSO): 7.63 (*d*, $J = 9.2$, NH); 6.40 (*s*, OH-C(2)); 5.01 (*d*, $J = 6.2$, OH); 4.84 (*d*, $J = 5.0$, OH); 4.28 (*dd*, $J = 10.5, 1.2$, H-C(6)); 3.95 (*ddd*, $J = 10.5, 9.2, 3.0$, H-C(5)); 3.87 (*ddd*, $J = 5.8, 5.8, 3.0$, H-C(4)); 3.71 (*dd*, $J = 9.8, 4.0$, H-C(9)); 3.69–3.59 (*m*, H-C(8)); 3.61 (*dd*, $J = 9.5, 1.2$, H-C(7)); 3.43 (*dd*, $J = 9.8, 8.2$, H-C(9)); 1.92 (*d*, $J = 3.0$, 2 H-C(3)); 1.80 (*s*, CH₃); 1.42 (*s*, *t*-Bu); 1.23 (*s*, CH₃); 1.19 (*s*, CH₃). CI-MS: 406 ($M^{++} + 1$), 388 ($(M^{++} + 1) - 18$), 350 ($(M^{++} + 1) - 56$); 332 ($(M^{++} + 1) - 74$). Anal. calc. for C₁₈H₃₁NO₉ (405.46): C 53.32, H 7.71, N 3.46; found: C 53.51, H 7.79, N 3.26.

tert-Butyl 5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (**22**). a) From **18**. To a suspension of 10% Pd/C (150 mg) in MeOH/H₂O 4:1 (20 ml) and AcOH (0.6 ml) was added a soln. of **18** (610 mg, 1.35 mmol) in MeOH (5 ml). The mixture was hydrogenated (24 h) until **18** had disappeared (TLC, CHCl₃/MeOH 9:1). The mixture was filtered through *Celite*, washed with MeOH and the filtrate was evaporated. A soln. of the residue in anh. MeOH (10 ml) was filtered through cotton and evaporated. For crystallization, a stirred soln. of the residual colourless oil in anh. MeOH (5 ml) was diluted with AcOEt (7 ml) and then with Et₂O until persistence of a slight turbidity. Additional Et₂O (5 ml) was added after 16 h, and the mixture was kept in the refrigerator (1 d). The crystals were dried (P₂O₅, 10⁻⁵ mbar, 2 d) affording **22** (422 mg, 85%). The mother liquors were chromatographed (AcOEt/MeOH/H₂O 170:27:3) on a preconditioned column (20 g of SiO₂, AcOEt/MeOH 98:2) to give additional **22** (45 mg, 9%). M.p. 178–179° (dec.). $[\alpha]_D^{25} = -24.8^\circ$ ($c = 1.0$, H₂O). IR (KBr): 3460s, 2980m, 2940m, 1726s, 1635s, 1555s, 1370s, 1312s, 1135s, 1030s. ¹H-NMR (400 MHz, D₂O): 4.10–4.01 (*m*, H-C(4), H-C(6)); 3.91 (*dd*, $J = 10.4, 10.4$, H-C(5)); 3.86 (*dd*, $J = 12.0, 2.4$, H-C(9)); 3.76 (*ddd*, $J = 9.4, 6.4, 2.4$, H-C(8)); 3.63 (*dd*, $J = 12.0, 6.4$, H-C(9)); 3.57 (*br. d*, $J = 9.4$, H-C(7)); 2.69 (*dd*, $J = 12.0, 4.8, 0.1$ H-C(3)); 2.31 (*dd*, $J = 13.0, 4.8, 0.9$ H-C(3)); 2.06 (*s*, 0.9 CH₃); 2.05 (*s*, 0.1 CH₃); 1.87 (*dd*, $J = 13.0, 12.0$, H-C(3)); 1.53 (*s*, 0.1 *t*-Bu); 1.52 (*s*, 0.9 *t*-Bu). ¹³C-NMR (100.6 MHz, CD₃OD): β -D-anomer: 175.05 (*s*); 170.84 (*s*); 96.71 (*s*); 83.85 (*s*); 72.26 (*d*); 72.11 (*d*); 70.47 (*d*); 68.01 (*d*); 64.91 (*r*); 54.43 (*d*); 40.83 (*r*); 28.09 (*q*); 22.68 (*q*); α -D-anomer: 175.32 (*s*); 170.84 (*s*); 97.38 (*s*); 84.59 (*s*); 74.90 (*d*); 72.71 (*d*); 70.29 (*d*); 68.90 (*d*); 64.81 (*r*); 54.05 (*d*); 42.47 (*r*); 28.09 (*q*); 22.68 (*q*). CI-MS: 366 ($M^{++} + 1$), 310, 292, 274. Anal. calc. for C₂₅H₂₇NO₉ (365.39): C 49.31, H 7.45, N 3.83; found: C 49.26, H 7.50, N 3.99.

b) From **20**. An ice-cold soln. of **20** (160 mg, 0.395 mmol) in CH₂Cl₂/MeOH 1:1 (5 ml) was treated with CF₃COOH (100 μ l). The mixture was slowly warmed to r.t. (melting-ice bath). After 6 h, TLC indicated the disappearance of **20**. The solvent was removed, the residue was dissolved in H₂O (7 ml) and extracted with CH₂Cl₂. The aq. layer was freeze-dried giving crude **22** (148 mg, quant.). Crystallization from anh. MeOH (1.5 ml)/AcOEt (1.5 ml)/Et₂O (*cf.* **18** → **22**) yielded **22** (124 mg, 86%). M.p. 176–177°. $[\alpha]_D^{25} = -25.0^\circ$ ($c = 1.0$, H₂O).

5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic Acid (= *N-Acetylneuraminic Acid*; Neu5Ac, **1**). A soln. of **22** (300 mg, 0.821 mmol) in MeOH/H₂O 3:2 (15 ml) was stirred with K₂CO₃ (150 mg) at r.t. (4 h) until TLC (AcOEt/MeOH/0.1N HCl 2:2:1) indicated the disappearance of **22**. MeOH was evaporated, H₂O (5 ml) was added to the residue, the soln. was acidified to pH 4 (*Dowex 50 W 4* (H⁺)) and stirred at r.t. for 30 min. The resin was filtered off, resuspended in H₂O (3 ml), and stirred for another 30 min. The filtrate and washings were combined and freeze-dried to give crude **1** (254 mg, 100%). Crystallization (0.4 ml of H₂O, 6.0 ml of AcOH, 3 d, 4°) [8] [10] gave, after drying (P₂O₅, KOH, 10⁻⁵ mbar), **1** (130 mg, 51%). M.p. 180–182° (dec.) ([10]: 181–183°). $[\alpha]_D^{20} = -33.0^\circ$ (*c* = 1.0, H₂O); ([10]: -32.1° (*c* = 1.3, H₂O)).

Methyl 5-Acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonate (23). A sample of **22** (100 mg, 0.274 mmol) was treated with K₂CO₃ as described for **1**. A soln. of crude **1** (90 mg) in anh. MeOH (9 ml), was stirred with CF₃COOH (40 μ l) at r.t. for 24 h. The solvent was removed and the residual oil was dried at 10⁻² mbar for 2 h. A soln. of the residue in anh. MeOH (0.7 ml) and AcOEt (0.7 ml) was treated with Et₂O and kept in the refrigerator (5 d). The crystals were dried (P₂O₅, 10⁻⁵ mbar) yielding **23** (52 mg, 59%). M.p. 176–178° ([38]: 179–180°); $[\alpha]_D^{25} = -28.6^\circ$ (*c* = 1.1, H₂O), ([38]: -28° (*c* = 1.0, H₂O)).

tert-Butyl 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-nonulosonate (24). a) *From 19*. A sample of **19** (130 mg, 0.287 mmol) was hydrogenated as described for **18**→**22** (TLC: CH₂Cl₂/EtOH 9:1). The crude **24** (104 mg) was crystallized from MeOH (1 ml), AcOEt (1.5 ml), and Et₂O (10 ml) affording pure **24** (73 mg, 70%). The mother liquors were chromatographed (AcOEt/MeOH/H₂O 170:27:3) on a preconditioned column (15 g of SiO₂, AcOEt) to give **24** (27 mg, 25%). M.p. 157–158°. $[\alpha]_D^{25} = -76.0^\circ$ (*c* = 1.0, H₂O). IR (KBr): 3500 (sh), 3360s, 2980w, 2940w, 1733s, 1668s, 1520m, 1430m, 1370m, 1309m, 1136s, 1089s, 1050m, 1027m, 1012m, 918m, 843m. ¹H-NMR (400 MHz, D₂O): 4.38 (br. *d*, *J* = 11.0, 0.7 H–C(6)); 4.34 (br., *d*, *J* = 11.0, 0.3 H–C(6)); 4.22–4.17 (*m*, H–C(4)); 4.14 (*dd*, *J* = 11.0, 3.0, 0.7 H–C(5)); 4.08 (*dd*, *J* = 11.0, 2.5, 0.3 H–C(5)); 3.97–3.78 (*m*, 2 H; including a *dd* at 3.87, *J* = 11.8, 2.6 for 0.7 H–C(9) and a *ddd* at 3.81, *J* = 9.0, 6.3, 2.6 for 0.7 H–C(8)); 3.71–3.62 (*m*, 1 H; including a *dd* at 3.65, *J* = 11.8, 6.3 for 0.7 H–C(9)); 3.61 (br. *d*, *J* = 9.0, 0.7 H–C(7)); 3.57 (br. *d*, *J* = 9.0, 0.3 H–C(7)); 2.68 (*dd*, *J* = 14.0, 3.6, 0.3 H–C(3)); 2.17 (*d*, *J* = 3.0, 0.7 2 H–C(3)); 2.06 (*s*, 0.7 CH₃); 2.04 (*s*, 0.3 CH₃); 1.89 (*dd*, *J* = 14.0, 2.7, 0.3 H–C(3)); 1.52 (*s*, *t*-Bu). ¹³C-NMR (25 MHz, CD₃OD): β -D-anomer: 172.57 (*s*); 170.13 (*s*); 95.36 (*s*); 83.70 (*s*); 71.12 (*d*); 68.59 (*d*); 66.86 (*d*); 66.19 (*d*); 62.91 (*t*); 47.98 (*d*); 36.44 (*t*); 26.70 (*q*); 21.34 (*q*); α -D-anomer: 172.86 (*s*); 170.46 (*s*); 94.12 (*s*); 71.68 (*d*); 70.24 (*d*); 69.03 (*d*); 65.75 (*d*); 63.35 (*t*); 48.32 (*d*); 40.28 (*t*); 26.70 (*q*); 21.34 (*q*). CI-MS: 366 (*M*⁺ + 1), 348, 310, 292, 274. Anal. calc. for C₁₅H₂₇NO₉ (365.39): C 49.31, H 7.45, N 3.83; found: C 49.07, H 7.70, N 4.01.

b) *From 21*. Similarly to **20**, deprotection of **21** (280 mg, 0.690 mmol) and crystallization of the product, gave **24** (164 mg, 65%). M.p. 156–158° (dec.). $[\alpha]_D^{25} = -76.4^\circ$ (*c* = 1.0, H₂O).

Sodium N-Acetamido-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (Sodium N-Acetyl-4-epineuraminatate; 2). Similarly to **22**, **24** (350 mg, 0.958 mmol) was saponified (TLC: CHCl₃/MeOH 3:1 and PrOH/MeOH/0.1N HCl 5:3:2). Crude **2** (295 mg) was purified by anion-exchange chromatography (35 ml of *Dowex 1* $\times$ 8 (HCOO⁻), elution by 0–0.04N aq. HCOOH). Fractions containing **2** (*R* = H) were collected, concentrated, and finally freeze-dried. The red-brown residual oil was decolorized by activated charcoal¹⁰). The residual slightly yellow oil was transformed to the Na salt by passing it through a cation exchanger (10 ml of *Dowex 50 W* $\times$ 4 (Na⁺), H₂O) to give, after freeze drying, **2** (146 mg, 46%; 5d 10⁻⁵ mbar) as a colourless solid. $[\alpha]_D^{25} = -71.4^\circ$ (*c* = 1.0, H₂O). ¹H-NMR (400 MHz, D₂O): α -D/ β -D = 1:9; β -D-anomer: 4.30 (*dd*, *J* = 10.8, 1.2, H–C(6)); 4.19 (*ddd*, *J* = 3.3, 3.2, 3.0, H–C(4)); 4.14 (*dd*, *J* = 10.8, 3.0, H–C(5)); 3.87 (*dd*, *J* = 11.5, 2.8, H–C(9)); 3.86–3.80 (*m*, H–C(8)); 3.64 (*dd*, *J* = 11.5, 6.1, H–C(9)); 3.55 (*dd*, *J* = 8.9, 1.2, H–C(7)); 2.12 (*dd*, *J* = 14.7, 3.3, H–C(3)); 2.08 (*dd*, *J* = 14.7, 3.2, H–C(3)); 2.05 (*s*, CH₃); α -D-anomer: 2.49 (*dd*, *J* = 14.7, 3.2, H–C(3)); 2.04 (*s*, CH₃); 1.99 (*dd*, *J* = 14.7, 3.7, H–C(3)); the signals for H–C(4) to 2 H–C(9) are covered by the signals of the β -D-anomer. ¹³C-NMR (50 MHz, D₂O): β -D-anomer: 177.10 (*s*); 174.33 (*s*); 96.54 (*s*); 70.52 (*d*, C(8)); 69.05 (*d*, C(7)); 66.62 (*d*, C(4)); 66.07 (*d*, C(6)); 63.63 (*t*); 48.11 (*d*); 36.76 (*t*); 22.34 (*q*); α -D-anomer: 176.52 (*s*); 174.54 (*s*); 95.96 (*s*); 71.22 (*d*); 70.03 (*d*); 63.39 (*t*); 48.67 (*d*); 38.41 (*t*); the signals for C(4) and C(6) were determined by selective ¹H, ¹³C-decoupling experiments. FAB-MS: 332 (*M*⁺ + 1). Anal. calc. for C₁₁H₁₈NNaO₉ (331.28): C 39.88, H 5.48, N 4.24; found: C 39.59, H 5.71, N 3.99.

Methyl 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-2-nonulosonate (25). A suspension of **24** (120 mg, 0.328 mmol) in chlorobutane (1.5 ml) was treated with CF₃COOH (1 ml) and stirred at r.t. (6 h). TLC (CHCl₃/MeOH 3:1) indicated then the disappearance of **24**. The solns. was concentrated and twice co-evaporated with anh. MeOH (2 $\times$ 3 ml). The residual oil was redissolved in anh. MeOH (3 ml), treated with CF₃COOH (100 μ l), and stirred at r.t. (2 d). The soln. was concentrated and chromatographed (20 g of SiO₂, CHCl₃/MeOH 9:1) on a preconditioned

¹⁰) Activated by treating it with 0.5N HCOOH (2 h) and washing with H₂O (bidest.).

column (24 h). Fractions containing **25** were combined, concentrated, and extracted with H₂O. The H₂O layer was freeze-dried to give **25** (100 mg, 94%) as a microcrystalline solid. An anal. sample was obtained by crystallization from anh. MeOH (1 ml), AcOEt (1 ml), and Et₂O (12 ml). M.p. 166–168° (dec.). $[\alpha]_D^{20} = -67^\circ$ (*c* = 1.0, H₂O). IR (KBr): 3380s (br.), 1740m, 1655m, 1545m. ¹H-NMR (400 MHz, D₂O): α-D/β-D = 1:3; β-D-anomer: 4.39 (dd, *J* = 10.6, 1.4, H-C(6)); 4.20 (ddd, *J* = 3.0, 3.0, 3.0, H-C(4)); 4.15 (dd, *J* = 10.6, 3.0, H-C(5)); 3.85 (dd, *J* = 11.6, 2.4, H-C(9)); 3.83 (s, CH₃); 3.79 (dd, *J* = 9.0, 6.2, 2.4, H-C(8)); 3.63 (dd, *J* = 11.6, 6.2, H-C(9)); 3.58 (dd, *J* = 9.0, 1.4, H-C(7)); 2.19 (d, *J* = 3.0, 2 H-C(3)); 2.05 (s, CH₃); α-D-anomer: 4.33 (dd, *J* = 11.0, 1.8, H-C(6)); 4.09 (dd, *J* = 11.0, 3.0, H-C(5)); 3.82 (s, CH₃); 2.69 (dd, *J* = 14.4, 3.6, H-C(3)); 2.03 (s, CH₃); 1.96 (dd, *J* = 14.4, 2.6, H-C(3)); the signals for H-C(4) and H-C(7) to 2 H-C(9) were overlapped by the signals of the β-D-anomer. ¹³C-NMR (50 MHz, D₂O): β-D-anomer: 174.58 (s); 171.96 (s); 95.66 (d); 70.52 (d); 68.90 (d); 66.57 (d); 66.06 (d); 63.69 (t); 54.03 (q); 48.01 (d); 36.57 (t); 22.45 (q); α-D-anomer: 174.77 (s); 94.61 (s); 71.67 (d); 70.69 (d); 69.01 (d); 63.51 (t); 53.58 (q); 48.28 (d); 39.81 (t). FAB-MS: 324 (*M*⁺ + 1). Anal. calc. for C₁₂H₂₁NO₉ (323.31): C 44.58, H 6.55, N 4.33; found: C 44.34, H 6.69, N 4.14.

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