128. Deoxy-nitrosugars

15th Communication[1])

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 NO_2 group gave the 4-nonulosonate tautomers **6**/**7** and **8**/**9**, respectively *(Scheme)*. Stereoselective reduction of **6**/**7** and **8**/**9** with $NaBH_4$/AcOH in dioxane/H_2O 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

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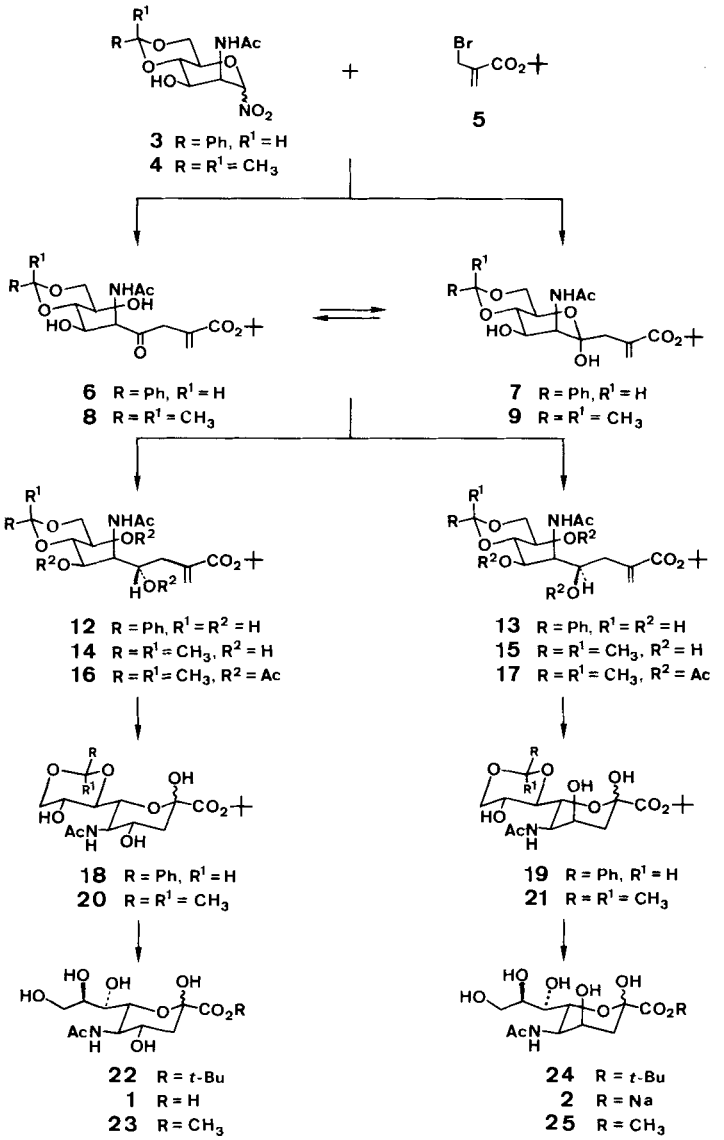
naturally occurring *N*,4-*O*-diacetylneuraminic acid [16] [17], which was also hardly cleaved by acylneuraminate pyruvate-lyase [18]. Furthermore, the synthetic *N*-acetyl-4-*O*-methylneuraminic acid was resistant to acylneuraminate pyruvate-lyase and did not inhibit the aldol cleavage of **1** by this 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-enoate[3]) (**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 NO_2 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 ^{13}C-NMR spectra ((D_6)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 ^{1}H-NMR spectra ((D_6)DMSO) and confirmed by the integrals in the ^{13}C-NMR spectra. The same ratio **6**/**7** was observed for a (D_8)dioxane/D_2O 4:1 solution even after addition of 2 equiv. of AcOD. A 65:35 ratio of **8**/**9** was also observed for a $CDCl_3$ solution.

Reduction of **6**/**7** with $NaBH_4$ in dioxane/H_2O 4:1 in the presence of 2 mol-equiv. of AcOH[5])[6]) 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 $NaBH_4$ 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 $NaBH_4$ in EtOH (95%) or with $Zn(BH_4)_2$ [31] in THF (95% combined yield). The diastereoselectivity of

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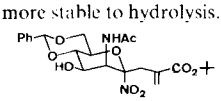
[2]) The overall yields of **4** from D-mannose (35%) [1] [21] were not affected by upscaling; most intermediates crystallized easily. The overall yield of **3** from D-glucose were 28% for batches up to 10 g [1] [21]; batches of 50 to 80 g gave a slightly lower yield (21%), mainly due to the formation 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 $\tilde{\nu}(NO_2)$ 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.



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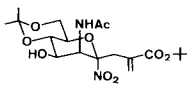

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[5]) The diastereoselectivity in the reduction of ketones depends upon the proportions of hydroxylic and non-hydroxylic components of the solvent [27]. For the use of sodium acyloxyborohydrides in diastereoselective reductions of cyclic ketones, see [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}H-NMR ((D_6)DMSO) and ^{13}C-NMR spectra (CD_3OD) of the neuraminic-acid derivative **18** indicated the presence of only the β-D-anomer. In the ^{1}H-NMR spectrum ((D_6)DMSO) of the 4-epineuraminic-acid derivate **19** also, only the β-D-anomer was detected. The ^{13}C-NMR (CD_3OD) and the ^{1}H-NMR spectrum (CD_3OD) of freshly dissolved **19** showed, however, signals for both anomers (α/β = 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}H-NMR ((D_6)DMSO) and their ^{13}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}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}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}H-NMR spectrum it is a 9:1 mixture of the β-D- and α-D-anomers.

Similarly, sodium *N*-acetyl-4-epineuraminate (**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-epineuraminate **2** (46%)[7]) as a 1:9 mixture of the α- and β-D-anomers (^{1}H-NMR).

Comparison of the ^{13}C-NMR spectrum of **2** with the one of sodium *N*-acetyl-β-neuraminate (**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}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, anh. 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*, 60–230 μm; flash chromatography (FC): 40–63 μm; MPLC [30]: 15–40 μm. 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: CH_2Cl_2/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, CH_2Cl_2/MeOH 97:3) gave further **6**/**7** (5.0 g, total yield 64%). M.p. 143–144°; $[\alpha]_D^{25} = +20.5°$ (*c* = 1.0, DMSO). IR: 3435*m*, 3320*m* (br.), 3000*m*, 2985*m*, 2935*w*, 2870*w*, 1715 (sh), 1680*s*, 1626*m*, 1502*m*, 1392*m*, 1370*s*, 1356*m*, 1152*s*, 1090*s*, 1030*m*. ^1H-NMR (400 MHz, (D_6)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 ArC*H*); 5.50 (*d*, *J* = 1.2, 0.65 olef. H); 5.40 (*s*, 0.65 ArC*H*); 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*, = 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, (D_6)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 ($M^{+\cdot}$ + 1), 432 (($M^{+\cdot}$ + 1) − 18), 390 (($M^{+\cdot}$ + 1) − 60), 376, 239, 209, 91. Anal. calc. for $C_{23}H_{31}NO_8$ (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 (CH_2Cl_2/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 (CH_2Cl_2/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, CH_2Cl_2/MeOH 20:1) afforded **8**/**9** (2.3 g, 83%) as a colourless foam. $[\alpha]_D^{25} = -11.4°$ (*c* = 1.0, $CHCl_3$). IR: 3420*m* (br.), 2980*m*, 2930*w*, 2870*w*, 1720 (sh), 1680*s*, 1632 (sh), 1502*m*, 1370*s*, 1318*m*, 1150*s*, 1064*s*, 955*w*, 876*m*, 856*m*. ^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 (2*d*, *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, (D_6)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/H_2O 4:1 (200 ml) was added a soln. of AcOH (0.80 g, 13.34 mmol) in dioxane/H_2O 4:1 (10 ml). To this mixture, 150 ml of a 0.13M soln. of $NaBH_4$[8]) in dioxane/H_2O 4:1 were added dropwise during 12 h (= 1 drop per 8 s). According to TLC ($CHCl_3$/MeOH 9:1), **6**/**7** had disappeared. The mixture was diluted with AcOEt (200 ml), excess $NaBH_4$ 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*, CH_2Cl_2/MeOH 97:3, 254 nm), which were separated by MPLC (500 g of SiO_2, CH_2Cl_2/MeOH 95:5) affording **12** (2.604 g, 86.5%) and **13** (0.166 g, 5.5%) as colourless foams.

Data of **12**: $[\alpha]_D^{25} = -37.3°$ (c = 1.1, $CHCl_3$). IR: 3430*m*, 3005*m*, 2985*m*, 2935*w*, 2870*w*, 1703*m*, 1676*s*, 1630*m*, 1508*m*, 1370*s*, 1152*s*, 1086*s*, 1075*s*, 1030*m*, 1016 (sh). ^{1}H-NMR (400 MHz, (D_6)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*, ArC*H*); 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*, CH_3); 1.42 (*s*, *t*-Bu). ^{13}C-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 $C_{23}H_{33}NO_8$ (451.53): C 61.18, H 7.37, N 3.10; found: C 61.41, H 7.25, N 3.30.

Data of **13**: $[\alpha]_D^{25} = -18.1°$ (c = 1.1, $CHCl_3$). IR: 3420*s* (br.), 3004*m*, 2985*m*, 2935*m*, 2870*m*, 1704 (sh), 1670*s*, 1632*m*, 1520*m*, 1370*s*, 1150*s*, 1086*s*, 1075*s*, 1015*m*. ^{1}H-NMR (400 MHz, (D_6)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*, ArC*H*); 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*, CH_3); 1.40 (*s*, *t*-Bu). ^{13}C-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 $C_{23}H_{33}NO_8$ (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 $NaBH_4$/AcOH. After 10 h, TLC (CH_2Cl_2/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 SiO_2, CH_2Cl_2/MeOH 19:1) gave a 92:8 mixture **14**/**15** (1.25 g, 83%) (anal. HPLC (*Zorbax-Sil*), CH_2Cl_2/MeOH 193:7, $\lambda_{obs.}$ 228 nm). ^{1}H- and ^{13}C-NMR showed only signals of **14**. ^{1}H-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 D_2O); 3.32–3.18 (*m*, OH); 2.52–2.38 (*m*, 2 H–C(3)); 2.04 (*s*, CH_3); 1.49 (*s*, *t*-Bu); 1.45 (*s*, CH_3); 1.39 (*s*, CH_3). ^{13}C-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 $C_{19}H_{33}NO_8$ (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/H_2O 4:1 (1.5 ml) was treated with $NaBH_4$ (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 $NaBH_4$ gave after extractive workup a 15:85 mixture **14**/**15** (16 mg, 73%) as determined by ^{1}H-NMR. ^{1}H-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*, CH_3); 1.55–1.36 (*t*-Bu, 2 CH_3).

Reduction of **6**/**7** *with* $NaBH_4$ *in EtOH*. To an ice-cold soln. of **6**/**7** (100 mg, 0.223 mmol) in EtOH (95%) (2 ml) was added $NaBH_4$ (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* $Zn(BH_4)_2$ *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 $Zn(BH_4)_2$ [31] in THF (2 ml). After 10 h, TLC indicated the disappearance of **6**/**7**.

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[8]) Addition of solid $NaBH_4$ to the mixture diminished the diastereoselectivity of the reduction.

Excess of $Zn(BH_4)_2$ 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 (CH_2Cl_2/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, CH_2Cl_2/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. 104–106°. $[\alpha]_D^{25} = -52.9°$ (c = 1.2, $CHCl_3$). IR: 3435*m*, 2985*m*, 2935*w*, 2880*w*, 1740*s*, 1702 (sh), 1687*s*, 1630*w*, 1502*m*, 1370*s*, 1316*w*, 1220*s* (br.), 1150*s*, 1068*m*, 1045*s*, 1030*s*, 850*m*. ^{1}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_3). ^{13}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, $M^{+\cdot} - 15$); 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 $C_{25}H_{39}NO_{11}$ (529.60): C 56.70, H 7.42, N 2.65; found: C 56.44, H 7.55, N 2.49.

Data of **17**: $[\alpha]_D^{25} = +20.3°$ (c = 1.2, $CHCl_3$). IR: 3425*m*, 2995*m*, 2985*m*, 2935*w*, 2880*w*, 1743*s*, 1705*s*, 1680*s*, 1632*w*, 1510*m*, 1370*s*, 1343*w*, 1310*w*, 1238*s*, 1153*s*, 1052*s*. ^{1}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_3). ^{13}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, $M^{+\cdot} - 15$), 471 (3). Anal. calc. for $C_{25}H_{39}NO_{11}$ (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 blue[9]). 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, CH_2Cl_2/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. 167–168° (dec.). $[\alpha]_D^{25} = -62.8°$ (20 h, c = 1.0, $CHCl_3$). IR (KBr): 3400*s* (br.), 2975*m*, 2905*w*, 2855*w*, 1740*s*, 1632*s*, 1567*m*, 1384*s*, 1370*s*, 1328*m*, 1142*s*, 1124*s*, 1088*s*, 1036*s*, 1026*s*. ^{1}H-NMR (400 MHz, (D_6)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*, ArC*H*); 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 $D_2O \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}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 $C_{22}H_{31}NO_9$ (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). CH_2Cl_2/MeOH 97:3 eluted Ph_3P and Ph_3PO; CH_2Cl_2/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. 164–165° (dec.). $[\alpha]_D^{25} = -80.9°$ (c = 1.0, $CHCl_3$). IR (KBr): 3500*s*, 3415*s*, 2985*w*, 1738*s*, 1640*s*, 1536*m*, 1385*m*, 1370*m*, 1320*m*, 1310*m*, 1150*s*, 1095*s*, 1080*s*, 1060*m*, 1020*m*, 850*w*, 770*m*. ^{1}H-NMR (400 MHz, (D_6)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*, ArC*H*); 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 $D_2O \rightarrow$br. *d*); 3.78 (*dddd*, J = 10.5, 9.5, 5.6, 5.0; H–C(8); addn. of $D_2O \rightarrow$*ddd*); 3.57 (*dd*, J = 9.5,

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[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*, CH_3); 1.40 (*s*, *t*-Bu). ^{1}H-NMR (200 MHz, CD_3OD); β-D/α-D = 3:1; β = D-anomer: 7.60–7.26 (*m*, 5 arom. H); 5.44 (*s*, ArC*H*); 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*, CH_3); 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*, CH_3); 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. ^{13}C-NMR (50 MHz, CD_3OD): 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 (*t*); 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 (*t*); 28.05 (*q*); 22.73 (*q*). Anal. calc. for $C_{22}H_{31}NO_9$ (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-nonulosonate* (**20**) *and* tert-*Butyl 5-Acetamido-3,5-dideoxy-7,9-O-isopropylidene*-D-glycero-D-talo-*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 SiO_2), CH_2Cl_2/MeOH 15:1 eluted **21** (70 mg, 7%) as a colourless foam and CH_2Cl_2/MeOH 12:1 **20** (861 mg, 85%) as an oil, which solidified after a few hours.

Data of **20**: $[\alpha]_D^{25} = -44.8°$ (c = 1.0, MeOH). IR (KBr): 3410*s* (br.), 2985*w*, 2940*w*, 1734*m*, 1643*s*, 1553*w*, 1370*s*, 1315*w*, 1227*w*, 1150*s*, 1125*s*, 1070*s*, 1030*m*. ^{1}H-NMR (400 MHz, (D_6)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 D_2O→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*, CH_3); 1.71 (*dd*, J = 12.6, 11.0, H–C(3)); 1.44 (*s*, *t*-Bu); 1.29 (*s*, CH_3); 1.25 (*s*, CH_3). ^{13}C-NMR (50 MHz, CD_3OD): 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 (*t*); 62.78 (*d*); 52.72 (*d*); 40.22 (*t*); 28.62 (*q*); 28.05 (*q*); 22.98 (*q*); 19.06 (*q*). Anal. calc. for $C_{18}H_{31}NO_9$ (405.46): C 53.32, H 7.71, N 3.46; found: C 53.06, H 7.90, N 3.54.

Data of **21**: $[\alpha]_D^{25} = -63.7°$ (c = 1.0, $CHCl_3$). IR: 3420*m* (br.), 2985*s*, 2930*m*, 2870*w*, 1730*s*, 1670*s*, 1505*m*, 1370*s*, 1306*m*, 1136*s*, 1090*s*, 1064*s*, 1024*m*. ^{1}H-NMR (400 MHz, (D_6)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*, CH_3); 1.42 (*s*, *t*-Bu); 1.23 (*s*, CH_3); 1.19 (*s*, CH_3). CI-MS: 406 ($M^{+\cdot}$ + 1), 388 (($M^{+\cdot}$ + 1) – 18), 350 (($M^{+\cdot}$ + 1) – 56); 332 (($M^{+\cdot}$ + 1) – 74). Anal. calc. for $C_{18}H_{31}NO_9$ (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/H_2O 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, $CHCl_3$/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 Et_2O until persistance of a slight turbidity. Additional Et_2O (5 ml) was added after 16 h, and the mixture was kept in the refrigerator (1 d). The crystals were dried (P_2O_5, 10^{-5} mbar, 2 d) affording **22** (422 mg, 85%). The mother liquors were chromatographed (AcOEt/MeOH/H_2O 170:27:3) on a preconditioned column (20 g of SiO_2, AcOEt/MeOH 98:2) to give additional **22** (45 mg, 9%). M.p. 178–179° (dec.). $[\alpha]_D^{25} = -24.8°$ (c = 1.0, H_2O). IR (KBr): 3460*s*, 2980*m*, 2940*m*, 1726*s*, 1635*s*, 1555*s*, 1370*s*, 1312*s*, 1135*s*, 1030*s*. ^{1}H-NMR (400 MHz, D_2O): 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 CH_3); 2.05 (*s*, 0.1 CH_3); 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). ^{13}C-NMR (100.6 MHz, CD_3OD): 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 (*t*); 54.43 (*d*); 40.83 (*t*); 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 (*t*); 54.05 (*d*); 42.47 (*t*); 28.09 (*q*); 22.68 (*q*). CI-MS: 366 ($M^{+\cdot}$ + 1), 310, 292, 274. Anal. calc. for $C_{25}H_{27}NO_9$ (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 CH_2Cl_2/MeOH 1:1 (5 ml) was treated with CF_3COOH (100 µl). 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 H_2O (7 ml) and extracted with CH_2Cl_2. The aq. layer was freeze-dried giving crude **22** (148 mg, quant.). Crystallization from anh. MeOH (1.5 ml)/AcOEt (1.5 ml)/Et_2O (*cf.* **18**→**22**) yielded **22** (124 mg, 86%). M.p. 176–177°. $[\alpha]_D^{25} = -25.0°$ (c = 1.0, H_2O).

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/H_2O 3:2 (15 ml) was stirred with K_2CO_3 (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, H_2O (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 H_2O (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 H_2O, 6.0 ml of AcOH, 3 d, 4°) [8] [10] gave, after drying (P_2O_5, KOH, 10^{-5} mbar), **1** (130 mg, 51%). M.p. 180–182° (dec.) ([10]: 181–183°). $[\alpha]_D^{20} = -33.0°$ (c = 1.0, H_2O); ([10]: −32.1° (c = 1.3, H_2O)).

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 K_2CO_3 as described for **1**. A soln. of crude **1** (90 mg) in anh. MeOH (9 ml), was stirred with CF_3COOH (40 µl) 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 Et_2O and kept in the refrigerator (5 d). The crystals were dried (P_2O_5; 10^{-5} mbar) yielding **23** (52 mg, 59%). M.p. 176–178° ([38]: 179–180°); $[\alpha]_D^{25} = -28.6°$ (c = 1.1, H_2O), ([38]: −28° (c = 1.0, H_2O)).

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: CH_2Cl_2/EtOH 9:1). The crude **24** (104 mg) was crystallized from MeOH (1 ml), AcOEt (1.5 ml), and Et_2O (10 ml) affording pure **24** (73 mg, 70%). The mother liquors were chromatographed (AcOEt/MeOH/H_2O 170:27:3) on a preconditioned column (15 g of SiO_2, AcOEt) to give **24** (27 mg, 25%). M.p. 157–158°. $[\alpha]_D^{25} = -76.0°$ (c = 1.0, H_2O). IR (KBr): 3500 (sh), 3360*s*, 2980*w*, 2940*w*, 1733*s*, 1668*s*, 1520*m*, 1430*m*, 1370*m*, 1309*m*, 1136*s*, 1089*s*, 1050*m*, 1027*m*, 1012*m*, 918*m*, 843*m*. ^{1}H-NMR (400 MHz, D_2O): 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 CH_3); 2.04 (*s*, 0.3 CH_3); 1.89 (*dd*, J = 14.0, 2.7, 0.3 H–C(3)); 1.52 (*s*, *t*-Bu). ^{13}C-NMR (25 MHz, CD_3OD): 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*); 82.34 (*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 ($M^{+\cdot}$ + 1), 348, 310, 292, 274. Anal. calc. for $C_{15}H_{27}NO_9$ (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.). $[\alpha]_D^{25} = -76.4°$ (c = 1.0, H_2O).

Sodium 5-Acetamido-3,5-dideoxy-D-glycero-D-talo-*2-nonulosonate* (*Sodium* N-*Acetyl-4-epineuraminate;* **2**). Similarly to **22**, **24** (350 mg, 0.958 mmol) was saponified (TLC: $CHCl_3$/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* × *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 decolourized by activated charcoal[10]). The residual slightly yellow oil was transformed to the Na salt by passing it through a cation exchanger (10 ml of *Dowex 50 W* × *4* (Na^+), H_2O) to give, after freeze drying, **2** (146 mg, 46%; 5d 10^{-5} mbar) as a colourless solid. $[\alpha]_D^{25} = -71.4°$ (c = 1.0, H_2O). ^{1}H-NMR (400 MHz, D_2O): 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*, CH_3); α-D-anomer: 2.49 (*dd*, J = 14.7, 3.2, H–C(3)); 2.04 (*s*, CH_3); 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. ^{13}C-NMR (50 MHz, D_2O): 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 ^{1}H, ^{13}C-decoupling experiments. FAB-MS: 332 ($M^{+\cdot}$ + 1). Anal. calc. for $C_{11}H_{18}NNaO_9$ (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 CF_3COOH (1 ml) and stirred at r.t. (6 h). TLC ($CHCl_3$/MeOH 3:1) indicated then the disappearance of **24**. The solns. was concentrated and twice co-evaporated with anh. MeOH (2 × 3 ml). The residual oil was redissolved in anh. MeOH (3 ml), treated with CF_3COOH (100 µl), and stirred at r.t. (2 d). The soln. was concentrated and chromatographed (20 g of SiO_2, $CHCl_3$/MeOH 9:1) on a preconditioned

[10]) Activated by treating it with 0.5N HCOOH (2 h) and washing with H_2O (bidest.).

column (24 h). Fractions containing **25** were combined, concentrated, and extracted with H_2O. The H_2O 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 Et_2O (12 ml). M.p. 166–168° (dec.). $[\alpha]_D^{20} = -67°$ (c = 1.0, H_2O). IR (KBr): 3380*s* (br.), 1740*m*, 1655*m*, 1545*m*. ^{1}H-NMR (400 MHz, D_2O): 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*, CH_3); 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*, CH_3); 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*, CH_3); 2.69 (*dd*, J = 14.4, 3.6, H–C(3)); 2.03 (*s*, CH_3); 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. ^{13}C-NMR (50 MHz, D_2O): 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 ($M^{+\cdot}$ + 1). Anal. calc. for $C_{12}H_{21}NO_9$ (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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