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Synthesis of 5-*N*- and 9-*N*-Thioacylated Sialic Acids[1]

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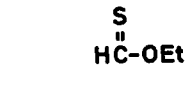
Abstract: *N*-Thioacylation of neuraminic acid methyl α-glycoside (**5**) with *O*-ethyl thioformate (**1**), methyl dithioacetate (**2**), and methyl dithiopropionate (**3**) afforded *N*-thioformyl, *N*-thioacetyl, and *N*-thiopropionyl neuraminic acid derivatives **6a-c** in high yield. Cleavage of the glycosides was accomplished either by acid hydrolysis or by sialidase treatment. Alternatively, 5-*N*-thioacylneuraminic acids were produced from the corresponding *N*-thioacyl-D-mannosamines **7a-c** and pyruvate employing *N*-acetylneuraminate pyruvate lyase. The sialidase-resistant methyl α-thioglycoside of *N*-thioacetylneuraminic acid (**10**) was also prepared. *N*-Acetyl-9-deoxy-9-thioacetamido neuraminic acid (**19**) was obtained either directly by reaction of *N*-acetyl-9-amino-9-deoxyneuraminic acid (**17**) with methyl dithioacetate (**2**) or *via* its methyl α-glycoside **18**. Compound **17** was produced from the methyl α-glycoside of 9-*O*-tosylated methyl ester **12** *via* the azide **13**. — For the 5-*N*-thioacyl sialic acids **6a-c** and **8a-c** as well as for the 9-deoxy-9-thioacetamido derivatives **18** and **19** some biological properties are reported.

Sialic acids occur as the terminal units of the carbohydrate moiety of many glycoproteins and glycolipids and take part in a large variety of biological functions including recognition phenomena. An important number of pathogens, for example, recognize sialic acids as receptor determinant. Particularly well studied are the influenza viruses. Here, a prerequisite for infection is the attachment of the virus to the surface sialic acid of the host cell mediated by the viral hemagglutinin.[2]

Obviously, synthetic analogs of naturally occurring sialic acids which may act as hemagglutinin inhibitors are of special interest. Moreover, such analogs are potential probes for the study of sialic acid recognizing proteins and of substrate specifities of the enzymes involved in sialic acid metabolism. Despite the fact that many different *N*-acylated sialic acids have been synthesized and tested in biological systems, *N*-thioacylated derivatives are still unknown.

In a preceding communication[3], we described the synthesis of unprotected 2-*N*-thioacylated hexosamines. These hitherto unknown compounds were obtained by the reaction of 2-amino-2-deoxy hexoses with *O*-ethyl thioformate (**1**), methyl dithioacetate (**2**), and methyl dithiopropionate (**3**), and are of



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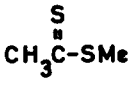

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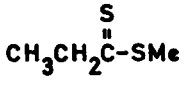


3

interest as analogs of the naturally occurring *N*-acetyl amino sugars. We here report on the extension of this work to sialic acids.

Our first aim was to prepare *N*-thioacetylneuraminic acid (Neu5ThAc) as the direct analog of the most abundant sialic acid, *N*-acetylneuraminic acid (Neu5Ac). The latter is known to be the receptor determinant

of influenza A and B viruses.[4] In addition, the corresponding *N*-thioformyl and *N*-thiopropionyl compounds might be of interest. Suitable starting materials for the synthesis of *N*-thioacylneuraminic acids are: (a) neuraminic acid glycosides possessing a free amino group that can be thioacylated, and (b) *N*-thioacyl-D-mannosamines, which might be condensed enzymatically with pyruvate.

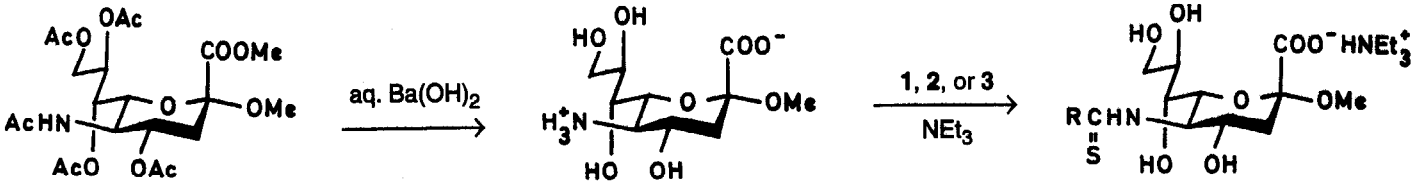
Further, we intended to obtain an α-thioglycoside of Neu5ThAc. Thioglycosides cannot be cleaved by sialidases but are known to act as competitive inhibitors.[5] These compounds might also be useful for studies with influenza viruses.

Finally, we wished to prepare a sialic acid bearing a thioacetamido substituent at the C-9 position. Influenza C virus requires *N*-acetyl-9-*O*-acetylneuraminic acid (9-OAc-Neu5Ac) as the attachment point on the host cell.[6] In a recent investigation, it was shown that 9-acetamido-*N*-acetyl-9-deoxyneuraminic acid (9-NAc-Neu5Ac) is also able to function as receptor determinant but is resistant to the viral receptor-destroying enzyme.[7] This prompted us to prepare the 9-thioacetamido analog (9-NThAc-Neu5Ac) which may contribute to get more insight into the role of this enzyme in the infection cycle.

For the preparation of the 5-*N*-thioacylneuraminic acids, we first investigated the chemical route starting from *N*-acetylneuraminic acid, which was converted into the known derivative **4**[8] (see Scheme 1, next page). Treatment of **4** with saturated aqueous barium hydroxide at reflux afforded the *N*-unsubstituted methyl α-glycoside **5**[9] in 89% yield. Thioacylation of **5** with reagents **1**, **2**, and **3** readily gave methyl α-glycosides of *N*-thioformylneuraminic acid (Neu5ThFo-2αMe, **6a**), *N*-thioacetylneuraminic acid (Neu5ThAc-2αMe, **6b**), and *N*-thiopropionylneuraminic acid (Neu5ThPr-2αMe, **6c**), respectively. The reactions were performed at room temperature in aqueous methanol in the presence of triethylamine and an excess of thioacylation reagent. As indicated by TLC, complete conversion of **5** to the strongly UV positive products **6a-c** required 2 to 14 hours, depending upon the thioacylation reagent employed. After this time, the mixture was concentrated in vacuum, and **6b** as well as **6c**, were obtained without laborious work-up as crystalline triethylammonium salts in analytically pure form (yield 82% and 84%, respectively). In the reaction of **5** with *O*-ethyl thioformate (**1**), TLC showed two well-separated spots due to the existence of *Z,E* rotamers about the NH-CHS bond[3, 10] of *N*-thioformyl derivative **6a**. In contrast to **6b** and **6c**, compound **6a** could not be crystallized but was obtained in pure form by silica gel chromatography (yield 85%).[11]

As expected, ^{1}H NMR spectra of **6b** and **6c** showed the characteristic downfield shift for proton H-5 that is attached to the carbon atom bearing the thioacylamino group (~4.60 ppm for H-5 of **6b** and **6c** *vs.* ~3.80 ppm for H-5 of the corresponding acylamino compounds[12, 13]). A related downfield shift was observed for the *N*-thioacetyl methyl group (2.58 ppm) and the *N*-thiopropionyl methylene group (2.74 ppm). The values for the corresponding *N*-acyl groups are ~2.03 ppm[12] and 2.32 ppm[13], respectively. In the ^{13}C NMR spectra, the thiocarbonyl carbon signal appeared at 205.4 ppm (for **6b**; *cf.* ~176 ppm for amide carbon of Neu5Ac-2αMe[14]) and at 211.6 ppm (for **6c**), respectively. The signal for carbon C-5 of these compounds was shifted downfield by ~6 ppm relative to that of Neu5Ac-2αMe (~59 ppm for **6b**, **6c** *vs.* ~53 ppm for Neu5Ac-2αMe[14]).

The ^{1}H NMR spectrum of Neu5ThFo-2αMe **6a** showed *Z* and *E* rotamers in the ratio 3:2. The rotamers can be distinguished on the basis of the coupling constants between NH and CHS protons in DMSO-d_6 ($J_{NH,CHS}$ ~7.5 Hz for *Z*-**6a** *vs.* 14.3 Hz for *E*-**6a**) and by means of the chemical shifts of thioformyl proton (9.51 ppm for *Z*-**6a** *vs.* 9.24 ppm for *E*-**6a**) and proton H-5 (4.72 ppm for *Z*-**6a** *vs.*

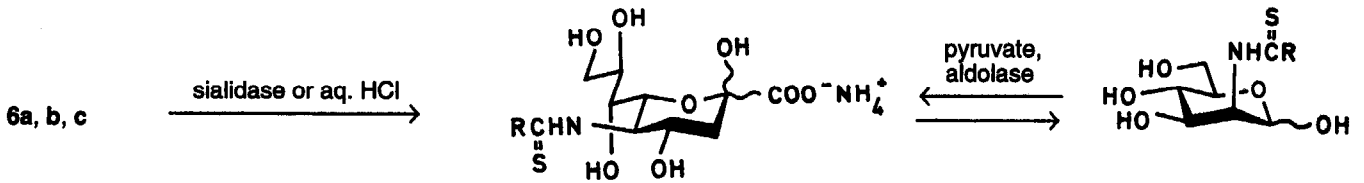


4

5

6a : R = H
6b : R = CH_3
6c : R = CH_2CH_3

6a, b, c



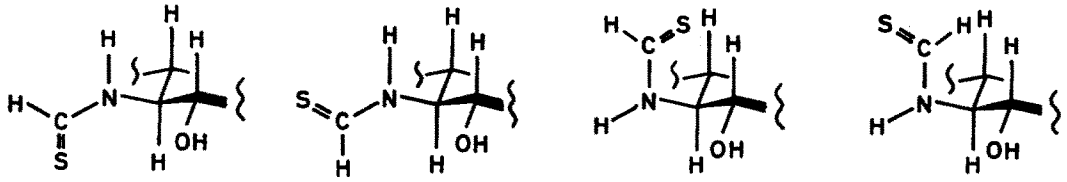
α : β ~ 7 : 93

8a : R = H
8b : R = CH_3
8c : R = CH_2CH_3

7a : R = H
7b : R = CH_3
7c : R = CH_2CH_3

Scheme 1.

~3.65 ppm for *E*-**6a**).[3, 9] Moreover, the fact that the shift of H-5 is dependent upon the rotameric disposition strongly suggests the conformation about the sugar-NHCHS bond to be antiperiplanar (see Scheme 2, Fig. I and II). In this case, the observed interaction of the axially oriented H-5 with thiocarbonyl sulfur (for *Z*-rotamer, Fig. I) and thioformyl proton (for *E*-rotamer, Fig. II), respectively, should be possible. On the other



I II III IV

Scheme 2. Conformations of *N*-thioformylneuraminic acid derivatives. Fig. I: antiperiplanar, *Z*-rotamer; II: antiperiplanar, *E*-rotamer; III: synperiplanar, *Z*-rotamer; IV: synperiplanar, *E*-rotamer.

hand, no significant influence of the rotameric conformation upon protons H-4 and H-6 can be observed. In a synperiplanar disposition about the sugar-NHCHS bond (Scheme 2, Fig. III and IV), such an influence seems to be likely due to the proximity of H-4 and H-6 to the thioformyl group. Furthermore, the shift of proton H-5 of *Z*-**6a** is similar to that of proton H-5 of **6b** and **6c**. For these compounds, in each case only one rotamer could be identified, presumably possessing the *Z*-conformation with the alkyl group directed away from the ring (Scheme 2, Fig. I, CH_3 or CH_3CH_2 instead of H). Such a conformation has been found for the acetamido group of Neu5Ac derivatives using NMR spectroscopy[15, 16] and x-ray analysis[17]. As observed previously for *N*-thioformyl hexosamines, ^{13}C NMR spectroscopy showed only slightly different signals for the thiocarbonyl carbon of *Z* and *E* rotamers (194.5 ppm for *E*-**6a** *vs.* 192.6 ppm for *Z*-**6a**). For carbon C-5, the shift difference between the rotamers was more significant (63.9 ppm for *E*-**6a** *vs.* 55.8 ppm for *Z*-**6a**).[3, 10]

For cleavage of the glycoside (see Scheme 1), the *N*-thioformyl derivative **6a** was hydrolyzed by 0.033 N hydrochloric acid. The ammonium salt of *N*-thioformylneuraminic acid (**8a**) was obtained in 75% yield after anion exchange chromatography on DEAE-Sephadex A-25 (HCO_3^-). As observed for **6a**, TLC of **8a** showed two well-separated spots corresponding to *Z,E* rotamers. The rotameric ratio of **8a** and **6a** were identical. In the first fractions, ammonium *N*-formylneuraminate was identified as a by-product in ~6% yield. In the ^1H NMR spectrum, signals for *N*-formyl protons of the predominating β-anomer appeared at 8.23 ppm (*Z*-rotamer) and at 8.04 ppm (*E*-rotamer; ratio 2.5:1). However, in an earlier investigation methyl β-glycoside of methyl *N*-formylneuraminate exhibited a ratio *Z/E* of 7:1.[15] Neu5ThFo-2αMe **6a** proved to be a poor substrate for *Arthrobacter ureafaciens* sialidase. Even with excess enzyme, cleavage was only 50% after five days of incubation.

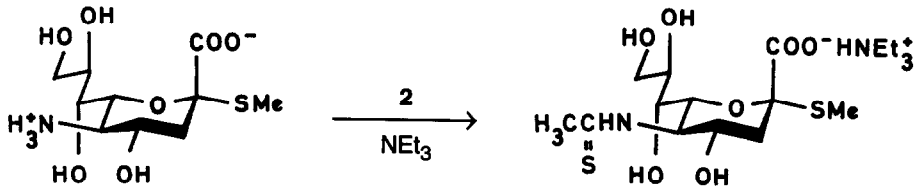
In contrast to **6a**, attempts to achieve hydrolysis of *N*-thioacetyl and *N*-thiopropionyl analogs **6b** and **6c** by dilute hydrochloric acid resulted in extensive decomposition. However, enzymatic cleavage using *Arthrobacter ureafaciens* sialidase turned out to be the method of choice.[18] Under the same conditions as applied for **6a**, compounds **6b** and **6c** were completely hydrolyzed within 22 hours. Chromatography on

DEAE-Sephadex afforded *N*-thioacetylneuraminic acid (Neu5ThAc, **8b**) and *N*-thiopropionylneuraminic acid (Neu5ThPr, **8c**) as ammonium salts in 83% and 74% yield, respectively.

The sialidase reactions of **6a-c** showed a similar dependence of the enzymatic cleavage rate upon the number of carbon atoms of the *N*-substituent as observed with parent *N*-acyl compounds. Whereas Neu5Fo-2αBn is poorly cleaved[19], Neu5Pr-2αBn is split somewhat slower than Neu5Ac-2αBn[20]. Compared to Neu5Ac-2αMe, the thio analog Neu5ThAc-2αMe **6b** was hydrolyzed by *Arthrobacter ureafaciens* sialidase ~ 30% slower.

In the second approach for the synthesis of 5-*N*-thioacylneuraminic acids, we studied the enzyme catalyzed aldol reaction between mannosamine derivatives and pyruvate[21] (see Scheme 1). Thus, the incubation of *N*-thioacetyl-D-mannosamine (**7b**) with sodium pyruvate in the presence of *N*-acetylneuraminate pyruvate lyase (Neu5Ac aldolase, E.C. 1.4.3.3), gave Neu5ThAc **8b** in 55% yield after purification by anion exchange chromatography. Compared to the synthesis of parent Neu5Ac, the reaction proceeded about one-third slower. Additional enzyme did not improve the yield. In order to suppress *S/O* exchange, the reaction was performed at pH 6.8 instead of pH 7.5 which is the optimum for aldolase reactions. Nevertheless, on prolonged incubation time (>24 h), *N*-acetylmannosamine and *N*-acetylneuraminate were obtained as side products, thereby limiting the yield and complicating the purification. The enzymatic reactions of mannoses **7a** and **7c** with pyruvate were carried out on a milligram scale and monitored by TLC. The results were similar to that obtained for **7b** under the same conditions. The desired products **8a-c** proved to be identical according to TLC and ^{1}H NMR spectroscopy (for **8b**) with those synthesized from zwitterion **5** *via* **6a-c**.

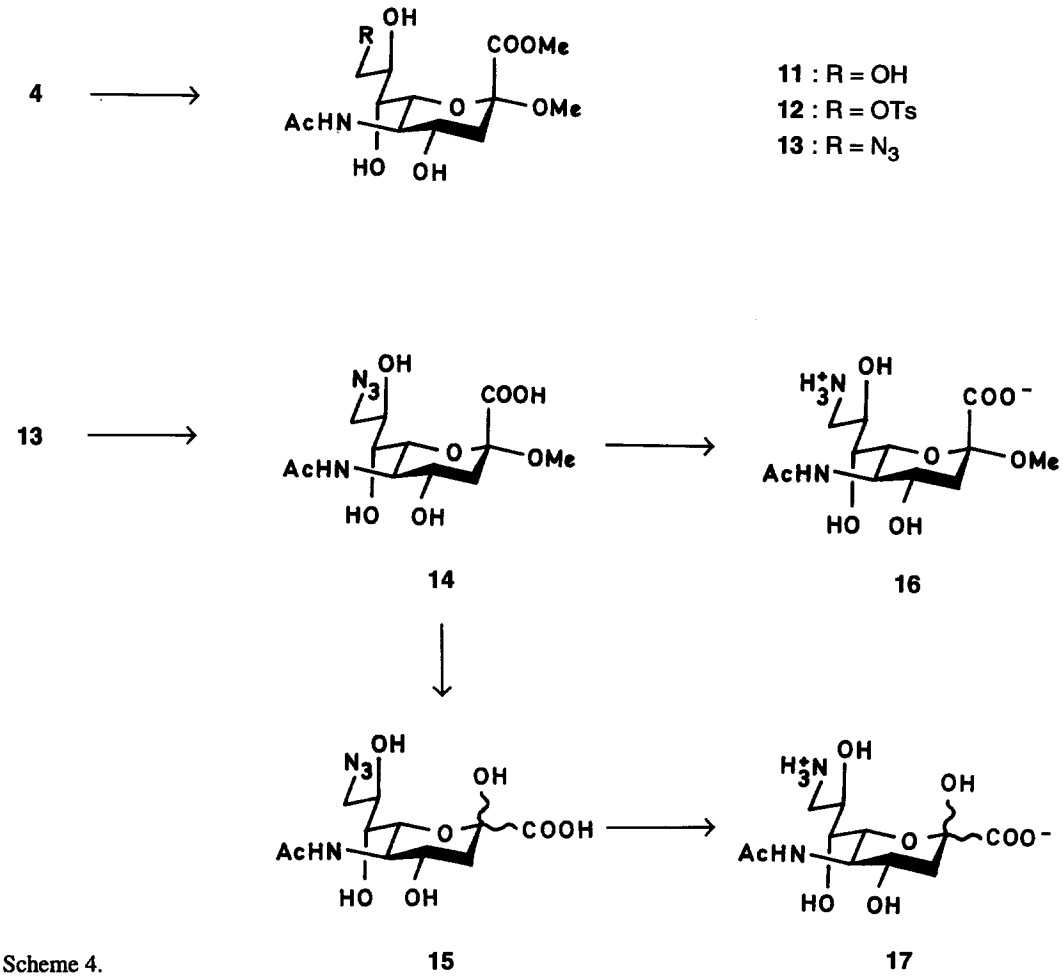
For the synthesis of a sialidase-resistant *N*-thioacetylneuraminic acid derivative, methyl α-thioglycoside **9**[22] was used (see Scheme 3). Reaction of **9** with methyl dithioacetate (**2**) was carried out in the same way as the reaction of the corresponding *O*-glycoside **5** and afforded the triethylammonium salt of Neu5ThAc-2αSMe **10** as pale yellow crystals in 85% yield. As expected, **10** turned out to be resistant against sialidases of *Vibrio cholerae*, *Clostridium perfringens*, *Arthrobacter ureafaciens*, and fowl plague virus.



Scheme 3. **9** **10**

As starting material for the synthesis of *N*-acetyl-9-deoxy-9-thioacetamido neuraminic acid derivatives, we employed methyl α-glycoside of *N*-acetylneuraminic acid methyl ester **11**[8b, 8c, 23] obtained from **4** by Zemplén deacetylation (see Scheme 4, next page). Treatment of **11** with *p*-toluenesulfonyl chloride in pyridine provided the 9-*O*-tosyl derivative **12**, which was converted into azide **13** by reaction with sodium azide in aqueous acetone. Saponification of methyl ester of **13** under mild conditions yielded the free acid **14** as a crystalline solid (yield 56%, based on **4**). Subsequent careful hydrolysis of **14** with Dowex 50W-X8 (H^+)

gave *N*-acetyl-9-azido-9-deoxyneuraminic acid **15**.[24] Catalytic hydrogenation (PdO) of the azide function of **14** and **15** in weakly acidic solution afforded the respective amines **16** and **17** in an almost quantitative yield.

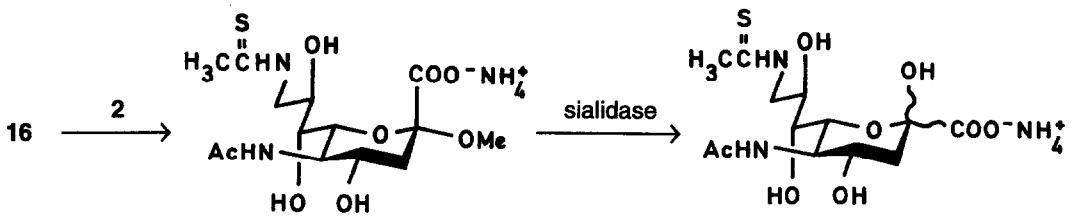


Scheme 4.

Both, **16** and **17** are monobasic amino acids. Due to the zwitterionic character (pK_2 9.7, for **17**) biological properties are quite different in comparison to the respective parent compounds. Thus, the additional amino group at C-9 renders **17** poorly cleavable by aldolase, and **16** is, for the same reason, no substrate for sialidases.[25, 26] Therefore **17**, though being readily activated and transferred onto glycoconjugates,[25, 27, 28] is enzymatically not attacked[26] which may be important for the half-life of such glycoconjugates. *N*-Acetyl-9-azido-9-deoxyneuraminic acid (**15**) carries a photoreactive group and may be useful for photoaffinity labelling. Activation and transfer of **15** have already been demonstrated.[24, 28]

The reaction of methyl α-glycoside **16** with methyl dithioacetate (**2**) in the presence of triethylamine

afforded 9-deoxy-9-thioacetamido neuraminic acid derivative **18** in 76% yield after silica gel chromatography and transformation into the ammonium salt (see Scheme 5). ^{1}H NMR spectroscopy indicated the conversion of the protonated amino group of **16** into the thioamide function of



Scheme 5. **18** **19**

9-NThAc-Neu5Ac-2αMe **18** by the downfield shift of protons H-9 and H-9' (4.02 ppm and 3.78 ppm, respectively, for **18** *vs.* 3.48 ppm and 3.09 ppm, respectively, for **16**). Attempts to obtain *N*-acetyl-9-deoxy-9-thioacetamido neuraminic acid (**19**) by acid hydrolysis of glycoside **18** resulted in extensive decomposition, as observed for Neu5ThAc-2αMe **6b**. However, the cleavage of **18** was readily accomplished by *Arthrobacter ureafaciens* sialidase and furnished the ammonium salt of **19** in 76% yield after purification on DEAE-Sephadex.

Compound **19** could also be obtained directly by *N*-thioacetylation of the unsubstituted zwitterion **17**. Chromatography as described above afforded **19** in 71% yield.

The new sialic acids with the amide oxygen replaced by sulfur were studied in a number of biological tests. In addition to the results obtained with sialidases and Neu5Ac aldolase (*vide supra*), derivatives Neu5ThFo **8a** and Neu5ThAc **8b** as well as 9NThAc-Neu5Ac **19** could be readily activated by the CMP-sialate synthase from bovine brain. This and the subsequent enzymatic transfer onto glycoconjugates will be subject of a separate report.

Studies with the hemagglutinin of influenza A virus X-31 strain showed Neu5ThAc-2αMe **6b** to bind about 8-fold stronger compared to the natural receptor and to have higher affinity than any other sialic acid methyl α-glycoside so far tested.[29] This change in the biological activity may be due to the higher polarizability of the thioacetamido group, resulting in an increased ability to form the hydrogen bond with glycine 135 of the hemagglutinin.[30] In addition, the thioamide sulfur will modify the hydrophobic interaction with leucine 194.[30]

Influenza C virus recognized 9-NThAc-Neu5Ac **19** as receptor determinant after enzymatic transfer onto the host cell. Most notably, in contrast to 9-NAc-Neu5Ac the thio analog **19** did not allow the cell to be infected.[31]

In another investigation with a sialic acid recognizing protein, the lectin from *Cepaea hortensis* was shown to be inhibited only by Neu5Ac-2αMe whereas the 5-thioacetyl analog **6b** was inactive.[32]

The potential of *N*-thioacylated sialic acids to affect additional biological systems is evident. At present we are extending our investigations to the introduction of thioacyl groups into glycolipids and to the preparation of *N*-thioacylated sialic acid polymers.

EXPERIMENTAL

General methods

Melting points were determined with a Tottoli-Büchi apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter after 24 hours keeping at ambient temperature (c 0.5, H_2O, unless otherwise stated). UV spectra were recorded in aqueous solution with a Hitachi U-2000 spectrophotometer. TLC was performed on aluminium sheets coated with Silica Gel 60 F_{254} (Merck) using the following solvent combinations (v/v) and others specifically mentioned: 1:1 ethyl acetate - methanol (A), 5:1 *n*-propanol - water (B), 8:1 *n*-propanol - water (C), 5:2:3 *n*-butanol - acetic acid - water (D), 5:1 chloroform - methanol (E). Compounds were detected by UV light, if possible, and by spraying TLC plates with 2 M H_2SO_4 and charring at 200°C for a few minutes. Column chromatography was performed on Silica Gel Merck 60 (70-230 mesh). Unless otherwise stated, ^1H NMR spectra were recorded in D_2O at 25°C with a Bruker AC 300, AM 360, or AM 500 spectrometer, and ^{13}C NMR spectra in D_2O at 25°C with a Bruker AC 300 spectrometer at 75 MHz. Chemical shifts are reported in ppm relative to the solvent; HOD in D_2O at 4.76 ppm, $D_2HCSOCD_3$ in DMSO-d_6 at 2.50 ppm, CD_3OH in methanol-d_4 at 4.82 ppm, and $CHCl_3$ in $CDCl_3$ at 7.25 ppm for the proton spectra. For carbon spectra, the reference is CD_3OD = 49.0 ppm in D_2O. FAB MS (matrix: glycerol; ion energy 15 kV) were recorded on a MAT 95 mass spectrometer (Finnigan MAT, Bremen, Germany). *Arthrobacter ureafaciens* sialidase (E.C. 3.2.1.18) was purchased from Boehringer Mannheim (Germany), Neu5Ac aldolase of *Escherichia coli* (E.C. 1.4.3.3) from Serva (Heidelberg, Germany).

Methyl 5-amino-3,5-dideoxy-D-glycero-α-*D*-galacto-*2-nonulopyranosidonic acid* (*Neu-2αMe*, **5**). The peracetylated methyl glycoside **4** (1.0 g, 1.98 mmol) was refluxed in a saturated aqueous solution of barium hydroxide (40 ml) for 16 hours, cooled to room temperature, filtered, and the filtrate was brought to pH ~7 by addition of small pieces of dry ice. After removal of the precipitate by filtration, the solution was cooled to 0°C and Amberlite IR 120 (H^+) cation exchange resin was added until Ba^{2+} could no longer be detected. (No precipitation of $BaSO_4$ occurred when aqueous H_2SO_4 was added to a diluted sample of the filtrate.) The resin was removed by filtration, washed exhaustively with water, and the collected solutions were lyophilized to give a first fraction of **5** (320 mg, 58%). Since a considerable amount of **5** adhered to the resin, it was finally rinsed with 0.5 M aqueous ammonia (40 ml) to afford, after freeze-drying, a second fraction of **5** (170 mg, 31%). An analytical sample of **5** was obtained by crystallization from ethanol - water, m.p. 195°C; R_F 0.21 (B); $[\alpha]_D^{20}$ -12.4°; ^1H NMR (300 MHz): δ 3.91 (dd, $J_{6,7}$ 2.0, $J_{5,6}$ 10.3 Hz, 1 H, H-6), 3.85 (ddd, $J_{8,9}$ 2.4, $J_{7,8}$ 8.9, $J_{8,9'}$ 5.6 Hz, 1 H, H-8), 3.79 (dd, $J_{9,9'}$ 12.0 Hz, 1 H, H-9), 3.67 (dd, 1 H, H-7), ~3.66 (m, 1 H, H-4), 3.60 (dd, 1 H, H-9'), 3.23 (s, 3 H, OCH_3), 3.10 (app. t, $J_{4,5}$ ~10.2 Hz, 1 H, H-5), 2.63 (dd, $J_{3eq,4}$ 4.7, $J_{3eq,3ax}$ 12.6 Hz, 1 H, H-3eq), 1.55 (app. t, $J_{3ax,4}$ ~12.2 Hz, 1 H, H-3ax). Anal. Calc. for $C_{10}H_{19}NO_8$ (281.26): C 42.70, H 6.81, N 4.98. Found: C 42.57, H 7.02, N 4.65.

Methyl 3,5-dideoxy-5-thioformamido-D-glycero-α-*D*-galacto-*2-nonulopyranosidonic acid* (*Neu5ThFo-2αMe*, **6a**). To a suspension of **5** (100 mg, 0.36 mmol) in aqueous methanol (2.7 ml, 16:1 methanol - water), triethylamine (0.1 ml, 0.72 mmol) was added at 0°C followed by **1** (0.05 ml, 0.55 mmol). The cold bath was removed after 1 hour, and stirring was continued at ambient temperature. After seven hours, TLC showed complete disappearance of **5** and the formation of two UV positive compounds (R_F 0.67 [*E*-**6a**] and 0.58 [*Z*-

6a], [A]; **5**: R_F 0.08 [A]) in a ratio of ~1:1. The reaction mixture was evaporated *in vacuo* and purified by column chromatography on silica gel (8:5 ethyl acetate - methanol).[11] Fractions containing **6a** were combined and freed from the solvent. The residue was taken up in water and passed at 4°C through a column of Amberlite IR 120 (H^+) resin. The solution was lyophilized to give **6a** as a colourless powder. Yield 104 mg (85%, for **6a**·0.75 H_2O); R_F 0.42 (*E*-rotamer) and 0.29 (*Z*-rotamer), (C); $[\alpha]_D^{20}$ +14.5° (NH_4^+ salt, measured after 48 h; steadiness of optical rotation indicated that the equilibrium between the rotamers has been reached); λ_{max} 266.0 nm (ε_M 13180); ^1H NMR (500 MHz, NH_4^+ salt, recorded after 48 h in D_2O solution; prolonged keeping in aqueous solution did not alter the rotameric ratio): δ 9.51 (s, 0.6 H, *Z*-HC(S)), 9.24 (s, 0.4 H, *E*-HC(S)), 4.72 (app. t, $J_{5,6}$ ~ $J_{4,5}$ ~10.2 Hz, 0.6 H, *Z*-H-5), 3.98-3.87 (m, ~3.4 H, presumably *Z*-H-6, 8, 9 and *E*-H-4, 6, 8, 9), 3.81 (ddd, $J_{3eq,4}$ 4.7, $J_{3ax,4}$ 11.7 Hz, 0.6 H, *Z*-H-4), 3.70-3.63 (m, ~1.8 H, presumably *Z*-H-9' and *E*-H-5, 7, 9'), 3.56 (dd, $J_{6,7}$ 1.2, $J_{7,8}$ 8.9 Hz, 0.6 H, *Z*-H-7), 3.38 (s, 1.8 H, *Z*-OCH_3), 3.37 (s, 1.2 H, *E*-OCH_3), 2.76 (broadened dd, $J_{3eq,4}$ ~4.6, $J_{3ax,3eq}$ ~12.4 Hz, 1 H, *E,Z*-H-3eq), 1.72 (app. t, 0.6 H, *Z*-H-3ax), 1.65 (app. t, $J_{3ax,4}$ ~12.2 Hz, 0.4 H, *E*-H-3ax); selected data in DMSO-d_6 (300 MHz, NH_4^+ salt): δ 10.84 (app. t, $J_{NH,CHS}$ ~ $J_{NH,5}$ ~7.5 Hz, 0.7 H, *Z*-NH), 10.05 (dd, $J_{NH,CHS}$ 14.3, $J_{NH,5}$ 8.2 Hz, 0.3 H, *E*-NH), 9.32 (d, 0.7 H, *Z*-HC(S)), 8.92 (d, 0.3 H, *E*-HC(S)), 2.68 (dd, $J_{3eq,4}$ ~4.8, $J_{3ax,3eq}$ ~11.8 Hz, 1H, *E,Z*-H-3eq), 1.31 (app. t, 0.7 H, *Z*-H-3ax), 1.23 (app. t, 0.3 H, *E*-H-3ax). ^{13}C NMR (NH_4^+ salt): δ 194.5 (*E*-C=S), 192.6 (*Z*-C=S), 174.2 (*E,Z*-C-1), 101.7 (*E,Z*-C-2), 72.7, 72.3, 69.0, 68.3 (*E*-C-4, 6, 7, 8), 72.6, 72.4, 69.3, 69.0 (*Z*-C-4, 6, 7, 8), 63.9 (*E*-C-5), 63.5 (*Z*-C-9), 63.4 (*E*-C-9), 55.8 (*Z*-C-5), 52.5 (*E,Z*-OCH_3), 40.8 (*Z*-C-3), 40.7 (*E*-C-3). Negative FAB MS: m/z 671 [3.3%, $(2M-2H+Na)^-$], 649 [1.6%, $(2M-H)^-$], 416 [8.9%, $(M-H+glycerol)^-$], 346 [11.8%, $(M+Na-2H)^-$], 324 [100%, $(M-H)^-$]. Anal. Calc. for $C_{11}H_{19}NO_8S$·0.75 H_2O (325.34+13.51): C 38.99, H 6.10, N 4.13, S 9.46. Found: C 39.11, H 6.15, N 4.28, S 9.71. - For storage, the free acid was transformed into the ammonium salt by addition of a slight excess of diluted aqueous ammonia to its aqueous solution and subsequent lyophilization.

Triethylammonium (methyl 3,5-dideoxy-5-thioacetamido-D-glycero-α-*D*-galacto-*2-nonulopyranosid)onate* (*Neu5ThAc-2αMe*, **6b**). A suspension of **5** (100 mg, 0.36 mmol) in water (0.2 ml) was diluted with methanol (2.5 ml). After cooling to 0°C, triethylamine (0.40 ml, 2.9 mmol) and reagent **2** (0.31 ml, 2.9 mmol) were added. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. TLC (solvent A) then indicated complete conversion into **6b**. Volatile material was removed under reduced pressure and the residue was taken up in solvent A (~3 ml). After filtration, addition of ethyl acetate followed by diethyl ether caused crystallization. Recrystallization from methanol - ethyl acetate - diethyl ether afforded **6b** as colourless crystals, m.p. 169°C. Yield 136 mg (86%); R_F 0.41 (A), 0.54 (B); $[\alpha]_D^{20}$ +51.5°; λ_{max} 265.0 nm (ε_M 11070); ^1H NMR (500 MHz): δ 4.61 (app. t, $J_{4,5}$ ~ $J_{5,6}$ ~10.1 Hz, 1 H, H-5), 3.95-3.86 (m, 4 H, H-4, 6, 8, 9), 3.65 (dd, $J_{8,9'}$ 6.2, $J_{9,9'}$ 11.9 Hz, 1 H, H-9'), 3.54 (dd, $J_{6,7}$ 1.6, $J_{7,8}$ 8.9 Hz, 1 H, H-7), 3.38 (s, 3 H, OCH_3), 3.22 (q, J 7.3 Hz, 6 H, 3 $NC\underline{H}_2CH_3$), 2.76 (dd, $J_{3eq,4}$ 4.7, $J_{3eq,3ax}$ 12.5 Hz, H-3eq), 2.58 (s, 3 H, $C(S)CH_3$), 1.71 (app. t, $J_{3ax,4}$ ~12.2 Hz, 1 H, H-3ax), 1.30 (t, 9 H, 3 $NCH_2C\underline{H}_3$); selected data in DMSO-d_6 (300 MHz): δ 10.39 (d, $J_{NH,5}$ 8.1 Hz, 1 H, NH), 2.63 (dd, $J_{3eq,4}$ 4.7, $J_{3eq,3ax}$ 11.7 Hz, 1 H, H-3eq), 2.44 (s, 3 H, $C(S)CH_3$), 1.29 (app. t, 1 H, H-3ax); ^{13}C NMR (NH_4^+ salt): δ 205.4 (C=S), 174.1 (C-1), 101.6 (C-2), 72.8, 72.4, 69.28, 69.26 (C-4, 6, 7, 8), 63.6 (C-9), 59.1 (C-5), 52.5 (OCH_3), 40.9 (C-3), 33.7 ($C(S)\underline{C}H_3$). Anal. Calc. for $C_{18}H_{36}N_2O_8S$ (440.56): C 49.07, H 8.24, N 6.36, S 7.28. Found: C 49.28, H 8.52, N 6.15, S 7.02.

Triethylammonium (methyl 3,5-dideoxy-5-thiopropionamido-D-glycero-α-*D*-galacto-*2-nonulopyranosid)onate (Neu5ThPr-2αMe*, **6c**). Reaction of **5** to **6c** was carried out as described for the preparation of **6b**, using reagent **3** (0.35 ml, 2.9 mmol) instead of **2**. Compound **6c** had m.p. 158°C. Yield 151 mg (92%); R_F 0.44 (A), 0.58 (B); $[\alpha]_D^{20}$ +54.2°; λ_{max} 264.5 nm (ε_M 11210); ^{1}H NMR (300 MHz): δ 4.62 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.1 Hz, 1 H, H-5), 3.99-3.81 (m, 4 H, H-4, 6, 8, 9), 3.65 (dd, $J_{8,9'}$ 6.3, $J_{9,9'}$ 12.0 Hz, 1 H, H-9'), 3.52 (dd, $J_{6,7}$ 1.3, $J_{7,8}$ 9.0 Hz, 1 H, H-7), 3.38 (s, 3 H, OCH_3), 3.22 (q, J 7.3 Hz, 6 H, 3 NCH_2CH_3), 2.77 (dd, $J_{3eq,4}$ 4.6, $J_{3eq,3ax}$ 12.4 Hz, 1 H, H-3eq), 2.74 (app. q, J 7.6 Hz, 2 H, $C(S)CH_2$), 1.72 (app. t, $J_{3ax,4}$ ~12.2 Hz, 1 H, H-3ax), 1.31 (t, 9 H, 3 NCH_2CH_3), 1.28 (t, 3 H, $C(S)CH_2CH_3$); ^{13}C NMR (NH_4^+ salt): δ 211.6 (C=S), 174.1 (C-1), 101.6 (C-2), 72.9, 72.5, 69.3, 69.2 (C-4, 6, 7, 8), 63.7 (C-9), 58.9 (C-5), 52.5 (OCH_3), 41.1, 40.4 (C-3, $C(S)CH_2$), 14.6 (CH_2CH_3). Anal. Calc. for $C_{19}H_{38}N_2O_8S$ (454.58): C 50.20, H 8.43, N 6.16, S 7.05. Found C 50.25, H 8.50, N 6.17, S 7.33.

Ammonium 3,5-dideoxy-5-thioformamido-D-glycero-*D*-galacto-*2-nonulopyranosonate (Neu5ThFo*, **8a**). A solution of **6a** (50 mg, 148 mmol) in aqueous 0.033 M HCl (2.5 ml) was heated at 70°C under nitrogen for 3 hours. The solution was evaporated under reduced pressure to a small volume and then coevaporated with water. The remaining residue was taken up in water and passed through a column (10 x 1 cm) filled with DEAE-Sephadex A-25 (HCO_3^-). After rinsing with water, **8a** was eluted with 0.006 M NH_4HCO_3 solution. Fractions containing the product were collected and lyophilized three times to remove NH_4HCO_3. **8a** remained as a white powder. Yield 36 mg (70%); R_F 0.45 (*E*-**8a**) and 0.37 (*Z*-**8a**), (B); R_F 0.39 (*E*-**8a**) and 0.32 (*Z*-**8a**), (D), *cf.* R_F 0.23 for Neu5Fo (no separation of the rotamers could be observed) and 0.24 for Neu5Ac (D)[19]; $[\alpha]_D^{20}$ -31.2° (measured after 48 hours, *cf.* **6a**); λ_{max} 266.5 nm (ε_M 13860); ^{1}H NMR (500 MHz, measured after 48 h, *cf.* **6a**): β-anomer (~92%) δ 9.514 (s, 0.6 H, *Z*-HC(S)), 9.230 (s, 0.4 H, *E*-HC(S)), 4.806 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.2 Hz, 0.6 H, *Z*-H-5), 4.210 (ddd, $J_{3eq,4}$ 5.1, $J_{3ax,4}$ 11.7 Hz, 0.6 H, *Z*-H-4), 4.188 (dd, $J_{5,6}$ 10.4, $J_{6,7}$ 1.1 Hz, 0.4 H, *E*-H-6), 4.149 (ddd, $J_{4,5}$ 10.0, $J_{3eq,4}$ 5.0, $J_{3ax,4}$ 11.6 Hz, 0.4 H, *E*-H-4), 4.125 (d, 0.6 H, *Z*-H-6), 3.854 (dd, $J_{8,9}$ 2.8, $J_{9,9'}$ 11.7 Hz, 0.4 H, *E*-H-9), 3.832 (dd, $J_{8,9}$ 2.7, $J_{9,9'}$ 11.7 Hz, 0.6 H, *Z*-H-9), 3.81-3.75 (m, 1 H, *Z*,*E*-H-8), 3.725 (app. t, 0.4 H, *E*-H-5), 3.623 (dd, $J_{8,9'}$ 6.5 Hz, 0.4 H, *E*-H-9'), 3.603 (dd, $J_{8,9'}$ 6.3 Hz, 0.6 H, *Z*-H-9'), 3.553 (dd, $J_{7,8}$ 9.4 Hz, 0.4 H, *E*-H-7), 3.467 (d, $J_{7,8}$ 8.9 Hz, 0.6 H, *Z*-H-7), 2.263 (dd, $J_{3ax,3eq}$ 13.0 Hz, 0.4 H, *E*-H-3eq), 2.249 (dd, $J_{3ax,3eq}$ 12.8 Hz, 0.6 H, *Z*-H-3eq), 1.895 (dd, 0.6 H, *Z*-H-3ax), 1.823 (dd, 0.4 H, *E*-H-3ax); α-anomer (~8%) δ 9.49 (s, 0.65 H, *Z*-HC(S)), 9.21 (s, 0.35 H, *E*-HC(S)), 2.751 (dd, $J_{3eq,4}$ 4.8, $J_{3ax,3eq}$ 12.6 Hz, 1 H, *Z*,*E*-H-3eq), 1.691 (app. t, $J_{3ax,4} \sim J_{3ax,3eq}$ ~12.2 Hz, 0.65 H, *Z*-H-3ax), 1.621 (app. t, $J_{3ax,4} \sim J_{3ax,3eq}$ ~12.2 Hz, 0.35 H, *E*-H-3ax). ^{13}C NMR: β-anomers δ 194.3 (*E*-C=S), 192.5 (*Z*-C=S), 177.1 (*Z*,*E*-C-1), 97.2 (*Z*,*E*-C-2), 71.3, 70.2, 69.5, 67.3 (*E*-C-4, 6, 7, 8), 71.2, 70.4, 69.5, 68.3 (*Z*-C-4, 6, 7, 8), 64.6 (*E*-C-5), 64.2 (*Z*,*E*-C-9), 56.3 (*Z*-C-5), 40.2 (*Z*-C-3), 40.1 (*E*-C-3). Negative FAB MS: m/z 621 [3.6%, $(2M-H)^-$], 402 [6.2%, (M-H+glycerol)$^-$], 310 [100%, $(M-H)^-$]. Anal. Calc. for $C_{10}H_{20}N_2O_8S \cdot H_2O$ (328.34+18.02): C 34.68, H 6.40, N 8.09, S 9.26. Found: C 34.72, H 6.38, N 7.85, S 8.85.

Ammonium 3,5-dideoxy-5-thioacetamido-D-glycero-*D*-galacto-*2-nonulopyranosonate (Neu5ThAc*, **8b**). - *From* **6b**. Compound **6b** (50 mg, 0.11 mmol) was dissolved in water (0.5 ml, containing 0.01% sodium azide), which has been brought to pH 5.0 by addition of dry ice. (Results obtained in this way did not differ from those obtained when sodium acetate buffer was used, which is the conventional medium for sialidase reactions.) After addition of *Arthrobacter ureafaciens* sialidase (0.2 U) and incubation of the solution for 10

hours at 37°C, a further amount of sialidase (0.1 U) was added and the incubation was continued for 14 hours. TLC indicated complete conversion of **6b** into **8b**. The mixture was transferred onto a column of DEAE-Sephadex A-25 (HCO_3^-), washed with water, and eluted with 0.006 M NH_4HCO_3 solution. The fractions containing **8b** were collected and freed from NH_4HCO_3 by three times repeated lyophilization. **8b** remained as a white powder. Yield 34 mg (83%, for **8b**·H_2O); R_F 0.41 (B); $[\alpha]_D^{20}$ +4.6°; λ_{max} 265.0 nm (ε_M 11100); ^{1}H NMR (300 MHz): β-anomer (~92%) δ 4.62 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.4 Hz, 1 H, H-5), 4.12 (ddd, $J_{3ax,4}$ 11.8, $J_{3eq,4}$ 5.0 Hz, 1H, H-4), 4.02 (d, 1 H, H-6), 3.75 (dd, $J_{8,9}$ 2.6, $J_{9,9'}$ 11.7 Hz, 1 H, H-9), 3.70 (ddd, $J_{7,8}$ 9.0, $J_{8,9'}$ 6.2 Hz, 1 H, H-8), 3.51 (dd, 1 H, H-9'), 3.36 (d, 1 H, H-7), 2.49 (s, 3 H, C(S)CH_3), 2.19 (dd, $J_{3ax,3eq}$ 12.8 Hz, 1 H, H-3eq), 1.80 (dd, 1 H, H-3ax); α-anomer (~8%) δ 4.52 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.2 Hz, 1 H, H-5), 2.68 (dd, $J_{3ax,3eq}$ 13.5, $J_{3eq,4}$ 5.4 Hz, 1 H, H-3eq), 2.47 (s, 3 H, C(S)CH_3), 1.60 (dd, $J_{3ax,4}$ 12.0 Hz, 1 H, H-3ax). ^{13}C NMR: β-anomer δ 205.2 (C=S), 176.9 (C-1), 97.2 (C-2), 71.1, 70.5, 69.5, 68.3 (C-4, 6, 7, 8), 64.2 (C-9), 59.5 (C-5), 40.2 (C-3), 33.7 (C(S)$\underline{C}H_3$). Negative FAB MS: m/z 649 [12.5%, $(2M-H)^-$], 324 [100%, $(M-H)^-$]. Anal. Calc. for $C_{11}H_{22}N_2O_8S \cdot H_2O$ (342.37+18.02): C 36.66, H 6.71, N 7.77, S 8.90. Found: C 36.87, H 6.63, N 7.36, S 8.90.

- *From* **7b**. A solution of *N*-thioacetyl-D-mannosamine[3] (**7b**; 40 mg, 0.17 mmol), sodium pyruvate (187 mg, 1.7 mmol) and *N*-acetylneuraminate pyruvate lyase (Neu5Ac aldolase, E.C. 1.4.3.3; 1.4 U) in 0.05 M phosphate buffer (pH 6.7; 2.0 ml, containing 0.01% sodium azide) was incubated at 37°C. The progress of the reaction was monitored by TLC (9:2:1 acetone - methanol - water, $R_{F,7b}$ 0.87, $R_{F,8b}$ 0.50; $R_{F,7b}$ 0.83, $R_{F,8b}$ 0.41 [B]). After 9 hours, another 1.4 U of the enzyme were added and the incubation was continued for 15 hours. (Ratio **8b/7b** ~2:1, by ^{1}H NMR; further incubation caused appearance of two additional spots [R_F 0.59 and R_F 0.13, solvent B] due to ManNAc and Neu5Ac.) For purification, the whole solution was loaded onto a column filled with DEAE-Sephadex A-25 (HCO_3^-), rinsed thoroughly with water followed by elution of the product with 0.006 M NH_4HCO_3 solution. Repeated freeze-drying of the combined fractions containing the product gave amorphous **8b** (34 mg, 56%), which was identical according to TLC and ^{1}H NMR spectroscopy with the substance obtained from **6b**.

Ammonium 3,5-dideoxy-5-thiopropionamido-D-glycero-*D*-galacto-*2-nonulopyranosonate* (*Neu5ThPr*, **8c**). Compound **6c** (50 mg, 0.11 mmol) was converted into **8c** as described for the synthesis of **8b** from **6b**. Yield 30 mg (74%, for **8c**·0.5 H_2O); R_F 0.44 (B); $[\alpha]_D^{20}$ +7.4°; λ_{max} 264.5 nm (ε_M 11435); ^{1}H NMR (300 MHz): β-anomer (~93%) δ 4.64 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.5 Hz, 1 H, H-5), 4.14 (ddd, $J_{3eq,4}$ 4.9, $J_{3ax,4}$ 12.3 Hz, 1 H, H-4), 4.04 (d, 1 H, H-6), 3.76 (dd, $J_{8,9}$ 2.2, $J_{9,9'}$ 11.5 Hz, 1 H, H-9), 3.73 (ddd, $J_{8,9'}$ 6.0, $J_{7,8}$ 9.0 Hz, 1 H, H-8), 3.53 (dd, 1 H, H-9'), 3.35 (d, 1 H, H-7), 2.66 (app. q, J 7.5 Hz, 2 H, C(S)CH_2), 2.20 (dd, $J_{3ax,3eq}$ 12.9 Hz, 1 H, H-3eq), 1.80 (dd, 1 H, H-3ax), 1.20 (t, 3 H, $CH_2C\underline{H}_3$); α-anomer (~7%) δ 4.55 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.4 Hz, 1 H, H-5), 1.63 (app. t, $J_{3ax,4} \sim J_{3ax,3eq}$ ~12.3 Hz, H-3ax), characterization of proton H-3eq was not possible due to coincidence with the signal for $C\underline{H}_2$C(S). ^{13}C NMR: β-anomer δ 211.5 (C=S), 176.8 (C-1), 97.1 (C-2), 71.1, 70.5, 69.4, 68.1 (C-4, 6, 7, 8), 64.2 (C-9), 59.1 (C-5), 40.5 (C(S)$\underline{C}H_2$), 40.2 (C-3), 14.6 ($CH_2\underline{C}H_3$). Negative FAB MS: m/z: 677 [14.1%, $(2M-H)^-$], 338 [100%, $(M-H)^-$]. Anal. Calc. for $C_{12}H_{24}N_2O_8S \cdot 0.5\ H_2O$ (356.39+9.01): C 39.44, H 6.90, N 7.67, S 8.78. Found: C 39.15, H 6.87, N 7.51, S 8.72.

Triethylammonium (methyl 3,5-dideoxy-2-thio-5-thioacetamido-D-glycero-α-*D*-galacto-*2-nonulo-pyranosid)onate* (*Neu5ThAc-2αSMe*, **10**). Compound **9**[22] (100 mg, 0.34 mmol) was converted into **10** as

described for the synthesis of **6b** from **5**. For **10**, m.p. 184°C. Yield 132 mg (85%); R_F 0.45 (A), 0.59 (B); $[\alpha]_D^{20}$ +89.2°; λ_{max} 265.0 nm (ε_M 11450); ^{1}H NMR (300 MHz): δ 4.61 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.2 Hz, 1 H, H-5), 3.92-3.84 (m, 3 H, H-4, 8, 9), 3.76 (dd, $J_{6,7}$ 1.4 Hz, 1 H, H-6), 3.63 (dd, $J_{8,9}$ 6.4, $J_{9,9'}$ 12.1 Hz, 1 H, H-9'), 3.53 (dd, $J_{7,8}$ 9.0 Hz, 1 H, H-7), 3.21 (q, J 7.3 Hz, 6 H, 3 NCH_2CH_3), 2.83 (dd, $J_{3eq,4}$ 4.8, $J_{3ax,3eq}$ 12.7 Hz, 1 H, H-3eq), 2.56 (s, 3 H, $C(S)CH_3$), 2.16 (s, 3 H, SCH_3), 1.82 (app. t, $J_{3ax,4}$ ~12.1 Hz, 1 H, H-3ax), 1.29 (t, 9 H, 3 NCH_2CH_3). Anal. Calc. for $C_{18}H_{36}N_2O_7S_2$ (456.62): C 47.35, H 7.95, N 6.14, S 14.04. Found: C 47.70, H 8.20, N 6.29, S 14.23.

*Methyl (methyl 5-acetamido-3,5-dideoxy-9-*O*-p-toluenesulfonyl-D-*glycero-α-*D*-galacto-2-nonulopyranosid)onate* (**12**). To remove traces of water, compound **11** (2.0 g, 5.93 mmol) was coevaporated twice with dry pyridine, and then dissolved in the same solvent (50 ml). The solution was cooled to 0°C and *p*-toluenesulfonyl chloride (1.13 g, 5.93 mmol) was added in small portions. The reaction mixture was allowed to warm up to room temperature and kept overnight at 20°C. After evaporation of the solvent *in vacuo*, ice-water (4 ml) and ethyl acetate (40 ml) were added, and the organic phase was separated. The aqueous solution was extracted once again with ethyl acetate (40 ml), and the combined organic phases were concentrated. Addition of diethyl ether followed by *n*-hexane caused crystallization of **12**, m.p. 150°C (dec.). Yield 2.28 g (78%); R_F 0.23 (*cf.* $R_{F,11}$ 0.05, 20:1 ethyl acetate - methanol); $[\alpha]_D^{20}$ +1.0 (c 0.5, methanol); ^{1}H NMR (300 MHz, CD_3OD): δ 7.80-7.43 (m, 4 H, ar), 4.31 (dd, $J_{9,9'}$ 9.9, $J_{8,9}$ 1.8 Hz, 1 H, H-9), 4.07 (dd, $J_{8,9'}$ 6.0 Hz, 1 H, H-9'), 3.98 (ddd, $J_{7,8}$ 8.5 Hz, 1 H, H-8), 3.81 (s, 3 H, $COOCH_3$), 3.70 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.0 Hz, 1 H, H-5), 3.61 (ddd, $J_{3eq,4}$ 4.7, $J_{3ax,4}$ 11.7 Hz, 1 H, H-4), 3.53 (dd, $J_{6,7}$ 1.2 Hz, 1 H, H-6), 3.44 (dd, 1 H, H-7), 3.27 (s, 3 H, OCH_3), 2.61 (dd, $J_{3ax,3eq}$ 12.9 Hz, 1 H, H-3eq), 2.45 (s, 3 H, ar-CH_3), 1.99 (s, 3 H, $NC(O)CH_3$), 1.66 (dd, 1 H, H-3ax). Anal. Calc. for $C_{20}H_{29}NO_{11}S$ (491.51): C 48.87, H 5.95, N 2.85, S 6.52. Found: C 48.83, H 6.18, N 2.67, S 6.48.

*Methyl (methyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero-α-*D-*galacto-*2-nonulopyranosid)onate* (**13**). A mixture of **12** (1.84 g, 3.74 mmol), sodium azide (1.09 g, 16.8 mmol), water (8 ml), and acetone (24 ml) was refluxed for 40 hours. TLC showed complete conversion of **12** into **13** ($R_{F,13}$ 0.48, $R_{F,12}$ 0.58 [E]). The solvents were removed completely under reduced pressure, and the residue was taken up in solvent E (~12 ml), filtered, and concentrated. After column chromatography on silica gel (10:1 chloroform - methanol), **13** was crystallized from methanol - diethyl ether, m.p. 168-170°C. Yield 1.11 g (82%); R_F 0.22 (12:1 ethyl acetate - methanol); $[\alpha]_D^{20}$ +4.2° (c 0.5, methanol); ν_{max} (KBr) 2120 cm^{-1} (azide); ^{1}H NMR (300 MHz, CD_3OD): δ 3.99 (ddd, $J_{8,9}$ 2.7, $J_{7,8}$ 8.8, $J_{8,9'}$ 6.4 Hz, 1 H, H-8), 3.84 (s, 3 H, $COOCH_3$), 3.75 (app. t, $J_{4,5} \sim J_{5,6}$ ~10.0 Hz, 1 H, H-5), 3.64 (ddd, $J_{3eq,4}$ 4.4, $J_{3ax,4}$ 11.9 Hz, 1 H, H-4), 3.60 (dd, $J_{6,7}$ 1.4 Hz, 1 H, H-6), 3.54 (dd, $J_{9,9'}$ 12.6 Hz, 1 H, H-9), 3.47 (dd, 1 H, H-7), 3.37 (dd, 1 H, H-9'), 3.34 (s, 3 H, OCH_3), 2.64 (dd, $J_{3ax,3eq}$ 12.8 Hz, 1 H, H-3eq), 2.00 (s, 3 H, $NC(O)CH_3$), 1.70 (dd, 1 H, H-3ax). Anal. Calc. for $C_{13}H_{22}N_4O_8$ (362.34): C 43.09, H 6.12, N 15.46. Found: C 43.12, H 6.41, N 15.09.

*Methyl 5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero-α-*D-*galacto-*2-nonulopyranosidonic acid* (**14**). Compound **13** (1.11 g, 3.06 mmol) was dissolved in methanol (90 ml), and 0.1 N NaOH (90 ml) was added. After keeping at room temperature for 1 hour, the solution was passed through a column of Dowex 50W-X8 (H^+) resin at 4°C, and then lyophilized. The residue crystallized from methanol - diethyl ether - *n*-hexane, m.p. 159-161°C (dec.). Yield 0.94 g (88%); R_F 0.44 (D); $[\alpha]_D^{20}$ +14.5°; ν_{max} (KBr) 2120 cm^{-1} (azide); ^{1}H

NMR (300 MHz): δ 4.06 (ddd, $J_{8,9}$ ~2.5, $J_{7,8}$ 9.0, $J_{8,9'}$ 6.2 Hz, 1 H, H-8), 3.86 (app. t, $J_{5,6} \sim J_{4,5}$ ~10.0 Hz, 1 H, H-5), ~3.78 (dd, $J_{6,7}$ ~1.2 Hz, 1 H, H-6), 3.76-3.67 (m, 2 H, H-4, 9), 3.64 (dd, 1 H, H-7), 3.53 (dd, $J_{9,9'}$ 13.2 Hz, 1 H, H-9'), 3.38 (s, 3 H, OCH_3), 2.75 (dd, $J_{3eq,4}$ 4.6, $J_{3eq,3ax}$ 12.4 Hz, 1 H, H-3eq), 2.08 (s, 3 H, $NC(O)CH_3$), 1.67 (app. t, $J_{3ax,4}$ ~12.1 Hz, 1 H, H-3ax). Anal. Calc. for $C_{12}H_{20}N_4O_8$ (348.31): C 41.38, H 5.79, N 16.09. Found: C 41.52, H 6.10, N 15.58.

5-Acetamido-9-azido-3,5,9-trideoxy-D-glycero-*D*-galacto-*2-nonulopyranosonic acid* (**15**). To a solution of **14** (0.40 g, 1.15 mmol) in water (120 ml), Dowex 50W-X8 (H^+) resin was added, and the mixture was heated at 80°C with stirring for 1 hour. TLC (2:3:1 methanol - ethyl acetate - 20% acetic acid) showed $R_{F,15}$ 0.56 (*cf.* $R_{F,14}$ 0.73). After removal of the resin by filtration, the solution was lyophilized and subsequently purified on a column of DEAE-Sephadex A-25 (HCO_3^-) (eluent 0.08 M NH_4HCO_3). The fractions containing the product were combined, treated with Amberlite IR 120 (H^+) resin, and lyophilized after removal of the resin. Yield 0.37 g (91%, for **15**·H_2O); R_F 0.38 (6:1:2 *n*-propanol - 25% ammonia - water); $[\alpha]_D^{20}$ -16.3°; ν_{max} (KBr) 2120 cm^{-1} (azide); ^1H NMR (300 MHz): β-anomer (~92%) δ 4.15 (ddd, $J_{4,5}$ 10.0, $J_{3eq,4}$ 4.9, $J_{3ax,4}$ 11.3 Hz, 1 H, H-4), 4.12 (dd, $J_{5,6}$ 10.0, $J_{6,7}$ 1.1 Hz, 1 H, H-6), 3.99 (app. t, 1 H, H-5), 3.97 (ddd, $J_{8,9}$ 2.7, $J_{7,8}$ 8.9, $J_{8,9'}$ 5.9 Hz, 1 H, H-8), 3.69 (dd, $J_{9,9'}$ 12.7 Hz, 1 H, H-9), 3.64 (dd, 1 H, H-7), 3.55 (dd, 1 H, H-9'), 2.38 (dd, $J_{3ax,3eq}$ 13.0 Hz, 1 H, H-3eq), 2.13 (s, 3 H, $NC(O)CH_3$), 1.95 (dd, 1 H, H-3ax); α-anomer (~8%) δ 2.78 (dd, $J_{3eq,4}$ ~4.5, $J_{3ax,3eq}$ ~12.5 Hz, 1 H, H-3eq), 2.12 (s, 3 H, $NC(O)CH_3$), 1.77 (dd, $J_{3ax,4}$ ~11.0 Hz, 1 H, H-3ax). Anal. Calc. for $C_{11}H_{18}N_4O_8 \cdot H_2O$ (334.29+18.02): C 37.50, H 5.72, N 15.90. Found: C 37.35, H 5.49, N 15.67.

Methyl 5-acetamido-9-amino-3,5,9-trideoxy-D-glycero-α-*D*-galacto-*2-nonulopyranosidonic acid* (**16**). A solution of **14** (0.40 g, 1.15 mmol) in water (20 ml) was hydrogenated in the presence of PdO (50 mg) at room temperature for 2 hours, then filtered, and lyophilized. Yield 0.41 g (~quant., for **16**·$2H_2O$); R_F 0.31 (D); $[\alpha]_D^{20}$ +2.8°; ^1H NMR (360 MHz): δ 4.12 (app. dt, $J_{7,8} \sim J_{8,9'}$ ~8.9, $J_{8,9}$ 2.9 Hz, 1 H, H-8), 3.87-3.72 (m, 3 H, H-4, 5, 6), 3.61 (broadened d, 1 H, H-7), 3.48 (dd, $J_{9,9'}$ 13.2 Hz, 1 H, H-9), 3.39 (s, 3 H, OCH_3), 3.09 (dd, 1 H, H-9'), 2.78 (dd, $J_{3eq,4}$ 4.5, $J_{3eq,3ax}$ 12.5 Hz, 1 H, H-3eq), 2.10 (s, 3 H, $NC(O)CH_3$), 1.71 (app. t, $J_{3ax,4}$ ~11.8 Hz, 1 H, H-3ax). Anal. Calc. for $C_{12}H_{22}N_2O_8 \cdot 2H_2O$ (322.31+36.03): C 40.22, H 7.31, N 7.82. Found: C 40.32, H 7.67, N 8.02.

5-Acetamido-9-amino-3,5,9-trideoxy-D-glycero-*D*-galacto-*2-nonulopyranosonic acid* (**17**). A solution of **15**·H_2O (0.37 g, 1.05 mmol) in water (30 ml) was brought to pH ~2 by addition of acetic acid and shaken with PdO (50 mg) under a hydrogen atmosphere for 1 hour at ambient temperature. After removal of the catalyst by filtration, the solution was lyophilized. Crystallization from water - acetone afforded 33 mg (91%, for **17**·$2H_2O$), m.p. ~190°C (dec.). R_F 0.24 (5:3 n-propanol - water); $[\alpha]_D^{20}$ -24.1°; ^1H NMR (300 MHz): β-anomer (~92%) δ 4.10-3.90 (m, 4 H, H-4, 5, 6, 8), 3.54 (broadened d, $J_{7,8}$ 8.9 Hz, 1 H, H-7), 3.41 (dd, $J_{8,9}$ 3.1, $J_{9,9'}$ 13.2 Hz, 1 H, H-9), 2.98 (dd, $J_{8,9'}$ 9.7 Hz, 1 H, H-9'), 2.25 (dd, $J_{3eq,4}$ 4.8, $J_{3ax,3eq}$ 12.8 Hz, 1 H, H-3eq), 2.08 (s, 3 H, $NC(O)CH_3$), 1.86 (dd, $J_{3ax,4}$ 11.2 Hz, 1 H, H-3ax); α-anomer (~8%) d 2.77 (dd, $J_{3eq,4}$ ~4.4, $J_{3ax,3eq}$ ~12.5 Hz, 1 H, H-3eq), 2.06 (s, 3 H, $NC(O)CH_3$), 1.66 (dd, $J_{3ax,3eq}$ ~11.5 Hz, 1 H, H-3ax). Anal. Calc. for $C_{11}H_{20}N_2O_8 \cdot 2H_2O$ (308.29+36.03): C 38.37, H 7.03, N 8.14. Found: C 38.32, H 6.72, N 7.99.

*Ammonium (methyl 5-acetamido-3,5,9-trideoxy-9-thioacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9-NThAc-Neu5Ac-2αMe,* **18**). Compound **16** (100 mg, 0.31 mmol) was suspended in aqueous methanol (2.0 ml, 14:1 methanol - water). At 0°C, triethylamine (0.25 ml, 1.8 mmol) was added followed by **2** (0.25 ml, 2.3 mmol). The mixture was allowed to warm up to room temperatur and after 14 hours, TLC showed complete conversion into product **18**. Removal of volatile material *in vacuo* and subsequent column chromatography on silica gel (6:5 ethyl acetate - methanol) gave the desired compound, which then was dissolved in water and passed through a column of Amberlite IR 120 (H^+) resin. During the last procedure the temperature was maintained below 4°C. The ammonium salt of **18** was obtained by neutralization to pH ~7 with dilute aqueous ammonia and subsequent lyophilization. Yield 103 mg (76%, for **18**·$2H_2O$); R_F 0.37 (A), R_F 0.37 (C); $[\alpha]_D^{20}$ -11.9° ; λ_{max} 259.5 nm (ε_M 10640); ^{1}H NMR (500 MHz): δ 4.14 (ddd, $J_{8,9}$ 3.0, $J_{8,9'}$ 8.0, $J_{7,8}$ 8.8 Hz, 1 H, H-8), 4.02 (dd, $J_{9,9'}$ 14.1 Hz, 1 H, H-9), 3.84 (app. t, $J_{5,6}$ ~ $J_{4,5}$ ~10.1 Hz, 1 H, H-5), 3.78 (dd, 1 H, H-9'), 3.76 (dd, $J_{6,7}$ 1.8 Hz, 1 H, H-6), 3.70 (ddd, $J_{3eq,4}$ 4.7, $J_{3ax,4}$ 11.8 Hz, 1 H, H-4), 3.58 (dd, 1 H, H-7), 3.36 (s, 3 H, OCH_3), 2.73 (dd, $J_{3ax,3eq}$ 12.5 Hz, 1 H, H-3eq), 2.57 (s, 3 H, $C(S)CH_3$), 2.06 (s, 3 H, $NC(O)CH_3$), 1.66 (app. t, 1 H, H-3ax); ^{13}C NMR: δ 202.9 (C=S), 176.0 ($NC(O)CH_3$), 174.3 (C-1), 101.6 (C-2), 73.4, 70.8, 70.2, 69.1, (C-4, 6, 7, 8), 52.9 (C-5), 52.5 (OCH_3), 50.3 (C-9), 41.0 (C-3), 33.3 ($C(S)\underline{C}H_3$), 23.0 ($NC(O)\underline{C}H_3$). Anal. Calc. for $C_{14}H_{27}N_3O_8S\cdot 2H_2O$ (397.45+36.03): C 38.79, H 7.21, N 9.69, S 7.40. Found: C 38.47, H 7.22, N 10.04, S 7.40.

*Ammonium 5-acetamido-3,5,9-trideoxy-9-thioacetamido-D-*glycero*-D-*galacto*-2-nonulopyranosonate (9-NThAc-Neu5Ac,* **19**). - *From* **18**. The enzymatic cleavage of glycoside **18** (50 mg, 0.115 mmol) to **19** was performed with *Arthrobacter ureafaciens* sialidase (0.3 U in all) as described for the synthesis of **8b** from **6b**. After purification by anion exchange chromatography (see **8b**) and removal of NH_4HCO_3 by repeated lyophilization, **19** remained as a white powder. Yield 36 mg (76%, for **19**·1.5 H_2O); R_F 0.29 (C); $[\alpha]_D^{20}$ -23.3°; λ_{max} 260.5 nm (ε_M 11540); ^{1}H NMR (360 MHz): β-anomer (~93%) δ 4.08-3.96 (m, 4 H, H-4, 6, 8, 9), 3.93 (app. t, $J_{4,5}$ ~ $J_{5,6}$ ~10.1 Hz, 1 H, H-5), 3.73 (dd, $J_{8,9'}$ 7.7, $J_{9,9'}$ 13.8 Hz, 1 H, H-9'), 3.52 (dd, $J_{6,7}$ 1.0, $J_{7,8}$ 8.9 Hz, 1 H, H-7), 2.545 (s, 3 H, $C(S)CH_3$), 2.24 (dd, $J_{3eq,4}$ 5.0, $J_{3ax,3eq}$ 13.0 Hz, 1 H, H-3eq), 2.064 (s, 3 H, $NC(O)CH_3$), 1.86 (dd, $J_{3ax,4}$ 11.5 Hz, 1 H, H-3ax); α-anomer (~7%) δ 2.74 (dd, $J_{3eq,4}$ 4.9, $J_{3ax,3eq}$ 12.6 Hz, 1 H, H-3eq), 2.554 (s, 3 H, $C(S)CH_3$), 2.045 (s, 3 H, $NC(O)CH_3$), 1.73 (app. t, $J_{3ax,4}$ ~12.2 Hz, 1 H, H-3ax); ^{13}C NMR (free acid): β-anomer δ 203.0 (C=S), 175.8 ($NC(O)CH_3$), 174.3 (C-1), 96.3 (C-2), 71.3, 70.7, 69.0, 67.6 (C-4, 6, 7, 8), 53.1 (C-5), 50.6 (C-9), 39.8 (C-3), 33.4 ($C(S)\underline{C}H_3$), 23.0 ($NC(O)\underline{C}H_3$). Negative FAB MS: m/z 731 [7.6%, $(2M-H)^-$], 365 [100%, $(M-H)^-$]. Anal. Calc. for $C_{13}H_{25}N_3O_8S\cdot 1.5\ H_2O$ (383.42+27.02): C 38.04, H 6.88, N 10.24, S 7.81. Found: C 38.40, H 6.66, N 9.73, S 7.54.

- *From* **17**. The reaction of **17** (80 mg, 0.26 mmol) to **19** was carried out in the same way as described for the synthesis of **18** from **16**. For purification, the reaction mixture was concentrated *in vacuo* and then dissolved in a small volume of methanol (~1 ml). Addition of ethyl acetate (~3 ml) caused precipitation of crude **19** which was separated by filtration. Further purification was accomplished by anion exchange chromatography on DEAE-Sephadex A-25 (HCO_3^-). After rinsing with water, **19** was eluted with 0.006 M NH_4HCO_3 solution. The fractions containing the product were pooled and lyophilized three times. The analytical data of the remaining compound **19** (76 mg, 71%) were identical with those of **19** obtained from **17**.

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