Approaches to intramolecular sialylation
2.* Synthesis of per-*O*-acetylated thioglycosides of *N*-acetylneuraminic acid containing an unprotected carboxy group

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Various approaches to the synthesis of per-*O*-acetylated thioglycosides of *N*-acetylneuraminic acid (Neu5Ac) containing an unprotected carboxy group starting from the corresponding methyl esters were comparatively studied. One-step demethylation of methyl thioglycoside (LiI, Py, reflux) proceeded inefficiently in contrast to the analogous smooth reaction of phenyl thioglycoside. An indirect route to derivatives with a free carboxy group involving saponification followed by acetylation of hydroxy groups proved to be more efficient for methyl α-thioglycoside of Neu5Ac.

Key words: sialic acids, *N*-acetylneuraminic acid, thioglycosides, monosaccharides, NMR spectroscopy.

Oligosaccharides containing residues of sialic acids, in particular, of *N*-acetylneuraminic acid (Neu5Ac), are responsible for various immunological, neurobiological, oncological, and other biological processes,[2] and therefore the development of new efficient procedures for the synthesis of such compounds is an important problem of the synthetic carbohydrate chemistry. One of the possible approaches to the synthesis of Neu5Ac glycosides involves intramolecular glycosylation, which has been used for the preparation of glycosides of other sugars (see the review[3]). Since intramolecular glycosylation proceeds as a monomolecular reaction, the target formation of the glycosidic bond is expected to more efficiently compete with the side reaction giving rise to Neu5Ac-derived glycal.

Of all available methods, the use of the carboxy group of Neu5Ac seems to be the simplest way of linking a glycosyl donor (Neu5Ac derivative) to a glycosyl acceptor by a temporary bond. Earlier,[1] we have used peracetylated Neu5Ac derivative (**1**) (Scheme 1) bearing an unprotected carboxy group for this purpose. At the same time, our experiments have provided evidence that it is apparently more advantageous to perform intramolecular sialylation using Neu5Ac derivatives in which the anomeric position can be activated under much milder conditions than those required for activation of acetate **1**. As an example, we refer to thioglycoside derivatives of Neu5Ac. In the present study, we report the synthesis of acetylated methyl and phenyl thioglycosides of Neu5Ac (**5** and **7**, respectively) containing an unprotected carboxy group. The corresponding Neu5Ac derivatives bearing a methyl ester-protected carboxy group (esters **3** [4] and **6** [5]) are well known and find wide use[6] in the synthesis of Neu5Ac glycosides.

A comparative study of different procedures for the preparation of target compounds from methyl esters of per-*O*-acetylated thioglycosides of Neu5Ac (**3** and **6**) demonstrated that one-step demethylation (LiI, Py, reflux)[7] proceeded smoothly only in the case of phenyl thioglycoside **6** to give (after chromatographic purification) the corresponding acid **7** in high yield (92%). An analogous reaction of methyl thioglycoside **3** proceeded less efficiently and afforded (after chromatographic purification) the corresponding acid **5** in only 55% yield. The structures of demethylation products **5** and **7** were confirmed by their ^{1}H and ^{13}C NMR spectra, which are, on the whole, similar to the spectra of the starting methyl esters **3** and **6**. It should be noted that the signals of the C(3)H_2 group in the ^{1}H NMR spectrum of the α isomer of phenyl thioglycoside **7** are observed at unusually low

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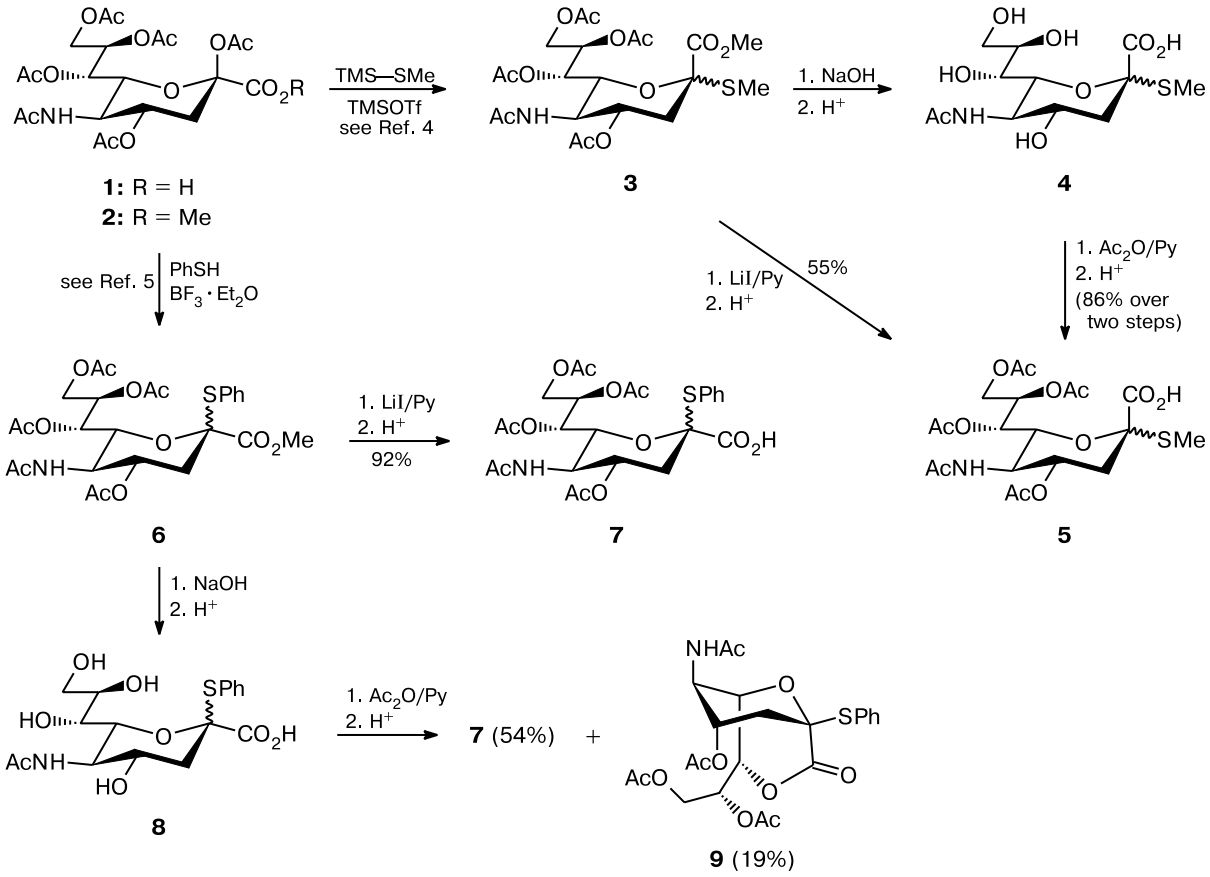
Scheme 1



field (δ_H 2.55 and 3.12), the signal for H(5) is observed at high field (δ_H 3.31), and the spin-spin coupling constants between H(3), H(4), and H(5) are changed substantially. These facts suggest a distortion of the pyranose ring in molecule **7** due apparently to an intramolecular hydrogen bond between the acetamido group C(5)NHAc and the carboxy group.

An indirect route (**3** → **4** → **5**) to derivatives with the unprotected carboxy group involving saponification (NaOH) followed by acetylation (Ac_2O/Py) of hydroxy groups proved to be more efficient for methyl α-thioglycoside of Neu5Ac (acetylated acid **5** was prepared in 86% yield starting from methyl ester **3**). Acetylation of the completely deprotected phenyl thioglycoside **8** afforded the target acid **7** in 54% yield along with the previously unknown 1,7-lactone of phenyl thioglycoside of Neu5Ac **9** (19%). It is known that acetylation of unprotected *N*-acetylneuraminic acid existing in the β configuration gives rise to peracetylated Neu5Ac 1,7-lactone containing the acetoxy group in the anomeric position.[8] The NMR spectra of lactone **9** are similar to those of lactone of peracetylated Neu5Ac, which suggests the structural similarity of these compounds. The structure of compound **9** was additionally confirmed by the NOE experiments. Thus, the NOESY spectrum of lactone **9** has (in addition to trivial correlations) the $H(3)_{ax}$—HNAc, H(4)—H(7), H(5)—H(8), and H(6)—H(8) cross-peaks, which cannot occur in the acid and isomeric lactones.

Earlier, analogous facts have been described[9] for acetylation of the corresponding ethyl thioglycosides. However, the experimental details (including NMR spectra) have not been reported in the cited study.

To summarize, we examined various approaches to per-*O*-acetylated derivatives of *N*-acetylneuraminic acid containing an unprotected carboxy group and compared their efficiency.

Experimental

All experiments were carried out in anhydrous solvents purified according to standard procedures. The reactions were performed with the use of commercial reagents (Aldrich and Fluka). Thin-layer chromatography was carried out on plates with silica gel 60 on aluminum foil (Merck). Column chromatography was performed on silica gel 60 (40—63 μm, Merck). The ^1H and ^{13}C NMR spectra were recorded on JEOL EX-270 and Bruker

AC-200 instruments relative to Me_4Si (internal standard) in $CDCl_3$. The assignment of the signals in the NMR spectra was made based on the DEPT-135 experiments and 2D correlation $^1H—^1H$ (COSY) and $^{13}C—^1H$ spectra (HETCOR, LRHETCOR). The NMR spectroscopic data are given in Tables 1—3. The optical rotation was measured on a JASCO

Table 1. ^{13}C NMR spectra (δ_C, $CDCl_3$) of the compounds synthesized

Compound	Configuration	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	C(9)	Ar	MeCN	MeCOO	C=O	SMe
1	β	168.4	97.3	36.2	68.7	49.2	72.4	67.8	71.2	62.0	—	22.95	20.77, 20.86, 20.92	170.3, 170.4, 170.8, 171.08, 171.15	—
5 (HETCOR) (acetone-d_6)	α	168.8	83.3	38.9	70.8	49.5	74.6	68.3	69.0	63.0	—	22.9	20.7, 20.9, 21.0	170.3, 170.4, 170.5, 170.6, 170.7	12.1
	β	169.3	85.3	38.0	70.4	49.8	72.5	69.3	72.0	62.9	—	22.9	20.6, 20.8, 20.9, 21.2	170.3, 170.4, 170.5, 170.6, 170.7	11.6
7	α	*	88.3	36.7	69.8	51.6	79.2	68.9	72.0	61.9	125.3, 128.8, 129.7, 129.8, 136.4	23.1	21.2, 21.5	169.7, 170.0, 170.6, 171.2, 171.5	—
	β	*	86.3	37.1	68.6	49.4	73.2	69.2	72.2	62.9	128.2, 129.0, 129.3 (2 C), 136.2	23.1	20.7, 20.84, 20.88, 21.1	170.2, 170.8, 171.03, 171.07, 171.4	—
9 (HETCOR + LRHETCOR)	β	166.5	85.0	33.9	67.3	47.9	70.4	77.2	70.7	60.8	127.6, 129.1 (2 C), 130.3, 137.0 (2 C)	23.2	20.7, 20.9	168.9, 169.4, 169.8, 170.4	—

* Undetermined.

Table 2. ^1H NMR spectra (δ_H, $CDCl_3$) of the compounds synthesized

Compound	Configuration	H(3)	H(4)	H(5)	H(6)	H(7)	H(8)	H(9)	Ar	MeCN	MeCOO	SMe
5 (COSY)	α [a]	2.16 (m); 2.78 (dd)	4.92 (m)	3.98 (m)	3.98 (m)	5.38 (m)	5.38 (m)	4.10 (dd); 4.36 (dd)	—	1.86 (s)	2.01, 2.03, 2.13, 2.16 (all s)	2.13 (s)
	α [b]	1.83 (m); 2.76 (dd)	4.96 (ddd)	4.13 (m)	4.00 (br.d)	5.35 (m)	5.35 (m)	4.08 (m); 4.27 (dd)	—	1.80 (s)	1.97, 2.09 (both s, 6 H)	2.12 (s)
	β [b]	2.05 (m); 2.55 (dd)	5.21 (ddd)	4.12 (m)	4.43 (dd)	5.47 (dd)	5.26 (m)	4.15, 4.62 (both dd)	—	1.81 (s)	2.01, 2.05, 2.06, 2.10 (all s)	2.08 (s)
7 (COSY)	α	2.55 (dd); 3.12 (d)	4.90 (d)	3.31 (dd)	4.12 (m)	5.09 (dd)		4.28, 4.51 (both dd)	7.15—7.65 (m)	1.91 (s)	1.96, 2.03, 2.05, 2.097, 2.10, 2.15 (all s)	—
	β	2.10 (m); 2.62 (dd)	5.43 (m)	4.16 (q (ddd))	4.60 (dd)	5.43 (m)	4.94 (m)	3.89 (dd); 4.58 (m)	7.15—7.65 (m)	1.91 (s)	1.96, 2.03, 2.05, 2.097, 2.10, 2.15 (all s)	—
9 (COSY)	β	2.20 (dd); 2.53 (dd)	5.04 (m)	4.17 (br.d)	4.00 (s)	4.54 (d)	4.76 (ddd)	3.56, 4.12 (both dd)	7.15—7.65 (m)	[c]	2.05 (9 H), 2.06, 2.08 (all s)	—

[a] In a 2 : 1 $CD_3OD—CDCl_3$ mixture.
[b] In acetone-d_6.
[c] Undetermined.

Table 3. Spin-spin coupling constants in the ^{1}H NMR spectra (J/Hz, $CDCl_3$) of the compounds synthesized[a]

Compound	Configuration	$J_{3,3'}$	$J_{3',4}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$	$J_{5,NH}$
5	α [b]	12.2	4.6	[c]	[c]	[c]	[c]	[c]	4.0	2.0	8.4	[c]
	α [d]	11.6	4.5	11.9	10.3	~10.7	~2	[c]	[c]	2.1	12.3	[c]
	β [d]	13.5	5.3	10.9	10.3	10.3	2.3	4.7	8.0	2.5	12.3	[c]
7	α	13.5	0	5.3	0	9.9	~1.0	7.0	5.0	2.3	12.5	7.3
	β	[c]	5.9	~11.2	10.2	10.2	2.3	[c]	8.3	[c]	12.2	10.2
9	β	15.2	1.3	3.3	~2.6	<1	0	7.9	4.6	2.3	12.5	~6.9

[a] Primed labels correspond to lower-field signals for the protons of the $C(3)H_2$ and $C(9)H_2$ groups.
[b] In a 2 : 1 CD_3OD—$CDCl_3$ mixture.
[c] Undetermined.
[d] In acetone-d_6.

DIP-370 polarimeter at ~20—30 °C. Mass spectra were recorded on JEOL JMS-HX-110 (FAB, 3-nitrobenzyl alcohol as a matrix) and Finnigan MAT LCQ mass spectrometers (atmospheric pressure chemical ionization, APCI).

(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-1-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onic acid (5). ***A.*** A mixture of methyl ester **3** (prepared from **2** according to a known procedure[4]) (96 mg, 0.18 mmol; α : β ≈ 1.5 : 1) and LiI (125 mg, 0.92 mmol) was dissolved in dry Py (3 mL) under argon. The reaction mixture was refluxed for 15 h and then concentrated after which toluene was added to, and distilled from, the residue (×2), and then THF (20 mL) was added to the residue. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in THF (3×2 mL) and purified by gel chromatography on a column (44×1.5 cm) with BioBeads SX-8 (THF as the eluent). Fractions containing the reaction product were concentrated, the residue was dissolved in MeOH (20 mL), and water (4 mL) was added. Then the mixture was treated with Amberlyst 15 (H^+) cation-exchange resin and concentrated. The residue was dissolved in CH_2Cl_2. Chromatography on silica gel (acetone—toluene—AcOH, 20 : 80 : 5, as the eluent) afforded the target acid **5** (50 mg, 55%; α : β ≈ 10 : 1, ^{1}H NMR spectroscopic data), $[\alpha]_D^{25}$ +4.2 (*c* 1.0, MeOH). MS (FAB, detection of negative ions): found, *m/z* 506.45 [M − H]. $C_{20}H_{28}NO_{12}S$. Calculated, *m/z* 506.13 [M − H].

B. A 1 *M* NaOH solution (1 mL) was added to a solution of methyl ester **3** [4] (59 mg, 0.11 mmol; α : β ≈ 1 : 1.1) in MeOH (4 mL) with cooling (ice water). The reaction mixture was kept at 22 °C for 15 h and then treated with Amberlyst 15 (H^+) cation-exchange resin to pH 2.5—3.0. The resin was filtered off and washed with water and MeOH. The combined filtrates were neutralized with Py to pH 7.0—7.5 and concentrated. Then 99.5% EtOH was added to, and distilled from, the residue (×2). Toluene was added to, and distilled from, the residue (×3). A solution of the residue in Py (1 mL) was cooled (ice water) and Ac_2O (1 mL) was added. The mixture was kept at +4 °C for 24 h and treated with Amberlyst 15 (H^+) cation-exchange resin to pH 3—4 until the Py smell disappeared. The resin was filtered off and washed with water. The filtrate was lyophilized and dried over KOH *in vacuo* until the AcOH smell disappeared. After purification on a BondElut C18 cartridge (gradient from 0.1% TFA in water to MeOH), acid **5** was obtained in a yield of 49.7 mg (86%; α : β ≈ 1 : 1.3, ^{1}H NMR spectroscopic data).

(Phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-1-thio-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onic acid (7) and (phenyl 5-acetamido-4,8,9-tri-*O*-acetyl-3,5-dideoxy-1-thio-β-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onic acid 1,7-lactone (9). ***A.*** A mixture of methyl ester **6** (prepared from **2** according to a known procedure[5]) (60 mg, 0.10 mmol; pure β-anomer) and LiI (174 mg, 1.3 mmol) was dissolved in dry Py (2 mL) under argon and refluxed for 3 h. The reaction mixture was concentrated. Toluene was added to, and distilled from, the residue (×2). Then 1 *M* H_2SO_4 (20 mL) and CH_2Cl_2 (10 mL) were added to the residue and the aqueous phase was extracted with CH_2Cl_2 (×7). The combined organic extracts were washed with brine, filtered through a cotton plug, and concentrated. Then CH_2Cl_2 was added to the residue, the mixture was filtered, and the filtrate was chromatographed on silica gel (acetone—toluene—AcOH, 10 : 90 : 5, as the eluent) to isolate the target acid **7** (54 mg, 92%), $[\alpha]_D^{20}$ −64.5 (*c* 1.0, $CHCl_3$). MS (APCI, detection of negative ions): found, *m/z* 568.7 [M − H], 569.5 [M + 1 − H]. $C_{25}H_{30}NO_{12}S$. Calculated, *m/z* 568.2 [M − H].

B. A 1 *M* NaOH solution (9 mL) was added to a solution of methyl ester **6** (104 mg, 0.18 mmol; α : β ≈ 1 : 4.6) in MeOH (9 mL) with cooling (ice water). The reaction mixture was kept at 21 °C for 24 h and then treated with Amberlyst 15 (H^+) cation-exchange resin to pH 3.5. The resin was filtered off and washed with water and MeOH. The combined filtrates were concentrated, 99.5% EtOH was added to, and distilled from, the residue (×2). Toluene was added to, and distilled from, the residue (×3). A solution of the residue in Py (2 mL) was cooled (ice water), Ac_2O (2 mL) was added, and the mixture was kept at +4 °C for 48 h. Then crushed ice was added to the reaction mixture. The reaction mixture was heated to ~20 °C (1 h) and treated with Amberlyst 15 (H^+) cation-exchange resin to pH 3.5—4 until the Py smell disappeared. The resin was filtered off and washed with water. The filtrate was lyophilized and purified by chromatography on silica gel (acetone—toluene—AcOH, 10 : 90 : 5, as the eluent) to isolate the target acid **7** (55 mg, 54%; α : β ≈ 1 : 2.7, ^{1}H NMR spectroscopic data) and lactone **9** (16 mg, 19%), $[\alpha]_D^{27}$ +15.9 (*c* 0.7, $CHCl_3$). MS (FAB, detection of negative ions): found, *m/z* 510.1466 [M + H]. $C_{23}H_{28}NO_{10}S$. Calculated, *m/z* 510.1434 [M + H].

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