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SYNTHESIS OF *N*-THIOACETYL GANGLIOSIDES GM_1 AND GM_3

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Abstract. Reaction of de-*N*-acetyl gangliosides GM_1 (**1b**) and GM_3 (**2b**) with methyl dithioacetate (**3**) afforded *N*-thioacetyl GM_1 (**1c**) and *N*-thioacetyl GM_3 (**2c**) with the oxygen of the acetamido group of *N*-acetylneuraminic acid residue replaced by sulfur.

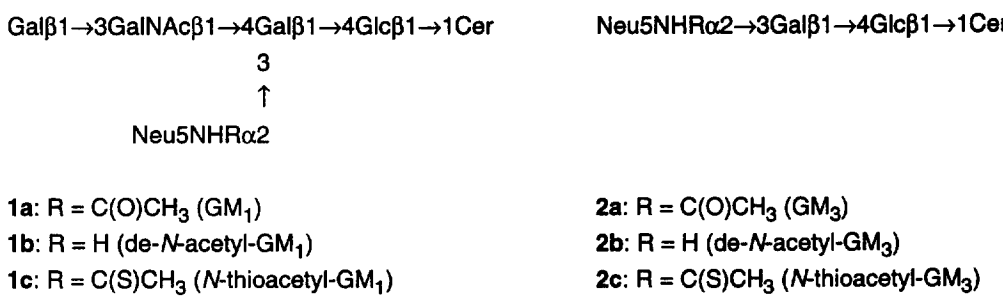
Gangliosides are sialic acid containing glycosphingolipids of the plasma membrane and are known to function as membrane receptors for viruses and bacteria and their toxins, also as antigenic determinants, and as mediators of cell interactions, to name only a few examples[1].

Some gangliosides do not contain the most abundant sialic acid, *N*-acetylneuraminic acid, but various modifications thereof. For instance, the *N*-acetyl group can be replaced by an *N*-glycoloyl group[2], and de-*N*-acetyl-GM_1 as well as de-*N*-acetyl-GM_3 with a free amino functionality have been isolated[3,4]. De-*N*-acetyl ganglioside GM_3 was found to have an opposite effect to *N*-acetyl-GM_3 on the phosphorylation of epidermal growth factor receptor in a cultured cell line[5]. *N*-Glycoloyl-GM_3 from small intestine epithelial cells binds enteropathogenic *E.coli* K99 bacteria whereas *N*-acetyl-GM_3 from the same origin is not recognized[6]. These observations reveal an important role of the 5-*N*-substituent of the sialic acid in signal transduction and recognition processes. The fact that not much is known about the exact function of gangliosides bearing modified sialic acids prompted us to synthesize two very close analogs of GM_1 and GM_3, namely *N*-thioacetyl-GM_1 and *N*-thioacetyl GM_3 with sulfur instead of oxygen in the amide side chain of neuraminic acid. The new compounds may allow to gain more insight into the importance of this sialic acid region for structure-function relationship.

Recently, we established a convenient method for the synthesis of *N*-thioacyl amino sugars[7,8]. These formerly unknown compounds with the amide oxygen replaced by sulfur are of biological interest as thio analogs of widely distributed *N*-acetyl amino sugars. Such a 'slight' change of the chemical structure was found to cause decisive alterations in some biological systems. Thus, both *N*-thioacetylglucosamine and methyl α-glycoside of *N*-thioacetylneuraminic acid abrogate binding to wheat germ agglutinin[9]. *N*-Thioacetylneuraminic acid was found to have a much higher affinity to the hemagglutinin of influenza A virus than the oxygen-containing parent compound and, moreover, copolymerized with acrylamide, it turned out to be a strong inhibitor of influenza A virus infection[10,11].

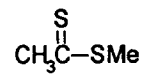
Thioacylation was accomplished by reaction of the free amino group of the unprotected sugar with methyl dithiocarboxylates and *O*-ethyl thioformate, respectively. In the case of neuraminic acid, the methyl α-glycoside was employed. In a subsequent step, the glycoside could be cleaved[8]. In all of our studies, thioacylation took place under mild conditions, without noticeable side reactions and gave, after simple work-up, the target compounds in high yields.

In the present communication we describe the synthesis of *N*-thioacetyl-GM_1 (**1c**) and *N*-thioacetyl-GM_3 (**2c**) from de-*N*-acetyl-GM_1 (**1b**) and de-*N*-acetyl-GM_3 (**2b**), respectively. **1b** and **2b** were obtained by alkaline hydrolysis of the natural gangliosides as described for GM_1[12]. Both GM_1 and GM_3 were isolated from calf brain[13,14]. Purification of de-*N*-acetyl gangliosides was achieved by silica gel chromatography[12,14]. Thioacetylation of **1b** and **2b** on a micromole scale turned out to be efficient when a large excess (~20-fold) of methyl dithioacetate (**3**) was employed[15]. Thus, after 24 h at ambient temperature, TLC revealed complete disappearance of **1b** and **2b**, and the formation of the faster-moving and UV-positive thioamides **1c** and **2c**, respectively. Silica gel chromatography afforded pure compounds **1c** (yield 68%) and **2c** (yield 62%). Densitometric HPTLC analysis showed **1c** and **2c** to contain ~1% of the parent oxygen compound **1a** and **2a**, respectively, originating from S/O-exchange during the purification procedure. As observed earlier, acetylamino sugars are more polar than their thioacetylamino analogs. Thus, using 6:1 *n*-propanol - water as the eluent, **1a** had R_F 0.22 whereas **1c** had R_F 0.37. For GM_3 derivatives, in the same solvent system, **2a** had R_F 0.44, and **2c** 0.51.



1a: R = $C(O)CH_3$ (GM_1)
1b: R = H (de-*N*-acetyl-GM_1)
1c: R = $C(S)CH_3$ (*N*-thioacetyl-GM_1)

2a: R = $C(O)CH_3$ (GM_3)
2b: R = H (de-*N*-acetyl-GM_3)
2c: R = $C(S)CH_3$ (*N*-thioacetyl-GM_3)



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360 MHz ^1H NMR spectra[16] of the products showed for both *N*-thioacetyl GM_1 (**1c**) and *N*-thioacetyl GM_3 (**2c**) the signal of the *N*-thioacetyl methyl protons at 2.53 ppm. As expected, the spectrum of **2c** totally lacked a signal for methyl protons of an *N*-acetyl group at ~2.00 ppm, and **1c** showed only one singlet at 1.99 ppm (3H) due to the acetamido group of *N*-acetylgalactosamine residue. Further, proton

H-5 of *N*-thioacetylneuraminic acid appeared at 4.44 (for **2c**) and 4.43 ppm (for **1c**), each ~t, $J_{4,5} \sim J_{5,6} \sim$ 10 Hz, *i.e.* in the region of the anomeric protons. A related characteristic downfield position of this proton that is attached to the carbon atom bearing the thioacetamido group was found for methyl α-glycoside of *N*-thioacetylneuraminic acid (4.60 ppm compared to 3.80 ppm for H-5 of the corresponding acetamido compound[8,17]).

In the negative ion FAB mass spectrum of **1c**, the characteristic pseudo molecular ions $(M-H)^-$ appeared at *m/z* 1588 and 1560 (100% and 76%), that means they were found to be higher by 16 mass units than those for the oxygen containing parent compound (*m/z* 1572 and 1544 for **1a**). The existence of two peaks differing by 28 mass units reflects the C_{18} and C_{20} sphingosine content of de-*N*-acetyl precursor **1b** which had been obtained from calf brain ganglioside GM_1.

The mass spectrum (electrospray procedure) of **2c** showed the $(M-H)^-$ peak at *m/z* 1195.8 (100%). In addition, the sulfur containing sialic acid residue was easily split to its glycal, giving rise to a fragment ion at *m/z* 306.1. A satellite peak at *m/z* 308 containing ^{34}S confirmed the presence of sulfur in the fragment. The appearance of only one molecular peak indicates **2c** to be composed of mainly C_{18} sphingosine and C_{16} fatty acid.

Results on studies of the biological activity of the *N*-thioacetylated gangliosides will be reported in due course.

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15. To a stirred suspension of **1b** or **2b** (6.0 mg, 3.9 μmol for **1b**, 5.1 μmol for **2b**) in methanol - water (220 μl; 20:1 by volume), at room temperature were added triethylamine (10 μl, 70 μmol) and methyl dithioacetate (10 μl, 90 μmol). After 24 h, TLC (6:1 *n*-propanol - water, $R_{F,1b}$ 0.15, $R_{F,1c}$ 0.37; $R_{F,2b}$ 0.21, $R_{F,2c}$ 0.51) indicated the reaction to be complete. Removal of volatile material *in vacuo* followed by column chromatography on silica gel (eluent, 14:1 *n*-propanol - water, v/v) yielded pure **1c** (4.1 mg, 68%) and **2c** (3.7 mg, 62%), respectively. The products where freed from traces of propanol by freeze-drying and keeping over P_2O_5 *in vacuo* for two days.
16. ^{1}H NMR spectra of **1c**, **2c**, and **1a** (for comparison) were measured at 30°C in CD_3OD (reference: D_2CHOD = 3.31 ppm) on a Bruker AM 360 spectrometer. All of the spectra showed the alkyl chain methyl protons at 0.90 ppm, the methylene protons at 1.29 ppm, and the olefinic protons at 5.45 and 5.68 ppm. Selected data: **1a**, δ 4.93 (d, $J_{1c,2c}$ 8.7 Hz, 1 H, H-1 of GalNAc), 4.44 (d, $J_{1d,2d}$ 7.4 Hz, 1 H, H-1 of Gal1→3), 4.41 (d, $J_{1b,2b}$ 7.8 Hz, 1 H, H-1 of Gal1→4), 4.30 (d, $J_{1a,2a}$ 7.8 Hz, 1 H, H-1 of Glc), 2.74 (dd, $J_{3eq,4}$ 4.7, $J_{3ax,3eq}$ ~12.7 Hz, 1 H, H-3eq of Neu5Ac), 2.17 (t, J 7.5 Hz, 2 H, $NC(O)CH_2$ of fatty acid), 2.01 and 1.99 (2 s, each 3 H, NHAc methyl of Neu5Ac and GalNAc), 1.91 (~t, $J_{3ax,4}$ ~11.3 Hz, 1 H, H-3ax of Neu5Ac); **1c**, δ 4.93 (d, $J_{1c,2c}$ 8.7 Hz, 1 H, H-1 of GalNAc), 4.45 (d, $J_{1d,2d}$ 7.4 Hz, 1 H, H-1 of Gal1→3), 4.43 (~t, $J_{4e,5e}$~$J_{5e,6e}$ ~10.0 Hz, 1 H, H-5 of Neu5ThAc), 4.42 (d, $J_{1b,2b}$ 7.9 Hz, 1 H, H-1 of Gal1→4), 4.30 (d, $J_{1a,2a}$ 7.8 Hz, 1 H, H-1 of Glc), 2.75 (dd, $J_{3eq,4}$ 4.6, $J_{3ax,3eq}$ ~12.8 Hz, 1 H, H-3eq of Neu5ThAc), 2.53 (s, 3 H, methyl of ThAc), 2.17 (t, J 7.5 Hz, 2 H, $NC(O)CH_2$ of fatty acid), 2.00 (s, 3 H, NHAc methyl of GalNAc), 1.96 (~t, $J_{3ax,4}$ ~11.3 Hz, 1 H, H-3ax of Neu5ThAc); **2c**, δ 4.44 (~t, $J_{4c,5c}$~$J_{5c,6c}$ ~10.0 Hz, 1 H, H-5 of Neu5ThAc), 4.43 (d, $J_{1b,2b}$ 7.8 Hz, 1 H, H-1 of Gal), 4.31 (d, $J_{1a,2a}$ 7.8 Hz, 1 H, H-1 of Glc, assignments of the anomeric protons may be interchanged), 3.43 (dd, $J_{6,7}$ 1.3, $J_{7,8}$ 8.9 Hz, 1 H, H-7 of Neu5ThAc), 2.88 (dd, $J_{3eq,4}$ 4.7, $J_{3ax,3eq}$ 12.4 Hz, 1 H, H-3eq of Neu5ThAc), 2.53 (s, 3 H, methyl of ThAc), 2.17 (t, J 7.5 Hz, 2 H, $NC(O)CH_2$ of fatty acid), 1.77 (~t, $J_{3ax,4}$ ~11.8 Hz, 1 H, H-3ax of Neu5ThAc).
17. In the spectrum of **1a**, H-5 of Neu5Ac could not be identified unambiguously; from comparison of the integrals of **1a** and **1c** it seems likely that H-5 appears at 3.40 ppm. Ref. 18 gives a value of 3.46 ppm (500 MHz, DMSO/D_2O).
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