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Note

Identification of an acetyl disulfide derivative in the synthesis of thiosialosides

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ABSTRACT

The first report of the formation of an acetyl disulfide sialoside during the synthesis of thioglycosides is described. This compound is a by-product in the synthesis of the 2-thioacetyl sialoside commonly used in thioglycoside preparation. Our investigations into the identification of this novel disulfide are described.

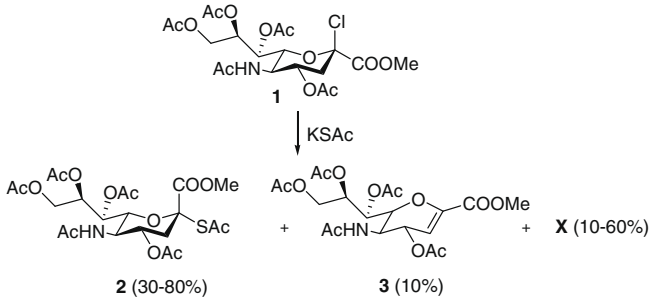
Thioacetates are commonly employed as a means by which to introduce sulfur into organic molecules, on route to thiols or disulfides. They are typically synthesised by the reaction of alkyl halides with potassium thioacetate.[1] Our interest in thiols arises from work in the area of thioglycoside synthesis,[2] and our experience with disulfides.[3] Glycosyl disulfides have received considerable interest recently due to their utility as glycosyl donors.[4] The observations described here result from our efforts to synthesise thiosialosides.

N-Acetylneuraminic acid (sialic acid, Neu5Ac) is commonly found on the surface of mammalian cells and is of particular importance in cellular recognition processes, cell adhesion and disease states.[5,6] There is therefore great interest in the synthesis of sialosides. Furthermore, due to stronger chemical and enzymatic stability than their *O*-glycosyl counterparts, sialosides bearing a sulfur atom at the anomeric carbon have been extensively studied as glycosyl mimics[7] or as synthetic intermediates.[8]

Typically, the key step in the synthesis of thiosialosides is the aforementioned conversion of 2-chlorosialoside **1** into 2-thioacetylsialoside **2** by reaction with potassium thioacetate (Scheme 1).[9] This reaction is known to commonly result in the formation of an unsaturated by-product (per-*O*-acetyl Neu5Ac2en1Me, **3**), a protected form of Neu5Ac2en, which is of considerable interest in its own right, both chemically[10] and biologically.[11–17] Glycal **3** is notoriously difficult to separate from desired compound **2** and other thiosialosides, often necessitating complex purification procedures and/or HPLC. This was first noted by the von Itzstein group, who additionally suggested that this by-product was often not detected.[18]

In our efforts to synthesise both alkyl and aryl thiosialosides via this route, formation of a third product (compound **X**) was observed, in addition to glycal **3** (Scheme 1). The synthesis of thiosialosides is routinely accomplished via the established method of simultaneous *S*-acetate de-protection and alkylation,[19] as exemplified in the case of ethyl thiosialoside **4**[20] (Scheme 2). In our experience, removal of **3** from reaction mixtures is easier following this alkylation step. To our surprise, the third product **X** also appeared to be affected during this reaction, yielding **Y** (Scheme 2). The reaction outlined in Scheme 1 was therefore re-visited in an attempt to isolate and identify unknown by-product **X**.

Preparative HPLC allowed separation of the components of this reaction mixture. NMR and low resolution mass spectrometry confirmed the presence of compounds **2** and **3**. Whilst NMR analysis proved inconclusive at this stage, compound **X** exhibited a mass



Scheme 1. Synthesis of 2-thioacetyl sialoside **2**.

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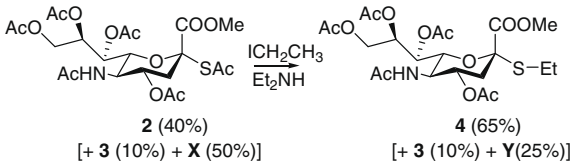
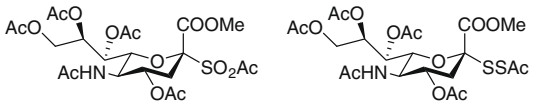


2 (40%) [+ **3** (10%) + **X** (50%)] **4** (65%) [+ **3** (10%) + **Y**(25%)]

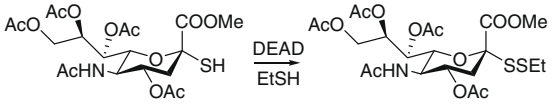
Scheme 2. Synthesis of ethyl thiosialoside **4**.

32 amu higher than **2**. It was therefore hypothesised that compound **X** could either be a product of oxidation of the thioacetate to the α-oxo-sulfone **5**[21,22] or acetyl disulfide **6** (Fig. 1)—both of which would result in a mass increase of 32. Analysis of compound **X** by high resolution mass spectrometry gave the ammonium adduct $[M+NH_4]^+$ at 599.1575. The respective calculated masses for the α-oxo-sulfone **5** and acetyl disulfide **6** were 599.1753 and 599.1575, respectively. Whilst not definitive, this strongly suggested the presence of the acetyl disulfide derivative **6**. This compound has not been reported previously. The IR spectrum also lacked a strong absorption signal at 1700 cm^{-1}, characteristic of α-oxo-sulfones.[21]



5 **6**

Figure 1. Structures of α-oxo-sulfone **5** and acetyl disulfide **6**.



7 **8**

Scheme 3. Synthesis of ethyl disulfide **8**.

Table 2
HPLC analysis of thiosialosides

Compound	Retention time[a] (min)
2 (SAc)	6.3
4 (SEt)	7.3
6 (SSAc)	6.9
8 (SSEt)	8.5
Y + **Z**	7.2, 8.5
Co-injection: (**Y** + **Z**), **4**, **8**	7.2, 8.5

[a] HPLC analyses were performed using an Agilent Technologies 1200 system, with diode array detection, using a C18 reverse phase column (Agilent Eclipse XDB: 4.6 × 100 mm).

We observed that the ratio of compound **2** to by-product **X** varied quite markedly with differing batches of KSAc, including newly purchased material from various suppliers. Given the potential difficulty in synthesising pure acetyl disulfide **6** and α-oxo-sulfone **5** (the latter being particularly unstable[21,22]), we decided to investigate further by subjecting purified compound **X** to the alkylation conditions outlined in Scheme 2. For comparison, ethyl disulfide **8** (the expected alkylation product if **X** were acetyl disulfide **6**) was purposely synthesised from 2-thiosialoside **7**[23] using methodology previously developed in our laboratory[3] (Scheme 3). Compound **8** was previously synthesised by Hummel and Hindsgaul via a different route,[24] but the characterisation was not reported. NMR (Table 1) and HPLC analysis (Table 2) showed **8** to be identical to compound **Y**. It was therefore concluded that compound **X** does indeed correspond to novel disulfide **6**.

Interestingly, alkylating pure acetyl disulfide **6** (**X**) resulted in a second compound in addition to ethyl disulfide **8** (**Y**). On isolation and close inspection of the NMR spectra (Table 1), this was identified as ethyl thioglycoside **4**. This was further confirmed by HPLC (Table 2). Given that thioacetate **2** was absent from the starting material, we deduced that ethyl sulfide **4** produced in this case resulted from disulfide **6** (Scheme 4).

SSAc **6** was found to be unstable to diethylamine treatment (routinely used in such reactions), leading to disulfide cleavage. Subsequent alkylation of the resulting 2-thiosialoside (**7**) gave **4**.

Table 1
^{1}H NMR data

	Chemical shift (ppm), multiplicity *Coupling constants[a] (Hz)*				
	2 (SAc)	**4** (SEt)	**6** (SSAc)	**8** (SSEt)	**Y** + **Z**[b]
H-9_b	4.37, dd	4.29, dd	4.34, d	4.36, dd	
	2.4, 12.4	*2.5, 12.5*	*12.1*	*2.4, 12.4*	
H-9_a	4.02, dd	4.10, dd	4.12, dd	4.11, dd	
	5.8, 12.4	*5.1, 12.5*	*4.7, 12.1*	*5.0, 12.4*	
H-8	5.20–5.22, m	5.35–5.38, m	5.29–5.33, m	5.26–5.30, m	
H-7	5.34, dd	5.30, dd	5.29–5.33, m	5.26–5.30, m	
	2.2, 6.7	*2.0, 8.4*			
H-6	4.65, dd	3.83, dd	3.86, d	3.96, dd	
	2.2, 10.8	*2.0, 10.8*	*9.4*	*1.3, 10.6*	
H-5	4.05–5.01, m	4.00–4.06, m	4.00–4.05, m	4.00–4.05, m	
H-4	4.87–4.91, m	4.82–4.87, m	4.83–4.88, m	4.85–4.90, m	
H-3eq	2.60, dd	2.70, dd	2.81, dd	2.68, dd	2.68, dd; 2.70, dd
	4.6, 12.9	*4.6, 12.7*	*4.4, 12.2*	*4.8, 12.7*	*4.8, 12.7; 4.6, 12.7*
H-3ax	2.09, dd	1.97, dd	2.07, dd	2.25, dd	2.25, dd; 1.97, dd
	9.2, 12.9	*12.0, 12.7*	*9.4, 12.2*	*12.0, 12.7*	*12.0, 12.7; 12.0, 12.7*
NH	5.16, d	5.24, d	5.21, d	5.24, d	
		9.9	*10.8*	*9.9*	
NAc	1.88, s	1.89, s	1.88, s	1.90, s	
SAc	2.27, s		2.46, s		
CH_2		2.52, dq; 2.75, dq		2.75–2.85, m	2.52, dq; 2.75–2.85, m
CH_3		1.18, dd		1.30, t	1.18, dd; 1.30, t

[a] NMR spectra were recorded using a JEOL ECA-600 (600 MHz) spectrometer at room temperature in $CDCl_3$. Chemical shifts are reported in ppm downfield relative to Me_4Si. The additional signals of *O*-acetates and the methyl ester are omitted.
[b] Significant peaks noted only.

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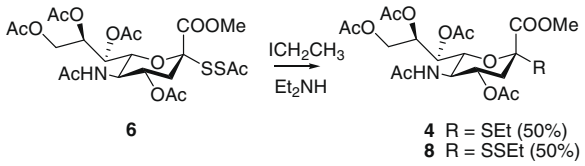

Scheme 4. Alkylation of acetyl disulfide **6**.

The mechanism by which acetyl disulfide **6** is formed in the reaction outlined in Scheme 1 is not certain. It was hypothesised, however, that oxidation of KSAc produces diacetyl disulfide, which further reacts to yield $AcSS^-$ and AcSAc.

It is possible that chlorosialoside **1** may undergo nucleophilic attack by the acetyl disulfide anion, to yield SSAc **6** directly. Preparation of AcSSAc[25] and subsequent reaction with chlorosialoside **1** proved this not to be the case. However, on repeating this reaction in the presence of KSAc, SSAc **6** was obtained almost exclusively. Interestingly, treatment of purified thioacetate **2** with a batch of KSAc that had promoted extensive disulfide formation also led to the formation of SSAc **6**, suggesting that the source of **6** is actually thioacetate **2**. This would require either reaction of AcSSAc with thioacetate **2**, via a radical-based mechanism, or by reaction with AcSAc. Auto-oxidation of thioacetic acid by air and light has also been observed[26] and may be significant. Further studies are required to understand this reaction further.

All three compounds (i.e., **2**, **3** and **6**) co-elute on thin layer chromatography plates and during flash chromatography. Preparative HPLC is required for effective separation. SSAc **6** has since been synthesised and purified from thiosialoside **7** using a similar methodology employed in the synthesis of ethyl disulfide **8**, and further confirmed the identity of compound **X**.

In summary, unexpected observations during the synthesis of thiosialosides are described. The synthesis of thiosialosides using KSAc leads to disulfide by-product formation, which is extremely variable and dependent on the commercial source of KSAc.

1. Experimental

1.1. Characterisation of compounds 6 and 8

1.1.1. Methyl 2-(acetylsulfanyl)-5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-α-D-glycero-D-galacto-2-nonulo pyranosonate (6)

$[\alpha]_D^{20}$ +155.0 ± 1.0 (*c* 0.85, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.88 (s, 3H, NAc), 2.02, 2.04, 2.12 (3s, 12H, 4OAc), 2.07 (dd, 1H, H-3ax, $J_{3ax,4}$ 9.4 Hz, $J_{3ax,3eq}$ 12.2 Hz), 2.46 (s, 3H, SAc), 2.81 (dd, 1H, H-3eq, $J_{3eq,4}$ 4.4 Hz, $J_{3ax,3eq}$ 12.2 Hz), 3.77 (s, 3H, COOMe), 3.86 (d, 1H, H-6, $J_{5,6}$ 9.4 Hz), 4.00–4.05 (m, 1H, H-5), 4.12 (dd, 1H, H-9a, $J_{8,9a}$ 4.7 Hz, $J_{9a,9b}$ 12.1 Hz), 4.34 (d, 1H, H-9b, $J_{9a,9b}$ 12.1 Hz), 4.83–4.88 (m, 1H, H-4), 5.21 (d, 1H, NH, $J_{5,NH}$ 10.8 Hz), 5.31 (br s, 2H, H-7, and H-8); ^{13}C NMR ($CDCl_3$) δ 20.87 (2C), 20.90, 21.24, 23.20, 28.81, 37.07, 49.45, 53.50, 62.01, 67.23, 69.04, 69.57, 74.44, 84.72, 166.85, 170.00, 170.18, 170.80, 170.89, 192.51; ES^+ MS $C_{22}H_{31}NO_{13}S_2$ (581.14) *m*/*z* (%) 582.2 $[M+H]^+$ (30), 604.2 $[M+Na]^+$ (100); HRMS (ES^+) found 599.1575, calcd for $C_{22}H_{35}O_{13}N_2S_2$ 599.1575 $[M+NH_4]^+$.

1.1.2. Methyl 2-(ethylsulfanyl)-5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosonate (8)

$[\alpha]_D^{20}$ +33.0 ± 1.0 (*c* 0.63, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, *J* 7.4 Hz), 1.90 (s, 3H, NAc), 2.02, 2.03, 2.10, 2.14 (4s, 12H, 4OAc), 2.25 (dd, 1H, H-3ax, $J_{3ax,4}$ 12.0 Hz, $J_{3ax,3eq}$ 12.7 Hz), 2.68 (dd, 1H, H-3eq, $J_{3eq,4}$ 4.8 Hz, $J_{3ax,3eq}$ 12.7 Hz), 2.75–2.85 (m, 2H), 3.80 (s, 3H, COOMe), 3.96 (dd, 1H, H-6, $J_{6,7}$ 1.3 Hz, $J_{5,6}$ 10.6 Hz), 4.00–4.05 (m, 1H, H-5), 4.11 (dd, 1H, H-9a, $J_{8,9a}$ 5.0 Hz, $J_{9a,9b}$ 12.4 Hz), 4.36 (dd, 1H, H-9b, $J_{8,9b}$ 2.4 Hz, $J_{9a,9b}$ 12.4 Hz), 4.85–4.90 (m, 1H, H-4), 5.24 (d, 1H, NH, $J_{5,NH}$ 9.9 Hz), 5.26–5.30 (m, 2H, H-7 and H-8); ^{13}C NMR ($CDCl_3$) δ 13.69, 20.81, 20.88, 20.92, 21.19, 23.30, 33.78, 37.10, 49.57, 53.15, 62.12, 67.41, 69.51, 69.72, 88.93, 74.77, 168.11, 170.10, 170.25, 170.78, 171.09, 171.12; ES^+ MS $C_{22}H_{33}O_2NS_2$ (567.63) *m*/*z* (%) 590 $[M+Na]^+$ (100); HRMS (ES^+) found 585.1783, calcd for $C_{22}H_{37}O_{12}N_2S_2$ 585.1782 $[M+NH_4]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.10.017.

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