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. © 2009 Published by Elsevier Ltd.

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 mimics⁷ or as synthetic intermediates.⁸

Typically, the key step in the synthesis of thiosialosides is the aforementioned conversion of 2-chlorosialoside 1 into 2-thioacetyl sialoside 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 chemically¹⁰ 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 (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

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-sulphone 5 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 \([\text{M+NH}_4]^+\) at 599.1575. The respective calculated masses for compound \(X\) which would result in a mass increase of 32 amu higher than 2 were 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.\(^2\)

![Figure 1. Structures of α-oxo-sulphone 5 and acetyl disulfide 6.](image)

![Scheme 2. Synthesis of ethyl thiosialoside 4.](image)

![Scheme 3. Synthesis of ethyl disulfide 8.](image)

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift (ppm), multiplicity</th>
<th>Coupling constants (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-9(_b)</td>
<td>4.37, dd</td>
<td>4.34, d</td>
</tr>
<tr>
<td>H-9(_a)</td>
<td>2.4, 12.4</td>
<td>2.4, 12.4</td>
</tr>
<tr>
<td>H-8</td>
<td>4.02, dd</td>
<td>4.12, dd</td>
</tr>
<tr>
<td>H-7</td>
<td>5.8, 12.4</td>
<td>4.7, 12.1</td>
</tr>
<tr>
<td>H-6</td>
<td>5.20–5.22, m</td>
<td>5.29–5.38, m</td>
</tr>
<tr>
<td>H-7</td>
<td>5.34, dd</td>
<td>5.29–5.33, m</td>
</tr>
<tr>
<td>H-6</td>
<td>2.2, 6.7</td>
<td>5.26–5.30, m</td>
</tr>
<tr>
<td>H-5</td>
<td>4.65, dd</td>
<td>5.26–5.30, m</td>
</tr>
<tr>
<td>H-4</td>
<td>4.87–4.91, m</td>
<td>5.29–5.33, m</td>
</tr>
<tr>
<td>H-3eq</td>
<td>2.60, dd</td>
<td>5.26–5.30, m</td>
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<tr>
<td>H-3ax</td>
<td>4.6, 12.9</td>
<td>5.29–5.33, m</td>
</tr>
<tr>
<td>NH</td>
<td>9.2, 12.9</td>
<td>5.29–5.33, m</td>
</tr>
<tr>
<td>NAc</td>
<td>1.88, s</td>
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<tr>
<td>CH(_2)</td>
<td>2.52, dq</td>
<td>5.29–5.33, m</td>
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<tr>
<td>CH(_3)</td>
<td>1.18, d</td>
<td>5.29–5.33, m</td>
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</table>

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (SAc)</td>
<td>6.3</td>
</tr>
<tr>
<td>4 (SEt)</td>
<td>7.3</td>
</tr>
<tr>
<td>6 (SSAc)</td>
<td>6.9</td>
</tr>
<tr>
<td>8 (SSEt)</td>
<td>8.5</td>
</tr>
<tr>
<td>Y + Z</td>
<td>7.2, 8.5</td>
</tr>
<tr>
<td>Co-injection: (Y + Z), 4, 8</td>
<td>7.2, 8.5</td>
</tr>
</tbody>
</table>

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-sulphone 5 (the latter being particularly unstable), 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 using methodology previously developed in our laboratory (Scheme 3). Compound 8 was previously synthesised by Hummel and Hindsgaul via a different route, 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 analysis (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.

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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 AcSSAc and AcSac.

It is possible that chlorosialoside 1 may undergo nucleophile attack by the acetyl disulfide anion, to yield SSAc 6 directly. Preparation of AcSSAc 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, or via a radical-based mechanism, or by reaction with AcSac.

Auto-oxidation of thioacetic acid by air and light has also been observed 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 been synthesised and purified from thiosialoside in the presence of KSAc, SSAc and AcSac. Auto-oxidation of thioacetic acid by air and light has also been observed and may be significant. Further studies are required to understand this reaction further.

1. Experimental

1.1. Characterisation of compounds 6 and 8

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

\[
\left[\text{d}^{15}O\right]_2\text{C}_{22}H_{31}NO_{13}S_2 (581.14) \quad \text{ES}^+ \text{MS} \quad m/z (%) \quad 582.2 
\]

\[
\left[\text{d}^{15}O\right]_2\text{C}_{22}H_{33}O_2NS_2 (567.63) \quad \text{HRMS} \quad \text{(ES+)} \quad \text{found} 585.1783, \text{calcd for C}_{22}H_{37}O_{12}N_2S_2 585.1782 \text{[M+NH}_4^+]^+.
\]

**Acknowledgements**

This work was supported by the EPSRC (G.R.M.), the Association for International Cancer Research (A.J.H.) and Yorkshire Cancer Research (R.A.F.). The authors thank Andrew Healey for running low resolution mass spectra and the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea, for high resolution accurate mass measurements. Dr. Kamyar Afarinia, Dr. Klaus Pors and Dr. Helen Sheldrake are thanked for useful discussions.

**Supplementary data**

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

**References**


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

**Table 1.**

<table>
<thead>
<tr>
<th>R</th>
<th>AcO</th>
<th>COOMe</th>
<th>SAc</th>
<th>Et2NH</th>
<th>COOMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = SSAc</td>
<td>AcO</td>
<td>COOMe</td>
<td>SAc</td>
<td>Et2NH</td>
<td>COOMe</td>
</tr>
<tr>
<td>R = SSAc</td>
<td>AcO</td>
<td>COOMe</td>
<td>SAc</td>
<td>Et2NH</td>
<td>COOMe</td>
</tr>
</tbody>
</table>

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