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Synthesis of Unsymmetric Trisulfides from 9-Fluorenylmethyl Disulfides

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Abstract

A convenient method for the preparation of unsymmetrical trisulfides using 9-fluorenylmethyl (Fm) disulfide as the precursors is reported. This method gives the desired trisulfides in good yields under mild conditions.

Graphical abstract

Trisulfide compounds ($R-S-S-R'$) belong to the polysulfide family. Naturally occurring trisulfides can be found in plants such as the Allium and Brassica family,^{1–3} as well as some fungi.^{4,5} Various natural products such as enediyne antibiotics calicheamicin⁶ and esperamicin⁷ also contain unique trisulfide functionality. Moreover, natural product- and protein-derived trisulfides are also known.^{8–10} Figure 1 shows two such examples. Trisulfides have decent stability in aqueous solutions and biological systems. They are involved in sulfur-related redox biology and can serve as nonenzymatic precursors of hydrogen sulfide, an important gasotransmitter in signal transduction. Synthetically, trisulfides are produced as food flavors or as byproducts in oil refining.¹¹ Biologically, organic trisulfides possess interesting properties such as antitumor activity,^{12,13} inducing reactive oxidative species (ROS) formation,¹⁴ and protection against toxic chemicals.¹⁵

Chemical syntheses of trisulfides, especially symmetric ones ($R-S-S-S-R$), have been well studied. Representative methods include reactions of thiols with sulfur dichloride¹⁶ or sulfur,¹⁷ reactions of thiol-sulfonates with metal sulfide,¹⁸ and sulfur insertion of disulfides.¹⁹ In

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ASSOCIATED CONTENT

Supporting Information

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Experimental and characterization of each compound (PDF)

The authors declare no competing financial interest.

contrast, the preparation of unsymmetric trisulfides ($R-S-S-R'$, $R \neq R'$) remains challenging. To date, only a few methods have been reported. For example, sequential coupling of two different thiols with sulfur dichloride²⁰ or direct coupling between one thiol and another thiol sulfonyl chloride^{21,22} could lead to the formation of unsymmetric trisulfides (Scheme 1a). A more recent method utilizing phosphorodithioic disulfides and acyl disulfides to prepare unsymmetric trisulfide is also known (Scheme 1b).²³ Although useful for some substrates, these methods still suffer from limitations such as difficult preparation of starting materials, narrow substrate scopes, low yields of the desired trisulfides, and often the formation of symmetric trisulfides as byproducts. The use of a strong base such as NaOMe (Scheme 1b) could be a concern for certain base-sensitive substrates. It can lead to epimerization on amino acid or peptide based substrates. Therefore, more efficient methods for the preparation of unsymmetric trisulfides are still needed.

Recently our group developed a method to generate persulfides (RSSH) using 9-fluorenylmethyl (Fm) disulfide precursors (RSSFm).²⁴ This convenient RSSH generation method could allow us to explore the biological and synthetic usefulness of RSSH. We envisioned that RSSH generated from RSSFm could readily react with certain sulfur-based electrophiles ($R'-S-LG$) to furnish trisulfide products (Scheme 1). As such, this would be a unique and convenient method for the preparation of unsymmetric trisulfides. Herein we report the development and application of this method.

Sulfur-based electrophiles ($RS-LG$) are commonly used to react with nucleophilic reagents to introduce an RS moiety in the structures. In this study, *S*-succinimide **2**, benzothiazole disulfide **3**, and trityl *S*-nitrosothiol **4** were employed to test their reactions with RSSH, which was *in situ* generated from 9-fluorenylmethyl disulfide. As shown in Table 1, a model RSSFm **1** was first mixed with the sulfur-based electrophile. To this mixture was added DBU to promote the formation of RSSH, which was then trapped by the electrophile. As expected, the reaction between **1** and **2** gave the desired trisulfide **5** in high yield (82%). The reaction of **1** with **3** also yielded **5**, although with a decreased yield (33%). Interestingly the reaction between **1** and **4** did not provide trisulfide **5**. Instead, a disulfide **6** was obtained as the major product. These results suggest that *S*-succinimide and benzothiazole disulfide could be suitable electrophiles to trap RSSH while *S*-nitrosothiols are not.

The formation of the crossed disulfide **6** from **1** and *S*-nitrosothiol **4** indicated a possible reaction mechanism as shown in Scheme 2. The proposed mechanism consists of a *trans*-nitrosation between the persulfide **7** and TrSNO **4**, resulting in the formation of TrS⁻ and persulfide SNO **8**. Then these two species further react to form the stable cross disulfide **6**.

Having proved the feasibility of our design, we then tested the scope of the method using a variety of RSSFm substrates together with different *S*-succinimide and benzothiazole disulfide substrates. The results are shown in Tables 2 and 3. In general, the reactions worked well and the desired trisulfides were obtained in good to excellent yields. The reactions with a tertiary electrophile or nucleophile sometimes gave lower yields, presumably due to steric hindrance. Nevertheless, it is demonstrated that this is a quite effective method for the preparation of unsymmetric trisulfides.

To further explore the scope of this reaction, a series of fluorenylmethyl disulfides derived from L-cysteine, with different *N*-protection groups (Boc, Bz, and Cbz), were also tested (Table 4). Again these substrates gave satisfactory results of trisulfide formation. No obvious epimerization was observed in these reactions.

It should be noted that, in some cases, crossed disulfides were obtained as the side products. For example, in entry 1 of Table 3, the corresponding disulfide was obtained in 10% yield. The disulfides normally showed very similar polarity as their trisulfide analogs. The separation and structure characterization of trisulfides could be challenging. To exclusively demonstrate the products we obtained were indeed trisulfides, we prepared the corresponding disulfide compounds using authentic methods²⁵ for each substrate and carefully compared the NMR spectra of disulfides and trisulfides. Some representative results are shown in Table 5. It is known that the chemical shifts of protons adjacent to trisulfides are more downfield than those of disulfides.²⁶ As shown in Table 5, protons (*H_a* and *H_b*) in trisulfides indeed showed higher chemical shifts than their disulfide counterparts. The difference ranges from 0.37 to 0.12, which is very obvious for ¹H NMR. However, the chemical shift difference in ¹³C NMR was found not to be significant. Therefore, ¹H NMR could be used to identify the formation of trisulfides. To further confirm the identity of trisulfides, we also tested their responses toward SSP4, a fluorescent probe that detects sulfane sulfurs.²⁷ As trisulfides belong to the sulfane sulfur family, all of them showed significant fluorescence increases (an example is shown in Figure S1, Supporting Information). These experiments confirmed the compounds we made with this method were indeed trisulfides.

The mechanism of crossed disulfide formation was interesting. We first speculated the disulfides could come from trisulfides. The other byproducts in the reaction, e.g. 2-mercaptobenzothiazole (BTSH) and succinimide, might act as nucleophiles under basic conditions to react with trisulfides to form disulfides. We carried out NMR studies to test this possibility. However, no reaction was observed when **5** was treated with BTSH or succinimide in the presence of DBU (Scheme 3). This result further demonstrated that the reaction conditions were compatible to the stability of trisulfides. The plausible mechanism of crossed disulfide formation is the partial decomposition of persulfides to form the corresponding thiolates,²⁸ which then react with RS–LG to form the disulfides. Therefore, the stability of persulfides should control their reaction productivity with electrophiles. The more stable they are, the higher the yields of trisulfides.

In summary, herein we report a facile method for the preparations of unsymmetric trisulfides from easily available 9-fluorenylmethyl disulfide substrates. In this process, highly reactive persulfide intermediates are generated under mild conditions. In theory, these intermediates can be captured by a variety of electrophiles or other reactive species. Other synthetic applications of these persulfide intermediates are currently being studied in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Representative trisulfide molecules.

Previous methods: **This work:**

Scheme 1.
Synthetic Methods for Trisulfides

Scheme 2.
Proposed Mechanism for the Generation of 6

Scheme 3.
Proposed Mechanism for Disulfide Formation

Table 1

Screen of Electrophiles

$ \begin{array}{c} \text{S-S-Fm} \\ \\ \text{AcHN}-\text{CH}-\text{C}(=\text{O})\text{NHBu} \\ \textbf{1} \end{array} + \text{RS-LG} \xrightarrow[\text{CHCl}_3]{\text{DBU (2 equiv)}} \begin{array}{c} \text{S-S-S-R} \\ \\ \text{AcHN}-\text{CH}-\text{C}(=\text{O})\text{NHBu} \end{array} $		
entry	RS-LG	product (yield)
1	2	5 (82%)
2	3	5 (33%)
3	4	6 (81%)

Table 2Generation of Unsymmetric Trisulfides from *S*-Succinimide Substrates

entry	disulfide	R'	product (yield)
1	1	Bn	5 (82%)
2	1	t-Bu	9 (32%)
3	1	Cy	10 (78%)
4	1	Ph	11 (75%)
5	1	Et	12 (93%)
6	13	Bn	14 (55%)
7	13	Cy	15 (75%)
8	16	Et	17 (95%)
9	16	t-Bu	18 (75%)

Table 3

Generation of Unsymmetric Trisulfides from Benzothiazole Disulfide Substrates

$\text{R-S-S-Fm} \xrightarrow[\text{DBU, CHCl}_3]{\text{Benzothiazole-2-disulfide-R'}}$ R-S-S-S-R'			
entry	disulfide	R'	product (yield)
1	1	Bn	5 (33%)
2	1	t-Bu	9 (35%)
3	1	Cy	10 (78%)
4	1	Ph	11 (71%)
5	1	Et	12 (54%)
6	19	Et	20 (81%)
7	19	t-Bu	21 (65%)

Table 4

Generation of Unsymmetric Trisulfides from L-Cysteine Based Substrates

$\text{R-S-S-Fm} \xrightarrow[\text{DBU, CHCl}_3]{\text{Cyclic sulfonamide}} \text{R-S-S-S-R'}$

entry	disulfide	R'	product (yield)
1		Ph	23 (85%)
2	22	Et	24 (91%)
3		Et	26 (quant.)
4		Et	28 (75%)
5		Et	30 (70%)
6		Et	32 (65%)
7	31	Cy	33 (79%)
8	31	Bn	34 (66%)

Table 5

NMR Differences between Disulfides and Trisulfides

trisulfide

chemical shift

disulfide

chemical shift

5

H_a 3.20, H_b 4.09
 C_a 39.41, C_b 43.21

35

H_a 2.68, H_b 3.90
 C_a 39.73, C_b 43.16

9

H_a 3.27
 C_a 41.45, C_b 49.91

36

H_a 3.01
 C_a 41.84, C_b 48.46

10

H_a 3.22, H_b 2.98
 C_a 41.75, C_b 48.44

37

H_a 3.00, H_b 2.77
 C_a 40.92, C_b 49.41

11

H_a 3.17
 C_a 41.00, C_b 136.22

38

H_a 3.05
 C_a 41.60, C_b 136.10

12

H_a 3.25, H_b 2.91
 C_a 41.40, C_b 32.73

39

H_a 2.99, H_b 2.71
 C_a 40.02, C_b 32.40