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# Dynamic Thiol Exchange with *β*-Sulfido-α,*β*-Unsaturated Carbonyl Compounds and Dithianes

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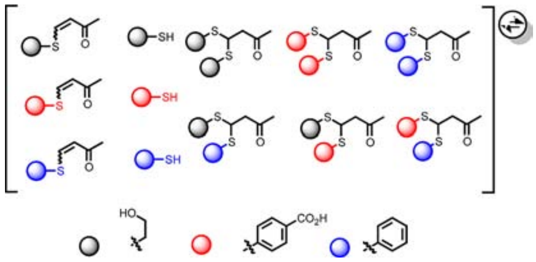
## ABSTRACT



**A reversible covalent bond exchange of thiols, *β*-sulfido-α,*β*-unsaturated carbonyls, and dithianes has been studied in DMSO and $D_2O$/DMSO mixtures. The equilibrium between thiols and *β*-sulfido-α,*β*-unsaturated carbonyls is obtained within a few hours, while the equilibration starting with the *β*-dithiane carbonyls and thiols requires a few days. This time scale makes the system ideal for utilization in dynamic combinatorial chemistry.**

The field of Dynamic Combinatorial Chemistry (DCC) has expanded dramatically since it was first established over a decade ago.[1] The popularity of DCC is due to the simplicity with which hosts and ligands,[2] or enzyme inhibitors,[3] can be discovered with minimal synthetic effort as compared to traditional approaches. There are, however, only a handful of dynamic covalent motifs that exchange components within a few hours or days, the most common being disulfide,[4] transimination,[5] and hydrazone[6] as well as thioester exchange.[7] Thus, there is interest in expanding the 'tool kit' available to the DCC community by developing new dynamic covalent chemistry.

A lesser used reaction for DCC is the thia-Michael addition, which takes approximately a week to achieve equilibria in slightly basic conditions. For example, Greaney used this

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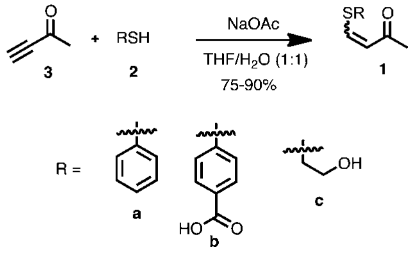
reaction for the discovery of inhibitors for glutathione-S-transferase.[3d] Recently, Taunton used α-cyano acrylamides as the conjugate acceptor to target noncatalytic cysteines, thereby creating kinase inhibitors.[8] In a slightly different approach, Che has used thia-Michael additions to *β*-sulfido-α,*β*-unsaturated carbonyls (Vinyl Sulfides Carbonyls, VSCs) to modify cysteines,[9] and as sensors for cysteine.[10] He found the reaction to be dynamic, but he did not explore the mechanistic details of the exchange.

Herein, we present experiments aimed at uncovering the time scale and mechanism of thia-Michael additions to VSCs, thereby creating new VSCs, and we report the formation of *β*-dithiane carbonyls. We find that the thia-conjugate addition is kinetically fast to exchange thiols and VSCs, typically complete in less than an hour, while equilibration with the *β*-dithiane carbonyls (BdTCs) can require a few days. Importantly, the reactions proceed in DMSO and DMSO water mixtures at physiological pH.

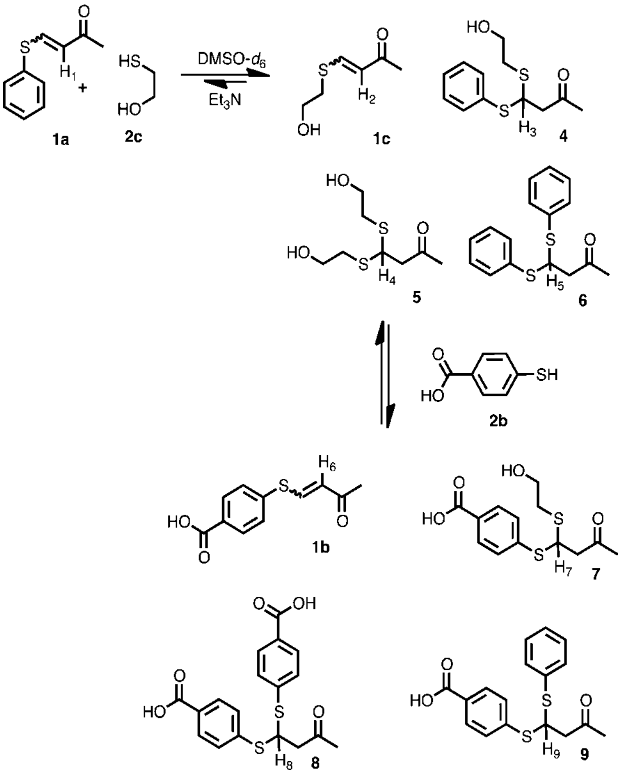
To examine the reversible covalent bond exchange, a series of *β*-sulfido-α,*β*-unsaturated ketones (i.e., VSCs) were required. The syntheses of **1a**−**c** were achieved by treating the butynone **3** with 1 equiv of thiols **2a**−**c** in good yields (Scheme 1). The conjugate addition yields both *E* and *Z* isomers, but the *Z* isomer dominates, as has been reported earlier.[11]

Thiol exchange with the VSCs was first carried out in DMSO-$d_6$ and monitored by $^1$H NMR spectroscopy. The addition of 1 equiv of **2c** with **1a** in the presence of $Et_3N$ (Scheme 2) quickly showed an equilibrating mixture of VSCs **1a** and **1c** (doublets between 5.8 and 6.8 ppm), while within 1 h the BdTCs **4**, **5**, and **6** (triplets at 4.7, 4.4, and 5.1 ppm respectively)[12] were present, with all five species in a ratio of 3:33:19:8:37, respectively, as determined by $^1$H NMR integration (for the VSCs, the ratios are inclusive of both *E* and *Z* isomers). The spectra did not further change with time, indicative that the mixture had reached equilibrium (Figure 1). Although, thiols are known to oxidize in DMSO under basic conditions, we found that the equilibrium between the VSC's and the thiols is attained faster (hours) than the disulfide-thiol formation (days).[13]

**Scheme 1.** Synthesis of **1a**−**c**



**Scheme 2.** Exchange Studies with Vinyl Sulfide **1a** and Thiols **2c** and **2b**



The subsequent addition of 4-mercaptobenzoic acid (**2b**) to the equilibrated mixture further generated **1b**, **7**, **8**, and **9** as depicted in Scheme 2, while the first products decreased but remained. This confirmed that all the species are interconverting. Interestingly, we find an equilibrating mixture of VSCs and BdTCs, even though excess thiols are present in the solution. Thus, the thermodynamic stability of vinyl sulfide carbonyls and *β*-dithiane carbonyls are comparable under the experimental conditions.

We sought to confirm the dynamic nature of the BdTCs by treating pure **6** with several equivalents of **2c** in DMSO-$d_6$ and $Et_3N$, monitoring the reaction by $^1$H NMR spectroscopy (Scheme 3). Within 1 h, the formation of **1a**, **1c**, **4**, and **5** was confirmed by the appearance of proton resonances at $\delta$ = 5.9, 6.1, 4.7, 4.4 ppm, respectively.[14] While acid catalyzed dithiane exchange was previously reported by Sutton and co-workers,[15] to our knowledge this is the first report of a base catalyzed dithiane exchange, which is undoubtedly due to the *β*-keto group.

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(12) The assignments were made based on the $^1$H NMR of **5** and **6**. The dithiane **6** was synthesized and purified, while the dithiane **5** was synthesized in the NMR tube from **1c** and **2c** in DMSO-$d_6$ and $Et_3N$. See Supporting Information for the $^1$H NMR spectra.

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(14) See Supporting Information, Figure S7.

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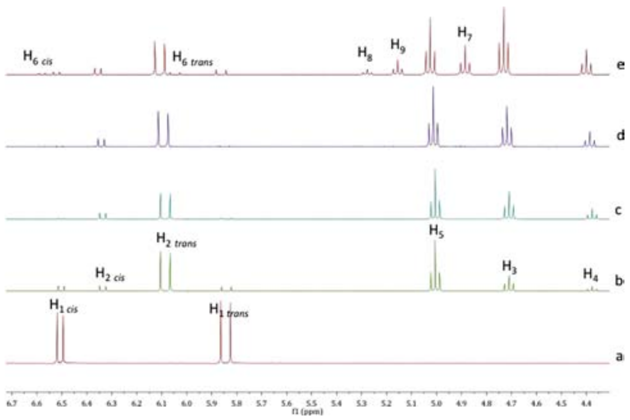
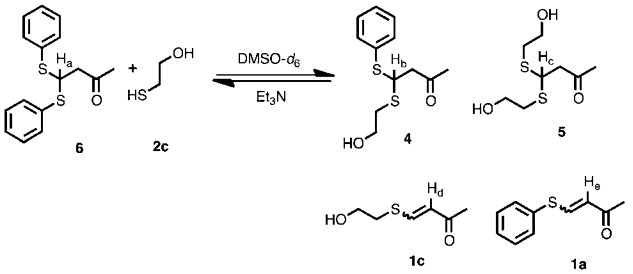
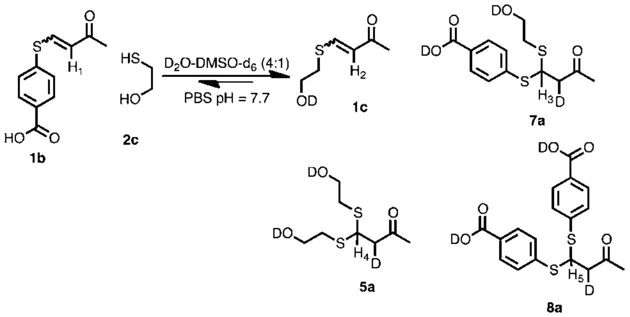


**Figure 1.** $^1$H NMR monitoring of thiol scrambling in DMSO-$d_6$ and $Et_3N$; (a) Partial spectra of **1a**. (b) 5 min after the addition of **2c**. (c) 50 min after the addition of **2c**. (d) 5 min after the addition of **2b**. (e) 14 h after the addition of **2b**. The labels correspond to the H's in Scheme 2.

**Scheme 3.** Base Catalyzed Thioacetal Exchange



**Scheme 4.** Thiol Scrambling in $D_2O$/DMSO-$d_6$ (4:1) in PBS and Deuteration of the α-Carbon



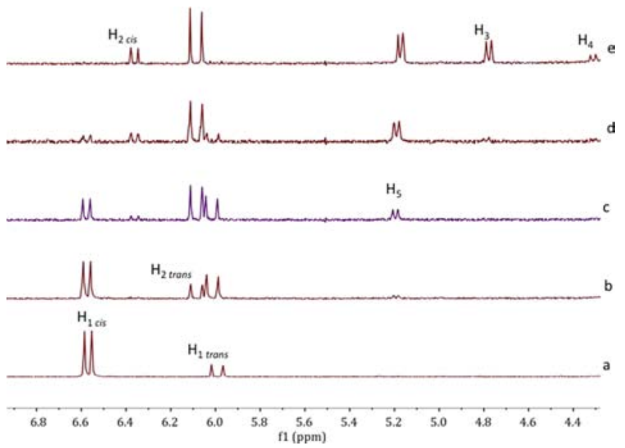
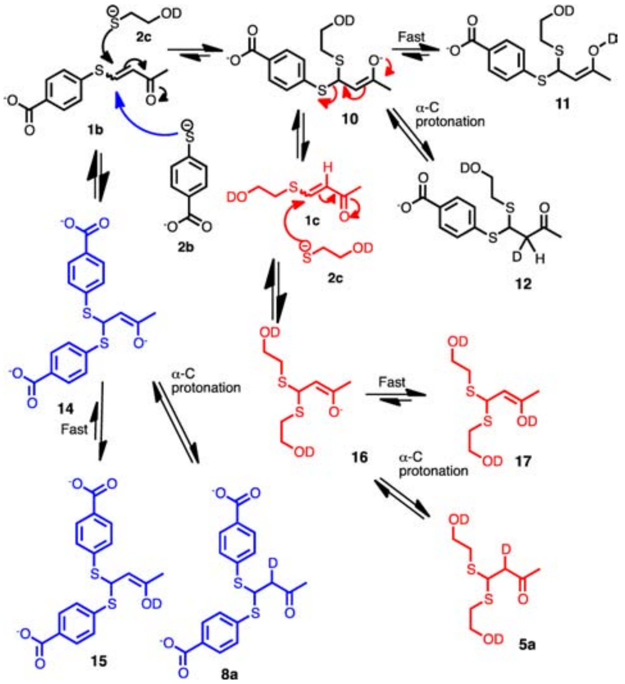


**Figure 2.** $^1$H NMR monitoring of thiol scrambling in $D_2O$: DMSO-$d_6$ (4:1) in PBS at pD = 7.7; (a) Partial spectra of **1b**. (b) 2 min after the addition of **2c**. (c) 10 min after the addition of **2c**. (d) 1 h after the addition of **2c**. (e) 2 h after the addition of **2c**. The labels correspond to H's in Scheme 4.

**Scheme 5.** Mechanism for the Thiol Exchange with **1b**.



To elucidate the mechanism of the dynamic exchange of thiols, VSCs, and BdTCs, we carried out studies using $D_2O$/DMSO-$d_6$ (4:1) at pD 7.7 in phosphate buffer (Scheme 4), and the reaction was monitored by $^1$H NMR spectroscopy and LCMS.[16] This solvent system allows one to follow the formation of any enol or enolate intermediates because they will necessarily be deuterated. When 1 equiv of **2c** was added to a solution of **1b**, a proton resonance at $\delta$ = 6.1 ppm appeared in less than 2 min, which is indicative of formation of the product **1c** (Figure 2). A resonance at $\delta$ = 5.2 ppm appeared a few minutes later, assigned to the methine proton of product **8a** (α-deuterio-**8**),

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(16) The reaction for LCMS was carried out in $H_2O$/DMSO (4:1) and diluted with methanol. The LCMS traces are included in the Supporting Information.

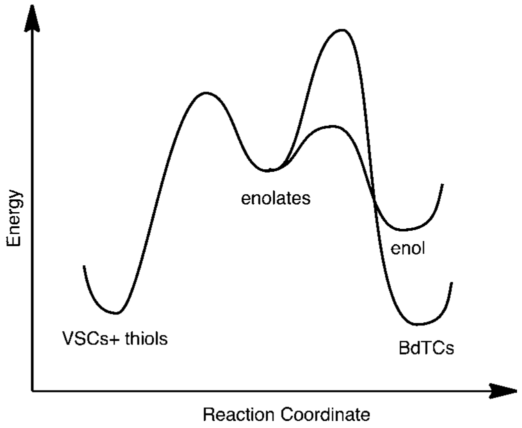

**Figure 3.** Reaction coordinate diagram showing relative energies of reactions, intermediates, products, and barriers that are consistent with the experimental observations for rates of conjugate additions, thiol scrambling, and dithiane exchange.

and exists as a doublet rather than a triplet due to monodeuteration of the α-carbon. After 2 h, products **7a** and **5a** were observed (see Scheme 4); the corresponding $\beta$-proton resonances were at $\delta = 4.8$ and 4.3 ppm, respectively.

The results of Figures 1 and 2 show that the scrambling of thiols and VSCs occurs faster than the formation, as well as the scrambling, of BdTCs. We expected the BdTCs to be the intermediates through which the thiols and VSCs would exchange. Hence, we wondered - How can VSCs exchange thiols faster than BdTCs if BdTCs are the intermediates? Therefore, the results show that these species must not be the correct intermediates.

Instead, the results force us to the following conclusions (Scheme 5). The attack of thiolate **2c** at the $\beta$-position of **1b** is a slow step. The attack generates an enolate **10**, which is ultimately deuterated at the α-carbon to create **12**. However, it is well-known that protonation of enolates, such as **10**, occurs faster on oxygen.[17] Hence, enol **11** would be the first formed neutral intermediate. The enol can regenerate the enolate leading to a thiolate leaving group departure, which can rapidly scramble thiols and the stereochemistry of the alkene as observed. Any other VSCs would undergo the same fate, leading to enols **15** and **17**. However, ultimately protonation at the α-carbon gives **12**, **5a**, and **8a**, which must revert slowly to the enolates because BdTC scrambling is the slowest process.

From the conclusions above, a qualitative energy profile diagram can be derived as shown in the Figure 3. In order for the BdTCs to be the slowest species to exchange thiols, one must conclude that the deprotonation of the α-carbon is the slowest step in the entire sequence, including the original conjugate addition of the thiols. The relative energy levels of the enolates and enols fall into place as shown. Future computational studies can put quantitative numbers on this qualitative diagram.

In summary, we report that $\beta$-sulfido-α,$\beta$-unsaturated carbonyls will reversibly and rapidly exchange thiols in organic and aqueous media, ultimately with an equilibrated mixture of thiols, vinyl sulfide carbonyls, and $\beta$-dithiane carbonyls. These latter species equilibrate the slowest, leading to the conclusion that enols are the neutral intermediates through which vinyl sulfide carbonyls exchange thiols rather than dithiane carbonyls.

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**Supporting Information Available.** General experimental procedures and spectroscopic data for all the compounds, experimental protocol for thiol exchange, and LCMS traces are mentioned in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.