Degenerate Intermolecular Thiolate–Disulfide Interchange Involving Cyclic Five-Membered Disulfides Is Faster by $\sim 10^3$ Than That Involving Six- or Seven-Membered Disulfides

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Abstract: The rate constants for degenerate intermolecular thiolate–disulfide interchange involving 1,2-dithiolane ($\text{S(CH}_2\text{)}_n\text{S}^-$) are higher than those involving 1,2-dithiane ($\text{S(CH}_2\text{)}_n\text{S}^-$) by a factor of $\sim 650$ in mixtures of DMSO-$d_6$ and D$_2$O. The extrapolated rate constant for 1,2-dithiolane in DMSO-$d_6$ is fast ($k \sim 10^7$ M$^{-1}$ s$^{-1}$). The rate constants for cyclic six- and seven-membered disulfides are similar to those for acyclic disulfides. Rate constants for self-exchange were measured by dynamic $^1$H NMR line-shape analysis. The evolutionary selection of lipase as the cofactor in 2-oxo acid dehydrogenases may reflect the fast rate of ring opening of the dithiolane ring by nucleophiles.

**Introduction**

We have used dynamic NMR spectroscopy to determine the rate constants for degenerate thiolate–disulfide interchange of cyclic disulfides and dithiols in mixtures of DMSO-$d_6$ and D$_2$O (eq 1). We had two objectives in this work: First, we wished to confirm that the thiolate–disulfide interchange reaction of a cyclic five-membered disulfide (1,2-dithiolane) is significantly faster than that of a cyclic six-membered disulfide (1,2-dithiane) and of a cyclic seven-membered disulfide (1,2-dithiopane) and to establish the magnitude of the difference in rate.1,2 We hoped this observation might help to rationalize the evolutionary selection in 2-oxo acid dehydrogenases of lipase with its 1,2-dithiolane moiety as cofactor rather than of a cofactor with a 1,2-dithiane or a 1,2-dithiopane group. Second, we wished to test a prediction of a cyclic seven-membered disulfide (1,2-dithiepane) and to cyclic five-membered disulfide (1,2-dithiolane) is significantly faster than the transition state by ring strain from destabilization of the ground state of the cyclic five-membered disulfide relative to the transition state by ring strain from destabilization of the ground state of thiolate anion in DMSO due to energetically less favorable solvation in DMSO.

Thiol–disulfide interchange is of broad importance in biochemistry.4-10 The mechanism of the reaction involves the nucleophilic attack of thiolate anion along the S–S bond axis of the disulfide.11 The reaction is kinetically second order: first order in thiolate and in disulfide. The rate constants follow a Bronsted relationship in the values of $pK_r$ of both the nucleophilic thiol and of the thiol being displaced.12-19

**Methods**

The rate constants for degenerate thiolate–disulfide interchange of samples of dithiolate and disulfide in mixtures of DMSO-$d_6$ and D$_2$O were determined by $^1$H NMR line-shape analysis. Typical $^1$H NMR spectra are shown in Figure 1. The accuracy of this line-shape analysis was aided by two factors: (1) The rate of the reaction, and width to confirm that the thiolate–disulfide interchange reaction of a cyclic five-membered disulfide (1,2-dithiolane) is significantly faster than that of a cyclic six-membered disulfide (1,2-dithiane) and of a cyclic seven-membered disulfide (1,2-dithiopane) and to establish the magnitude of the difference in rate.1,2 We hoped this observation might help to rationalize the evolutionary selection in 2-oxo acid dehydrogenases of lipase with its 1,2-dithiolane moiety as cofactor rather than of a cofactor with a 1,2-dithiane or a 1,2-dithiopane group. Second, we wished to test a prediction of a cyclic seven-membered disulfide (1,2-dithiepane) and to cyclic five-membered disulfide (1,2-dithiolane) is significantly faster than the transition state by ring strain from destabilization of the ground state of the cyclic five-membered disulfide relative to the transition state by ring strain from destabilization of the ground state of thiolate anion in DMSO due to energetically less favorable solvation in DMSO than in water.

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**Table 1. Second-Order Rate Constants ($k$, M$^{-1}$ s$^{-1}$) for Degenerate Thiolate–Disulfide Interchange for $\text{S(CH}_2\text{)}_n\text{S}^-$/S($\text{CH}_2\text{)}_n\text{S}$ in Mixtures of D$_2$O and DMSO-$d_6$ at 24 °C**

<table>
<thead>
<tr>
<th>mol % D$_2$O</th>
<th>$n = 3$</th>
<th>$n = 4$</th>
<th>$n = 5$</th>
<th>$k_{a35}/k_{a44}$</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>90</td>
<td>19</td>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>13000</td>
<td>22</td>
<td>590</td>
<td></td>
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<tr>
<td>25</td>
<td>18</td>
<td></td>
<td>710</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>6000</td>
<td>9.8</td>
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<td></td>
</tr>
<tr>
<td>33</td>
<td>3600</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2100</td>
<td>3.2</td>
<td>660</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>930</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>300</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*The uncertainties in $k$ are $\pm 10^9$. The rate constants are reported per mole of disulfide and of dithiolate they are not corrected statistically for the presence of two symmetry-equivalent sulfur centers in each reactant.

\[ \text{S(CH}_2\text{)}_n\text{S}^- + \text{S(CH}_2\text{)}_n\text{S} = \text{S(CH}_2\text{)}_n\text{S}^- + \text{S(CH}_2\text{)}_n\text{S}^- \] (1)

Figure 1. Representative experimental and calculated line shapes for the methylene protons adjacent to sulfur in 500-MHz 1H NMR spectra of (A) potassium 1,4-butanedithiolate and 1,2-dithiane (46 mM) in a solvent mixture consisting of 15 mol % D2O in DMSO-d6; (B) potassium 1,4-butanedithiolate and 1,2-dithiane (41 mM) in 40 mol % D2O-DMSO-d6; (C) potassium 1,3-propanedithiolate and 1,2-dithiolane (5.6 mM) in 20 mol % D2O-DMSO-d6; (D) potassium 1,3-propanedithiolate and 1,2-dithiolane (14 mM) in 50 mol % D2O-DMSO-d6. The peak due to 1H in the DMSO is marked with an asterisk.

Results and Discussion

Kinetic Data. Table I summarizes the values for rate constants \( k \) (M\(^{-1}\) s\(^{-1}\)) of degenerate thiolate-disulfide interchange reactions of cyclic disulfides in mixtures of D2O and DMSO-d6. The rate constant for 1,2-dithiole is higher than that of 1,2-dithiane by a factor of \( \sim 650 \). The rate constant of 1,2-dithiane is higher than of 1,2-dithiole by a factor of \( \sim 5 \). The rate constants for 1,2-dithiole and 1,2-dithiane are comparable (within a factor of 2) to that for the noncyclic dibutyl disulfide. The value of the rate constant for thiolate-disulfide interchange of potassium 1-butanedithiol and dibutyl disulfide in DMSO-d6 is \( k = 54 \times 10^3 \) M\(^{-1}\) s\(^{-1}\). This value must be multiplied by 2 for it to be comparable to the rate constants for interchange between di- and cyclic disulfides to account for the presence of two symmetry-equivalent thiolate groups in dibutyl disulfide.

Comparison of the kinetic data for degenerate thiolate-disulfide interchange of cyclic five- and six-membered disulfides with the equilibrium data for 1,4-butanedithiol and 1,2-dithiolane (eqs 2 and 3) provides an independent check on validity of these values.

\[
K_{eq} = \frac{k_{h2}}{k_{h1}}
\]

We expect the ratio \( k_{h2}/k_{h1} \) to be close to the ratio of rate constants (\( \sim 650 \)) of degenerate thiolate-disulfide interchange of cyclic five- and six-membered disulfides. The value of the equilibrium con-

(25) Creighton et al. have reported that the rate of reduction of lipic acid by dithiothreitol in water (\( k = 56 \text{ M}^{-1} \text{s}^{-1} \)) is 160-fold greater than expected by the Bronsted relationship for unstrained linear disulfides (\( k_{\text{proted}} = 0.35 \text{ M}^{-1} \text{s}^{-1} \)). Fava et al. have reported that the rate constant for the interchange of lipic acid and 2-carboranethiolate and dibutyl disulfide in DMSO-d6 is \( k = 54 \times 10^3 \) M\(^{-1}\) s\(^{-1}\). The value of the rate constant for the interchange of lipic acid and dibutyl disulfide in DMSO-d6 is \( k = 5.3 \times 10^3 \) M\(^{-1}\) s\(^{-1}\) in 25 mol % D2O. The difference between the observed factor of 3–4 and the expected factor of 2 may reflect stabilization of the S(CH3)2SH by intramolecular H bonding, or enhanced nucleophilicity for the dianion.

(26) The rate constants for samples of 1,4-butanedithiolate/1,2-dithiolane were indistinguishable at different concentrations (22 and 44 mM); the reaction is therefore second order overall.

(27) The values of rate constants for samples of 1,4-butanedithiol and 1,2-dithiole with 1 equiv of potassium tert-butoxide were lower by a factor of 3–4 than those with 2 equiv of base; for 1 equiv of potassium tert-butoxide present, \( k = 16 \times 10^3 \) M\(^{-1}\) s\(^{-1}\) in 15 mol % D2O, and \( k = 5.3 \times 10^3 \) M\(^{-1}\) s\(^{-1}\) in 25 mol % D2O. The difference between the observed factor of 3–4 and the expected factor of 2 may reflect stabilization of the S(CH3)2SH by intramolecular H bonding, or enhanced nucleophilicity for the dianion.

(28) The rate constants for thiolate-disulfide interchange should be divided by 2 to be directly comparable to reactions in which a single thiolate attacks a single electrophilic center (e.g., CH3S– + CH3Br → CH3SCH3 + Br\(^{-}\)).
stant \( (K_{eq}) \) for the thiol–disulfide interchange of 1,4-butane-dithiole and 1,2-dithiolane (eqs 2 and 3) in DMSO is \( \sim 30 \). Thus (eq 3) \( k_{-1} \sim 20 k_2 \). At first glance, this conclusion is surprising, since \( k_2 \) is making a strained dithiole ring and \( k_{-1} \) an unstrained dithiolane ring. We suggest later, however, that the ring strain in the dithiole ring effectively disappears in the transition state for thiolate–disulfide reactions involving it, presumably because the S–S bond is weakened and stretched. Thus, the factor of 20 can be rationalized as the entropic advantage to closing five-membered rings relative to six-membered rings. In the analogous ring-closure reactions of \( o-(\omega\text{-bromoalkyl})\text{phenoxide} \), the ratio can be rationalized as the entropic advantage to closing five-membered cyclic ethers is formed 20 times faster than the six-membered one. The effective molarity for an intramolecular thiolate–disulfide interchange reaction involving formation of six-membered oxidized dithiothreitol is estimated as \( 10^8 \) M, and for the formation of two cystine bonds in bovine pancreatic trypsin inhibitor as \( 10^2 \) and \( 10^3 \) M. Plots of \( \log k \) vs Mole Percent D:O Are Linear. For thiolate–disulfide interchange in mixtures of DMSO-\( d_2 \) and D:O, the plots of \( \log k \) vs mole percent D:O are linear (Figure 2). The slopes for 1,2-dithiane (0.050) and for 1,2-dithiolane (0.041) are similar and are comparable to that for bis(2-hydroxyethyl) disulfide (0.035).3 The rate constant for thiolate–disulfide interchange between oxidized dithiothreitol and dithioerythritol in D:O \( (k = 5 \text{ M}^{-1} \text{s}^{-1}; \log k = 0.69) \) correlates well with the plot for 1,2-dithiane in Figure 2; we infer that the plot of \( \log k \) vs mole percent D:O is linear over the entire solvent range.3 The relative rate constants reported in Table I would hold approximately for the entire range of mixtures from DMSO-\( d_2 \) to D:O. The rate constant for degenerate thiolate–disulfide interchange involving 1,2-dithiolane is expected to be \( \sim 10^6 \) faster than that involving 1,2-dithiane in D:O from extrapolation of Figure 1.

**The Rate of Thiolate–Disulfide Interchange of 1,2-Dithiolane in DMSO-\( d_2 \) Is Fast.** The extrapolated rate constant \( (k \sim 10^9 \text{ M}^{-1} \text{s}^{-1}) \) for thiolate–disulfide interchange of 1,3-propanedithiolate and 1,2-dithiolane in DMSO-\( d_2 \) is fast compared to the rate constant for interchange of 1,4-butane-dithiole and 1,2-dithiolane in D:O \( (k \sim 5 \times 10^{-5} \text{ M}^{-1} \text{s}^{-1}) \). We propose that this large difference in rates arises from two factors (Figure 3): (i) The ground state of 1,2-dithiolane is destabilized relative to the transition state for thiolate–disulfide interchange because of ring strain. This conclusion.

**Ring Strain in the Ground State of the 1,2-Dithiolane Ring Is Released in the Transition State for Degenerate Thiolate–Disulfide Interchange.** The rate of thiolate–disulfide interchange increases with increasing ring strain in the ground state of cyclic disulfides.

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**References**


(31) The equilibrium constant was determined for a mixture of 1,2-dithiane and 1,3-propanedithiol in both DMSO-\( d_2 \) and equimolar DMSO-\( d_2 \)-D:O with 4 mol% potassium tert-butoxide by \( ^{1} \mathrm{H} \) NMR spectroscopy after quenching with DCl; this value of the equilibrium constant (30) is similar to that reported for CDOD-\( d_2 \)-D:O (33).30


(35) For the thiolate–disulfide interchange of lipic acid and 2-carboxy-1,3-propanedithiol in D:O at 297 K, only a lower limit for the rate constant could be determined \( (k > 2000 \text{ M}^{-1} \text{s}^{-1}; \log k > 2.3) \).


(38) The rate constants of \( S_{2}S_{2} \) and \( S_{2}A_{2} \) reactions involving anionic nucleophiles increase by a factor of \( 10^{2} \) on going from a protic polar to a nonpolar solvent: Reichardt, C. Solvents and Solvent Effects in Organic Chemistry, VCH: Weinheim, 1988. Although the effects of hydration on the reaction kinetics of an \( S_{2}A_{2} \) reaction \( (\text{CF}_{3} \text{CH}_{2} \text{OH}) \) are profound, the structure of the transition state is only slightly distorted: Jorgensen, W. L.; Buckner, J. K. J. Phys. Chem. 1986, 90, 4651–4654.

(39) The MM2 calculations were done with the program MacroModel V2.0: Burns, J. A.; Whitesides, G. M. J. Am. Chem. Soc., submitted. The difference in steric energy (\( \Delta \)SE) between the disulfide and the dithiol was calculated with reference to 1,2-dithiane (\( \Delta \)SE(dithiane) = 0), and the free energy change was predicted for the reaction 1,2-dithiane + HSRSH \( \rightarrow \) 1,4-butane-dithiole + S:RS. MacroModel V2.0 is available from Prof. W. C. Still et al., Department of Chemistry, Columbia University, New York.

Degenerate Intermolecular Thiolate–Disulfide Interchange

Table II. X-ray Crystallographic Data for Selected Disulfides

<table>
<thead>
<tr>
<th>disulfide</th>
<th>ring size</th>
<th>CSSC dihedral</th>
<th>CSS$'$ bond length</th>
<th>ref</th>
</tr>
</thead>
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<tr>
<td>$\text{N,N'-digenlyl-t-cystine}$</td>
<td></td>
<td>84</td>
<td>103</td>
<td>2.04</td>
</tr>
<tr>
<td>$\text{(Me,NCH}_2\text{CH}_2\text{SH})_2$</td>
<td></td>
<td>82</td>
<td>103</td>
<td>2.04</td>
</tr>
<tr>
<td>$\text{covalent-Cys-Gly-Pro-Phe}_2$</td>
<td>6</td>
<td>92</td>
<td>96</td>
<td>2.10</td>
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<td>$\text{covalent-Cys-Gly-Pro-Phe}_2$</td>
<td>7</td>
<td>93</td>
<td>96</td>
<td>2.05</td>
</tr>
<tr>
<td>$\text{(HOOCCH}_2\text{CH}_2\text{SH})_2$</td>
<td>6</td>
<td>27</td>
<td>99</td>
<td>2.03</td>
</tr>
<tr>
<td>$\text{(Me}_2\text{NCH}_2\text{CH}_2\text{SH})_2$</td>
<td>6</td>
<td>94</td>
<td>99</td>
<td>2.03</td>
</tr>
<tr>
<td>$\text{cyclo(Cys-Gly-Pro-Phe)_2}$</td>
<td>17</td>
<td>89</td>
<td>104</td>
<td>2.03</td>
</tr>
</tbody>
</table>


1,2-dithiolane than by noncyclic cystine.

Rationalization for the Evolutionary Selection of Lipoamide in 2-Oxo Acid Dehydrogenases. Lipoic acid is a cofactor of the 2-oxo acid dehydrogenase complexes, which catalyze the oxidative decarboxylation of 2-oxo acids and the formation of the corresponding acyl-CoA and of NADH (Figure 4). The pyruvate dehydrogenase complex comprises three enzymes—pyruvate decarboxylase (E1p), dihydrolipoamide acetyltransferase (E2p), and dihydrolipoamide dehydrogenase (E3p). The E2p chain of Escherichia coli has three lipoyl domains, which are linked to each other by highly mobile peptide chains. The distances between the enzymes in the complex have been shown by fluorescence-transfer measurements to be long and cannot be traversed by a single lipoyl arm. It has been suggested that facile acyl transfer and redox coupling (by thiolate–disulfide interchange) among the lipoyl arms help to coordinate the functions of the three enzymes and to maintain high flux through this important enzyme system.

During the enzymatic cycle the disulfide ring of lipoamide is formed or cleaved at three stages: (i) hydroxyethylthiamine pyrophosphate (the cofactor of pyruvate decarboxylase) reacts with lipoamide and transfers the acetyl group to reduced lipoamide, (ii) the lipoyl arms are involved in thiolute–disulfide interchange; (iii) a reduced lipoamide close to the dihydrolipoamide dehydrogenase is oxidized to regenerate lipoamide. The extrapolated rate constant for thiolate–disulfide interchange between 1,2-dithiolane and 1,2-dithiole in water is $10^4 \text{ M}^{-1} \text{s}^{-1}$ (Figure 2). The value of the first thiol $pK_a$, of 1,3-propanedithiol is, however, 10, and only a small fraction of reduced lipoamide would be present as thiole anion. The rate of thiol–disulfide interchange at pH 7 would thus be slow ($\sim 10^4 \text{ M}^{-1} \text{s}^{-1}$) even for the 1,2-dithiolane system; rates for 1,2-dithianes or 1,2-dithiole will be even slower (by a factor of $\sim 650$). The evolutionary selection of lipoamide in 2-oxo acid dehydrogenases may thus be due to the significantly faster rates for thiolate–disulfide interchange than for those involving cyclic five-membered disulfides.

Lipoic acid is ubiquitous in nature. Halophilic archaeobacteria have dihydrolipoamide dehydrogenase activity but lack
the 2-oxo acid dehydrogenase multienzyme complexes. In these organisms, the reduced lipoamide may be involved in another high-flux metabolic pathway; that is, sulfate reduction (by reaction of reduced lipoamide with 3'-phosphoadenosine 5'-phosphosulfate, PAPS) (Figure 5). The large effective molarity for the attack of the second thiol group of lipoamide on the thiosulfate, and the rapid regeneration of reduced lipoamide from oxidized lipoamide by dihydrolipoamide dehydrogenase, may help in maintaining the flux through these coupled reactions.

Conclusions

The rate for thiolate-disulfide interchange of 1,2-dithiolane is only a factor of approximately 10^9 slower than the diffusion-limited rate in DMSO; the theoretical value of diffusion limit for the second-order rate constant in DMSO is \( k = 3 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \). It may be possible to increase the rates for thiolate-disulfide interchange further by introducing more strain into the ground state of the cyclic disulfide, by using a solvent in which the thiolate is less solvated, or by making the geometry of the ground state resemble more closely that of the transition state. The strategy of increasing ring strain in the ground state of 1,2-dithiolane may, however, be limited by polymerization of the disulfide. Attempts to lower the solvation of the thiolate by changing the solvent may lead to the problems of ion pairing and insolubility. We do not know the relative contributions of dielectric constant and hydrogen bonding to destabilizing the thiolate in going from DMSO to DMSO.

Experimental Section

General Methods. \( ^{1} \)H NMR spectra were recorded with a Bruker AM-500 spectrometer. Argon was deoxygenated and dried by passing over a mixture of copper(II) nitrate and molecular sieves before use. Distilled water from a Corning AG-1b still was used to wash all glassware.

Materials. Aldrich supplied 1,3-propanedithiol, 1,4-butanedithiol, 1,5-pentanedithiol, 4,5-dihydroxy-1,2-dithiane, 5,5'-dithiobis(2-nitrobenzoic acid) and potassium tert-butoxide. 1,4-Dithioerythritol was purchased from Fluka. DMSO-d6 and D2O were purchased from MSD Isotopes. 2-(Mercaptomethyl)-3-mercaptop-1-propene was prepared from 2-(hydroxymethyl)-1-propen-3-ol by a literature procedure.

Calculation of Rate Constants for Thiolate-Disulfide Interchange by Dynamic \( ^{1} \)H NMR Line-Shape Analysis. In the degenerate thiolate-disulfide interchange involving \( \alpha \)-dithiols and cyclic disulfides (eq 4), the intermolecular rate constant, \( k_{\text{int}} \), is the rate-determining step (eq 5).

\[
\begin{align*}
\text{S-S} & \rightarrow \text{S-S} \\
\text{S-S} & \rightarrow \text{S-S} \\
\text{S-S} & \rightarrow \text{S-S} \\
\text{S-S} & \rightarrow \text{S-S}
\end{align*}
\]

\[
k_{\text{int}} = \frac{k_{\text{dithiolate}}}{} \]

(54) DNMR, written by Prof. C. H. Bushweller et al. (Program No. 466) is available from the Quantum Chemistry Program Exchange, Department of Chemistry, Indiana University.

(55) The \( ^{1} \)H NMR chemical shifts of 2,7-dithiaoctane in 33 mol % D2O-DMSO-d6 are 2.43 (t, CH3), 2.13 (s, CH3S), and 1.56 (br, C112).


(5) The equilibrium constant for the reduction of NAD+ by reduced lipoamide is 0.088 in water at pH 7.8, 30 °C.

(57) The theoretical value of the rate constant for a diffusion-limited second-order reaction is \( k = 3 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) in DMSO (\( \eta = 19.8 \text{ mPa} \cdot \text{s} \)) and \( 6.5 \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) in water (\( \eta = 10.1 \text{ mPa} \cdot \text{s} \)) by using the equation \( k = \frac{8RT}{30000} \) (Gordon, A. J.; Ford, R. A. The Chemist's Companion; Wiley: New York, 1972). The rate constants for the displacement reaction of thiol radicals with disulfides (RS' + RSSR) are also lower than the diffusion-limited rate constants; the values of the rate constants are 3.8 \( \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) (R = CH3) and 7.7 \( \times 10^9 \text{ M}^{-1} \text{s}^{-1} \) (R = cysteine); Bonifacic, M.; Asmus, K.-D. J. Phys. Chem. 1984, 88, 6286-6290.

(53) Thiolates are oxidized rapidly by oxygen; the oxidation is catalyzed by metal ions. In the degenerate thiolate-disulfide interchange involving \( \alpha \)-dithiols and cyclic disulfides (eq 4), the intermolecular rate constant, \( k_{\text{int}} \), is the rate-determining step (eq 5).

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(51) The equilibrium constant for the reduction of NAD+ by reduced lipoamide is 0.088 in water at pH 7.8, 30 °C.
Degenerate Intermolecular Thiolate-Disulfide Interchange

1,2-dithiolane. The oxidation of 1,3-propanedithiolate to 1,2-dithiolane was, however, a more severe problem in the transfer of a stock solution of dithiolate and disulfide to the NMR tube than in the transfer of a stock solution of dithiol and disulfide. In order to ensure the absence of polymeric forms, the solution was quenched with methyl iodide; the 1H NMR spectrum showed only monomeric 1,2-dithiolane and 2,6-dithiaheptane.\(^{56}\) The procedure for preparing samples of 4-exo-methylene-1,2-dithiolate and potassium 2-methylene-1,3-propanedithiolate in DMSO-\(d_6\) was similar. Exchange broadening was seen in the 1H NMR spectra; the quenching of the samples by methyl iodide, however, resulted in complex 1H NMR spectra, presumably due to polymerization. The sample of 4-exo-methylene-1,2-dithiolate and potassium 2-methylene-1,3-propanedithiolate (5 mM) in 50 mol % D\(2\)O-DMSO-\(d_6\) mixture was colorless and not pale, unlike the typical solutions of 1,2-dithiolane, which are pale; we infer that this solution contained oligomeric disulfides. The sample of 4-exo-methylene-1,2-dithiolate and potassium 2-methylene-1,3-propanedithiolate (5 mM) in DMSO-\(d_6\) was pale in color.

Preparation of a Sample Containing Potassium 1,5-Pentanediol and 1,2-Dithiopane in DMSO-\(d_6\), for Dynamic NMR Spectroscopy: Representative Procedure. Potassium tert-butoxide (0.0153 g, 0.136 mmol) was transferred into a flask in the glovebox, and deoxygenated DMSO-\(d_6\) (0.068 mL) was added to prepare a 200 mM stock solution of potassium tert-butoxide. 1,2-Dithiopane (0.0067 g, 0.050 mmol) and 1,5-pentanediol (8.0 \(\mu\)L, 0.060 mM) were placed in another flask, to this flask were added DMSO-\(d_6\) (1.0 mL) and a 0.50 \(\mu\)L aliquot of the stock solution of potassium tert-butoxide (0.10 mmol), and the suspension was sonicated for 15 min until the polymeric 1,2-dithiopane dissolved. To the NMR tube were added deoxygenated DMSO-\(d_6\) (425 \(\mu\)L) and a 75-\(\mu\)L aliquot of the stock solution of 1,5-pentanediol and 1,2-dithiopane (2.5 mmol). The solution in the NMR tube was 5 mM in 1,5-pentanediol and 1,2-dithiopane.

1,2-Dithiane.\(^{57}\) To a solution of 1,4-butanedithiol (5.0 mL, 43 mmol) in DMSO (85 mL) was added concentrated HCl (3.5 mL of a 37 wt % aqueous solution, 43 mmol). The solution was stirred at room temperature for 2 days. The reaction mixture was poured into an ice-water mixture (1 L) with vigorous stirring. The mixture was extracted with methylene chloride (2 \(\times\) 100 mL). The methylene chloride layer was washed with water (2 \(\times\) 500 mL), dried (Na\(\_2\)CO\(_3\)), and concentrated at reduced pressure to yield a yellow liquid (0.088 g, 67%). 1H NMR (CDCl\(_3\)) \(\delta\) 5.14 (s, 2 H), 3.71 (s, 3 H). The liquid polymerized and solidified on standing at room temperature for 2–3 h. Anal. Caled for C\(_4\)H\(_8\)S\(_2\): C 44.73; H 7.51. Found: C 44.63; H 7.56.

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(56) The 1H NMR chemical shifts of 2,6-dithiaheptane in 33 mol % D\(2\)O-DMSO-\(d_6\) are \(\delta\) 2.49 (CH\(_2\)S overlap with DMSO peak), 2.13 (s, CH\(_3\)S), and 1.73 (quint, CH\(_2\)). A concentrated solution of 1,3-propanedithiolate and 1,2-dithiolane (53 mM) in 50 mol % DMSO-\(d_6\)-toluene-\(d_8\) showed oligomeric dithiolate and 1,2-dithiolane in ratio of 1:1.5, when quenched with methyl iodide; the 1H NMR chemical shifts of bis(4-thiapentyl) disulfide are \(\delta\) 2.80 (br, CH\(_2\)SS), 2.54 (br, CH\(_2\)SCH\(_2\)), 2.08 (s, CH\(_2\)S), and 1.96 (br, CH\(_2\)).


1,2-Dithiolane. To a solution of 1,3-propanedithiol (1.25 g, 12 mmol) in DMSO (25 mL) was added concentrated HCl (1.0 mL of a 37 wt % aqueous solution, 12 mmol) and the mixture was stirred at room temperature for 15 h. The reaction mixture was poured into an ice-water mixture (200 mL) and filtered. The residue was repeatedly washed with water and was dried to yield white powder (0.93 g, 76%). Anal. Caled for C\(_4\)H\(_8\)S\(_2\): C 33.93; H 5.69. Found: C 34.39; H 5.69. A solution of the monomeric 1,2-dithiolane (25 mM) was prepared by sonication of a suspension of solid 1,2-dithiolane polymer with KCN (5 mol %) in DMSO-\(d_6\). 1H NMR (CDCl\(_3\)) \(\delta\) 3.10 (t, 2 H, J = 6.5 Hz), 2.20 (quint, 2 H, J = 6.5 Hz).

4-exo-Methylene-1,2-Dithiolane. To a solution of 2-(mercapto-methyl)-3-mercapto-1-propene\(^{59}\) (0.134 g, 1.11 mmol) in methylene chloride (50 mL) in an ice bath was added with stirring a solution of 5.5'-dithiobis(2-nitrobenzoic acid) (Filmar's reagent) (0.463 g, 1.17 mmol) in cold 10 wt % aqueous K\(_2\)CO\(_3\) solution (100 mL). The red reaction mixture was stirred in an ice bath for 1 h. The methylene chloride layer was separated, washed with water (100 mL), dried (MgSO\(_4\)), and concentrated at reduced pressure to yield a yellow liquid (0.088 g, 67%). 1H NMR (CDCl\(_3\)) \(\delta\) 5.14 (s, 2 H), 3.71 (s, 3 H). The liquid polymerized and solidified on standing at room temperature for 2–3 h. Anal. Caled for C\(_4\)H\(_8\)S\(_2\): C 40.64; H 5.12. Found: C 40.61; H 5.01.

Determinations of the Rate Constant for Thiol-Disulfide Interchange of 1,4-Dithioerythritol and trans-4,5-Dihydroxy-1,2-dithiane in D\(_2\)O. D\(_2\)O buffer (pD 7.7, 50 mM in phosphate) was deoxygenated by bubbling argon through it for 1 h. A stock solution of 1,4-dithioerythritol (20 mM) was prepared in a flask by deoxygenated D\(_2\)O buffer (1.0 mL) to 1,4-dithioerythritol (0.0031 g, 0.020 mmol). A stock solution of trans-4,5-dihydroxy-1,2-dithiane (20 mM) was prepared in another flask by adding deoxygenated D\(_2\)O buffer (1.0 mL) to trans-4,5-dihydroxy-1,2-dithiane (0.0030 g, 0.020 mmol). A NMR tube was added 250 \(\mu\)L of the stock solution of trans-4,5-dihydroxy-1,2-dithiane. The stock solution of 1,4-dithioerythritol (250 \(\mu\)L) was added to the NMR tube and the stop watch was started. The reaction was quenched after 1 min by addition of D\(_2\)O (20 \(\mu\)L of a 12 wt % solution in D\(_2\)O). The initial values of concentration of the NMR tubes were 1,4-dithioerythritol = trans-4,5-dihydroxy-1,2-dithiane = 0 M. The rate of reaction was determined by integration of 1,4-dithioerythritol and 1,4-dithioerythritol in the 1H NMR spectrum at -1.9 ppm. The molar rate constant \(k_{94}\) for thiol-disulfide interchange was therefore 1.5 \(M^{-1}\) s\(^{-1}\). The second-order rate constant for thiolate-disulfide interchange was calculated as 4.9 M\(^{-1}\) s\(^{-1}\) \((k_{94} = k_{14} = 1.0\times10^7 M^{-1} s^{-1})\).