

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

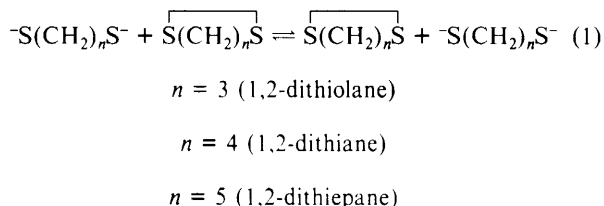
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**Abstract:** The rate constants for degenerate intermolecular thiolate–disulfide interchange involving 1,2-dithiolane ( $\overline{\text{S}(\text{CH}_2)_3\text{S}}$ ) are higher than those involving 1,2-dithiane ( $\overline{\text{S}(\text{CH}_2)_4\text{S}}$ ) by a factor of  $\sim 650$  in mixtures of DMSO- $d_6$  and  $\text{D}_2\text{O}$ . The extrapolated rate constant for 1,2-dithiolane in DMSO- $d_6$  is fast ( $k \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ). 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  $^1\text{H}$  NMR line-shape analysis. The evolutionary selection of lipoamide 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 dithiolates in mixtures of DMSO- $d_6$  and  $\text{D}_2\text{O}$  (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 of a cyclic six-membered disulfide (1,2-dithiane) and of a cyclic seven-membered disulfide (1,2-dithiepane) and to establish the magnitude of the difference in rate.<sup>1,2</sup> We hoped this observation might help to rationalize the evolutionary selection in 2-oxo acid dehydrogenases of lipoamide with its 1,2-dithiolane moiety as cofactor rather than of a cofactor with a 1,2-dithiane or a 1,2-dithiepane group. Second, we wished to test a prediction of previous work<sup>3</sup> that the rate of degenerate thiolate–disulfide interchange of 1,2-dithiolane in DMSO should be fast relative to the rates of interchange of noncyclic disulfides in water, because the rates of self-exchange reactions of 1,2-dithiolane would benefit both from destabilization of the ground state of the cyclic five-membered disulfide relative to the transition state by ring strain and from destabilization of the ground state of thiolate anion in DMSO due to energetically less favorable solvation in DMSO than in water.

Thiol–disulfide interchange is of broad importance in biochemistry.<sup>4–10</sup> The mechanism of the reaction involves the nu-

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

| mol % $\text{D}_2\text{O}$ | $10^{-3}k$ |         |         | $k_{n=3}/k_{n=4}$ |
|----------------------------|------------|---------|---------|-------------------|
|                            | $n = 3$    | $n = 4$ | $n = 5$ |                   |
| 0                          |            |         | 65      |                   |
| 10                         |            | 90      | 19      |                   |
| 15                         |            | 58      |         |                   |
| 20                         | 13000      | 22      |         | 590               |
| 25                         |            | 18      |         |                   |
| 30                         | 5500       | 8.8     |         | 630               |
| 33                         | 3600       | 5.1     |         | 710               |
| 35                         |            | 5.0     |         |                   |
| 40                         | 2100       | 3.2     |         | 660               |
| 50                         | 930        |         |         |                   |
| 60                         | 300        |         |         |                   |

<sup>a</sup> The uncertainties in  $k$  are  $\pm 10\%$ . 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.

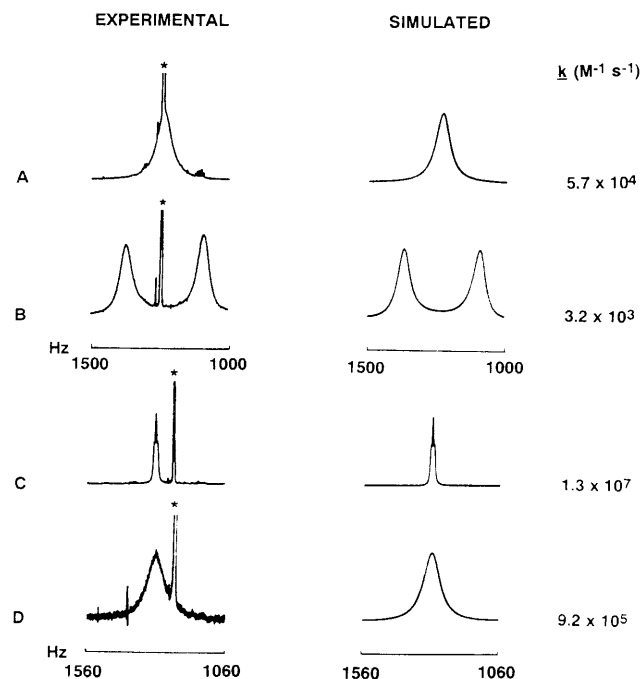
cleophilic attack of thiolate anion along the S–S bond axis of the disulfide.<sup>11</sup> The reaction is kinetically second order: first order in thiolate and in disulfide. The rate constants follow a Brønsted relationship in the values of  $\text{p}K_a$  of both the nucleophilic thiol and of the thiol being displaced.<sup>12–19</sup>

## Methods

The rate constants for degenerate thiolate–disulfide interchange of samples of dithiolate and disulfide in mixtures of DMSO- $d_6$  and  $\text{D}_2\text{O}$  were determined by  $^1\text{H}$  NMR line-shape analysis. Typical  $^1\text{H}$  NMR spectra are shown in Figure 1. The accuracy of this line-shape analysis<sup>20–24</sup> was aided by two factors: (i) The rate of the reaction, and width

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**Figure 1.** Representative experimental and calculated line shapes for the methylene protons adjacent to sulfur in 500-MHz  $^1\text{H}$  NMR spectra of (A) potassium 1,4-butanedithiolate and 1,2-dithiane (46 mM) in a solvent mixture consisting of 15 mol %  $\text{D}_2\text{O}$  in  $\text{DMSO}-d_6$ ; (B) potassium 1,4-butanedithiolate and 1,2-dithiane (41 mM) in 40 mol %  $\text{D}_2\text{O}$ - $\text{DMSO}-d_6$ ; (C) potassium 1,3-propanedithiolate and 1,2-dithiolane (5.6 mM) in 20 mol %  $\text{D}_2\text{O}$ - $\text{DMSO}-d_6$ ; (D) potassium 1,3-propanedithiolate and 1,2-dithiolane (4.9 mM) in 50 mol %  $\text{D}_2\text{O}$ - $\text{DMSO}-d_6$ . The peak due to  $^1\text{H}$  in the DMSO is marked with an asterisk.

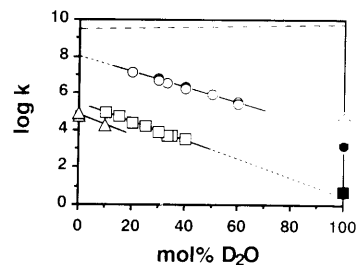
of the lines, could be adjusted over a range by adjusting the concentration of dithiolate and disulfide; the reaction is second order and line widths depend on the concentration of both dithiolate and disulfide. The line-shape analysis is more accurate for exchange-broadened lines than for narrow lines. (ii) The difference in chemical shifts between the methylene protons adjacent to sulfur in the dithiolate and disulfide is large (280–440 Hz at 500 MHz).

The range of mixtures of solvents studied (Table I) was dictated by two constraints: (i) insolubility or polymerization in samples of dithiolates and disulfides at the concentrations (>50 mM) required to obtain broad  $^1\text{H}$  NMR lines in mixtures of  $\text{D}_2\text{O}$  and  $\text{DMSO}-d_6$  containing more than 60 mol %  $\text{D}_2\text{O}$ ; and (ii) oxidation of thiolates at the concentrations (<1 mM) required to obtain broad lines for a sample of 1,3-propanedithiolate and 1,2-dithiolane in 100 mol %  $\text{DMSO}-d_6$ .

The samples of dithiolate and disulfide were prepared by addition of 2 equiv of potassium *tert*-butoxide to a mixture of dithiol and disulfide in  $\text{DMSO}-d_6$ - $\text{D}_2\text{O}$ . The strained cyclic disulfides (1,2-dithiolane and 1,2-dithiepane) are polymeric in neat form, but are monomeric in dilute solutions. The  $^1\text{H}$  NMR line shapes were indistinguishable on addition of 2, 4, or 6 equiv (10, 20, or 30 mM, respectively) of potassium *tert*-butoxide to a solution of 1,3-propanedithiol and 1,2-dithiolane (5 mM) in 50 mol %  $\text{D}_2\text{O}$ - $\text{DMSO}-d_6$ ; we infer that the dissociation of dithiol to dithiolate is essentially complete after addition of 2 equiv of potassium *tert*-butoxide to the dithiol in 50 mol %  $\text{D}_2\text{O}$ - $\text{DMSO}-d_6$ . The oxidation of dithiolate to disulfide was prevented most successfully by addition of the base (potassium *tert*-butoxide) to the mixture of dithiol and disulfide in the NMR tube under argon.

## Results and Discussion

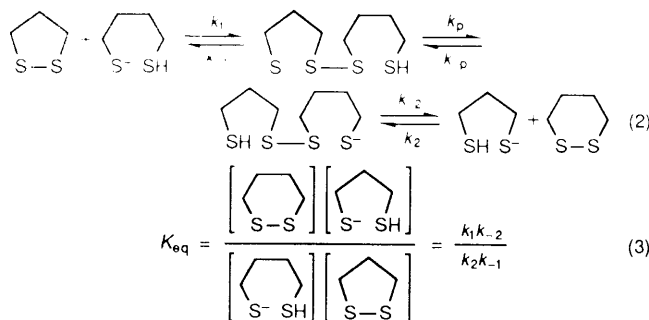
**Kinetic Data.** Table I summarizes the values for rate constants ( $k$ ) of degenerate thiolate–disulfide interchange reactions of cyclic disulfides in mixtures of  $\text{D}_2\text{O}$  and  $\text{DMSO}-d_6$ . The rate constant for 1,2-dithiolane is higher than that of 1,2-dithiane by a factor of  $\sim 650$ .<sup>25–27</sup> The rate constant of 1,2-dithiane is higher than



**Figure 2.** Effect of changing the mole fraction of  $\text{D}_2\text{O}$  on the second-order rate constant ( $k$ ,  $\text{M}^{-1} \text{s}^{-1}$ ) of thiolate–disulfide interchange of potassium 1,3-propanedithiolate and 1,2-dithiolane (O), potassium 1,4-butanedithiolate and 1,2-dithiane (□), and potassium 1,5-pentanedithiolate and 1,2-dithiepane (Δ) in mixtures of  $\text{DMSO}-d_6$  and  $\text{D}_2\text{O}$  at 297 K. The rate constant for the thiolate–disulfide interchange of dithioerythritol and oxidized dithiothreitol in  $\text{D}_2\text{O}$  at 297 K is indicated by ■. The lower limit of the rate constant for the interchange of lipoic acid and 2-carboxy-1,3-propanedithiol in  $\text{D}_2\text{O}$  is indicated by ●.<sup>35</sup> The line (---) represents approximately the diffusion-limited rate constant in this medium.

of 1,2-dithiepane by a factor of  $\sim 5$ . The rate constants for 1,2-dithiane and 1,2-dithiepane are comparable (within a factor of  $\sim 2$ ) to that for the noncyclic dibutyl disulfide. The value of the rate constant for thiolate–disulfide interchange of potassium 1-butanethiolate and dibutyl disulfide in  $\text{DMSO}-d_6$  is  $54 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>3</sup> This value must be multiplied by 2 for it to be comparable to the rate constants for interchange between dithiolates and cyclic disulfides to account for the presence of two symmetry-equivalent thiolate groups in dithiolate.<sup>28</sup>

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.<sup>29</sup>



We expect the ratio  $k_1/k_2$  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<sup>1</sup> has reported that the rate of reduction of lipoic acid by dithiothreitol in water ( $v = 56 \text{ M}^{-1} \text{ s}^{-1}$ ;  $k = 230 \text{ M}^{-1} \text{ s}^{-1}$ ) is 160-fold greater than expected by the Brønsted relationship for unstrained linear disulfides ( $v_{\text{predicted}} = 0.35 \text{ M}^{-1} \text{ s}^{-1}$ ). Fava et al.<sup>2</sup> have reported that the rate constant of the cleavage of 1,2-dithiolane by 1-buthylthiolate in methanol ( $k = 1400 \text{ M}^{-1} \text{ s}^{-1}$ ) is  $\sim 5400$  times higher than for the cleavage of dibutyl disulfide ( $k = 0.26 \text{ M}^{-1} \text{ s}^{-1}$ ). Both of these studies are indirect and we believe our data give a more direct measure of the rate constants for thiolate–disulfide interchange of cyclic disulfides.

(26) The rate constants for two samples of 1,4-butanedithiolate/1,2-dithiane 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-dithiane with 1 equiv of potassium *tert*-butoxide were lower by a factor of  $\sim 3$ –4 than those with 2 equiv of base; for 1 equiv of potassium *tert*-butoxide present,  $k = 16 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  in 15 mol %  $\text{D}_2\text{O}$ , and  $k = 5.3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  in 25 mol %  $\text{D}_2\text{O}$ . The difference between the observed factor of 3–4 and the expected factor of 2 may reflect stabilization of the  $^-\text{S}(\text{CH}_2)_2\text{SH}$  by intramolecular H bonding, or enhanced nucleophilicity for the dianion.

(28) The rate constants for dithiolate–disulfide interchange should be divided by 4 to be directly comparable to reactions in which a single thiolate attacks a single electrophilic center (e.g.,  $\text{CH}_3\text{S}^- + \text{CH}_3\text{Br} \rightarrow \text{CH}_3\text{SCH}_3 + \text{Br}^-$ ).

(29) Equation 3 is based on the assumptions that the first  $\text{pK}_a$  of 1,3-propanedithiol and 1,4-butanedithiol are equal, and that the proton-transfer steps are fast and equal in both directions ( $k_p = k_{-p}$ ).

(22) Binsch, G. *Top. Stereochem.* **1968**, *3*, 97–192.

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(24) Pleasants, J. C.; Guo, W.; Rabenstein, D. L. *J. Am. Chem. Soc.* **1989**, *111*, 6553–6558.

stant ( $K_{eq}$ ) for the thiol–disulfide interchange of 1,4-butanedithiol and 1,2-dithiolane (eqs 2 and 3) in DMSO is  $\sim 30$ .<sup>30,31</sup> Thus (eq 3)  $k_{-1} \sim 20 k_{-2}$ . At first glance, this conclusion is surprising, since  $k_{-1}$  is making a strained dithiolane ring, and  $k_{-2}$  an unstrained dithiane ring. We suggest later, however, that the ring strain in the dithiolane 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.<sup>32</sup> In the analogous ring-closure reactions of *o*-( $\omega$ -bromoalkyl)phenoxide, the five-membered cyclic ether is formed 20 times faster than the six-membered one.<sup>33</sup> The effective molarity for an intramolecular thiol–disulfide interchange reaction involving formation of six-membered oxidized dithiothreitol is estimated as  $10^4$  M, and for the formation of two cystine bonds in bovine pancreatic trypsin inhibitor as  $10^3$  and  $10^7$  M.<sup>34</sup>

**Plots of  $\log k$  vs Mole Percent D<sub>2</sub>O Are Linear.** For thiolate–disulfide interchange in mixtures of DMSO-*d*<sub>6</sub> and D<sub>2</sub>O, the plots of  $\log k$  vs mole percent D<sub>2</sub>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).<sup>3</sup> The rate constant for thiolate–disulfide interchange between oxidized dithiothreitol and dithioerythritol in D<sub>2</sub>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<sub>2</sub>O is linear over the entire solvent range.<sup>35</sup> The relative rate constants reported in Table I would hold approximately for the entire range of mixtures from DMSO-*d*<sub>6</sub> to D<sub>2</sub>O. The rate constant of degenerate thiolate–disulfide interchange involving 1,2-dithiolane is expected to be  $\sim 10^3$  faster than that involving 1,2-dithiane in D<sub>2</sub>O from extrapolation of Figure 1.

**The Rate of Thiolate–Disulfide Interchange of 1,2-Dithiolane in DMSO-*d*<sub>6</sub> Is Fast.** The extrapolated rate constant ( $k \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ) for thiolate–disulfide interchange of 1,3-propanedithiolate and 1,2-dithiolane in DMSO-*d*<sub>6</sub> is fast compared to the rate constant for interchange of 1,4-butanedithiolate and 1,2-dithiane in D<sub>2</sub>O ( $k \sim 3 \text{ M}^{-1} \text{ s}^{-1}$ ). We propose that this large difference in rate 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.<sup>36,37</sup> (ii) The dithiolate is less solvated and therefore more destabilized in DMSO than in D<sub>2</sub>O; the ground state of dithiolate with its more localized charge is more strongly destabilized than is the transition state.<sup>38</sup>

**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.

(30) Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825–6836.

(31) The equilibrium constant was determined for a mixture of 1,2-dithiane and 1,3-propanedithiol in both DMSO-*d*<sub>6</sub> and equimolar DMSO-*d*<sub>6</sub>-D<sub>2</sub>O with 4 mol % potassium *tert*-butoxide by <sup>1</sup>H NMR spectroscopy after quenching with DCl; this value of the equilibrium constant (30) is similar to that reported for CD<sub>3</sub>OD–D<sub>2</sub>O (33).<sup>30</sup>

(32) Some general references on effective molarities for ring-closure reactions: Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183–278. Mandolini, L. *Adv. Phys. Org. Chem.* **1986**, *22*, 1–111. Illuminati, G.; Mandolini, L. *Acc. Chem. Res.* **1981**, *14*, 95–102. Page, M. I. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 449–459. Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678–1683.

(33) Illuminati, G.; Mandolini, L.; Masci, B. *J. Am. Chem. Soc.* **1975**, *97*, 4960–4966.

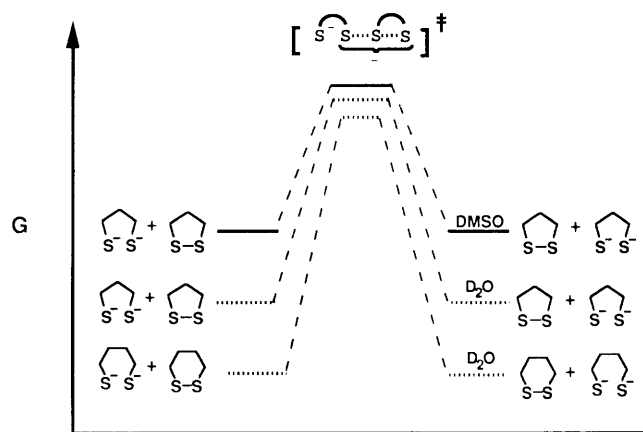
(34) Creighton, T. E. *Biopolymers* **1983**, *22*, 49–58.

(35) For the thiolate–disulfide interchange of lipoic acid and 2-carboxy-1,3-propanedithiol in D<sub>2</sub>O at 297 K, only a lower limit for the rate constant could be determined ( $k > 2000 \text{ M}^{-1} \text{ s}^{-1}$ ;  $\log k > 3.3$ ).

(36) Sunner, S. *Nature* **1955**, *176*, 217.

(37) Bartrop, J. A.; Hayes, P. M.; Calvin, M. *J. Am. Chem. Soc.* **1954**, *76*, 4348–4367.

(38) The rate constants of S<sub>N</sub>2 and S<sub>N</sub>Ar reactions involving anionic nucleophiles increase by a factor of  $10^2$ – $10^3$  on going from a protic polar to a nonprotic polar 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<sub>N</sub>2 reaction (Cl<sup>−</sup> + CH<sub>3</sub>Cl) 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.



**Figure 3.** Hypothetical plot of free energy vs reaction coordinate for thiolate–disulfide interchange of 1,3-propanedithiolate/1,2-dithiolane and 1,4-butanedithiolate 1,2-dithiane in DMSO-*d*<sub>6</sub> and in D<sub>2</sub>O.

By calorimetric measurement, the ring strain of 1,2-dithiolane is higher than that of 1,2-dithiane by 3.7 kcal/mol.<sup>36</sup> Calculation of this difference in ring strain (the steric energy,  $\Delta SE$ ) by molecular mechanics [MM2(85)] gave a value of 5.4 kcal/mol.<sup>39</sup> Correlations with experimental values of equilibrium constants indicate that values calculated by this procedure are systematically too large and suggest that a good estimate of the difference in free energy of formation is given by the empirical relationship  $\Delta G \sim 0.4 \Delta SE = 2.2 \text{ kcal/mol}$ .<sup>39</sup> By comparing the rates for thiolate–disulfide interchange of 1,2-dithiolane and 1,2-dithiane, we estimate  $\Delta \Delta G^\ddagger \sim 3.8 \text{ kcal/mol}$ . The agreement of the experimental values from calorimetry and kinetics suggests that the ring strain in the 1,2-dithiolane ring is completely released in the transition state. The rough agreement with the value estimated by semiempirical molecular mechanics calculations and after application of an empirical correction is consistent with this conclusion.

**Increase in Ring Strain of 1,2-Dithiolane Leads to Polymerization.** The ring strain of 4-*exo*-methylene-1,2-dithiolane is predicted to be  $\sim 0.7 \text{ kcal/mol}$  higher than of 1,2-dithiolane by MM2 calculations.<sup>39,40</sup> We therefore expected faster rates of degenerate thiolate–disulfide interchange reactions for 4-methylene-1,2-dithiolane than for 1,2-dithiolane. The polymer of 4-*exo*-methylene-1,2-dithiolane was more difficult to depolymerize than that of 1,2-dithiolane. We were able to prepare dilute (5 mM) homogeneous solutions of 4-*exo*-methylene-1,2-dithiolane and 2-methylene-1,3-propanedithiolate in DMSO-*d*<sub>6</sub>, and we infer that the rate constant for thiolate–disulfide interchange is  $\sim 4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  by using line-shape analysis. We cannot, however, rule out the possibility of oligomerization, and this rate constant value should therefore be taken as a lower limit. The strategy of increasing the rate constant for thiolate–disulfide interchange by increasing ring strain in the disulfide may therefore not be feasible beyond a certain point, due to polymerization of the disulfide.

**Geometry of the Transition State.** Thiolate–disulfide interchange may be facilitated if the geometry of the ground state resembles that of the transition state. In the transition state, the S–S bond is predicted to be longer than in the ground state of disulfides, and the CSS angle at the central carbon must be  $\sim 90^\circ$ .<sup>40</sup> Table II compares the CSSC dihedral angle, CSS angle, and S–S bond length for cyclic disulfides. The geometry expected for the transition state is better matched by the ground state of

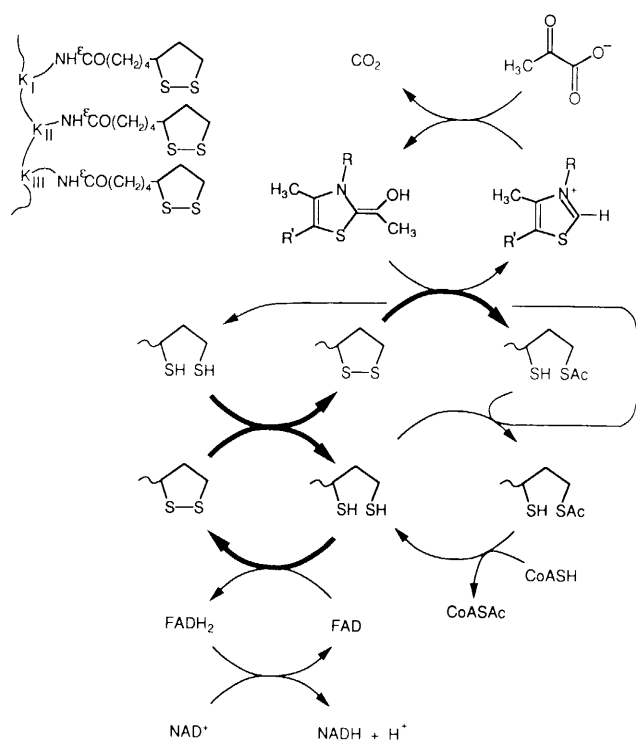
(39) The MM2 calculations were done with the program Macromodel V2.0: Burns, J. A.; Whitesides, G. M. *J. Am. Chem. Soc.*, submitted. The difference in steric energy ( $\Delta SE$ ) between the disulfide and the dithiol was calculated with reference to 1,2-dithiane [ $\Delta SE(\text{dithiane}) = 0$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

(40) Pappas, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 2926–2930. Schmidt, U.; Grafen, P.; Altland, K.; Goedde, H. W. *Adv. Enzymol.* **1969**, *32*, 423–469.

**Table II.** X-ray Crystallographic Data for Selected Disulfides

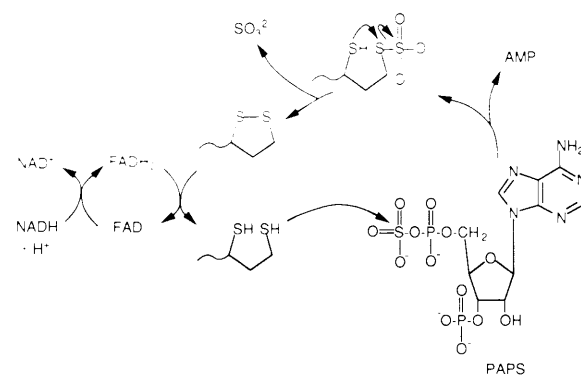
| disulfide   | ring size | angle, deg     |                  | bond length S–S, Å | ref      |
|---|-----------|----------------|------------------|--------------------|----------|
|   |           | CSSC, dihedral | CSS <sup>a</sup> |                    |          |
| <i>N,N'</i> -diglycyl-L-cystine   |           | 84             | 103              | 2.04               | <i>b</i> |
| (Me <sub>2</sub> NCH <sub>2</sub> CH <sub>2</sub> S) <sub>2</sub> ·2HCl |           | 82             | 103              | 2.04               | <i>c</i> |
| (HOOCCH <sub>2</sub> CH <sub>2</sub> S) <sub>2</sub>                    |           | 79             | 104              | 2.03               | <i>d</i> |
| L-cystine·2HCl  |           | 82             | 104              | 2.04               | <i>e</i> |
| 1,2-dithiolane-4-carboxylic acid  | 5         | 27             | 92, 96           | 2.10               | <i>f</i> |
| D,L-lipoic acid   | 5         | 35             | 93, 96           | 2.05               | <i>g</i> |
| 1 $\alpha$ ,5 $\alpha$ -epidithioandrostanediol                         | 5         | 3              | 94, 97           | 2.10               | <i>h</i> |
| 1,2-dithiane-(4 <i>R</i> ,5 <i>R</i> )-diol                             | 6         | 59             | 99               | 2.03               | <i>i</i> |
| 1,2-dithiane-3,6-dicarboxylic acid                                      | 6         | 60             | 99               | 2.07               | <i>j</i> |
| cyclo(Cys-Gly-Pro-Phe) <sub>2</sub>                                     | 17        | 89             | 104              | 2.03               | <i>k</i> |

<sup>a</sup> Two values of CSS angles are reported where those are different in each molecule. <sup>b</sup> Stallings, W. C.; Donohue, J. *Acta Crystallogr.* **1976**, *B32*, 1916–1917. <sup>c</sup> Ottersen, T.; Warner, L. G.; Seff, K. *Acta Crystallogr.* **1973**, *B29*, 2954–2958. <sup>d</sup> Prout, K.; Cassou, S. H. *Acta Crystallogr.* **1982**, *B38*, 338–340. Rao, G. V. N. A.; et al. *Acta Crystallogr.* **1982**, *B38*, 2852–2855. <sup>e</sup> Jones, D. D.; Bernal, I.; Frey, M. N.; Koetzle, T. F. *Acta Crystallogr.* **1974**, *B30*, 1220–1227. <sup>f</sup> Foss, O.; Hordvik, A.; Sletten, J. *Acta Chem. Scand.* **1966**, *20*, 1169–1171. <sup>g</sup> Stroud, R. M.; Carlisle, C. H. *Acta Crystallogr.* **1972**, *B28*, 304–307. <sup>h</sup> Neubert, L. A.; Carmack, M.; Huffman, J. C. *Acta Crystallogr.* **1977**, *B33*, 962–969. <sup>i</sup> Capasso, S.; Zagari, A. *Acta Crystallogr.* **1981**, *B37*, 1437–1439. <sup>j</sup> Foss, O.; Johnsen, K.; Reistad, T. *Acta Chem. Scand.* **1964**, *18*, 2345–2354. <sup>k</sup> Kopple, K. D.; Wang, Y. S.; Cheng, A. G.; Bhandary, K. K. *J. Am. Chem. Soc.* **1988**, *110*, 4168–4176.


**Figure 4.** Schematic diagram for the pyruvate dehydrogenase complex.

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).<sup>41</sup> 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.<sup>42,43</sup> 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.<sup>44–46</sup> It has been suggested that facile acyl


**Figure 5.** Reduction of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) by dihydrolipoamide.

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.<sup>41–46</sup>

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;<sup>47</sup> (ii) the lipoyl arms are involved in thiolate–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 1,3-propanedithiolate and 1,2-dithiolane in water is  $\sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$  (Figure 2). The value of the first thiol  $\text{pK}_a$  of 1,3-propanedithiol is, however, 10, and only a small fraction of reduced lipoamide would be present as thiolate anion. The rate of thiol–disulfide interchange at pH 7 would thus be slow ( $v \sim 10 \text{ M}^{-1} \text{ s}^{-1}$ ) even for the 1,2-dithiolane system; rates for 1,2-dithianes or 1,2-dithiepanes would 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 reactions (and perhaps of reductive acylation) involving cyclic five-membered disulfides than for those involving cyclic six- or seven-membered disulfides, and to the resulting ability of a dithiolane-derived system to maintain a high flux through the 2-oxo acid dehydrogenase complex.

Lipoic acid is ubiquitous in nature.<sup>41,48</sup> Halophilic archaeobacteria have dihydrolipoamide dehydrogenase activity but lack

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(46) Angelides, K. J.; Hammes, G. G. *Biochemistry* **1979**, *18*, 5531–5537.

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the 2-oxo acid dehydrogenase multienzyme complexes.<sup>49</sup> 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).<sup>50</sup> The large effective molarity<sup>32,34</sup> 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,<sup>51</sup> may help in maintaining the flux through these coupled reactions.

## Conclusions

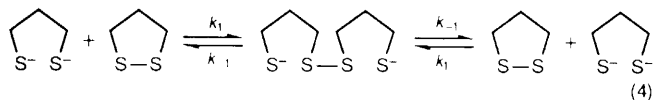
The rate for thiolate–disulfide interchange of 1,2-dithiolane is only a factor of approximately  $10^2$  slower than the diffusion-limited rate in DMSO; the theoretical value of diffusion limit for the second-order rate constant in DMSO is  $\sim 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>52</sup> 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 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  $\text{D}_2\text{O}$  to DMSO.

## Experimental Section

**General Methods.**  $^1\text{H}$  NMR spectra were recorded with a Bruker AM-500 spectrometer. Argon was deoxygenated and dried by passing over Ridox (Fisher Scientific) and molecular sieves before use. Distilled water from a Corning AG-1b still was used to wash all glassware.<sup>53</sup>

**Materials.** Aldrich supplied 1,3-propanedithiol, 1,4-butanedithiol, 1,5-pentanedithiol, *trans*-4,5-dihydroxy-1,2-dithiane, 5,5'-dithiobis(2-nitrobenzoic acid) and potassium *tert*-butoxide. 1,4-Dithioerythritol was purchased from Fluka.  $\text{DMSO-}d_6$  and  $\text{D}_2\text{O}$  were purchased from MSD Isotopes. 2-(Mercaptomethyl)-3-mercapto-1-propene was prepared from 2-(hydroxymethyl)-1-propen-3-ol by a literature procedure.<sup>30</sup>

**Calculation of Rate Constants for Thiolate–Disulfide Interchange by Dynamic  $^1\text{H}$  NMR Line-Shape Analysis.** In the degenerate thiolate–disulfide interchange involving  $\alpha,\omega$ -dithiolates and cyclic disulfides (eq 4), the intermolecular rate constant,  $k_1$ , is the rate-determining step ( $k_1$



$\gg k_1[\text{disulfide}]$ ). In dilute solutions, the oligomeric disulfide is absent and the only observable species present at equilibrium are the disulfide monomer and the dithiolate. In the degenerate thiolate–disulfide interchange, if we denote the rate of exchange seen by NMR as  $-d[\text{disulfide}]/dt$  and  $-d[\text{dithiolate}]/dt$  for the disulfide and dithiolate, respectively, we obtain  $-d[\text{disulfide}]/dt = -d[\text{dithiolate}]/dt = k_1[\text{disulfide}][\text{dithiolate}]$ . If  $\tau_{\text{disulfide}}$  and  $\tau_{\text{dithiolate}}$  are the lifetimes of the disulfide and dithiolate, we obtain  $(1/\tau_{\text{dithiolate}})[\text{dithiolate}] = (1/\tau_{\text{disulfide}})[\text{disulfide}] = k_1[\text{disulfide}][\text{dithiolate}]$ . In our experiments,  $[\text{disulfide}] = [\text{dithiolate}]$ . The second-order rate constant ( $k$ ) is therefore related to the pseudounimolecular rate constant ( $k' = 1/\tau_{\text{disulfide}} = 1/$

$\tau_{\text{dithiolate}}$ ) determined by dynamic NMR line-shape analysis:  $k = k'/[\text{dithiolate}] = k'/[\text{disulfide}]$ .

The rate constants ( $k'$ ) were determined by visual comparison of experimental and simulated  $^1\text{H}$  NMR spectra. The NMR spectra were simulated on a VAX 8600 by using the computer program DNMR4.<sup>54</sup> For the thiolate–disulfide interchange of potassium 1,3-propanedithiolate and 1,2-dithiolane, the NMR spectra of the methylene protons adjacent to sulfur were simulated by using the values  $\nu(\text{disulfide}) = 1534 \text{ Hz}$ ,  $\nu(\text{dithiolate}) = 1096 \text{ Hz}$ ,  $J = 7 \text{ Hz}$ , and  $T_2^* = 0.15 \text{ s}$ . For the thiolate–disulfide interchange of potassium 1,5-pentanedithiolate and 1,2-dithiepane, the NMR spectra of the methylene protons adjacent to sulfur were simulated by using the values  $\nu(\text{disulfide}) = 1375 \text{ Hz}$ ,  $\nu(\text{dithiolate}) = 1093 \text{ Hz}$ ,  $J = 7 \text{ Hz}$ , and  $T_2^* = 0.15 \text{ s}$ . For the thiolate–disulfide interchange of potassium 1,4-butanedithiolate and 1,2-dithiane, the NMR spectra of the methylene protons adjacent to sulfur were approximated as broad singlets, and the NMR spectra were simulated by using the values  $\nu(\text{disulfide}) = 1375 \text{ Hz}$ ,  $\nu(\text{dithiolate}) = 1093 \text{ Hz}$ , and  $T_2^* = 0.025 \text{ s}$ . For the thiolate–disulfide interchange of potassium 2-methylene-1,3-propanedithiolate and 4-*exo*-methylene-1,2-dithiolane, the NMR spectra of the methylene protons adjacent to sulfur were simulated by using the values  $\nu(\text{disulfide}) = 1869 \text{ Hz}$ ,  $\nu(\text{dithiolate}) = 1243 \text{ Hz}$ , and  $T_2^* = 0.23 \text{ s}$ . The values of  $T_2^*$  were estimated from the reciprocal of the product of  $\pi$  and the peak width (hertz) at half-height ( $T_2^* = 1/\pi\nu_{1/2}$ ). The relative populations of the disulfide and dithiolate were taken as 0.5 each. The relaxation delay between pulses (7.3 s) was significantly higher than  $T_1$ ; values of  $T_1$  for the methylene protons of dithiolate and disulfide were determined by the inversion–recovery method as  $\leq 2.2 \text{ s}$ .

**Preparation of a Sample Containing Dithiolate and Cyclic Disulfide in a Mixture of  $\text{DMSO-}d_6$  and  $\text{D}_2\text{O}$  for Dynamic NMR Spectroscopy: General Procedure.** All flasks and the NMR tube were stoppered with rubber septa and were flushed with argon before use. Gas-tight syringes were used for all transfers. In a flask,  $\text{DMSO-}d_6$  was deoxygenated by bubbling argon through it for 1 h. Deoxygenation of  $\text{DMSO-}d_6$ , either by bubbling argon through it, or by four cycles of freeze–pump–thaw was effective in preventing oxidation of the dithiolate to disulfide. After the sample had been prepared, the top of the NMR tube was sealed with paraffin wax.

**Preparation of a Sample Containing Potassium 1,4-Butanedithiolate and 1,2-Dithiane in a Mixture of  $\text{DMSO-}d_6$  and  $\text{D}_2\text{O}$  for Dynamic NMR Spectroscopy: Representative Procedure.** Potassium *tert*-butoxide (0.0800 g, 0.71 mmol) was transferred into a flask in the glovebox, and deoxygenated  $\text{DMSO-}d_6$  (1.49 mL) was added to prepare a 0.48 M stock solution of potassium *tert*-butoxide. In another flask a stock solution of 1,2-dithiane (0.0180 g, 0.15 mmol) and 1,4-butanedithiol (19  $\mu\text{L}$ , 0.16 mmol) was prepared in deoxygenated  $\text{DMSO-}d_6$  (2.5 mL). To a NMR tube were added  $\text{D}_2\text{O}$  (55  $\mu\text{L}$ ), a 400- $\mu\text{L}$  aliquot of the stock solution of 1,2-dithiane and 1,4-butanedithiol in  $\text{DMSO-}d_6$  (24  $\mu\text{mol}$ ), and a 100- $\mu\text{L}$  aliquot of the stock solution of potassium *tert*-butoxide (48  $\mu\text{mol}$ ). The  $^1\text{H}$  NMR spectrum was recorded. The solution in the NMR tube was 43 mM in both 1,4-butanedithiolate and 1,2-dithiane. The solvent mixture was 30 mol %  $\text{D}_2\text{O}$ – $\text{DMSO-}d_6$ . In order to ensure the absence of polymeric forms, the solution was quenched with methyl iodide; the  $^1\text{H}$  NMR spectrum showed only monomeric 1,2-dithiane and 2,7-dithiooctane.<sup>55</sup>

**Preparation of a Sample Containing Potassium 1,3-Propanedithiolate and 1,2-Dithiolane in a Mixture of  $\text{DMSO-}d_6$  and  $\text{D}_2\text{O}$  for Dynamic NMR Spectroscopy: Representative Procedure.** Potassium *tert*-butoxide (0.0100 g, 0.089 mmol) was transferred in the glovebox into a flask, and deoxygenated  $\text{DMSO-}d_6$  (0.30 mL) was added to prepare a 0.30 M stock solution of potassium *tert*-butoxide. To another flask containing 1,3-propanedithiol (2.8  $\mu\text{L}$ , 28  $\mu\text{mol}$ ) and polymeric 1,2-dithiolane (0.0027 g, 25  $\mu\text{mol}$ ) were added deoxygenated  $\text{DMSO-}d_6$  (1.02 mL) and a 3- $\mu\text{L}$  aliquot of the stock solution of potassium *tert*-butoxide in  $\text{DMSO-}d_6$  (0.9  $\mu\text{mol}$ ). The resulting suspension was sonicated. The suspension depolymerized and turned into a pale solution within 30 min. To a NMR tube containing deoxygenated  $\text{DMSO-}d_6$  (300  $\mu\text{L}$ ) and deoxygenated  $\text{D}_2\text{O}$  (106  $\mu\text{L}$ ) were added a 100- $\mu\text{L}$  aliquot of the stock solution of 1,3-propanedithiol and 1,2-dithiolane in  $\text{DMSO-}d_6$  (2.5  $\mu\text{mol}$ ) and a 17- $\mu\text{L}$  aliquot of the stock solution of potassium *tert*-butoxide (5.1  $\mu\text{mol}$ ). The  $^1\text{H}$  NMR spectrum was recorded. The resulting solution in the NMR tube was 4.8 mM in 1,3-propanedithiolate and 1,2-dithiolane. The solvent mixture was 50 mol %  $\text{D}_2\text{O}$ – $\text{DMSO-}d_6$ . Indistinguishable results were obtained using potassium cyanide to depolymerize 1,2-dithiolane, or by preparing a stock solution of potassium 1,3-propanedithiolate and

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(51) The equilibrium constant for the reduction of  $\text{NAD}^+$  by reduced lipoamide is 0.0858 in water at pH 7, 30  $^\circ\text{C}$ .<sup>12</sup>

(52) The theoretical value of the rate constant for a diffusion-limited second-order reaction is  $k \sim 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  in DMSO ( $\eta = 19.8 \text{ mP}$ ) and  $\sim 6.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  in water ( $\eta = 10.1 \text{ mP}$ ) by using the equation  $k = 8RT/3000\eta$ ; Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972. The rate constants for the displacement reaction of thyl radicals with disulfides ( $\text{RS}^{\cdot} + \text{RSSR}$ ) are also lower than the diffusion-limited rate constants; the values of the rate constants are  $3.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  ( $\text{R} = \text{CH}_3$ ) and  $7.7 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  ( $\text{R} = \text{cysteine}$ ); 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.<sup>10</sup>

(54) DNMR4, 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  $^1\text{H}$  NMR chemical shifts of 2,7-dithiooctane in 33 mol %  $\text{D}_2\text{O}$ – $\text{DMSO-}d_6$  are  $\delta$  2.43 (t,  $\text{CH}_2\text{S}$ ), 2.13 (s,  $\text{CH}_2\text{S}$ ), and 1.56 (br,  $\text{CH}_2$ ).

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  $^1\text{H}$  NMR spectrum showed only monomeric 1,2-dithiolane and 2,6-dithiaheptane.<sup>56</sup> The procedure for preparing samples of 4-*exo*-methylene-1,2-dithiolane and potassium 2-methylene-1,3-propanedithiolate in DMSO- $d_6$  was similar. Exchange broadening was seen in the  $^1\text{H}$  NMR spectra; the quenching of the samples by methyl iodide, however, resulted in complex  $^1\text{H}$  NMR spectra, presumably due to polymerization. The sample of 4-*exo*-methylene-1,2-dithiolane and potassium 2-methylene-1,3-propanedithiolate (5 mM) in 50 mol %  $\text{D}_2\text{O}$ –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-dithiolane 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-Pentanedithiolate and 1,2-Dithiepane 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- $d_6$  (0.68 mL) was added to prepare a 200 mM stock solution of potassium *tert*-butoxide. 1,2-Dithiepane (0.0067 g, 0.050 mmol) and 1,5-pentanedithiol (8.0  $\mu\text{L}$ , 0.060 mmol) were placed in another flask; to this flask were added DMSO- $d_6$  (1.0 mL) and a 0.50-mL 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-dithiepane dissolved. To the NMR tube were added deoxygenated DMSO- $d_6$  (425  $\mu\text{L}$ ) and a 75- $\mu\text{L}$  aliquot of the stock solution of 1,5-pentanedithiolate and 1,2-dithiepane (2.5  $\mu\text{mol}$ ). The solution in the NMR tube was 5 mM in 1,5-pentanedithiolate and 1,2-dithiepane.

**1,2-Dithiane.**<sup>57</sup> 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 ( $\text{Na}_2\text{CO}_3$ ), and concentrated at reduced pressure to yield a colorless liquid (4.4 g, 86%). By  $^1\text{H}$  NMR the crude liquid was a mixture of monomer and oligomers. The crude was distilled (30–60  $^\circ\text{C}$ , 1 Torr) and the distillate (1.5 g) was collected in a dry ice–acetone trap: mp 32  $^\circ\text{C}$  (lit.<sup>58</sup> mp 31–32  $^\circ\text{C}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.85 (s, 2 H), 1.97 (s, 2 H).

**1,2-Dithiepane.** To a solution of 1,5-pentanedithiol (2.0 mL, 15 mmol) in DMSO (35 mL) was added concentrated HCl (1.2 mL of a 37 wt % aqueous solution, 15 mmol), and the reaction mixture was stirred at room temperature for 36 h. The solution was poured into an ice–water mixture (1 L) with vigorous stirring. The mixture was extracted with methylene chloride ( $2 \times 75$  mL), dried ( $\text{Na}_2\text{CO}_3$ ), and concentrated at reduced pressure to yield a colorless liquid (2.0 g, 99%). Anal. Calcd for  $\text{C}_5\text{H}_{10}\text{S}_2$ : C, 44.73; H, 7.51. Found: C, 44.63; H, 7.56.

(56) The  $^1\text{H}$  NMR chemical shifts of 2,6-dithiaheptane in 33 mol %  $\text{D}_2\text{O}$ –DMSO- $d_6$  are  $\delta$  2.49 ( $\text{CH}_2\text{S}$ , overlaps with DMSO peak), 2.13 (s,  $\text{CH}_2\text{S}$ ), and 1.73 (quintet,  $\text{CH}_2$ ). 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  $^1\text{H}$  NMR chemical shifts of bis(4-thiapentyl) disulfide are  $\delta$  2.80 (br,  $\text{CH}_2\text{SS}$ ), 2.54 (br,  $\text{CH}_2\text{SCH}_3$ ), 2.08 (s,  $\text{CH}_3\text{S}$ ), and 1.96 (br,  $\text{CH}_2$ ).

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(58) Schoberl, A.; Grafje, H. *Justus Liebig's Ann. Chem.* **1958**, *614*, 66–83.

**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 a white powder (0.93 g, 76%). Anal. Calcd for  $\text{C}_3\text{H}_6\text{S}_2$ : 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- $d_6$ :  $^1\text{H}$  NMR  $\delta$  3.10 (t, 4 H,  $J = 6.5$  Hz), 2.20 (quintet, 2 H,  $J = 6.5$  Hz).

**4-*exo*-Methylene-1,2-Dithiolane.** To a solution of 2-(mercapto-methyl)-3-mercaptopropene<sup>59</sup> (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) (Fillman's reagent, 0.463 g, 1.17 mmol) in cold 10 wt % aqueous  $\text{KHCO}_3$  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 ( $\text{MgSO}_4$ ), and concentrated at reduced pressure to yield a yellow liquid (0.088 g, 67%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.14 (s, 2 H), 3.71 (s, 4 H). The liquid polymerized and solidified on standing at room temperature for 2–3 h. Anal. Calcd for  $\text{C}_4\text{H}_6\text{S}_2$ : C, 40.64; H, 5.12. Found: C, 40.61; H, 5.01.

**Determination of the Rate Constant for Thiol–Disulfide Interchange of 1,4-Dithioerythritol and *trans*-4,5-Dihydroxy-1,2-dithiane in  $\text{D}_2\text{O}$ .**  $\text{D}_2\text{O}$  buffer (pD 7.7,<sup>59</sup> 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 adding deoxygenated  $\text{D}_2\text{O}$  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  $\text{D}_2\text{O}$  buffer (1.0 mL) to *trans*-4,5-dihydroxy-1,2-dithiane (0.0030 g, 0.020 mmol). To an NMR tube was added 250  $\mu\text{L}$  of the stock solution of *trans*-4,5-dihydroxy-1,2-dithiane. The stock solution of 1,4-dithioerythritol (250  $\mu\text{L}$ ) was added to the NMR tube and the stopwatch was started. The reaction was quenched after 1 min by addition of DCl (20  $\mu\text{L}$  of a 12 wt % solution in  $\text{D}_2\text{O}$ ). The initial values of concentration in the NMR tube were  $[\text{1,4-dithioerythritol}] = [\textit{trans}\text{-4,5-dihydroxy-1,2-dithiane}] = 0.1$  mM. The extent of reaction was determined by integrations of peaks of 1,4-dithioerythritol and 1,4-dithioerythritol in the  $^1\text{H}$  NMR spectrum. After 1 min,  $[\text{1,4-dithioerythritol}] = [\textit{trans}\text{-4,5-dihydroxy-1,2-dithiane}] = 9.2$  mM. The apparent rate constant ( $k^{\text{app}}$  or  $\nu$ ) of thiol–disulfide interchange was therefore  $0.15$   $\text{M}^{-1} \text{s}^{-1}$ . The second-order rate constant for thiolate–disulfide interchange was calculated as  $4.9$   $\text{M}^{-1} \text{s}^{-1}$  [ $k = k^{\text{app}}(1 + 10^{\text{pA}_2 - \text{pH}})$ , and  $\text{pA}_2(\text{dithioerythritol}) = 9.2$ ]. Room temperature was 297 K.

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