Degenerate Intermolecular Thiolate–Disulfide Interchange Involving Cyclic Five-Membered Disulfides Is Faster by ~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 ($\overline{S(CH_2)_3S}$) are higher than those involving 1,2-dithiane ($\overline{S(CH_2)_4S}$) by a factor of ~650 in mixtures of DMSO-d_6 and D_2O. The extrapolated rate constant for 1,2-dithiolane in DMSO-d_6 is fast ($k \sim 10^8$ 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 ^1H 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 D_2O (eq 1). We had two objectives in this work: First, we wished

$$^-S(CH_2)_nS^- + \overline{S(CH_2)_nS} \rightleftharpoons \overline{S(CH_2)_nS} + {}^-S(CH_2)_nS^- \quad (1)$$

$n = 3$ (1,2-dithiolane)

$n = 4$ (1,2-dithiane)

$n = 5$ (1,2-dithiepane)

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.[1,2] 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[3] 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.[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 Brønsted relationship in the values of pK_a of both the nucleophilic thiol and of the thiol being displaced.[12-19]

Table I. Second-Order Rate Constants (k, M^{-1} s^{-1})[a] for Degenerate Thiolate–Disulfide Interchange for $^-S(CH_2)_nS^-/\overline{S(CH_2)_nS}$ in Mixtures of D_2O and DMSO-d_6 at 24 °C

	$10^{-3}k$			
mol % D_2O	$n = 3$	$n = 4$	$n = 5$	$k_{n=3}/k_{n=4}$
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			

[a] The uncertainties in k are ±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.

Methods

The rate constants for degenerate thiolate–disulfide interchange of samples of dithiolate and disulfide in mixtures of DMSO-d_6 and D_2O were determined by ^1H NMR line-shape analysis. Typical ^1H NMR spectra are shown in Figure 1. The accuracy of this line-shape analysis[20-24] was aided by two factors: (i) The rate of the reaction, and width

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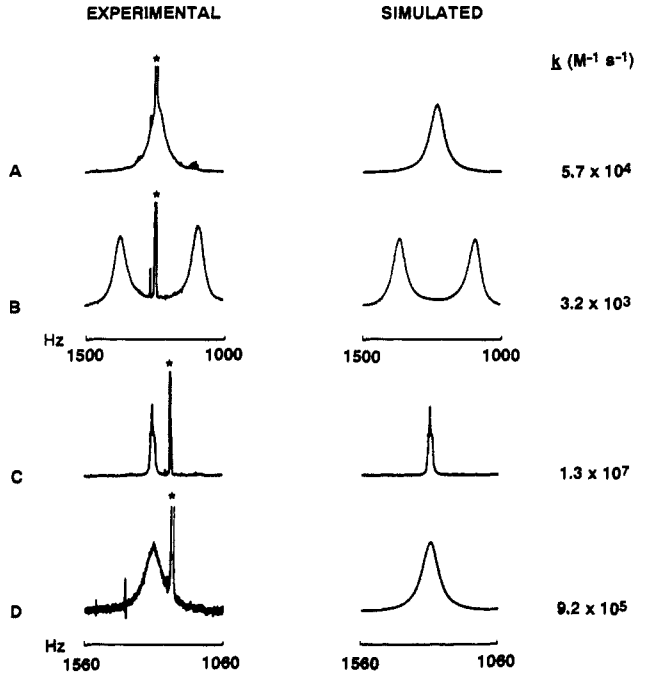


Figure 1. Representative experimental and calculated line shapes for the methylene protons adjacent to sulfur in 500-MHz ^1H NMR spectra of (A) potassium 1,4-butanedithiolate and 1,2-dithiane (46 mM) in a solvent mixture consisting of 15 mol % D_2O in DMSO-d_6; (B) potassium 1,4-butanedithiolate and 1,2-dithiane (41 mM) in 40 mol % D_2O–DMSO-d_6; (C) potassium 1,3-propanedithiolate and 1,2-dithiolane (5.6 mM) in 20 mol % D_2O–DMSO-d_6; (D) potassium 1,3-propanedithiolate and 1,2-dithiolane (4.9 mM) in 50 mol % D_2O–DMSO-d_6. The peak due to ^1H 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 lineshape 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 ^1H NMR lines in mixtures of D_2O and DMSO-d_6 containing more than 60 mol % D_2O; 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 % 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 DMSO-d_6–D_2O. The strained cyclic disulfides (1,2-dithiolane and 1,2-dithiepane) are polymeric in neat form, but are monomeric in dilute solutions. The ^1H 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 % D_2O–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 % D_2O–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 D_2O and DMSO-d_6. The rate constant for 1,2-dithiolane is higher than that of 1,2-dithiane by a factor of ~650.[25-27] The rate constant of 1,2-dithiane is higher than

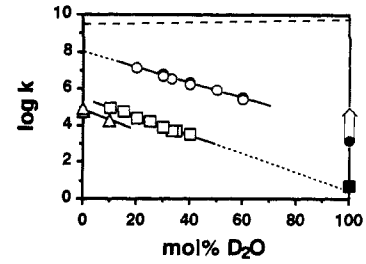
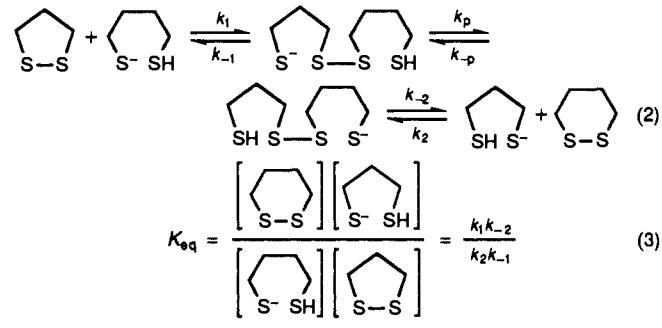


Figure 2. Effect of changing the mole fraction of D_2O on the second-order rate constant (k, M^{-1} s^{-1}) of thiolate–disulfide interchange of potassium 1,3-propanedithiolate and 1,2-dithiolane (○), potassium 1,4-butanedithiolate and 1,2-dithiane (□), and potassium 1,5-pentanedithiolate and 1,2-dithiepane (△) in mixtures of DMSO-d_6 and D_2O at 297 K. The rate constant for the thiolate–disulfide interchange of dithioerythritol and oxidized dithiothreitol in D_2O 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 D_2O is indicated by ●.[35] The line (---) represents approximately the diffusion-limited rate constant in this medium.

of 1,2-dithiepane by a factor of ~5. The rate constants for 1,2-dithiane and 1,2-dithiepane 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-butanethiolate and dibutyl disulfide in DMSO-d_6 is $k = 54 \times 10^3$ M^{-1} s^{-1}.[3] 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.[28]

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.[29]



We expect the ratio k_1/k_2 to be close to the ratio of rate constants (~650) of degenerate thiolate–disulfide interchange of cyclic five- and six-membered disulfides. The value of the equilibrium con-

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(25) Creighton[1] has reported that the rate of reduction of lipoic acid by dithiothreitol in water ($v = 56$ M^{-1} s^{-1}; $k = 230$ M^{-1} s^{-1}) is 160-fold greater than expected by the Brønsted relationship for unstrained linear disulfides ($v_{predicted} = 0.35$ M^{-1} s^{-1}). Fava et al.[2] have reported that the rate constant of the cleavage of 1,2-dithiolane by 1-butylthiolate in methanol ($k = 1400$ M^{-1} s^{-1}) is ~5400 times higher than for the cleavage of dibutyl disulfide ($k = 0.26$ M^{-1} 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 contants for samples of 1,4-butanedithiol and 1,2-dithiane 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 % D_2O, and $k = 5.3 \times 10^3$ M^{-1} s^{-1} in 25 mol % D_2O. The difference between the observed factor of 3–4 and the expected factor of 2 may reflect stabilization of the $^-S(CH_2)_4SH$ 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., $CH_3S^- + CH_3Br \rightarrow CH_3SCH_3 + Br^-$).
(29) Equation 3 is based on the assumptions that the first 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 ($k_p = k_{-p}$).

stant (K_{eq}) for the thiol–disulfide interchange of 1,4-butanedithiol and 1,2-dithiolane (eqs 2 and 3) in DMSO is ~30.[30,31] 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.[32] In the analogous ring-closure reactions of *o*-(ω-bromoalkyl)phenoxide, the five-membered cyclic ether is formed 20 times faster than the six-membered one.[33] 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.[34]

Plots of log *k* vs Mole Percent D_2O Are Linear. For thiolate–disulfide interchange in mixtures of DMSO-d_6 and D_2O, the plots of log *k* vs mole percent D_2O 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_2O ($k = 5\ M^{-1}\ s^{-1}$; 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_2O is linear over the entire solvent range.[35] The relative rate constants reported in Table I would hold approximately for the entire range of mixtures from DMSO-d_6 to D_2O. The rate constant of degenerate thiolate–disulfide interchange involving 1,2-dithiolane is expected to be ~10^3 faster than that involving 1,2-dithiane in D_2O from extrapolation of Figure 1.

The Rate of Thiolate–Disulfide Interchange of 1,2-Dithiolane in DMSO-d_6 Is Fast. The extrapolated rate constant ($k \sim 10^8\ M^{-1}\ s^{-1}$) for thiolate–disulfide interchange of 1,3-propanedithiolate and 1,2-dithiolane in DMSO-d_6 is fast compared to the rate constant for interchange of 1,4-butanedithiolate and 1,2-dithiane in D_2O ($k \sim 3\ M^{-1}\ s^{-1}$). 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.[36,37] (ii) The dithiolate is less solvated and therefore more destabilized in DMSO than in D_2O; the ground state of dithiolate with its more localized charge is more strongly destabilized than is the transition state.[38]

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.

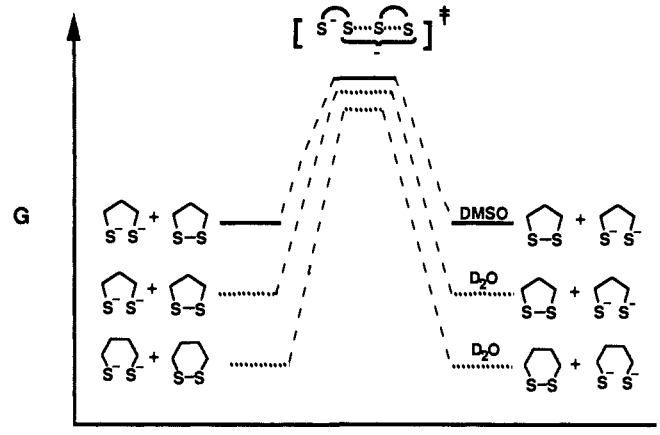

Figure 3. Hypothetical plot of free energy vs reaction coordinate for thiolate–disulfide interchange of 1,3-propanedithiolate/1,2-dithiolane and 1,4-butanedithiolate/1,2-dithiane in DMSO-d_6 and in D_2O.

By calorimetric measurement, the ring strain of 1,2-dithiolane is higher than that of 1,2-dithiane by 3.7 kcal/mol.[36] Calculation of this difference in ring strain (the steric energy, ΔSE) by molecular mechanics [MM2(85)] gave a value of 5.4 kcal/mol.[39] 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 $\Delta G \sim 0.4\ \Delta SE = 2.2$ kcal/mol.[39] By comparing the rates for thiolate–disulfide interchange of 1,2-dithiolane and 1,2-dithiane, we estimate $\Delta\Delta G^{\ddagger} \sim 3.8$ 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 ~0.7 kcal/mol higher than of 1,2-dithiolane by MM2 calculations.[30,39] 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_6, and we infer that the rate constant for thiolate–disulfide interchange is $\sim 4 \times 10^7\ M^{-1}\ s^{-1}$ 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 ~90°.[40] 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

(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_6 and equimolar DMSO-d_6–D_2O with 4 mol % potassium *tert*-butoxide by ^1H NMR spectroscopy after quenching with DCl; this value of the equilibrium constant (30) is similar to that reported for CD_3OD–D_2O (33).[30]

(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.

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(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_2O at 297 K, only a lower limit for the rate constant could be determined ($k > 2000\ M^{-1}\ s^{-1}$; log $k > 3.3$).

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(38) The rate constants of S_N2 and S_NAr reactions involving anionic nucleophiles increase by a factor of 10^2–10^7 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_N2 reaction ($Cl^- + CH_3Cl$) 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 (ΔSE) between the disulfide and the dithiol was calculated with reference to 1,2-dithiane [ΔSE(dithiane) = 0], and the free energy change was predicted for the reaction 1,2-dithiane + HSRSH → 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: CSSC, dihedral	angle, deg: CSS[a]	bond length S-S, Å	ref
N,N'-diglycyl-L-cystine		84	103	2.04	*b*
$(Me_2NCH_2CH_2S)_2 \cdot 2HCl$		82	103	2.04	*c*
$(HOOCCH_2CH_2S)_2$		79	104	2.03	*d*
L-cystine·2HCl		82	104	2.04	*e*
1,2-dithiolane-4-carboxylic acid	5	27	92, 96	2.10	*f*
D,L-lipoic acid	5	35	93, 96	2.05	*g*
1α,5α-epidithioandrostanediol	5	3	94, 97	2.10	*h*
1,2-dithiane-(4*R*,5*R*)-diol	6	59	99	2.03	*i*
1,2-dithiane-3,6-dicarboxylic acid	6	60	99	2.07	*j*
cyclo(Cys-Gly-Pro-Phe)$_2$	17	89	104	2.03	*k*

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

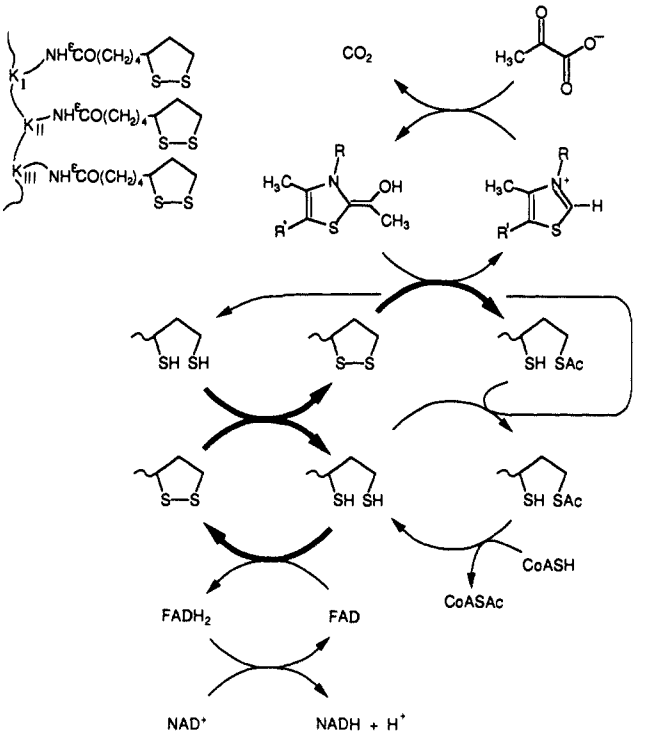

Figure 4. Schematic diagram for the pyruvate dehydrogenase complex.

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).[41] 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.[42,43] 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.[44-46] It has been suggested that facile acyl

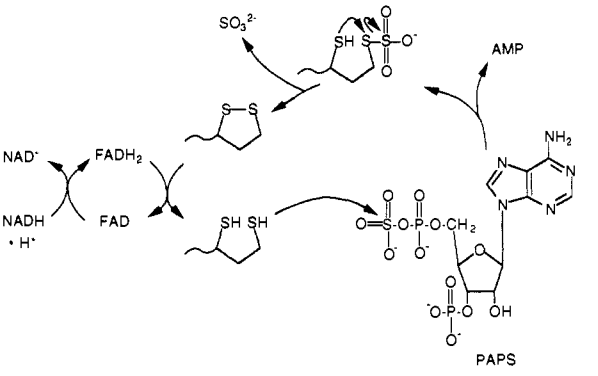

Figure 5. Reduction of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) by dihydrolipoamide.

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.[41-46]

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;[47] (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 $\sim 10^4$ M^{-1} 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 thiolate anion. The rate of thiol–disulfide interchange at pH 7 would thus be slow ($v \sim 10$ M^{-1} 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.[41,48] Halophilic archaebacteria have dihydrolipoamide dehydrogenase activity but lack

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

Conclusions

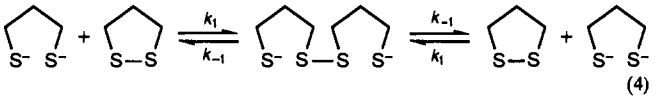
The rate for thiolate–disulfide interchange of 1,2-dithiolane is only a factor of approximately 10^2 slower than the diffusion-limited rate in DMSO; the theoretical value of diffusion limit for the second-order rate constant in DMSO is $\sim 3 \times 10^9$ M^{-1} s^{-1}.[52] It may be possible to increase 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 D_2O to DMSO.

Experimental Section

General Methods. ^1H 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.[53]

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. DMSO-d_6 and D_2O were purchased from MSD Isotopes. 2-(Mercaptomethyl)-3-mercapto-1-propene was prepared from 2-(hydroxymethyl)-1-propen-3-ol by a literature procedure.[30]

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



(4)

$\gg k_1$[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 $-d[\text{disulfide}]/dt = -d[\text{dithiolate}]/dt = k[\text{disulfide}][\text{dithiolate}]$. If $\tau_{\text{disulfide}}$ and $\tau_{\text{dithiolate}}$ are the lifetimes of the disulfide and dithiolate, we obtain $(1/\tau_{\text{dithiolate}})[\text{dithiolate}] = (1/\tau_{\text{disulfide}})[\text{disulfide}] = k[\text{disulfide}][\text{dithiolate}]$. In our experiments, [disulfide] = [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 ^1H NMR spectra. The NMR spectra were simulated on a VAX 8600 by using the computer program DNMR4.[54] 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 ν(disulfide) = 1534 Hz, ν(dithiolate) = 1096 Hz, J = 7 Hz, and T_2^* = 0.15 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 ν(disulfide) = 1375 Hz, ν(dithiolate) = 1093 Hz, J = 7 Hz, and T_2^* = 0.15 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 ν(disulfide) = 1375 Hz, ν(dithiolate) = 1093 Hz, and T_2^* = 0.025 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 ν(disulfide) = 1869 Hz, ν(dithiolate) = 1243 Hz, and T_2^* = 0.23 s. The values of T_2^* were estimated from the reciprocal of the product of π 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 ≤2.2 s.

Preparation of a Sample Containing Dithiolate and Cyclic Disulfide in a Mixture of DMSO-d_6 and D_2O 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, DMSO-d_6 was deoxygenated by bubbling argon through it for 1 h. Deoxygenation of 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 DMSO-d_6 and D_2O 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 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 μL, 0.16 mmol) was prepared in deoxygenated DMSO-d_6 (2.5 mL). To a NMR tube were added D_2O (55 μL), a 400-μL aliquot of the stock solution of 1,2-dithiane and 1,4-butanedithiol in DMSO-d_6 (24 μmol), and a 100-μL aliquot of the stock solution of potassium *tert*-butoxide (48 μmol). The ^1H 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 % D_2O–DMSO-d_6. 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-dithiane and 2,7-dithiaoctane.[55]

Preparation of a Sample Containing Potassium 1,3-Propanedithiolate and 1,2-Dithiolane in a Mixture of DMSO-d_6 and D_2O 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 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 μL, 28 μmol) and polymeric 1,2-dithiolane (0.0027 g, 25 μmol) were added deoxygenated DMSO-d_6 (1.02 mL) and a 3-μL aliquot of the stock solution of potassium *tert*-butoxide in DMSO-d_6 (0.9 μmol). The resulting suspension was sonicated. The suspension depolymerized and turned into a pale solution within 30 min. To a NMR tube containing deoxygenated DMSO-d_6 (300 μL) and deoxygenated D_2O (106 μL) were added a 100-μL aliquot of the stock solution of 1,3-propanedithiol and 1,2-dithiolane in DMSO-d_6 (2.5 μmol) and a 17-μL aliquot of the stock solution of potassium *tert*-butoxide (5.1 μmol). The ^1H 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 % D_2O–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 NAD^+ by reduced lipoamide is 0.0858 in water at pH 7, 30 °C.[12]

(52) The theoretical value of the rate constant for a diffusion-limited second-order reaction is $k \sim 3 \times 10^9$ M^{-1} s^{-1} in DMSO (η = 19.8 mP) and $\sim 6.5 \times 10^9$ M^{-1} s^{-1} in water (η = 10.1 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 thiyl radicals with disulfides (RS· + RSSR) are also lower than the diffusion-limited rate constants; the values of the rate constants are 3.8×10^6 M^{-1} s^{-1} (R = CH_3) and 7.7×10^5 M^{-1} 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.[10]

(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 ^1H NMR chemical shifts of 2,7-dithiaoctane in 33 mol % D_2O–DMSO-d_6 are δ 2.43 (t, CH_2S), 2.13 (s, CH_3S), and 1.56 (br, 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}H NMR spectrum showed only monomeric 1,2-dithiolane and 2,6-dithiaheptane.[56] 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}H NMR spectra; the quenching of the samples by methyl iodide, however, resulted in complex ^{1}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 % D_2O–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 μL, 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 μL) and a 75-μL aliquot of the stock solution of 1,5-pentanedithiolate and 1,2-dithiepane (2.5 μmol). The solution in the NMR tube was 5 mM in 1,5-pentanedithiolate and 1,2-dithiepane.

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 × 100 mL). The methylene chloride layer was washed with water (2 × 500 mL), dried (Na_2CO_3), and concentrated at reduced pressure to yield a colorless liquid (4.4 g, 86%). By ^{1}H NMR the crude liquid was a mixture of monomer and oligomers. The crude was distilled (30–60 °C, 1 Torr) and the distillate (1.5 g) was collected in a dry ice–acetone trap: mp 32 °C (lit.[58] mp 31–32 °C); ^{1}H NMR ($CDCl_3$) δ 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 × 75 mL), dried (Na_2CO_3), and concentrated at reduced pressure to yield a colorless liquid (2.0 g, 99%). Anal. Calcd for $C_5H_{10}S_2$: C, 44.73; H, 7.51. Found: C, 44.63; H, 7.56.

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 $C_3H_6S_2$: 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}H NMR δ 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-(mercaptomethyl)-3-mercapto-1-propene[30] (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) (Ellman's reagent; 0.463 g, 1.17 mmol) in cold 10 wt % aqueous $KHCO_3$ 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 ($MgSO_4$), and concentrated at reduced pressure to yield a yellow liquid (0.088 g, 67%): ^{1}H NMR ($CDCl_3$) δ 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 $C_4H_6S_2$: 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 D_2O. D_2O buffer (pD 7.7,[59] 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 D_2O 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_2O 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 μL of the stock solution of *trans*-4,5-dihydroxy-1,2-dithiane. The stock solution of 1,4-dithioerythritol (250 μL) was added to the NMR tube and the stopwatch was started. The reaction was quenched after 1 min by addition of DCl (20 μL of a 12 wt % solution in D_2O). The initial values of concentration in the NMR tube were [1,4-dithioerythritol] = [*trans*-4,5-dihydroxy-1,2-dithiane] = 10 mM. The extent of reaction was determined by integrations of peaks of 1,4-dithioerythritol and 1,4-dithiothreitol in the ^{1}H NMR spectrum. After 1 min, [1,4-dithioerythritol] = [*trans*-4,5-dihydroxy-1,2-dithiane] = 9.2 mM. The apparent rate constant (k^{app} or v) of thiol–disulfide interchange was therefore 0.15 M^{-1} s^{-1}. The second-order rate constant for thiolate–disulfide interchange was calculated as 4.9 M^{-1} s^{-1} [$k = k^{app}(1 + 10^{pK_a - pH})$, and pK_a(dithioerythritol) = 9.2]. Room temperature was 297 K.

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(56) The ^{1}H NMR chemical shifts of 2,6-dithiaheptane in 33 mol % D_2O–DMSO-d_6 are δ 2.49 (CH_2S, overlaps with DMSO peak), 2.13 (s, CH_3S), and 1.73 (quintet, 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}H NMR chemical shifts of bis(4-thiapentyl) disulfide are δ 2.80 (br, CH_2SS), 2.54 (br, CH_2SCH_3), 2.08 (s, CH_3S), and 1.96 (br, CH_2).

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(59) The pH meter reading of the D_2O buffer was corrected (pD = pH meter reading + 0.4): Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190.