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Striking changes in molecular recognition by deuteration of aqueous binary solvents

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ABSTRACT: Oxidation of a pair of associating thiols **1** and **2** with oxygen, each with a binding site [—C(═O)NHC(═O)NH—] and a recognition site (R^1 or R^2), is examined at various temperatures in aqueous binary solvents of water or deuterium oxide with ethanol, acetonitrile or methanol. The selectivity (r)—a measure of the degree of molecular recognition in the oxidation—represented by the logarithmic ratio of the yield of the unsymmetrical disulfide (**4**) to twice that of the symmetrical disulfide (**3**) was examined as a function of the mole fractions of water (x_w) and deuterium oxide (x_{do}). It is found that, on deuteration of aqueous binary solvents containing ethanol ($x_w = 0.50$ and 0.75), the temperature dependence of molecular recognition (r) alters strikingly from a clear bell-shaped type (a maximum at 35 °C) to a plateau-like type in the range 20–70 °C. It is clarified further that, in aqueous acetonitrile at $x_w = 0.20$ and 0.75, deuteration of water causes the temperature dependence of r to become fairly dull. In contrast, it is found that the deuteration effect in water–methanol is much less than that in water–ethanol or in water–acetonitrile. Furthermore, conversion of the NH groups in **1** and **2** to the ND groups occurs very quickly. A possible cause of the large deuterium effect is discussed. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: deuteration effect of aqueous binary solvents on molecular recognition; ethanol–deuterium oxide solvent; acetonitrile–deuterium oxide solvent; molecular recognition in oxidation of associating thiols; solute–solvent interaction; temperature dependence of molecular recognition; bell-shaped curve

INTRODUCTION

Specific and precise molecular recognition in aqueous media is essential to living systems. With regard to studies on molecular recognition, it is now very important to clarify the factors controlling discrimination:

(i) The steric relationship between solute molecules—this produces the most striking effects on discrimination, thus leading to specific and precise molecular recognition.[1]
(ii) Alterations in solute–solvent interactions on the deuteration.[2]
(iii) solvent–solvent interactions, especially in binary solvents of water with organic solvents capable of forming intermolecular hydrogen bonds with each other and/or with themselves, probably concerned with solvent structures.

Differences in physicochemical properties are known to be very slight between water and deuterium oxide. On the other hand, there have been reports published on differences in properties between binary H_2O and D_2O solutions: vapor pressures of tetramethylurea–H_2O and tetramethylurea–D_2O solutions;[3a] and liquid–liquid coexistence curves of re-entrant phase transitions beyond the asymptotic range: tetrahydrofuran–H_2O and tetrahydrofuran–D_2O.[3b]

There are also studies on the ability of deuterium oxide to self-associate[4] and on its effect on the association of hydrophobic polymers[5] and a protein.[6]

Molecular recognition has been reported in deuterium oxide[7] but, no reports have been published on molecular recognition in binary deuterium oxide solvents (and mixtures). In view of the significance of binary aqueous media in specific recognition,[8] studies on discrimination in binary deuterium oxide solvents should give some information on the effect of binary aqueous media on discrimination.

As a continuing study for elucidating environmental factors controlling specific recognition, the degree of molecular recognition in binary solvents containing water

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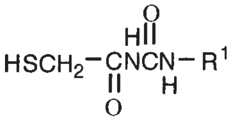
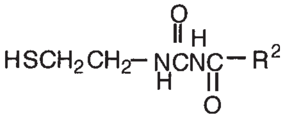
was compared with that in binary solvents containing D_2O at various temperatures. Here we report the first example in which deuteration of water in binary solvents causes the temperature dependence of the recognition to become fairly dull.

Our model compounds[8,9] for molecular recognition consist of a pair of acylurea derivatives **1** and **2**, which are open-chain analog of pyrimidine bases (e.g. uracil and thymine). Thiols **1** and **2** have three sites: the reaction site (SH group) where a model reaction takes place; the binding site [—C(═O)NHC(═O)NH—, acylurea bond] whose inner NHC(═O) unit participates in two NH···O intermolecular hydrogen bonds[10,11] and which extends in the opposite direction to each other; and the recognition site (R^1 or R^2) that participates in the discrimination through specific weak intermolecular interactions.[12] Thiol **1** has the same group as a cysteine side-chain ($HSCH_2$) and thiol **2** is a derivative of cysteamine (the decarboxylated compound of cysteine).



$HSCH_2{-}C(O)NHC(O)NH{-}R^1$

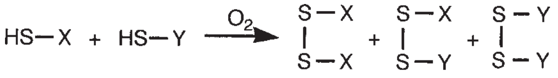
1 $R^1 = C_5H_{11}$



$HSCH_2CH_2{-}NHC(O)NHC(O){-}R^2$

2 $R^2 = C_5H_{11}$

Oxidation[13] of a pair of thiols **1** and **2** [Eqn (1)] was examined as a model reaction for discrimination because the correct pairing of half-cystine residues (S—S bond formation) has been suggested to depend upon specific non-covalent bonds. The selectivity (*r*) in the oxidation—a measure of the degree of molecular recognition of **1** by **2** (or of **2** by **1**)—is defined as the logarithmic ratio of the yield of the unsymmetrical disulfide (**4**) to twice that of the symmetrical disulfide (**3**): $r = \ln\{[\mathbf{4}]/2[\mathbf{3}]\}$.



$$HS{-}X + HS{-}Y \xrightarrow{O_2} \begin{matrix} S{-}X \\ | \\ S{-}X \end{matrix} + \begin{matrix} S{-}X \\ | \\ S{-}Y \end{matrix} + \begin{matrix} S{-}Y \\ | \\ S{-}Y \end{matrix} \qquad (1)$$

1 **2** **3** **4** **5**

Therefore, when the three disulfides (**3**, **4** and **5**) are formed in the statistical ratio (i.e. 1:2:1 ratio), then *r* becomes zero.

RESULTS AND DISCUSSION

Temperature dependence of molecular recognition in undeuterated and deuterated aqueous binary solvents

The temperature dependence of the selectivity (*r*) was examined in various undeuterated and deuterated binary aqueous solvents. The mole fractions of water (x_w) used were 0.50 and 0.75 for the H_2O–ethanol (EtOH) system, 0.20 and 0.75 for the H_2O–acetonitrile (MeCN) system and 0.20 and 0.75 for the H_2O–methanol (MeOH) system. These solvents were chosen because of the remarkable temperature dependence of *r* in undeuterated solvents,[14] thus the effect of deuteration on discrimination was likely to be observed.

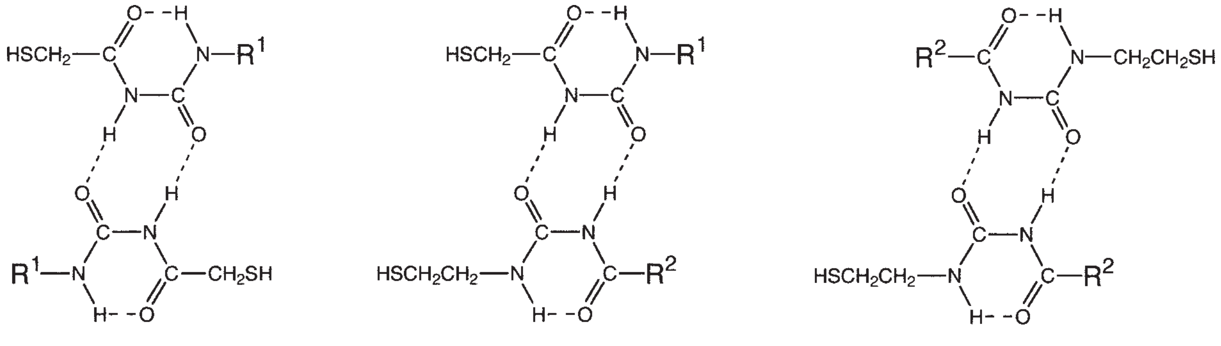
Plate 1 shows the temperature dependence of the selectivity (*r*) for $R^1 = R^2 = C_5H_{11}$ in undeuterated[14] and deuterated binary aqueous ethanol ($x_w = 0.50$). Upon deuteration of water in aqueous ethanol, the temperature dependence of *r* has been found to alter markedly from a clear bell-shaped curve (a maximum at 35 °C) to a nearly flat curve (20–35 °C), followed by a progressive decrease (35–70 °C) with increasing temperature. It is interesting that the *r* values at 35 °C remain practically unaltered regardless of the solvents used. Furthermore, deuteration of ethanol (i.e. the D_2O–EtOD system) only slightly changes the selectivity (*r*) compared with that in the D_2O–EtOH system.

Plate 2 displays the temperature dependence of *r* for $R^1 = R^2 = C_5H_{11}$ in undeuterated[14] and deuterated binary aqueous ethanol at $x_w = 0.75$. On deuteration of the H_2O–EtOH system, the temperature dependence has proved to alter radically from the very sharp bell-shaped curve (a maximum at 35 °C) to a plateau-like curve. As is evident from Plates 1 and 2, the deuteration effect on the temperature dependence at $x_w = 0.75$ becomes more remarkable than that at $x_w = 0.50$. Also in this case, the *r* values at 35 °C remain practically unaltered regardless of the solvents used. Furthermore, deuteration of ethanol (i.e. the D_2O–EtOD system) only slightly changes the selectivity (*r*) compared with that in the D_2O–EtOH system.

Plate 3 illustrates temperature dependence of *r* for $R^1 = R^2 = C_5H_{11}$ in undeuterated[14] and deuterated binary aqueous acetonitrile at $x_w = 0.20$ and 0.75. On deuteration of water at $x_w = 0.20$, the temperature dependence of *r* changes from a marked decrease to a dull decrease in the 20–50 °C range. Furthermore, deuteration of water at $x_w = 0.75$ causes the bell-shaped curve in the temperature–*r* value profiles to become fairly dull in the 20–70 °C range.

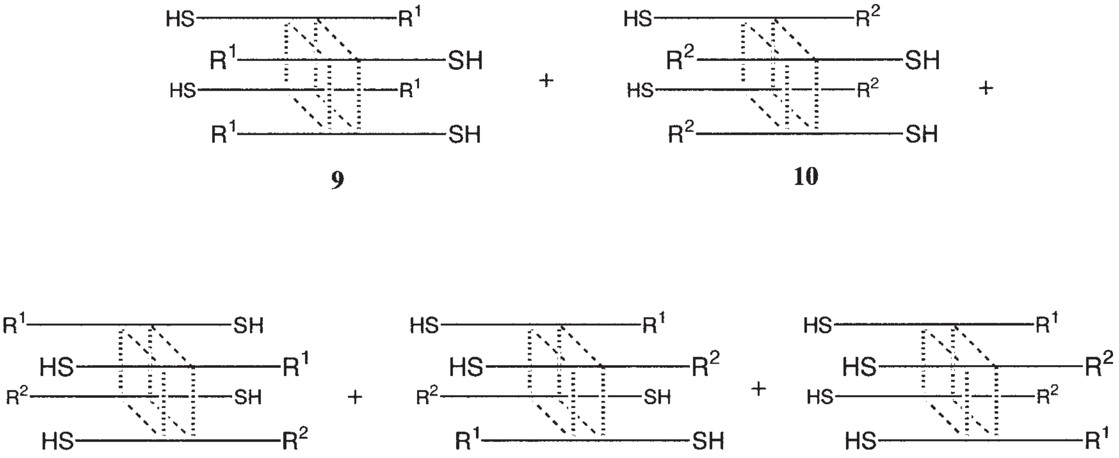
Plate 4 plots *r* for for $R^1 = R^2 = C_5H_{11}$ in undeuterated and deuterated binary aqueous methanol at $x_w = 0.20$ and 0.75. The deuteration effect in the H_2O–MeOH system has been found to be far slighter than that in the H_2O–EtOH and H_2O–MeCN system.

Product ratio in the oxidation.[8] It has been demonstrated that the product ratio in the oxidation is kinetically controlled.[9,15] Both ^1H NMR and IR studies of **1** and **2** revealed that they formed homodimers (**6** and **8**) and a heterodimer (**7**) through two NH···O intermolecular hydrogen bonds (Fig. 1).[10a] Moreover, based on experimental evidence, at least five tetramers (two homotetramers **9** and **10** and three heterotetramers **11**–**13**) (Fig. 2) formed by dimerization of the dimers[10a] have been suggested to be intermediates in this oxidation.[8,16] In addition, the product ratio is considered to depend on the relative concentrations of the tetramers.



6 **7** **8**

Figure 1. Association patterns of dimers **6**–**8**. Dashed lines depict hydrogen bonding



9 **10**

11 **12** **13**

Figure 2. Association schemes of tetramers **9**–**13** formed by dimerization of dimers **6**–**8**. Hydrogen bonding responsible for the stabilization of dimers is depicted by diagonal dashed lines. Non-covalent weak interactions responsible for the stabilization of tetramers are depicted by vertical dashed lines

Intermolecular association. Intermolecular association has been demonstrated to be the first requirement for molecular recognition.[9]

The decrease in *r* with increasing temperature (Plates 1–3), which is usually observed for selectivity in ordinary organic reactions, would be explained by weakening of intermolecular association between associating thiols **1** and **2** with increasing temperature.

Conversion of NH groups to ND groups by H-D exchange. In addition to the above findings that thiols **1** and **2** form two *intermolecular* NH···O hydrogen bonds between the inner —NHC(=O)— units in the —C(=O)NHC(=O)NH— groups, thiols **1** and **2** form two *intramolecular* NH···O hydrogen bonds between the outer —NH— and —C(=O)— units in the —C(=O)NHC(=O)NH— groups.[10b,17] These —NH— groups can be converted to —ND— groups by H–D exchange.

Indeed, NMR spectra of **1** or **2** at 500 MHz have clarified that signals for NH protons participating in the intermolecular and intramolecular hydrogen bonds are scarcely detected in EtOD after the time required to prepare a sample solution (ca. 20 min). In contrast, the reaction times in undeuterated and deuterated aqueous ethanol (e.g. $x_w = 0.50$) are ca. 520 h (20 °C), ca. 130 h (35 °C), ca. 50 h (50 °C) and 7 h (70 °C). Moreover, a long reaction time also is required for oxidation in undeuterated and deuterated aqueous ethanol ($x_w = 0.75$). Thus, it is suggested that almost all of the NH groups in **1** and **2** would be converted to ND groups, prior to the oxidation reaction, under the reaction conditions and that the NH–ND exchange would afford no significant change in the time course of the reaction.

Pattern change in temperature dependence of molecular recognition by deuteration of aqueous binary solvents. As mentioned above, deuteration of water in binary solvents causes the temperature dependence of the discrimination to become fairly dull.

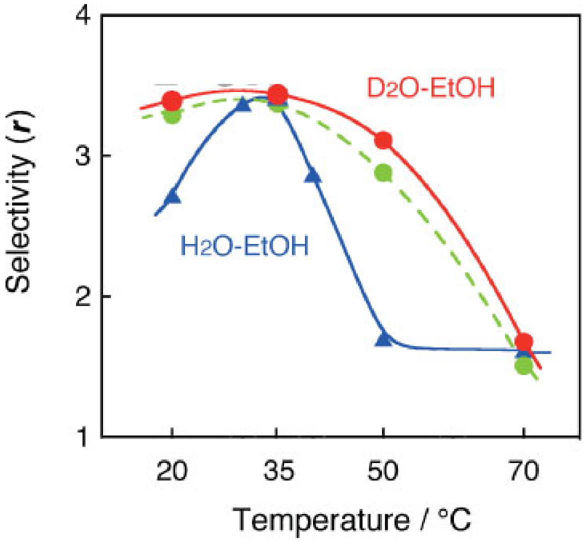


Plate 1. Temperature dependence of the selectivity (*r*) for $R^1 = R^2 = C_5H_{11}$ in undeuterated and deuterated aqueous ethanol ($x_w = 0.50$). Solvents used are H_2O–EtOH (–▲–), D_2O–EtOH (–●–) and D_2O–EtOD (–●–). Errors (three times the standard deviations) for *r* values range from ± 0.04 to ± 0.17

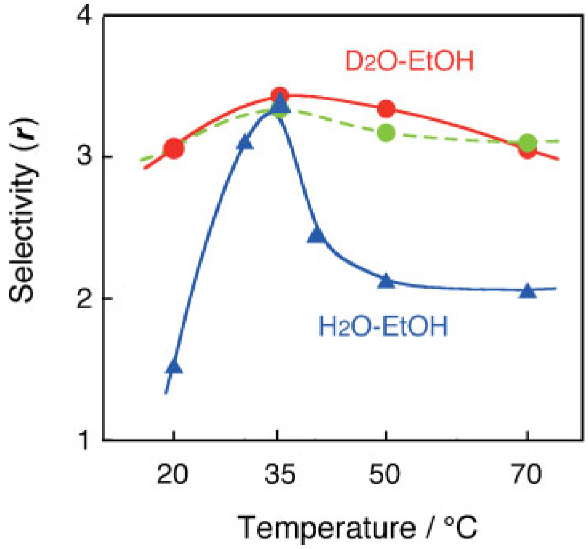


Plate 2. Temperature dependence of the selectivity (*r*) for $R^1 = R^2 = C_5H_{11}$ in undeuterated and deuterated aqueous ethanol ($x_w = 0.75$). Solvents used are H_2O–EtOH (–▲–), D_2O–EtOH (–●–), and D_2O–EtOD (–●–). Errors (three times the standard deviations) for *r* values range from ± 0.05 to ± 0.16

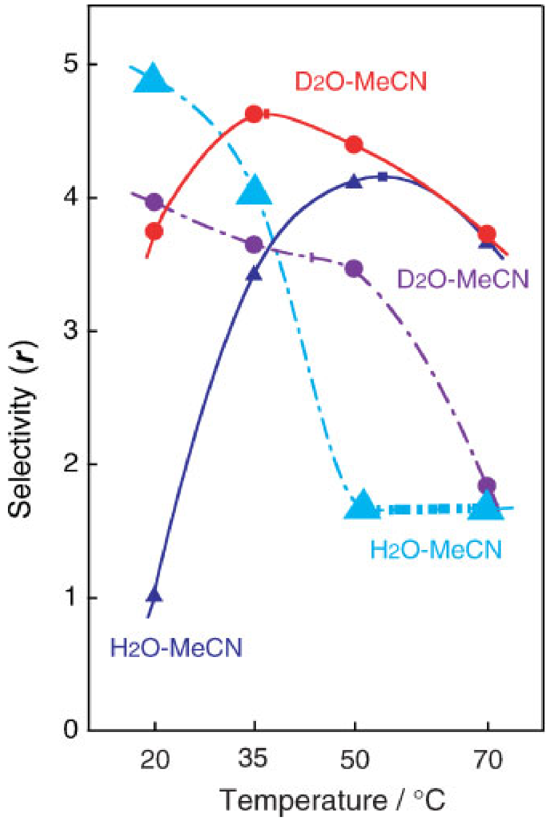


Plate 3. Temperature dependence of the selectivity (*r*) for $R^1 = R^2 = C_5H_{11}$ in undeuterated and deuterated aqueous acetonitrile. In the case of $x_w = 0.20$, solvents used are H_2O–MeCN (–▲–) and D_2O–MeCN (–●–); for $x_w = 0.75$, solvents used are H_2O—MeCN (–▲–) and D_2O–MeCN (–●–). Errors (three times the standard deviations) for *r* values range from ± 0.06 to ± 0.22

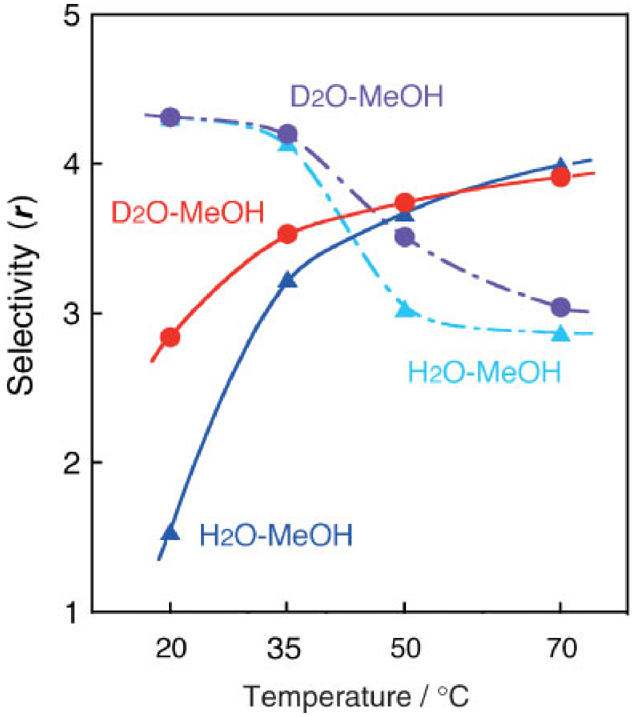


Plate 4. Temperature dependence of the selectivity (*r*) for $R^1 = R^2 = C_5H_{11}$ in undeuterated and deuterated aqueous methanol. In the case of $x_w = 0.20$, solvents used are H_2O–MeOH (–▲–) and D_2O–MeOH (–●–); for $x_w = 0.75$, solvents used are H_2O–MeOH (–▲–) and D_2O–MeOH (–●–). Errors (three times the standard deviations) for *r* values range from ± 0.02 to ± 0.12

The NH groups in the two thiols (**1** and **2**) had been converted to the corresponding ND groups when the oxidation started. In view of the above findings, it seems reasonable that the marked changes in the pattern of dependence of the selectivity (*r*) on x_w would result from changes in the properties of intermolecular association between the two thiols (**1** and **2**) due to the deuteration. This is because intermolecular association has been demonstrated to be the first requirement for molecular recognition (namely, for the *r* values becoming higher or lower than zero),[9] although it has not been clarified whether or not deuterium bonds are stronger than hydrogen bonds.[18]

It should be noted also that the temperature dependence of the discrimination differs markedly with the solvent systems employed, even in deuterated solvents (i.e. D_2O–EtOH and D_2O–MeCN). Therefore, it is necessary to consider the alterations in solute–solvent interactions on deuteration because they could change the solvent structures of binary aqueous solvents of the same x_w.

Our previous work indicated that the selectivity (*r*) varies strikingly with the solvent composition in undeuterated aqueous solvents;[14] this has been shown to be caused by alterations in the structures of binary aqueous solvents. Considering that similar composition–selectivity profiles are observed in deuterated solvents, although in a rather dull manner, the possibility that the above alterations in solvent structure would have marked effects on *r* should be taken into account in deuterated solvents.

CONCLUSION

Using a pair of associating model compounds each participating in two intermolecular hydrogen bonds and in shape-specific weak interactions, it has been demonstrated that: on deuteration of aqueous binary solvents, the pattern of temperature dependence of molecular recognition alters strikingly from a clear bell-shaped type (a maximum at 35 °C) to a plateau-like type in aqueous ethanol ($x_w = 0.50$ and 0.75) in the range 20–70 °C, and that in aqueous acetonitrile at $x_w = 0.20$ and 0.75 the deuteration of water causes the temperature dependence of the *r* to become fairly dull in the range 20–70 °C. At present, the reason for the unexpected deuteration effect observed in the present work remains to be elucidated.

EXPERIMENTAL

General procedures. The ^{1}H NMR spectra were recorded with a Jeol GX-270 spectrometer and a Varian Unity Plus 500 FT-NMR spectrometer. Chemical shifts (δ) are reported downfield from internal $SiMe_4$. Low- and high-resolution mass spectra were obtained on a Jeol JMS-DX303 mass spectrometer. Melting points were determined on a Yamato oil-immersion apparatus and are uncorrected. The HPLC separations were conducted on a Waters system (515 pump, 486 UV detector and Millennium 32).

Materials. The alcohols and acetonitrile used were all of HPLC grade (Wako, Tokyo) and were used as such. Water was purified through the Millipore Milli-Q water purification system, followed by distillation.

Preparation of thiols.[8] Thiols **1** were prepared by reaction of the corresponding *S*-esters [$MeC(=O)SCH_2C(=O)NHC(=O)NH-R^1$] with cysteamine, as described previously.[8] Their properties were reported previously.[8] Thiols **2** were prepared by addition of the corresponding acyl isocyanates to freshly sublimed cysteamine in tetrahydrofuran (THF) under argon at 0 °C, as described previously.[15] Their properties were reported previously.[19]

Preparation of disulfides.[15] Symmetrical disulfides **3** were obtained easily by treatment of **1** with O_2 in the presence of Et_3N in MeCN at room temperature and recrystallized from THF—dichloromethane. Unsymmetrical disulfides **4** were prepared by repeated recrystallization of the corresponding oxidation mixtures. The properties of disulfides **3** and **4** were reported previously.[8,20]

Oxidation of a pair of thiols. A mixture of **1** (0.50 mmol) and **2** (0.50 mmol) in a solvent (12.5 cm^3) was stirred vigorously under oxygen for 15 min in a well-stirred water bath that was thermostated to ± 0.1 °C for 20–50 °C and to ± 0.5 °C for 70 °C. To this mixture was added Et_3N (0.05 mmol), and vigorous stirring was continued for the time required to complete the oxidation (the oxidation was performed at least twice under the same conditions). When the oxidation was completed, the reaction mixture was evaporated to dryness. The yields of **3** and **4** were determined by the use of their absorption at 251 nm after separation of the three disulfides in the mixture by HPLC using LiChrosorb CN with hexane–isopropanol (98.5:1.5) as an eluent. The *r* values given in Plates 1–3 represent the mean values for two or more experiments, and were reproducible within the errors shown therein.

Determination of ^{1}H NMR spectra of a 1:1 mixture of 1 and 2. A 1:1 mixture of thiols **1** and **2**, which are difficult to dissolve in organic solvents, was dissolved in $CDCl_3$ for larger solubilities therein. To this solution, an excess amount of EtOD was added. About 20 min was required to prepare a sample solution. Immediately after the sample solution was prepared, NMR spectra for the NH protons were measured using a 500 MHz NMR spectrometer.

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