1974, 1258–1268.
(27) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1–19.
(28) Chipperfield, J. R. *Proc. R. Soc. London, Ser. B* **1966**, *164*, 401–410.
(29) Kresge, A. J., Chiang, Y. *J. Am. Chem. Soc.* **1973**, *95*, 803–806. Chwang, W. K.; Eliason, R.; Kresge, A. J. *Ibid.* **1977**, *99*, 805–808.
(30) The accuracy of these estimations, which are based on the ratio of the rate constants for N-protonation and deprotonation, depends on the accuracy of the estimated rate constant for the protonation of water by the N-protonated species. It is generally assumed that this can be taken as a rate constant of $10^{10}$ $s^{-1}$, with an effective $pK_a$ of zero for protonated water (Molday, R. S.; Kallen, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 6739–6745. Williams, A. *Ibid.* **1976**, *98*, 5645–5651. Martin, R. B.; Hutton, W. C. *Ibid.* **1973**, *95*, 4752–4754). These assumptions are uncertain and there is a corresponding uncertainty in the $pK_a$ estimates.
(31) Sayer, J. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1973**, *95*, 5637–5649.
(32) Cox, B. G.; de Maria, P. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1385–1387, and references cited therein.
(33) Wahlberg, A. *Acta Chem. Scand., Ser. A* **1976**, *30*, 433–436. Chatt, J.; Duncanson, L. A.; Venanzi, L. M. *Suom. Kemistil. B* **1956**, *29*, 75–84. However, see: Jensen, K. A.; Dahl, B. M.; Nielsen, P. H.; Borch, G. *Acta Chem. Scand.* **1971**, *25*, 2029–2038.
(34) Jencks, W. P.; Schaffhausen, B.; Tornheim, K.; White, H. *J. Am. Chem. Soc.* **1971**, *93*, 3917–3922.
(35) Takami, F.; Wakahara, S.; Maeda, T. *Tetrahedron Lett.* **1971**, 2645–2648.
(36) Wepster, B. M. *Recl. Trav. Chim. Pays-Bas* **1952**, *71*, 1171–1178.
(37) Ahrens, M.-L; Maass, G. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 818–819.
(38) Christenson, I. *Acta Chem. Scand.* **1964**, *18*, 904–922.
(39) It does not appear to be possible to account for the observed decrease in the rate of the pH-independent reaction at high pH by a kinetically equivalent mechanism in which the reaction at very high pH represents rate-determining N-protonation of the carbamate by the proton.
(40) Mader, P. M. *J. Org. Chem.* **1968**, *33*, 2253–2260.
(41) Salomaa, P. *Acta Chem. Scand.* **1971**, *25*, 367–368.
(42) Sauers, C. K., Jencks, W. P., Groh, S. *J. Am. Chem. Soc.* **1975**, *97*, 5546–5553.
(43) (a) Williams, A.; Jencks, W. P. *J. Chem. Soc., Perkins Trans. 2* **1974**, 1753–1759. (b) Jensen, M. B. *Acta Chem. Scand.* **1959**, *13*, 289–300.
(44) Armstrong, V. C.; Farlow, D. W.; Moodie, R. B. *Chem. Commun.* **1968**, 1362–1363, and footnote 21 of ref 4.
(45) Thornton, E. R. *J. Am. Chem. Soc.* **1967**, *89*, 2915–2927.
(46) Jencks, D. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 7948–7960.
(47) Funderburk, L. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 6708–6714.
(48) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 1228–1235.
(49) Eliason, R.; Kreevoy, M. M. *J. Am. Chem. Soc.* **1978**, *100*, 7037–7041.
(50) The comparison is based on $\alpha$ values calculated from rate constants reported in Tables I, IV, and V;[8] however, the $\alpha$ values differ from those given previously.[8]
(51) Hine, J. *J. Am. Chem. Soc.* **1972**, *94*, 5766–5771.
(52) Moodie, R. B.; Sansom, P. J., personal communication.

# Kinetics and Mechanism of the Thiolytic Removal of the Dithiasuccinoyl (Dts) Amino Protecting Group[1,2]

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**Abstract:** The dithiasuccinoyl (Dts) amino protecting group is removed by thiols through the intermediacy of open-chain carbamoyl disulfides. The elucidation of practical and effective conditions for carrying out the reductive deprotection was facilitated by a rapid, convenient, and quantitative method to directly measure starting materials, intermediates, and products on a standard amino acid analyzer. The apparent pseudo-first-order rate constants were determined as a function of thiol, base, and solvent composition and concentration. Both steps of the mechanism were first order in thiol. In anhydrous solutions, the rate of the second step, $k_2$, varied directly with tertiary amine concentration, suggesting that the active species is an association complex of the thiol and the base. In contrast, a more complex explanation is required to account for the fact that plots of log $k_1$ against log [base] had a slope of only 0.7–0.8. The ratio of rates, $\kappa = k_2/k_1$, was generally between 0.1 and 5 for neutral monofunctional aliphatic thiols, but with bifunctional thiols, where the second step can proceed intramolecularly because a cyclic disulfide is formed, $\kappa \geq 100$ and consequently carbamoyl disulfide intermediates could not be observed. Intermediates were also never observed for thiophenol, the most acidic thiol tested, nor for 2-mercaptopyridine, a compound existing primarily as its thione tautomer. For these two cases, $\kappa$ was estimated, by indirect means, as $\sim 10^5$ and $\geq 10^9$, respectively. The fastest overall rates were observed with thiols of intermediate acidity ($pK_a$ = 8.0–9.5) in polar aprotic media of high dielectric constant. In aqueous solutions, the first step of the mechanism was rate limiting ($\kappa \sim 375$ based on an independent measurement). The observed rates $k_1$ were directly proportional to the thiol anion concentration and the data for monofunctional thiols fit a Brønsted correlation of thiol anion reactivity against $pK_a$ with slope $\beta_{nuc} \simeq 0.9$. The two steps in the mechanism of thiolytic deprotection of dithiasuccinoyl amines have strikingly different electronic requirements, meaning that the transition states are different. The driving force for the first step appears to be relief of the ring strain of the Dts heterocycle, while the rate of the second step correlates with the ease of ionization of the thiocarbamate leaving group. Suitable conditions for the quantitative removal of the Dts protecting group from any amino acid residue at 25 °C include (1) $\beta$-mercaptoethanol (0.2 M)–triethylamine (0.5 M) in benzene for 5 min; (2) *N*-methylmercaptoacetamide or dithiothreitol (0.1 M) in neat pyridine for 5 min; (3) *N*-methylmercaptoacetamide or *N*-acetyl-$\beta$-mercaptoethylamine (0.1 M)–*N*-methylmorpholine (0.5 M) in acetonitrile for 1 min; (4) $\beta$-mercaptoethanol (0.1 M) and 2-mercaptopyridine or thiophenol (1.1 equiv over Dts amine) in *N,N*-dimethylformamide–pyridine (9:1) for 1 min; (5) *N*-methylmercaptoacetamide (0.2 M) in *N,N*-dimethylformamide–acetic acid (9:1) for 2 min; (6) dithiothreitol (10 mM) in pH 7.0 phosphate buffer for 2 min. The reductive deprotection of the Dts group and of carbamoyl disulfide intermediates is much more facile than the reduction of acyclic aliphatic disulfides.

The dithiasuccinoyl (Dts) amino protecting group[3–6] was developed for eventual application to *orthogonal* schemes[5,7,8] of peptide synthesis. On the one hand, the Dts group is stable under the acidolytic conditions used to remove *tert*-butyl and benzyl-based protecting groups, and it is also resistant to the photolytic conditions used to cleave the acid-stable *o*-nitrobenzyl and $\alpha$-methylphenacyl esters. Conversely, the Dts function is rapidly and quantitatively removed in the presence of the other mentioned groups through application of mild and specific treatments[5,6] with thiols, borohydrides, and trialkylphosphines. The present paper provides practical details for effectively carrying out the reductive deprotection of dithiasuccinoyl amines with thiols; such information is a prerequisite for optimizing the conditions of solid-phase peptide

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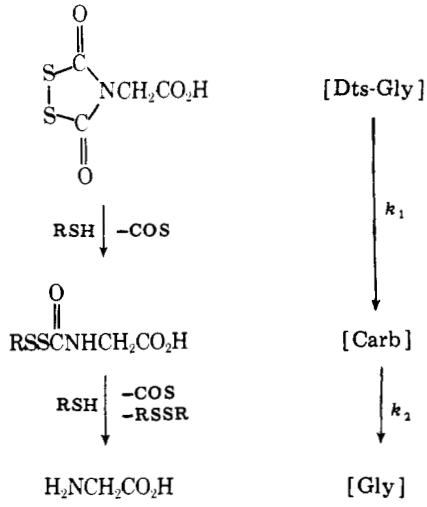
**Scheme I**




synthesis[8,9] with Dts amino acids.[4] All of the reported rates were derived for Dts-glycine because earlier work[6] established that rates with other Dts amino acids never differed by a factor of more than 3.

The kinetic pattern which was analyzed is outlined in Scheme I. The starting material, intermediates, and product were all quantitated[6] by a rapid and convenient chromatographic method utilizing an automated amino acid analyzer based on the design of Spackman, Stein, and Moore.[10] Detection of compounds with the standard ninhydrin-hydrindantin reagent[11] was possible[5,6] because the hydrindantin acts as a reducing agent and the released amino acid reacts in situ with the ninhydrin to give a purple (570 nm) color. The open-chain carbamoyl disulfide intermediates in the mechanism of reductive deprotection were demonstrated directly by chromatography; these species were resolved in the cases of three different amino acids[6] and three different thiols (see ref 6 and Experimental Section of this work). The elution times of the components in the glycine series (Scheme I) were such as to permit efficient and unequivocal analysis of a number of samples in a single chromatographic run by a serial injection protocol.[6]

On a molar basis, the Dts, carbamoyl disulfide, and free amino acid derivatives quantitatively accounted for all of the glycyl residue at all stages of the reductive deprotection. Kinetic runs were carried out under pseudo-first-order conditions with the thiol in approximately 20-fold molar excess over the starting Dts-glycine. The rate constants $k_1$ and $k_2$ were determined from the experimental data by a set of mathematical procedures described elsewhere,[12] and were then used to reconstruct theoretical curves[13] for the disappearance of starting Dts-glycine, the appearance and consumption of the intermediate carbamoyl disulfide, and the appearance of free glycine. The good fit between the observed kinetic patterns and the calculated curves (Figure 1) was considered to be excellent evidence for the validity of the mechanistic sequence shown in Scheme I.

In the following, a physical organic study of the effects of thiol, base, and solvent compositions and concentrations has been carried out to identify the factors responsible for fast reaction rates. The two steps in the mechanism have been shown to have strikingly different electronic requirements despite nominal chemical similarities. Thus, the results of the present work extend the understanding of thiol-disulfide exchange reactions.

## Results

**Rates of Reductive Deprotection of Dts-Glycine in Organic Media.** Both steps of the reaction mechanism (Scheme I) were

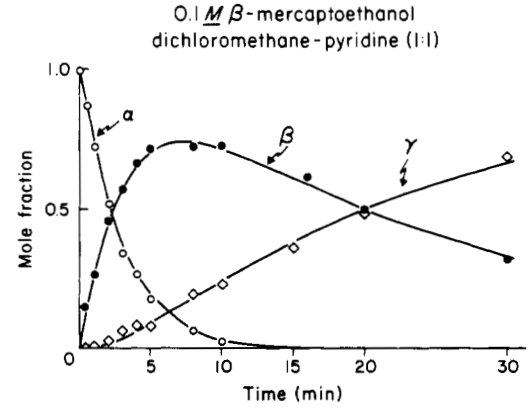



**Figure 1.** Kinetics of reductive deprotection of Dts-glycine (7 mM) with β-mercaptoethanol (0.1 M) in dichloromethane-pyridine (1:1). The pseudo-first-order rate constants for the first and second steps of the reaction mechanism were determined to be $k_1 = 5.3 \times 10^{-3}$ s$^{-1}$ and $k_2 = 6.9 \times 10^{-4}$ s$^{-1}$, respectively. These values were used (see ref 12 and 13 for equations) to calculate the theoretical curves (solid lines) of α, β, and γ, which are respectively the mole fractions of the three components of the reaction mechanism (Scheme I). The experimental points for Dts-glycine (○) have been plotted as obtained; those for the carbamoyl disulfide (●) and glycine (◇) have been slightly corrected from the raw data to compensate for an aqueous quench artifact (ref 12; see also legend to Figure 2). The correction parameter was $\mu = 1.15$, as compared to a value of $\mu = 1.0$ which would imply perfect agreement between theory and experiment.

shown to be first order in thiol.[14] Hence, it was justified to express the rates $k_1$ and $k_2$ as true second-order rate constants. Results from an extensive series of experiments in which the solvents and bases were varied have been compiled in Table I (35 entries; see supplementary material). With β-mercaptoethanol as the thiol, the ratio of rates, $\kappa = k_2/k_1$, was found to vary from essentially zero to over 100, and to be most typically in the range from 0.1 to 5.

Table II summarizes the relative rates at which Dts-glycine is reductively deprotected by β-mercaptoethanol as a function of solvent composition. Under comparable conditions, rates varied over three to four orders of magnitude, with the fastest rates observed in polar aprotic media with high dielectric constants. By comparison, the rates of aminolysis by *n*-butylamine (Scheme VI) varied in the same order, but only over two orders of magnitude.

Two separate factors were identified that promote rapid rates of reductive deprotection. A synergistic effect of polar, autoprotolyzable (Table II, column 3) solvents of which *N,N*-dimethylformamide is a prototype was indicated by the observation that rates in pyridine-dimethylformamide mixtures were much faster than rates in either neat pyridine or in neat dimethylformamide, and by the finding of appreciable rates in dimethylformamide-acetic acid mixtures. Relative rates were in the order "buffered"[15] dimethylformamide > dimethylformamide-pyridine (9:1) > pyridine ~ dimethylformamide-benzene (1:1) > pyridine-benzene (1:1) ~ dimethylformamide-acetic acid (9:1) in the approximate ratio 400:150:20:10:1:1. The observations of synergism with *N,N*-dimethylformamide were extended to the other amide solvents *N*-methylpyrrolidone and *N,N*-dimethylacetamide, and were also found to apply to dimethyl sulfoxide and at a less dramatic level to acetonitrile.

When less polar solvents such as dioxane, dichloromethane, benzene, and acetonitrile were tested in the absence of base (e.g., Table I, lines 1 and 2) or in the presence of acetic acid, the rates $k_1$ and particularly $k_2$ were very slow. Addition of tertiary amines such as pyridine ($pK_a$ in water = 5.25), *N*-methylmorpholine ($pK_a$ in water = 7.4), triethylamine ($pK_a$ in water = 10.8), and *N,N*-diisopropylethylamine (comparable basicity to triethylamine, but sterically hindered) resulted in

**Table II.** Relative Rates of Reactions of Dts-Glycine in Various Solvent Mixtures[a]

| solvent (X) | $\epsilon$ | $pK_S$ | $K_{BHA}$ | β-mercaptoethanol in neat X (no base)[c] | β-mercaptoethanol in neat X (with base)[d] | β-mercaptoethanol in pyridine–X 1:1 (v/v)[e] | β-mercaptoethanol in pyridine–X 9:1 (v/v) | *n*-butylamine[b] in neat X |
|---|---|---|---|---|---|---|---|---|
| dioxane | 2.2 | | **1** | **1** | **1** | **1** | | **1** |
| ethyl acetate | 6.0 | | 8 | | | 1.4 | | |
| chloroform | 4.8 | | 10 | | | 2.2 | | |
| dichloromethane | 8.9 | | 30 | | 35 | 6.2 | | 0.9 |
| benzene | 2.3 | | 2 | | 20 (7)[f] | 8.1 | | 0.9 |
| acetone | 20.7 | | NC | | | 23 | | |
| pyridine | 12.4 | | NC | $4 \times 10^4$ [g] | | 41 | **50**[h] | 20 |
| methanol | 32.7 | 16.7 | NC | | | 47 | | |
| water | 78.4 | 14.0 | NC | | | 100 (10)[i] | | |
| acetonitrile | 36.0 | 26.5 | 160 | 770 | 100 | 250 | 170 | 100 |
| *N*-methylpyrrolidone | 32.0 | NC | NC | | | | 300 | |
| *N,N*-dimethylformamide | 36.7 | 18.0 | >500 | $3 \times 10^4$ | | 1000 | 1200 | 125 |
| *N,N*-dimethylacetamide | 37.8 | NC | NC | | | | 1200 | |
| dimethyl sulfoxide | 46.7 | 17.3 | >500 | $1 \times 10^4$ | | | 2500 | |

[a] Rates are relative to dioxane = **1.0**, and have been compared on the basis of $k^{env} = k_1k_2/(k_1 + k_2)$, as explained in ref 12. The detailed rate data and conditions for some of the entries of this table are listed in Table I (supplementary material). Details on the necessary purification of solvents and bases are in the Appendix (part V). Dielectric constants ($\epsilon$) and autoprotolysis constants ($pK_S$), taken from Riddick and Bunger (ref 16), are usually measured at 25 °C, except for cases when only data at 20 °C is available. $K_{BHA}$ are the approximate relative base strengths of tertiary amines B (*N*-methylmorpholine or triethylamine) in the solvents indicated, as reflected by the equilibrium constant $K_{BHA} = [BH^+A^-]/[B][A]$ for association with HA (2,4-dinitrophenol; $pK_a$ in water = 4.0), and reported by Pearson and Vogelsong (ref 18), and Williams and Young (ref 19); see also Davis (ref 17). NC means not cited. [b] Depending on the solvent, the rates for *n*-butylamine ($pK_a$ in water = 10.6) were 5 (±2)-fold higher than those for benzylamine ($pK_a$ in water = 9.3). The $pK_a$ values are from Perrin (ref 20). The pseudo-first-order rate constant for aminolysis of Dts-glycine with *n*-butylamine (0.1 M) in dioxane was $5.5 \times 10^{-3}$ s$^{-1}$. [c] Since in dioxane $\kappa = 0$ (legend to Figure 2), the comparisons for this column have been made solely on the basis of $k_1$, not $k^{env}$. In the absence of base, $\kappa$ was 0.03 for acetonitrile, 0.5 for *N,N*-dimethylformamide, and 1.5 for dimethyl sulfoxide. See footnote *g* for data in neat pyridine. [d] Base was *N*-methylmorpholine or triethylamine (Table I, lines 23–29). The pseudo-first-order rate constants for thiolysis of Dts-glycine with β-mercaptoethanol (0.1 M) in dioxane were $k_1 = 1.3 \times 10^{-3}$ s$^{-1}$, $k_2 = 3.5 \times 10^{-4}$ s$^{-1}$, $\kappa = 0.27$, $t_{1/2}^{env} = 42$ min in the presence of *N*-methylmorpholine (0.05 M), and $k_1 = 5.5 \times 10^{-3}$ s$^{-1}$, $k_2 = 1.3 \times 10^{-3}$ s$^{-1}$, $\kappa = 0.24$, $t_{1/2}^{env} = 10.9$ min in the presence of triethylamine (0.05 M). [e] Under these conditions, the ratio $\kappa$ was found to be relatively insensitive (usually 0.08–0.13; somewhat higher in a few cases) to either the nature of the solvent or to the relative concentration of pyridine acting as a base. The large overall mole fraction of pyridine in the cosolvent mixtures causes the effects of the added cosolvent X to be less pronounced than they would be if X were being used alone as a neat solvent. The pseudo-first-order rate constants for thiolysis of Dts-glycine with β-mercaptoethanol (0.1 M) in dioxane–pyridine (1:1) were $k_1 = 1.0 \times 10^{-3}$ s$^{-1}$, $k_2 = 1.1 \times 10^{-4}$ s$^{-1}$, $\kappa = 0.11$, $t_{1/2}^{env} = 116$ min. [f] With triethylamine, 20-fold rate difference; with *N*-methylmorpholine, 7-fold difference. [g] Pyridine itself is the base. The pseudo-first-order rate constants for thiolysis of Dts-glycine with β-mercaptoethanol in neat pyridine are found in Table III, first entry, second set. The value of $\kappa$ was 0.12, with $t_{1/2}^{env} = 2.9$ min for 0.1 M thiol. [h] Since the data for dioxane–pyridine (9:1) were not available, all further data in this column have been arbitrarily normalized to the rate in neat pyridine (footnote *g*) = **50.0**. [i] As shown in Table I, line 17, $\kappa$ for reductive deprotection in pyridine–water (1:1) was 100. The rate relative to dioxane (footnote *e*) as based on $k^{env}$ is thus 100, but based on $k_1$ it is only 10.

rate enhancements of three to five orders of magnitude (Table I, lines 23–29). Neat pyridine itself was an especially effective solvent because of its basic properties (see Table III, second set, in main text). These data show the importance of base catalysis of the reductive deprotection.

The relative strengths of tertiary amine bases in aprotic solvents[17] have been estimated by Pearson and Vogelsong[18] and especially Williams and Young[19] (Table II, column 4 and footnote *a*). In dioxane, the base strengths varied in the order[19] NMM:*i*-$Pr_2EtN$:$Et_3N$ = 1:20:60, while the relative rates of thiolysis of the Dts group with these bases in dioxane were in the same order but over the much narrower range 1:2.3:4.0. In benzene, the ratio of rates of thiolysis in the presence of NMM, as compared to in the presence of triethylamine, was 1:10 for both steps ($k_1$ and $k_2$) of the reaction mechanism. As a different type of example, triethylamine was reported[18] to be twice as strong a base in benzene as in dioxane, whereas the relative rates of reductive deprotection with β-mercaptoethanol and triethylamine in these solvents were 5 for $k_1$ and 100 for $k_2$! Closer quantitative agreement was obtained by comparing base strengths (ratio[19] = 160) with reaction rates (ratio = 100; Table I, lines 23 vs. 25) in the cases of *N*-methylmorpholine in dioxane and acetonitrile, respectively.

Figure 2 is a log–log plot of the pseudo-first-order rate constants for the reductive deprotection of Dts-glycine with β-mercaptoethanol in anhydrous dioxane over a tenfold concentration range of the triethylamine catalyst. Nonparallel straight lines were observed, with slopes of 0.7 for the first step of the reaction mechanism (open circles) and 1.0 for the second step (closed circles). These findings mean that $\kappa$ is *not* constant as a function of base concentration, a surprising result that was carefully confirmed because of an internal control feature inherent in the analysis of the kinetics. Hence, the observed curves of carbamoyl disulfide intermediate plotted against $k_1t$ showed a continuous trend to lesser width with increasing triethylamine concentration, in contrast to the superimposability of these curves required by constant $\kappa$. In addition, the slopes of 0.7 and 1.0 for the first and second steps, respectively, were confirmed over a tenfold concentration range of *N*-methylmorpholine in dioxane; experiments performed under conditions giving high $\kappa$ (specifically, thiophenol with 0.05–0.5 M triethylamine in dioxane (compare to Table III, seventh line) and β-mercaptoethanol with 0.05–0.5 M *N*-methylmorpholine or 0.05–0.2 M triethylamine in benzene; $\kappa = 5$ (compare to Table I, lines 24 and 28)) all gave slopes for the first step of 0.7–0.8.

The direct variation of the rate $k_2$ with tertiary amine concentration (Figure 2), as well as with thiol concentration,[14] implied the kinetic scheme in anhydrous media signified by Scheme II, which is closely analogous to the proposals of Dmuchovsky and collaborators[21] on the mechanism of base-catalyzed addition of thiols to maleic anhydride in xylene so-

**Scheme II**

$$[RSH] + [NR'_3] \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [RS^- \cdots {}^+HNR'_3] \quad (1a)$$

(S) (N) (SN)

$$K_{SN} = (SN)/(S)(N) = k_3/k_4 \quad (1b)$$

$$[RS^- \cdots {}^+HNR_3'] + [Carb] \xrightarrow[rds]{k_5} [Gly] \quad (2)$$

(SN) (B) (C)

$$dC/dt = k_2^{obsd}B = k_5K_{SN}[B][S][N] \quad (3)$$

lution. It is assumed that the equilibrium 1 to give an association complex of the thiol and base (Dmuchovsky et al.[21a] suggested a non-hydrogen-bonded ion pair) is set up rapidly, so that reaction 2 is rate determining [$k_4 \gg k_5$(SN); easily satisfied because $k_4$ can be estimated[22] as $10^{10}$ L mol$^{-1}$ s$^{-1}$]. The observed third-order expression (3) then follows from the steady-state assumption d(SN)/d$t$ = 0. To account for the linearity (i.e., no saturation level of rates was ever observed) of eq 3 in the ranges of thiol (S) and tertiary amine (N) concentrations studied in this work, $K_{SN}$ (eq 1b) must be <0.1 L mol$^{-1}$. The equilibrium constant $K_{BHA}$ (Table II, footnote *a*) for ion-pair formation from triethylamine and 2,4-dinitrophenol was measured[18] to be 1460 L mol$^{-1}$ in dioxane. Linear extrapolation of these data to ion-pair formation from triethylamine and thiophenol (respectively the strongest base and most acidic thiol studied), based on p$K_a$ values measured in water, would give $K_{SN} \sim 2$ L mol$^{-1}$ in dioxane, but there is clearly much room for error in this kind of calculation.

An explanation of the experimentally well-established fractional order in base concentration for the first step of the reaction mechanism cannot be provided by manipulating a series of expressions such as (1) that are symmetrical in both thiol and tertiary amine concentration. One empirical proposal that is consistent with the data is outlined in Scheme III. The

**Scheme III**

$$[R_3'N] + [Dts\text{-}Gly] \underset{K_{NA}}{\rightleftharpoons} [R_3'N \cdots Dts\text{-}Gly] \quad (4)$$

(N) (A) (NA)

$$[RS^- \cdots {}^+HNR_3'] + \begin{cases} [Dts\text{-}Gly] \xrightarrow{k_6} \\ (A) \\ [R_3'N \cdots Dts\text{-}Gly] \xrightarrow{\omega k_6} \\ (NA) \end{cases} [Carb] \quad (B) \quad (5)$$

$$dA/dt = k_1^{obsd}[A + NA] = k_1^{obsd}[A_{tot}]$$

$$= k_6K_{SN}[A_{tot}][S][N] \underbrace{\left[\frac{1 + \omega K_{NA}[N]}{1 + K_{NA}[N]}\right]}_{\text{proportionality factor}} \quad (6)$$

required asymmetry in tertiary amine concentration was achieved by postulating an association complex (NA) with Dts-glycine (eq 4) that reacts with the *same* active species (eq 1) already discussed for the second step of the mechanism (Scheme II), although at a rate $\omega k_6$ which is somewhat slower than the reaction rate $k_6$ of unassociated Dts-glycine. Assuming $K_{NA}$ = 10 L mol$^{-1}$ and $\omega$ = 0.3, the apparent slope of a plot of log $k_1^{obsd}$ against log [base] computed from eq 6 will be 0.75 over a range of tertiary amine concentration from 0.05 to 1.0 M. This slope will approach 1.0 at both lower and higher base concentrations because the terms involving (N) in the proportionality factor of eq 6 will cancel out; other apparent slopes of the intermediate region of the log-log plot may be

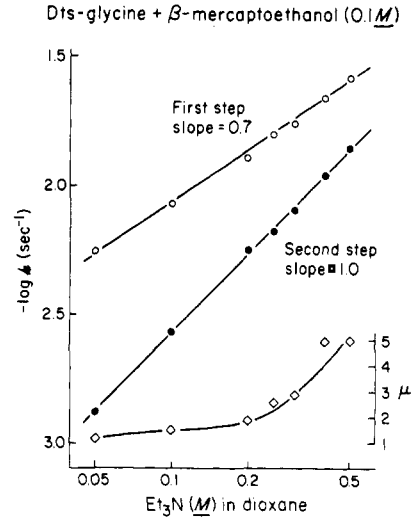



**Figure 2.** Rates of reductive deprotection of Dts-glycine (5 mM) as a function of triethylamine concentration (abscissa). The thiol used was β-mercaptoethanol (0.1 M) in anhydrous dioxane. Pseudo-first-order rate constants of the reaction mechanism: first step $k_1$ (○); second step $k_2$ (●). The parameter $\mu$ (◇) reflects the extent of an aqueous quench artifact, as developed in detail elsewhere (ref 12). The points at the extreme right of the figure were found to be the same when revised nonaqueous quench conditions giving $\mu$ = 1.0 were used. A value of $\mu$ = 1.0 implies perfect agreement between theory and experiment (compare to Figure 1). Note that the results of this figure, where the ratio $\kappa = k_2/k_1$ is *not* constant as a function of base concentration, even extrapolate accurately to zero base concentration, where $\kappa$ = 0 ($k_1 = 10^{-6}$ s$^{-1}$; $t_{1/2} \sim 8$ days; $k_2$ = negligible since no product glycine found after 2 weeks; see Table I, line 1, in supplementary material).

worked out with other assumed values of $K_{NA}$ and $\omega$. Finally, in the special case of Scheme III when $\omega$ = 1, the slope will be 1.0 throughout, as it was for Scheme II.

Table III presents the results of a comparative study on the relative rates of reductive deprotection of Dts-glycine as mediated by a variety of thiols. The observed trends were quite different for the first and second steps of the reaction mechanism (Scheme I), and these were a function of the thiol structure and not of the composition of the organic media in which the reactions were being carried out. By far the least reactive thiol was 2-mercaptopyridine, a compound existing primarily as its thione tautomer.[23] Excluding this special case, the rates of the first step, measured on nine other thiols, varied over a 20-fold range. The fastest rates, both of the first step ($k_1$) and overall ($k^{env}$), were observed with *N*-methylmercaptoacetamide, *N*-acetyl-β-mercaptoethylamine, and dithiothreitol. The p$K_a$ values of these three thiols [measured in water; see Appendix (part II) in supplementary material, as well as legend to Figure 4] are intermediate to the p$K_a$ values of the most slowly reacting thiols, which were thiophenol at the acidic end of the scale and ethanethiol and octadecanethiol at the other end. This biphasic pattern for $k_1$ contrasted to the clear trend for $k_2$, where the observed rates were faster with increasing acidity (lower p$K_a$) of the attacking thiol. Thus, carbamoyl disulfide intermediates were never observed for either 2-mercaptopyridine or thiophenol; these findings were followed up as described subsequently (see "Rates of Reactions of Carbamoyl Disulfides"). The value of $\kappa$ was also very high ($\geq$100) when reductive deprotections were mediated by bis(β-mercaptoethyl) ether or dithiothreitol. In these cases, the second step of Scheme I can proceed intramolecularly with respectively formation of a seven- or a six-membered cyclic disulfide.[24,25]

**Rates of Reductive Deprotection of Dts-Glycine in Aqueous Media.** The effect of water on reaction rates was first studied in mixed aqueous media under buffered conditions (Table I,

**Table III.** Rates of Reductive Deprotection of Dts-Glycine with Various Thiols[a]

| Thiol | $k_1$ | $k_2$ | $\kappa$ | $t_{1/2}^{env}$, min | $k_{rel}^{env}$ |
|---|---|---|---|---|---|
| | | In $Et_3N$ (0.1 M)–Dioxane | | | |
| β-mercaptoethanol | $8.5 \times 10^{-2}$ | $2.7 \times 10^{-2}$ | 0.32 | 5.6 | **1.0** |
| ethanethiol | $1.8 \times 10^{-2}$ | $5.8 \times 10^{-3}$ | 0.32 | 26.2 | 0.2 |
| octadecanethiol | $1.5 \times 10^{-2}$ | $7 \times 10^{-3}$ | 0.5 | 24.1 | 0.2 |
| methyl 3-mercaptopropionate | $3 \times 10^{-2}$ | $6 \times 10^{-3}$ | 2 | 5.8 | 1.0 |
| *N*-acetyl-β-mercaptoethylamine[b] | 0.4 | 2.0 | 5 | 0.3 | 16 |
| *N*-methylmercaptoacetamide[b] | 0.4 | 2.0 | 5 | 0.3 | 16 |
| thiophenol | $1.4 \times 10^{-2}$ | N | >30[c,d] | 8.2 | 0.7 |
| 2-mercaptopyridine | $8 \times 10^{-5}$ [e] | N | >20[c,f] | *e* | 0.004[g] |
| bis(β-mercaptoethyl) ether[a] | $2 \times 10^{-2}$ | N | >10[c] | 5.8 | 1.0[a] |
| dithiothreitol[a] | 0.18 | N | >20[c] | 0.6 | 8.7[a] |
| | | In Neat Pyridine | | | |
| β-mercaptoethanol | 0.38 | $4.5 \times 10^{-2}$ | 0.12 | 2.9 | **1.0** |
| methyl 3-mercaptopropionate | 0.15 | $9 \times 10^{-2}$ | 0.6 | 2.0 | 1.4 |
| thiophenol | $6.6 \times 10^{-2}$ | N | >30[c] | 1.7 | 1.6 |
| dithiothreitol[a] | 0.46 | N | >10[c] | 0.25 | 11.4[a] |
| | | In *N,N*-Dimethylformamide–Acetic Acid (9:1) | | | |
| β-mercaptoethanol | $7.5 \times 10^{-2}$ | $9.0 \times 10^{-3}$ | 0.12 | 14.3 | **1.0** |
| *N*-methylmercaptoacetamide | 1.0 | N[h] | ≥10 | 0.1 | 124[i] |
| | | In pH 7.0 Phosphate Buffer | | | |
| β-mercaptoethanol | 0.06[j,k] | N | 375[l] | 9.6 | **1.0** |
| ethanethiol*[,m] | 0.11 | N | >100[c,l] | 5.2 | 1.8 |
| *N*-acetyl-β-mercaptoethylamine | 0.13 | N | >100[c,l] | 4.6 | 2.2 |
| methyl 3-mercaptopropionate | 0.10 | N | >100[c,l] | 5.8 | 1.7 |
| *N*-methylmercaptoacetamide | 0.55[n] | N | >100[c,l] | 1.0 | 9.2 |
| methyl 2-mercaptoacetate* | 0.50 | N | >100[c,l] | 1.1 | 8.3 |
| thiophenol*[,o] | 0.09[p] | N | >100[c,l] | 6.4 | 1.5 |
| 2-mercaptopyridine | *q* | N | | ≥$3 \times 10^4$ | ≤$4 \times 10^{-4}$ |
| bis(β-mercaptoethyl) ether*[,a] | 0.37[a] | N | >100[c,l] | 1.6 | 6.1[a] |
| dithiothreitol[a] | 4.0[a,r] | N | >100[c,l] | 0.1[r] | 67[a] |

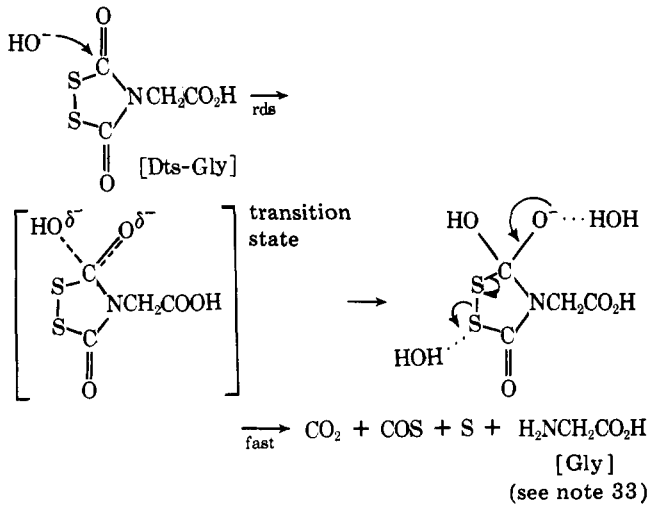
[a] For the first three sets, Dts-glycine (~5 mM) was treated with the appropriate monofunctional (100 mM) or bifunctional (50 mM) thiols under the indicated reaction conditions at 25 °C. For the last set, Dts-glycine (1 mM) was treated at 25 °C with the appropriate monofunctional (20 mM) or bifunctional (10 mM) thiol in pH 7.00 standard phosphate buffer, 0.036 M $Na_2HPO_4$–0.026 M $KH_2PO_4$, ionic strength ($I$) = 0.13, from Scientific Products. For thiols marked with an asterisk, the indicated concentrations of Dts-glycine and thiol were halved, and 10% (v/v) acetonitrile was added to ensure the complete aqueous solubility of the thiol (confirmed in separate experiments by Ellman's method, ref 69). Chromatographic properties of the thiols used, and of the corresponding carbamoyl disulfides, are given in the Experimental Section. N means that no carbamoyl disulfide intermediate was ever observed. Rate constants $k_1$ and $k_2$ were determined as outlined in the Experimental Section, and are expressed in L $mol^{-1}$ $s^{-1}$. The ratio of rates is defined by $\kappa = k_2/k_1$, and $k_{rel}^{env}$ is the envelope rate constant $k_1k_2/(k_1 + k_2)$, as explained in ref 12, relative to β-mercaptoethanol (first entry of each set) = **1.0.** The quantity $t_{1/2}^{env}$ (min) is given by $\ln 2/k^{env}$, and refers to the standard conditions with thiol concentrations specified in the first two sentences of this note. Observe that for effectively pseudo-first-order reactions, with high $\kappa$ (no carbamoyl disulfide intermediates ever observed = N), the equation for $t_{1/2}^{env}$ reduces to $t_{1/2}$, the half-time of that reaction in the usual sense. Second-order rate constants for reactions of bifunctional thiols have been calculated based *not* on the concentration of the reagent, but rather based on the total concentration of sulfhydryl groups which is *double* the previous figure. With the latter method of calculations, justified in the text, kinetic results obtained with bifunctional thiols were directly comparable to those obtained for monofunctional thiols. In each given set, thiols are listed in the following order: β-mercaptoethanol first; then monofunctional thiols in approximate descending order of $pK_a$ (measured in water; see legend to Figure 4); then 2-mercaptopyridine; then bifunctional thiols. All other footnotes to this table are included in the supplementary material.

lines 30–35). An increase of the proportion of pH 7.0 phosphate buffer in a dioxane-based reaction mixture from 10 to 30% (v/v) led to a progressive increase in the rate $k_1$ from 30 to 60% of the value observed in the entirely aqueous medium. On the other hand, when acetonitrile was the organic cosolvent, the rate $k_1$ was essentially independent of the amount of pH 7 buffer present. In the cases of both dioxane and acetonitrile, the second step of the reaction mechanism, $k_2$, was still observable in predominantly organic media. However, as the amount of water increased, so did $\kappa$ until the point was reached where the carbamoyl disulfide intermediate was no longer detectable. Hence, in entirely aqueous media, the first step of the mechanism of reductive deprotection is rate determining, and the constant $k_1$ was given directly by a simple pseudo-first-order treatment. An independent measurement (Table IV, line 5) revealed that in these cases $\kappa \sim 375$.

As shown in Figure 3, the rates of *reductive deprotection* of Dts-glycine mediated by 20 mM β-mercaptoethanol (solid line) were independent of buffer composition and varied directly with hydroxide ion concentration over a 2500-fold range at pH values below the $pK_a$ of the thiol. By comparison, the *hydrolysis* (dashed line) of Dts-glycine to glycine (Scheme IV)[27] proceeded at a $1.8 \times 10^4$-fold slower rate over a range from pH 8 to 10, and was subject to general base catalysis[28-32] (Appendix, part II).

Ten thiols with ionization constants varying over three orders of magnitude were compared in an aqueous pH 7.0 phosphate buffer to determine their relative efficiencies in mediating the reductive deprotection of Dts-glycine (Table III, last set). The reaction with 2-mercaptopyridine, which as already noted exists predominantly as its thione tautomer[23], was indistinguishable from background hydrolysis. Two of the thiols tested, namely, thiophenol and *N*-methylmercaptoacetamide, were sufficiently acidic to permit determination of reaction rates under conditions of complete thiol ionization. Complete "kinetic" titrations were carried out as in Figure 3 (solid line), and a saturation level of rates was reached at sufficiently high pH values. The apparent $pK_a$ values determined kinetically, which were 6.8 and 8.5, respectively, were at the high end and possibly even slightly in excess of the true $pK_a$ values reported in the

**Scheme IV**




literature for these thiols, as determined spectrophotometrically or potentiometrically [see Appendix (part I), in supplementary material, for range and references].

The data for the thiolytic deprotection of Dts-glycine under aqueous conditions were fully in accord with the well-accepted mechanism of thiol–disulfide exchange[24,34,35] involving thiol anions[36–39] as the reactive species (Scheme V). One difference

**Scheme V**

$$RSH + {}^{-}OH \underset{K_a}{\rightleftharpoons} RS^{-} + HOH \qquad (7)$$

$$RS^{-} + \text{Dts-Gly} \xrightarrow[\text{rds}]{k_{S^-}} \text{Carb} \xrightarrow[\text{fast}]{RS^-} \text{Gly} \qquad (8)$$

(A)

$$\partial(A)/\partial t|_{pH} = k_{pH}^{obsd}(A)(RSH_{tot}) = \frac{k_{S^-}}{[1 + \text{antilog}\,(pK_a - pH)]}(A)(RSH_{tot}) \qquad (9)$$

is that thiolysis of the Dts group, as well as of carbamoyl disulfides, is an irreversible reaction driven to completion by loss of carbonyl sulfide, whereas thiolysis of ordinary disulfides is an equilibrium process. Note that ionization of the thiol in aqueous solution (eq 7) is formally analogous to ion-pair formation in anhydrous media (eq 1, Scheme II), and that the linear variation of the first step of the reaction mechanism with pH at pH < p$K_a$ that was both predicted (eq 9) and found (Figure 3) made it unnecessary to invoke any additional equilibria involving Dts-glycine and the base (as in eq 4, Scheme III for anhydrous media). Finally, the specific base catalysis of the reaction implied by eq 7 was supported[30b,31] by a determination (Table III, footnote *k*) of a small inverse isotope effect[40] on the reaction rate of the mercaptoethanol anion with Dts-glycine, $k_{D_2O}/k_{H_2O} = 1.5$, in good agreement with theory.[21a]

The true rates, $k_{S^-}$, due to the thiol anion were either known directly or calculated by eq 9 from the observed rates at pH 7.0 (Table III) and have been plotted against the ionization constants of the thiols according to Brønsted[28,30–32] (eq 10 and

$$\log k_{S^-} = \beta_{nuc} pK_a + G \qquad (10)$$

$\beta_{nuc} = 0.9$ (notes 41 and 42)

$G = -7.1$, with units of *k* as L mol$^{-1}$ s$^{-1}$

Figure 4). A single straight line[41] fit the data for five monofunctional thiols ranging from thiophenol (**2**) to ethanethiol (**8**). It is in the nature of the arithmetic used to obtain Figure

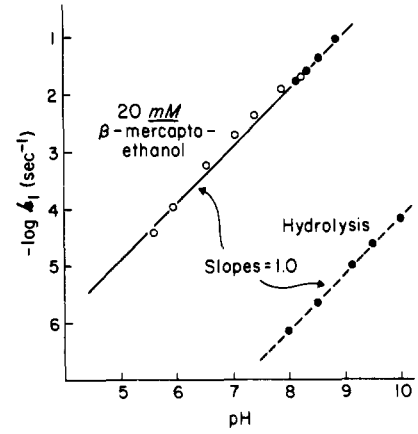



**Figure 3.** Rates of reactions of Dts-glycine in buffered aqueous media as a function of pH. Phosphate (○) and borate (●) buffers were prepared according to the instructions of Kolthoff (ref 26): 0.002–0.05 N NaOH was used to adjust the pH of 0.05 M $KH_2PO_4$ (gave buffers of pH 5.5–8.0, $I$ = 0.05–0.15) and of 0.05 M $H_3BO_3$–0.05 M KCl (gave buffers of pH 8.0–10.0, $I$ = 0.05–0.10). Dashed line, hydrolysis to glycine (Scheme IV and note 27); solid line, reductive deprotection to glycine mediated by 20 mM β-mercaptoethanol (20-fold molar excess over Dts-glycine). Upon completion of the reactions, the pH of the reaction mixtures was remeasured, and in all cases was found to be unchanged.

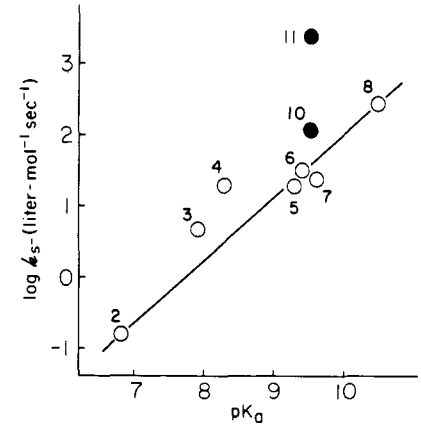



**Figure 4.** Brønsted correlation of the true rates of reductive deprotection of Dts-glycine mediated by thiol anions plotted against the ionization constants of these thiols. Mono- (○) and bifunctional (●) thiols are numbered as follows [p$K_a$ values, documented in appendix (Part I), are in parentheses]: **2** = thiophenol (6.8); **3** = methyl 2-mercaptoacetate (7.9); **4** = *N*-methylmercaptoacetamide (8.3); **5** = methyl 3-mercaptopropionate (9.3); **6** = *N*-acetyl-β-mercaptoethylamine (9.4); **7** = β-mercaptoethanol (9.6); **8** = ethanethiol (10.5); **10** = bis(β-mercaptoethyl) ether (9.5); **11** = dithiothreitol (9.5).

4 that the derived slope $\beta_{nuc}$ will be quite insensitive to uncertainties of up to ±0.5 units in the p$K_a$ values. Positive deviations of 0.6–0.9 log units from the Brønsted line were seen with methyl 2-mercaptoacetate (**3**) and the closely related derivative *N*-methylmercaptoacetamide (**4**); the former thiol anion has been tested under a variety of conditions[35,45] and has been systematically found to give positive deviations of 0.3–0.7 log units from the appropriate Brønsted plots.

Treatment of the rate data obtained for bifunctional thiols was performed as follows in a way designed to facilitate a direct comparison with data for monofunctional thiols. One mole of a bifunctional thiol with a measured first ionization constant p$K_1$ was considered to ionize and react with Dts-glycine in the same way as 2 mol of an imaginary "equivalent" monofunctional thiol with an ionization constant p$K_a$. At the pH values used in these experiments, which were much less than p$K_1$, only the monoanion of the bifunctional thiol must be taken into account. Using arguments of symmetry and mass balance, it is straightforward to derive the facts: (i) $pK_a = pK_1 + \log 2$;

(ii) second-order rate constants are properly calculated on the basis of the *total* sulfhydryl group concentration, which is twice the concentration of the bifunctional thiol (see Table III, footnote *a*). For the Brønsted plot, the equivalent rates and $pK_a$ must be used, and it was found (Figure 4, dark circles) that bis(β-mercaptoethyl) ether (**10**) and dithiothreitol (**11**) reacted respectively at rates 4- and 90-fold faster than expected. These same compounds were on the Brønsted line of the reactivity[24] of thiol anions with 5,5′-dithio(2-nitrobenzoic acid). The results of the work reported here might be empirically accommodated by inclusion into the Brønsted relation (eq 10) of another term reflecting the oxidation-reduction potential of the thiol-disulfide system (eq 11; oxibase scale formulated by Edwards[48] and also studied by Davies[49a]). Taking the equilibrium constant for exchange of dithiothreitol with cystine ($1.3 \times 10^4$), as reported by Cleland,[25] $\alpha$ may be estimated as 0.5. Interestingly, the special reactivity of bifunctional thiols noted in aqueous solutions did not carry over into anhydrous media (see Table III).

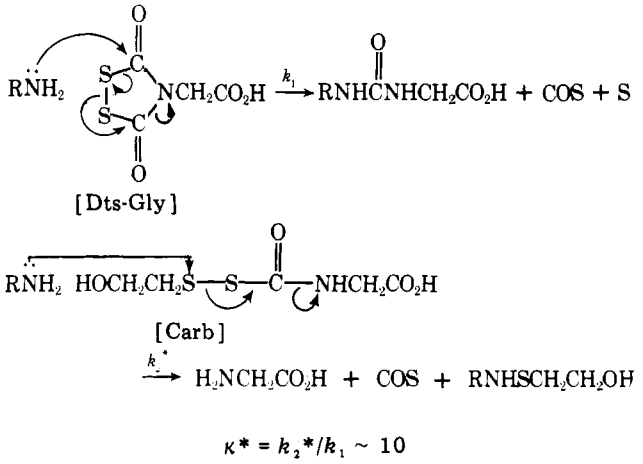
$$\log k_{S^-} = \beta_{nuc} pK_a + \alpha \log K_{ox} + \text{constants} \qquad (11)$$

**Rates of Reactions of Carbamoyl Disulfides.** The values of $\kappa$ reported in Tables I and III only give an indirect indication of the chemical properties and reactivities of the carbamoyl disulfide intermediates (Scheme I). Independent confirmation of the deduced rate constants $k_2$ might be obtained by treating an authentic sample of a carbamoyl disulfide under the various reaction conditions and directly observing simple pseudo-first-order kinetics. Although synthetic methods are available[50] which in principle ought to unambiguously give pure carbamoyl disulfides, these were not explored here. Rather, samples enriched in a carbamoyl disulfide, specifically β-hydroxyethyldithiocarbonylglycine, were obtained by the partial reaction of β-mercaptoethanol with Dts-glycine (see Experimental Section). Residual Dts-glycine in these preparations prevented determination of reaction rates with β-mercaptoethanol under *low* $\kappa$ conditions, because in that case new carbamoyl disulfide would be created at the same time as it was being transformed. Results on reaction rates of β-hydroxyethyldithiocarbonylglycine under conditions giving rise to high values of $\kappa$ are summarized in Table IV (13 entries; see supplementary material).

The aqueous quench procedure used in the majority of the kinetic studies was justified by the finding that both Dts and carbamoyl disulfide derivatives were sufficiently stable in acidic aqueous media, especially at pH 1.5, to permit accurate analysis within a reasonable period of time. Under mildly alkaline aqueous conditions, the carbamoyl disulfide was hydrolyzed to free glycine ($t_{1/2}$ = 3.3 min at pH 8) at a rate 5000-fold faster than the corresponding reaction (Scheme IV) of Dts-glycine. The rate of reductive deprotection of β-hydroxyethyldithiocarbonylglycine in aqueous media was directly measured (Table IV, line 5) and the calculated $\kappa$ = 375 was entirely consistent with the earlier results that no carbamoyl disulfide intermediates were detectable during the reaction of Dts-glycine with β-mercaptoethanol under these conditions.

The fastest rates for the thiolytic conversion of the carbamoyl disulfide to the free amino acid were observed in the presence of thiophenol and especially 2-mercaptopyridine (Table IV, lines 9 and 10), even in the absence of base (e.g., $t_{1/2}$ = 0.3 min with 0.1 *M* thiophenol in dioxane). Comparing these results with the rates of the first step of the reaction mechanism (Table III), the quantity $\kappa$ for these two thiols could be estimated as $10^5$ and $\geq 10^9$, respectively. Crossed experiments (Table IV, lines 7 and 8), discussed in detail in the Appendix (part III), revealed that thiolytic cleavage of carbamoyl disulfides occurred monodirectionally[51] to give the product amino acid and the mixed disulfide. Of further interest, nucleophilic aminolysis of Dts-glycine on the one hand[3,5] and

Scheme VI




$$\kappa^* = k_2^*/k_1 \sim 10$$

of β-hydroxyethyldithiocarbonylglycine on the other hand (Table IV, lines 11 and 12) led to different products formed at different rates (Scheme VI).

## Discussion

**Electronic Requirements of the Two Steps of the Reaction Mechanism.** The mechanism of the thiolytic deprotection of dithiasuccinoyl amines (Scheme I) formally involves two base-catalyzed thiol-disulfide interchange reactions, both with a thiocarbamate moiety as the leaving group. The ratio of rates $\kappa$ was found to vary widely depending on the precise reaction conditions, suggesting that the two steps have strikingly different electronic requirements. In the first step, the substrate of the thiolysis is a heterocyclic biscarbamoyl disulfide, while for the second step the substrate is an open-chain monocarbamoyl disulfide.

In *anhydrous* media, $\kappa$ was usually between 0.1 and 5, and the active species for *both* steps of the reaction mechanism was proposed to be an association complex of the thiol and a base (Schemes II and III). The observed solvent effects on the rates of reductive deprotection (Table II) suggested that the transition states of these reactions involve generation of species with ionic character and separations of charge, and are stabilized by polar aprotic media of high dielectric constant. The solvent effects qualitatively paralleled relative base strengths of the tertiary amines used as catalysts, as reflected by the association constants for ion-pair formation in aprotic media.[17-19] The latter criterion was also successful in predicting relative rates of thiolysis in the presence of different bases in the same solvent. A quantitative treatment would require precise knowledge of solvation, steric hindrance, and polarization effects, as well as other parameters that may not be readily amenable to experimental measurement or theoretical prediction.

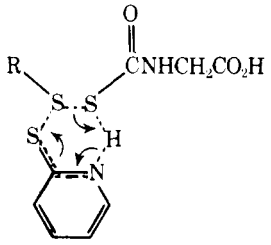
A subtle difference in the two steps of the mechanism in anhydrous media, namely, the observation of nonparallel lines in plots of log *k* against log [base], has already been discussed (Figure 2 and accompanying text). The most interesting fundamental differences between the two steps were in the values of $\kappa$ determined with different thiols (Tables III and IV). The fact that observed rates for the second step increased steadily with increasing acidity of the attacking thiol was subject to the following preliminary interpretation. It was assumed that the true rate $k_5$ (Scheme II) was subject to a Brønsted-type correlation (eq 12); substitution into eq 3 gave eq 13. A *small*

$$k_5 \propto (K_{SN})^{-\beta_{nuc}} \qquad (12)$$

$$k_2^{obsd} \propto (K_{SN})^{[1-\beta_{nuc}]} \qquad (13)$$

value of $\beta_{nuc}$ would mean that the intrinsic rate $k_5$ was rather insensitive to the ease of removal of a proton from the thiol. The observed rate (eq 13) would then be highest for the most acidic

thiols, since the concentration of the active species would be relatively the highest. While this argument qualitatively reproduced the observed trends, it still fell short of giving a quantitative accounting of the large values of $\kappa$ observed with thiophenol and 2-mercaptopyridine (Table IV). For the former purpose the polarizability and "softness" of thiophenol as a nucleophile could be invoked in the same manner as discussed at length by Hupe,[35] while in the latter case a transition state such as I might play a role. Finally, mention must be made of



I

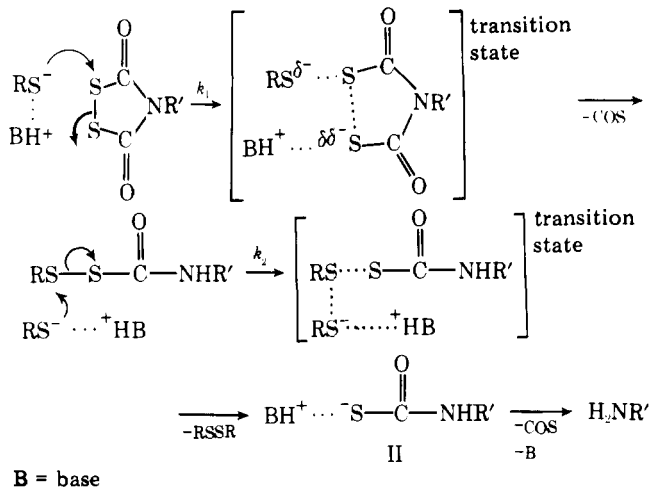
the general sensitivity of carbamoyl disulfides to nucleophiles, as typified by the fission to the free amine upon aminolysis (Scheme VI).

In contrast to the features of the second step just discussed, the observed rates of the first step of the reaction mechanism in anhydrous media were consistent with a relatively *high* value of $\beta_{nuc}$ (eq 12 and 13 with subscripts suitably altered to those of Scheme III). In qualitative terms, greater nucleophilicity was shown with the more basic thiols; the point was underlined by the negligible reactivity of 2-mercaptopyridine (contrast to transition state I accounting for the unusual reactivity of 2-mercaptopyridine ($\kappa \geq 10^9$) in the second step). The observed rates of the first step of the mechanism showed a biphasic pattern with regard to the ionization constants of the thiols, with the fastest overall rates found with thiols of intermediate acidity ($pK_a$ = 8.0–9.5). Since eq 13 predicts a monotonic pattern, it obviously does not give a complete description of all of the factors contributing to the observed reaction rates.

The active species in *aqueous* solutions was shown to be the thiol anion, and the data for the rates $k_1$ of five monofunctional thiols fit a Brønsted correlation (Figure 4) with $\beta_{nuc}$ = 0.9 (eq 10), consistent with the qualitative expectation in *anhydrous* media just outlined. The interpretation[30c,32,35,45] of the slopes of Brønsted correlations must be carried out with caution; however, it is generally accepted that these slopes may give a picture of the intimate charge distribution of the transition state. Scheme VII, meant to apply both in aqueous and anhydrous media, contrasts the electronic requirements of the two steps of the reaction mechanism. It is based on the dependencies of reaction rates on thiol structures, and no attempt has been made to take into account differing geometric constraints of nucleophilic displacements at acyclic[34,49b] and cyclic[21a] substrates. The lengths of the dotted lines have been adjusted inversely to the extent of bond formation in the transition state. The low $\beta_{nuc}$ value of the second step is consistent with the idea that the conjugate acid of the thiocarbamate II can be[33] much more readily ionized[52] than the conjugate acid of the attacking thiol anion or thiol–base ion pair; hence the resemblance[53] of the transition state for that step to the starting components. Yet for the first step apparently the opposite is true, as reflected by the large $\beta_{nuc}$.

A further insight into the driving forces of the two separate steps was provided by a quantitative comparison of our data with literature values on the approximate rates of reactions in protic solvents of thiol anions with a variety of cyclic[34] and acyclic aliphatic,[34,54-56] aromatic,[24,35] and mixed[24,35] disulfides (Scheme VIII). The ease of reduction of open-chain disulfides depends[49] markedly on the $pK_a$ of the thiol leaving group, and the position of the carbamoyl disulfide intermediate [Carb]

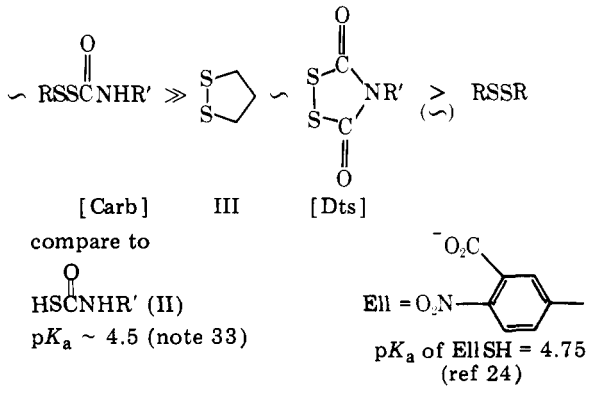
**Scheme VII**




B = base

**Scheme VIII**

in $H_2O$, relative rates of reaction with $RS^-$:




in Scheme VIII reflects the relative acidity in water[33,52,57] of the thiocarbamate leaving group from the thiolytic cleavage.

Fava et al.[34] found that the exchange of *n*-butylthiolate proceeded in methanol at a rate $10^4$-fold faster with 1,2-dithiolane (III) as the substrate as opposed to radiolabeled bis(*n*-butyl) disulfide. The absolute rate with III was 1400 L $mol^{-1}$ $s^{-1}$, which is of the same order as our calculated rate of 350 L $mol^{-1}$ $s^{-1}$ for the reaction of the ethanethiol anion ($pK_a$ 0.15 units less[58] than that of *n*-butanethiol) with Dts-glycine. Using the anion of methyl 2-mercaptoacetate, the rates were 2.2 L $mol^{-1}$ $s^{-1}$ with III and 4.5 L $mol^{-1}$ $s^{-1}$ with Dts-glycine. Competition experiments summarized in the Appendix (part IV) showed that reductive deprotection of Dts-glycine proceeded at least two orders of magnitude more rapidly than the reduction of aliphatic disulfides to the sulfhydryl compounds.

The quantitative similarity of the rates for the simple five-membered-ring dithiolane III, and for Dts-glycine, which contains two additional carbonyl groups and a nitrogen atom as part of the ring, and where the leaving group is more acidic by six orders of magnitude, suggests that electronic factors are not important in determining these reactivities.[52] Rather, by analogy to proposals already forwarded for the dithiolane case,[34,49b] we conclude that the driving force for the thiolysis of the Dts heterocycle appears to be relief of ring strain.

**Comparison of the Dts-Amino Protecting Group with Other Thiolyzable Groups Used in Peptide Synthesis.**[8,59] The $N^{\alpha}$-*o*-nitrophenylsulfenyl (Nps) group[60] is best removed[61-63] by polarizable, nucleophilic thiols and thiones, preferentially in

the presence of weak carboxylic acid catalysts. The reaction of aliphatic thiols with Dts amines may be estimated to occur $10^2$- to $10^5$-fold more rapidly than with Nps amines under comparable aqueous or anhydrous conditions, but selectivity in the converse direction is achieved[63] with 2-mercaptopyridine in dichloromethane, methanol, or *N,N*-dimethylformamide solvents with glacial acetic acid as the catalyst. These latter conditions, which lead to quantitative removal of the Nps group within 30 min, would also be conducive to rapid transformations of open-chain carbamoyl disulfides, but will *not* affect the Dts group. The $N^{im}$-2,4-dinitrophenyl (Dnp) protecting group[64] for histidine provides yet another example of a "low $\beta_{nuc}$" substrate for thiolysis in that reactions with $\beta$-mercaptoethanol or dithiothreitol proceed at least two orders of magnitude more slowly than the reductive deprotection of Dts-glycine, but reactions with thiophenol (0.1 M) in *N,N*-dimethylformamide for 30 min (the preferred conditions for removal of the Dnp group) occur at approximately the same rate with both the Dts and Dnp substrates.

The reductive deprotection of the Dts group, not only with thiols but also[6] with trialkylphosphines and borohydrides, is much more facile than the corresponding reactions of aliphatic mixed disulfides used to protect cysteine[65] or of the sulfoxide used to protect methionine.[62,66,67c] Finally, the chemistry of alkoxycarbonylsulfenyl derivatives of cysteine,[51,68] which are important intermediates in the directed formation of unsymmetrical disulfide bonds,[8,65] contains many mechanistic features closely related to the chemistry of dithiasuccinoyl and carbamoyl disulfide derivatives of amines as presented here.

**Quantitative Thiolytic Removal of the Dts-Amino Protecting Group.** The goal of the present study was to devise practical and effective conditions for rapidly carrying out repetitive thiolytic deprotection of dithiasuccinoyl amines as the "temporary" $N^{\alpha}$-protected derivatives for stepwise solid-phase peptide synthesis.[4,8,9] Most of the data reported herein were determined with Dts-glycine treated under conditions of thiol, base, and solvent composition and concentration where the reaction rates were easily measurable. These accurately known rates could then be validly extrapolated to much faster rates on the basis of the various thiol, base, and solvent dependencies derived in this work. Times necessary for completion of reactions were taken as $\geq 10t_{1/2}^{env} \times 5$, where 10 half-lives calculated by the envelope approximation[12] ensured $\gg$99.9% conversion to product, and the factor of 5 more than compensated[6] for any minor differences in reactivities due to variations in amino acid residue side chains.

Suitable conditions for quantitative removal of the Dts-amino protecting group at 25 °C, estimated on the basis of the considerations outlined in the previous paragraph, include (1) $\beta$-mercaptoethanol (0.2 M)–triethylamine (0.5 M) in benzene for 5 min; (2) *N*-methylmercaptoacetamide or dithiothreitol (0.1 M) in neat pyridine for 5 min; (3) *N*-methylmercaptoacetamide or *N*-acetyl-$\beta$-mercaptoethylamine (0.1 M)–*N*-methylmorpholine (0.5 M) in acetonitrile for 1 min; (4) $\beta$-mercaptoethanol (0.1 M) and 2-mercaptopyridine or thiophenol (1.1 equiv over Dts amine) in *N,N*-dimethylformamide–pyridine (9:1) for 1 min; (5) *N*-methylmercaptoacetamide (0.2 M) in *N,N*-dimethylformamide–acetic acid (9:1) for 2 min; (6) dithiothreitol (10 mM) in pH 7.0 phosphate buffer for 2 min. All of the listed conditions have been chosen so that the ratio of rates $\kappa$ will be $\geq 5$, meaning that the first step of the reaction mechanism (Scheme I) will essentially determine the overall rate.

The availability of such a wide range of suitable conditions for effecting the reductive deprotection has a number of advantages. For example, suppose that a low-level side reaction becomes identified; one possibility could be "wrong-way" thiolytic opening of the Dts heterocycle at the carbonyl group to give a thiourethane, by analogy to the aminolytic opening (Scheme VI) giving a urea derivative. All of the available data[5,6] rule out that particular side reaction at a level of $\leq$2%. Nonetheless, the reaction of the Dts carbonyl with nucleophiles, as well as any other putative side reactions, will in all likelihood have a quite differing set of electronic requirements ($\beta_{nuc}$, solvent dependencies, etc.) from those of the reduction of the Dts–disulfide bond. Thus, by making appropriate adjustments in the natures of the thiol, base, and solvent used, it should be possible to devise conditions which minimize the relative rate of the hypothetical side reaction in comparison to the rate of the desired reductive deprotection.

## Experimental Section

Some of the materials and methods used in this study have been detailed previously.[6] Purification of solvents and bases is described in the Appendix (part V). Stock solutions of Dts-glycine (10 mg/mL) prepared in purified dichloromethane, benzene, dioxane, acetonitrile, and pyridine were entirely stable for months (and where tested, for over 1 year) as judged by several exacting quantitative criteria including chromatography on the amino acid analyzer, IR, UV, and NMR spectra, and reproducibility of rate constants from kinetic experiments carried out in the given solvent under standardized conditions. Aqueous stocks of Dts-glycine underwent slow pH-dependent hydrolysis (see dashed line, Figure 3) and solvolysis also occurred in methanol (half-time ~3 weeks) to yield methoxycarbonylglycine. Stocks in dimethyl sulfoxide and in purified *N,N*-dimethylformamide were stable for 20 h or so, but decomposed thereafter in autocatalytic fashion to give breakdown products which included 10–30% free glycine. Decomposition of Dts-glycine in *N,N*-dimethylformamide was retarded somewhat by exclusion of light, or in the presence of oxygen acting as a free-radical inhibitor; decomposition was completely prevented (10 weeks) by addition of 10% (v/v) glacial acetic acid or by standing over resin beads of Amberlyst-15.

**Thiols.** The 11 thiols tested in this study were either prepared in the laboratory (see following procedures) or obtained commercially from Aldrich, Eastman, MCB, RSA (Ardsley, N.Y.; for dithiothreitol), or as a gift from Evans Chemetics, Inc. (Darien, Conn.), and were used without further purification. Stock solutions of a given concentration were prepared freshly, and the quantitative sulfhydryl content of all thiols was confirmed to be 97 ± 3% of theory by the procedures of Ellman[69] [with 5,5′-dithiobis(2-nitrobenzoic acid); measured at 412 nm, $\epsilon$ $1.36 \times 10^4$] and of Grassetti and Murray[70] [with 2,2′-dipyridyl disulfide; measured at 343 nm, $\epsilon$ $7.06 \times 10^3$]. The latter procedure was specifically preferred for accurate quantitation of thiophenol, ethanethiol, and octadecanethiol because of the greater reactivity of 2,2′-dipyridyl disulfide and the greater stability of the ensuing chromophore. Other evidence for the purity of the thiols came from the NMR spectra and from the extinction coefficients and absorption maxima of the thiol anions measured in 0.01 N NaOH. Thiol $pK_a$ values were estimated by either a potentiometric or a spectrophotometric technique (Appendix, part I).

In selected instances, the thiol concentration and extent of oxidation occurring in the course of a kinetic experiment were determined by periodic Ellman's titration.[69] An 0.1 M solution of $\beta$-mercaptoethanol in peroxide-free dioxane (Table I, line 1) was entirely stable for 3 weeks. In pH 7 phosphate buffer, 20 mM $\beta$-mercaptoethanol and 10 mM *N*-methylmercaptoacetamide oxidized with half-times of 86 and 4 h, respectively, corresponding to 0.2 and 1.0% of the rates of thiolysis of Dts-glycine under these conditions (Table III). Finally, a 50 mM solution of dithiothreitol in pyridine oxidized with a half-time of about 60 h, or 0.01% of the rate of reductive deprotection of Dts-glycine (Table III). The fact that all of the kinetics measured in this work faithfully adhered to the required pseudo-first-order expressions was taken as strong evidence for negligible oxidative losses of the thiols.

***N*-Methylmercaptoacetamide.**[67] Methyl 2-mercaptoacetate (150 mL, 1.7 mol) and 40% aqueous methylamine (300 mL, 3.8 mol) were combined at room temperature in a spontaneously exothermic reaction. Excess methylamine, water, and the produced methanol were removed at 50 °C (12 mm) and the product thiol was isolated by fractional vacuum distillation through a Vigreux column: yield 120 g (67%); bp 126–128 °C (5 mm) [lit.[67c] bp 71–72 °C (0.2 mm)]; NMR ($CDCl_3$) $\delta$ 3.50 (s, 4% by height, disulfide $CH_2$), 3.27 (s, 2 H, $CH_2$), 2.86 (d, $J$ = 5 Hz, 3 H, $NHCH_3$). The NMR spectrum just cited was recorded half a year after the synthesis and distillation of the title compound, which had subsequently been stored in a closed

amber bottle without special precautions. The molarity of the neat liquid was determined to be 10.8 M; further characterization was provided by conversion to the analytically pure disulfide, mp 122–123 °C from ethyl acetate (lit.[67a] mp 123–125 °C after repeated recrystallization from absolute ethanol).

***N*-Acetyl-β-mercaptoethylamine.** Following Kuhn and Quadbeck,[71] ethylenimine (16 g, 0.37 mol) was added dropwise at 10–15 °C over 1 h to a solution of thioacetic acid (25 mL, 0.35 mol) in methanol (200 mL). The mixture was refluxed for an additional 30 min, solvent was removed at 25 °C (12 mm), and the product thiol was purified (reddish forerun discarded) by vacuum distillation: yield 30 g (68%); bp 100 °C (0.9 mm) [lit.[71] bp 138–140 °C (7 mm)]; molarity of neat liquid = 9.1 M. The product obtained for this study was judged to be free (≤3%) of *S*-acetyl-β-mercaptoethylamine as well as 2-methyl-$\Delta^2$-thiazoline on the basis of the UV spectrum (ref 72 and 73), which showed only end absorption even half a year after the synthesis and distillation. This result is in contrast to reports[72,74] that the Kuhn–Quadbeck procedure gave rise to contaminated material which had a marginal shelf life. Other routes to the title compound have been reported.[72,75] NMR ($CDCl_3$): δ 3.40 (skewed q, 2 H), 2.65 (skewed t, 2 H), 2.00 (s, 3 H, $CH_3CO$). Reaction with 1-fluoro-2,4-dinitrobenzene (1.05 equiv) in aqueous sodium carbonate gave the *S*-dinitrophenyl derivative (93%), mp 131–132 °C, which was recrystallized from absolute ethanol, melting point unchanged.

Anal. Calcd for $C_{10}H_{11}N_3O_5S$ (mol wt 285.21): C, 42.11; H, 3.89; N, 14.73, Found: C, 42.13; H, 3.98; N, 14.78.

**β-Hydroxyethyldithiocarbonylglycine. Run I.** Dts-glycine (35 mg, 0.18 mmol) was dissolved in peroxide-free dioxane (7 mL), and triethylamine (100 μL, 0.7 mmol) and β-mercaptoethanol (50 μL, 0.7 mmol) were added. After 10 min, the reaction mixture was diluted into a mixture of ethyl acetate and 1 N hydrochloric acid (50 mL each). Amino acid analysis showed that at this point the reaction had proceeded to 65% conversion to glycine, and the ratio of Dts:Carb was 1:6. The aqueous phase removed all of the product glycine, but also about half of the carbamoyl disulfide. The organic phase was dried over magnesium sulfate, filtered, concentrated by rotary evaporation at 10 mm and 45 °C, and dissolved in chloroform (2 mL). In the course of the rotary evaporation, some carbamoyl disulfide was destroyed, so that the final ratio of Dts:Carb was 1.0:1.5 (ratio of peak heights = 1:5 due to differences in integration constants).

**Run II.** Dts-glycine (24 mg, 0.12 mmol) was dissolved in *N,N*-dimethylformamide–acetic acid (4:1 v/v, 0.75 mL) containing β-mercaptoethanol (15 μL, 0.21 mmol). Amino acid analysis after 4 h showed that all of the thiol had been consumed, and that the ratio of Dts:Carb:Gly was 1:8:4. The reaction mixture was concentrated by rotary evaporation at 0.5 mm and 45 °C. Acetic acid (2 × 5 mL) and then carbon tetrachloride (3 × 5 mL) were then added as the sample was successively redissolved and reconcentrated. Finally, the residue was dissolved in chloroform (2 mL) and this solution was clarified to remove insoluble glycine by filtration through a plug of glass wool and by low-speed centrifugation. An aliquot (10 μL) was diluted to 2 mL with citrate buffer and chromatographed. The only peak seen was that for the carbamoyl disulfide, but up to 25% residual Dts-glycine (not seen because of the low relative integration constant) was indirectly indicated by several experiments to determine the yield of glycine after certain treatments. The overall yield of the carbamoyl disulfide by run II was 15%, as it was calculated that 30% survived the rotary evaporation step.

The samples obtained by runs I and II were suitably enriched in carbamoyl disulfide to allow determinations of reaction rates (Table IV) by the usual chromatographic technique (see following). Portions of the stock solutions were brought to dryness by air evaporation or blowing a stream of nitrogen, and redissolved in the appropriate media. Conditions giving rise to high values of κ were particularly profitably studied by this approach. It was easy to establish that there was absolutely no residual β-mercaptoethanol in these samples (see Table IV, footnote *b*). Reactions rates observed on run I or run II carbamoyl disulfide were in good agreement.

**Chromatography.**[6] A Beckman Model 120B amino acid analyzer based on the design of Spackman, Stein, and Moore[10] was used. A 0.9 × 54 cm column was packed with Beckman AA-15 sulfonated polystyrene cation exchange resin and operated at a temperature of 57 °C. Samples of 1.0 mL were reproducibility applied (±2%) with an Altex rotary-valve injector and eluted at a buffer flow rate of 62 mL/h with 0.2 N sodium citrate buffer, pH 3.20. The ninhydrin flow rate was 31 mL/h.

Elution times and peak widths were found to vary slightly with the actual pH and amount of water-miscible organic cosolvent in the applied sample. For most of the experiments reported here, the elution times of Dts-glycine and glycine were about 37 and 140 min, respectively, with half-widths of 3.5 and 2.4 min, respectively. When these were the only species present from a kinetic run to be analyzed (e.g., reductive deprotection with thiophenol or dithiothreitol or studies of pH-dependent hydrolysis), it was possible to serially inject ten samples at 10-min intervals and still unambiguously identify each peak in the chromatogram. Thus, successive Dts-glycine peaks were separated by 10 min, whereas glycine peaks merged slightly but were still 6–7 min apart (enough time to reestablish a base line in between peaks). Too high an amount of pyridine in the applied sample (>1% v/v after dilution) led to chromatographic difficulties in that the series of glycine peaks became broad and fused.

When reductive deprotections were carried out with a variety of thiols, additional peaks were observed upon chromatography of the obtained samples. Carbamoyl disulfide intermediates were resolved from Dts-glycine when the thiol used was β-mercaptoethanol, methyl 3-mercaptopropionate, or ethanethiol; respective elution times of these peaks were 42, 57, and 78 min, and the $A_{570nm}/A_{440nm}$ ratio of ninhydrin absorbances was the normal value of ~6. Peaks were also seen due to the thiols themselves, with elution times very close to the ones due to the carbamoyl disulfide intermediates, but nonetheless readily distinguishable because the $A_{570nm}/A_{440nm}$ ratio for thiols was low, 0.1–0.2. When *N*-methylmercaptoacetamide or *N*-acetyl-β-mercaptoethylamine were tested, the thiol and carbamoyl disulfide peaks were not chromatographically resolved from Dts-glycine. When octadecanethiol was used, the thiol and the corresponding carbamoyl disulfide intermediate were both insoluble in the pH 2 citrate buffer used to apply samples. Finally, for 2-mercaptopyridine, thiophenol, bis(β-mercaptoethyl) ether, and dithiothreitol, no carbamoyl disulfide intermediate was ever observed. Except for bis(β-mercaptoethyl) ether, which gave a peak at 66 min, none of the thiols mentioned in the preceding sentence yielded a ninhydrin-positive peak under standard analytical conditions with pH 3.20 buffer (dithiothreitol eluted at 51 min with pH 6.40 buffer).

For studying reactions of β-mercaptoethanol, typically eight serial injections were performed at 12-min intervals. For other thiols, suitable injection protocols were derived via some easy arithmetic, e.g., 0, 10, 40, 50, 160, 170, min, etc., in the case of methyl 3-mercaptopropionate.

Further details of the analytical method have been described elsewhere.[6] As reported, the relative ninhydrin color yields (integration constants) of Dts-glycine, carbamoyl disulfide intermediates, and free glycine were in the approximate ratio (accurate to ±15%) of 0.3:1.0:1.

**General Procedures for Kinetic Experiments.** All reactions were performed at room temperature (25 °C). Some specific representative procedures have been outlined in previous reports.[1,6] Different permutations in the order of addition of reagents were used depending on what parameter was being varied. In general, reactions were initiated when three components (Dts-glycine, thiol, and base) were brought together, and stock solutions containing one or two of these components were usually stable over a reasonable time span. Specific initiation conditions, all of which led to the same rate data, included (i) combination in a 1:1 (by volume) ratio of two doubly concentrated stock solutions containing among them all the necessary ingredients of the reaction mixture and (ii) delivery of base or thiol, in a small relative volume, to a mixture of all the other ingredients at the proper concentrations. Reaction mixtures were briefly agitated on a Vortex mixer. Aliquots of 20–100 μL were periodically withdrawn by micropipetting and quenched into 2 or 3 mL of 0.2 N sodium citrate buffer, pH 1.5, and 1 mL of the quenched solutions was then chromatographed on the Beckman Model 120B amino acid analyzer (see previous section and ref 6). In an alternative, anhydrous, quench procedure, 50-μL aliquots of the reaction mixtures were diluted into 0.2 mL of *p*-toluenesulfonic acid monohydrate (1 M) in dioxane, followed by bringing the quenched solutions to volume in aqueous citrate buffer. For accurate end points, aliquots were taken at an early stage of the kinetic reactions, but quenched only later. The yield of glycine obtained this way agreed, within the experimental error of amino acid analysis (±2%), exactly with the values obtained by base hydrolysis (0.1 N NaOH, 10 min) of the starting amount of Dts-glycine.

To permit a complete accounting of the glycyl residue from among

the derivatives listed in Scheme I, and to circumvent a sampling problem in the determination of heterogeneous solutes,[6] entire kinetic experiments were occasionally carried out on a "same-tube" basis.[6] In these cases, the reaction medium to be tested was assembled from doubly concentrated stock solutions in a total volume of 50 or 100 $\mu$L at the bottom of a multitude of conically tipped centrifuge tubes. The entire contents of a given tube were quenched with citrate buffer at an appropriate time, and a sample was chromatographed as before.

As the first steps in the analysis of raw data from kinetic experiments, all peaks in the chromatogram were identified, integrated, and corrected for backgrounds and relative ninhydrin color yields. The conservation law [Dts] + [Carb] + [Gly] = [$Dts_{initial}$] = [$Gly_{final}$] was then verified for all stages of the reductive deprotection. Within the experimental uncertainty imposed in this calculation by the chosen values for the integration constants, the conservation law was always valid whenever kinetics were obtained on a "same-tube" basis, or when aliquots were taken from a solvent, such as water or near pyridine, in which the product glycine was fully soluble or from which glycine only crystallized out slowly. The heterogeneous solute sampling problem[6] led to low glycine values for aliquots taken at late stages of the kinetic reactions, but did not affect the accuracy of determination of Dts-glycine or the carbamoyl disulfide intermediate, which are the quantities needed to derive the rate constants. As a matter of fact, only the *relative,* as opposed to *absolute,* amounts of each of these two components are required for the calculations.

The mathematical procedures to determine the rate constants from the integrated and normalized raw data are described elsewhere.[12] All rate constants reported in this paper were determined on at least two separate occasions, and are reported to ±8% for $k_1$ and ±15% for $k_2$.

Very occasionally, autocatalytic reaction kinetics were observed; in these cases, the reported data refers to the initial phase of the consecutive reactions where valid pseudo-first-order kinetics still held. A complete discussion of these effects is found in the Appendix (part VI).

**Supplementary Material Available:** An appendix, in six parts: (I) Determination of Thiol p$K_a$ Values; (II) General Acid and Base Catalysis of Hydrolysis of Dts Group; (III) Monodirectional Thiolysis of Carbamoyl Disulfides; (IV) Reduction of Disulfides with Dithiothreitol; (V) Purification of Solvents and Bases; (VI) Conditions for Observation of Autocatalytic Reaction Kinetics. Also, Table I (35 entries), "Rates of Reductive Deprotection of Dts-Glycine by $\beta$-Mercaptoethanol as a Function of Organic Base and Solvent Composition", additional notes to Table III in text, and Table IV (13 entires), "Rates of Reactions of $\beta$-Hydroxyethyldithiocarbonylglycine" (9 pages). Ordering information is given on any current masthead page.

## References and Notes


(1) A preliminary report on this work was presented by G.B. at the Sixth American Peptide Symposium, Washington, D.C., June 17–22, 1979; in "Peptides: Structure and Biological Function", E. Gross and J. Meienhofer, Eds., Pierce Chemical Co., Rockford, Ill., 1979, pp 313–316. Supported by U.S. Public Health Service Research Fellowship 5 F 32 AM 05806-02 to G.B., and Grant AM 01260 to R.B.M.

(2) Abbreviations and nomenclature used: Dts, dithiasuccinoyl; Carb, open-chain carbamoyl disulfide intermediate of Scheme I; Gly, glycine; $\alpha$, $\beta$, $\gamma$, respective mole fractions of these three species (Figure 1); $I$, ionic strength; NMM, *N*-methylmorpholine; $Et_3N$, triethylamine; rds, rate-determining step; $\kappa = k_2/k_1$, ratio of rates of two steps of Scheme I; $k^{env} = k_1k_2/(k_1 + k_2)$, envelope rate constant (ref 6, 1, and 12); $t_{1/2}$, reaction half-time; $k_{S^-}$, intrinsic reaction rate of thiol anion; $\beta_{nuc}$, slope of Brønsted-type correlations (eq 10 and 12). The meanings of these and additional terminologies used are explained at the first appearance and subsequently in the accompanying text.

(3) G. Barany, Ph.D. Thesis, The Rockefeller University, New York, 1977; *Diss. Abstr.,* **38,** 5893-B.

(4) G. Barany and R. B. Merrifield, *Fed. Proc. Fed. Am. Soc. Exp. Biol.,* **36,** 864 (1977).

(5) G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.,* **99,** 7363–7365 (1977).

(6) G. Barany and R. B. Merrifield, *Anal. Biochem.,* **95,** 160–170 (1979).

(7) (a) R. B. Merrifield, G. Barany, W. L. Cosand, M. Engelhard, and S. Mojsov in "Peptides—Proceedings of the Fifth American Peptide Symposium", M. Goodman and J. Meienhofer, Eds., Wiley, New York, 1977, pp 488–502. (b) For a description of different efforts in other laboratories to achieve the same goal of a mild, orthogonal protection scheme, see E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Chem. Commun.,* 537–539 (1978); E. Atherton, R. C. Sheppard, and B. J. Williams in "Peptides 1978", I. Z. Siemion and G. Kupryszewski, Eds., Wroclaw University Press, Wroclaw, Poland, 1979, pp 207–210; C. D. Chang and J. Meienhofer, *Int. J. Pept. Protein Res,* **11,** 246–249 (1978).

(8) Review: G. Barany and R. B. Merrifield in "The Peptides", Vol. 2, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980, pp 1–284.

(9) R. B. Merrifield, *J. Am. Chem. Soc.,* **85,** 2149–2154 (1963).

(10) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.,* **30,** 1190–1206 (1958).

(11) (a) S. Moore and W. H. Stein, *J. Biol. Chem.,* **211,** 907–913 (1954); (b) S. Moore, *ibid.,* **243,** 6281–6283 (1968).

(12) G. Barany, to be published.

(13) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism", 2nd ed., Wiley, New York, 1961, pp 166–171, and references cited therein.

(14) A plot of $k_1$ over a tenfold range of $\beta$-mercaptoethanol concentration (0.05–0.5 M), in dioxane in the presence of triethylamine (0.1 M), was linear and passed through the origin, or equivalently, a plot of log $k_1$ against log [thiol] had a slope of 1.0 (compare to Figure 2 in text). The rate of the second step was only determined over a fourfold concentration range because with too great an excess of thiol interference of the mercaptoethanol peak in the elution region of the open-chain carbamoyl disulfide intermediate (ref 6 and Experimental Section, this work) was too great to be subtracted as background. Over the experimentally accessible range, again, the plot of log $k_2$ against log [thiol] had a slope = 1.0.

(15) J. D. Glass, A. Talansky, Z. Grzonka, I. L. Schwartz, and R. Walter, *J. Am. Chem. Soc.,* **96,** 6476–6480 (1974). The mixture of *N*-methylmorpholine (1 M) and glacial acetic acid (0.5 M) in *N,N*-dimethylformamide has been referred to as "buffered" DMF by these authors because the absorbance of a bromocresol green indicator remained constant upon addition of glycine ethyl ester hydrochloride from 0 to 0.16 M.

(16) J. A. Riddick and W. B. Bunger, "Organic Solvents—Physical Properties and Methods of Purification", 3rd ed., Wiley, New York, 1970.

(17) Review: M. M. Davis, "Acid-Base Behavior in Aprotic Organic Solvents", *Natl. Bur. Stand. (U.S.), Monogr.,* **No. 105** (1968).

(18) R. G. Pearson and D. C. Vogelsong, *J. Am. Chem. Soc.,* **80,** 1038–1043 (1958).

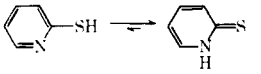
(19) A. W. Williams and G. T. Young, *J. Chem. Soc., Perkin Trans. 1,* 1194–1200 (1972).

(20) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution", Butterworths, London, 1965.

(21) (a) B. Dmuchovsky, B. D. Vineyard, and F. B. Zienty, *J. Am. Chem. Soc.,* **86,** 2874–2877 (1964); (b) B. Dmuchovsky, F. B. Zienty, and W. A. Vredenburgh, *J. Org. Chem.,* **31,** 865–869 (1966). See also references cited in these two papers.

(22) M. Eigen, *Angew. Chem., Int. Ed. Engl.,* **3,** 1–19 (1964).

(23) A. Albert and G. B. Barlin, *J. Chem. Soc.,* 2384–2396 (1959). In aqueous solution, the pyridine thione tautomeric structure of 2-mercaptopyridine is preferred in a ratio of 49 000:1.



(24) G. M. Whitesides, J. E. Lilburn, and R. P. Szajewski, *J. Org. Chem.,* **42,** 332–338 (1977).

(25) W. W. Cleland, *Biochemistry,* **3,** 480–482 (1964).

(26) I. M. Kolthoff, "Acid-Base Indicators", Macmillan, New York, 1937, p 245.

(27) The rates of hydrolysis of Dts-glycine (Scheme IV) derived by the amino acid analysis technique were confirmed in selected cases by UV quantitation of the remaining starting material ($\lambda_{max}$ 255 nm, $\epsilon$ 3 × $10^3$; see ref 5). The formation of 1 equiv of elemental sulfur ($\lambda_{max}$ 264 nm, $\epsilon$ 860) was also shown in this way.

(28) (a) J. B. Brønsted and K. J. Pedersen, *Z. Phys. Chem. (Leipzig),* **108,** 185–235 (1923); (b) J. N. Brønsted, *Chem. Rev.,* **5,** 231–338 (1928).

(29) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.,* **83,** 1743–1750 (1961).

(30) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. I, W. A. Benjamin, New York, 1966, especially (a) pp 287–288; (b) p 36; (c) pp 32–34.

(31) W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, 1969.

(32) R. P. Bell, "The Proton in Chemistry", 2nd ed., Cornell University Press, Ithaca, N.Y., 1973.

(33) It is possible that in basic solutions glycine may exist as its carbamate or thiocarbamate salt, which would have a limited lifetime at pH <10 and become instantly converted to free glycine upon acidification to pH ≤6.5. Cf. (a) R. Hirschmann, R. G. Strachan, H. Schwam, E. F. Schoenewaldt, H. Joshua, H. Barkemeyer, D. F. Veber, W. J. Paleveda, T. A. Jacob, T. E. Beesley, and R. G. Denkewalter, *J. Org. Chem.,* **32,** 3415–3425 (1967); (b) R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Paleveda, H. Schwam, H. Barkemeyer, B. H. Arison, D. F. Veber, R. G. Strachan, J. Milkowski, R. G. Denkewalter, and R. Hirschmann, *J. Org. Chem.,* **36,** 49–59 (1971). See also (c) M. M. Sharma, *Trans. Faraday Soc.,* **61,** 681–688 (1965); (d) S. J. Joris, K. I. Aspila, and C. L. Chakrabarti, *Anal. Chem.,* **41,** 1441–1445 (1969); (e) S. L. Johnson and D. L. Morrison, *J. Am. Chem. Soc.,* **94,** 1323–1334 (1972), and references cited therein, for data on rates of formation and hydrolysis of carbamates, thiocarbamates, and dithiocarbamates. The kinetically obtained ionization constants of these three species of salts have been estimated to be from p$K_{app}$ 5.3 to 4.5 to 3.4.

(34) A. Fava, A. Iliceto, and E. Camera, *J. Am. Chem. Soc.,* **79,** 833–838 (1957).

(35) J. M. Wilson, R. J. Bayer, and D. J. Hupe, *J. Am. Chem. Soc.,* **99,** 7922–7926 (1977).

(36) M. Calvin in "Glutathione Symposium", S. P. Colowick, Ed., Academic Press, New York, 1954, pp 3–26.

(37) H. Lindley, *Biochem. J.,* **74,** 577–584 (1960).

(38) J. P. Danehy and C. J. Noel, *J. Am. Chem. Soc.,* **82,** 2511–2515 (1960).

(39) J. W. Oglivie, J. T. Tildon, and B. S. Strauch, *Biochemistry,* **3,** 754–758 (1964).

(40) W. P. Jencks and K. Salvesen, *J. Am. Chem. Soc.,* **93,** 4433–4436 (1971).

(41) For the same reaction, namely, that of thiol anions with Ellman's reagent

5,5′-dithiobis(2-nitrobenzoic acid), Whitesides et al. (ref 24) found a single line with slope $\beta_{nuc} = 0.36$, whereas Wilson et al. (ref 35) found two parallel lines of slope $\beta_{nuc} = 0.48$ with the rates for aromatic thiols being about fivefold higher than those for aliphatic thiols of the same $pK_a$.
(42) For purposes of reference, some values of $\beta_{nuc}$ for reactions of thiol anions follow: with acetaldehyde (ref 43), 0.1; with benzene oxide (ref 44) or ethylene oxide (ref 38), 0.2 and 0.3; with *p*-nitrothiophenyl acetate (ref 45), 0.27; with *p*-nitrophenyl acetate (ref 39), 0.38; with 5,5′-dithiobis(2-nitrobenzoic acid) (note 41), 0.36 or 0.48; with *N*-*p*-2-benzimidazolylphenylmaleimide (ref 46), 0.42; with acrylonitrile (ref 47), 0.45; with chloroacetamide (ref 37), ~1; with 1,2-dithiolane (ref 34), ~1.
(43) (a) G. E. Lienhard and W. P. Jencks, *J. Am. Chem. Soc.*, **88,** 3982–3995 (1966); (b) R. E. Barnett and W. P. Jencks, *ibid.*, **91,** 6758–6765 (1969).
(44) D. M. Reuben and T. C. Bruice, *J. Am. Chem. Soc.*, **98,** 114–121 (1976).
(45) D. J. Hupe and W. P. Jencks, *J. Am. Chem. Soc.*, **99,** 451–464 (1977).
(46) T. Sekine, K. A. Kato, K. Takamori, M. Machida, and Y. Kanaoka, *Biochim. Biophys. Acta,* **354,** 139–147 (1974).
(47) M. Friedman, J. F. Cavins, and J. S. Wall, *J. Am. Chem. Soc.*, **87,** 3672–3681 (1965).
(48) J. O. Edwards, *J. Am. Chem. Soc.*, **76,** 1540–1547 (1954).
(49) R. E. Davies, *Surv. Prog. Chem.*, **2,** 189–238 (1964), especially (a) pp 199–212, (b) p 197.
(50) (a) J. F. Harris, Jr., *J. Am. Chem. Soc.*, **82,** 155–158 (1960). (b) Review: G. Zumach and E. Kühle, *Angew. Chem., Int. Ed. Engl.*, **9,** 54–63 (1970).
(51) I.e., $R^1SH + R^2SSCONHR \rightarrow R^1SSR^2 + COS + H_2NR$, with no $R^1SSCONHR$ formed. Note the close analogy of this result to the reported thiol-induced fragmentation of sulfenylthiocarbonates to give unsymmetrical disulfides: $R^1SH + R^2SSCOOCH_3 \rightarrow R^1SSR^2 + COS + CH_3OH$. See S. J. Brois, J. F. Pilot, and H. W. Barnum, *J. Am. Chem. Soc.*, **92,** 7629–7631 (1970).
(52) The trend of increased $k_2$ with increased mole fraction of water in mixed aqueous media (Table I, lines 31–35) supports the suggestion that the driving force of the second step has an electronic origin, namely, the acidity of the thiocarbamate leaving group, since ionization constants are lower under entirely aqueous conditions. By the same token, the relative insensitivity of $k_1$ to the mole fraction of water as demonstrated in the same experiments indicates the unimportance of electronic factors in governing reactivities of the first step of the reaction mechanism.
(53) J. E. Leffler, *Science,* **117,** 340–341 (1953).
(54) L. Eldjarn and A. Pihl, *J. Biol. Chem.*, **225,** 449–510 (1957).
(55) P. C. Jocelyn, *Eur. J. Biochem.*, **2,** 327–331 (1967).
(56) W. L. Zahler and W. W. Cleland, *J. Biol. Chem.*, **243,** 716–719 (1968).
(57) In water, $\kappa \sim 375$ is unusually high, meaning that the carbamoyl disulfide intermediate [Carb] reacts much more readily with thiol anions than does the starting Dts amine. As is described in the text, the factors governing the rates $k_1$ and $k_2$ are quite distinct, so it should not be surprising that under other conditions, specifically nonaqueous ones, $\kappa$ is often $\ll 1$.
(58) D. L. Yabroff, *Ind. Eng. Chem.*, **32,** 259–262 (1940).
(59) I. Photaki in "Topics in Sulfur Chemistry", Vol. 1, A. Senning, Ed., Georg Thieme Verlag, Stuttgart, 1976, pp 111–183.
(60) L. Zervas, D. Borovas, and E. Gazis, *J. Am. Chem. Soc.*, **85,** 3660–3666 (1963).
(61) (a) W. Kessler and B. Iselin, *Helv. Chim. Acta,* **49,** 1330–1344 (1966); (b) A. Fontana, F. Marchiori, L. Moroder, and E. Scoffone, *Tetrahedron Lett.*, 2985–2987 (1966); (c) M. Juillerat and J. P. Bargetzi, *Helv. Chim. Acta,* **59,** 855–866 (1976).
(62) K. P. Polzhofer and K. H. Ney, *Tetrahedron,* **27,** 1997–2001 (1971).
(63) (a) A. Tun-Kyi, *Helv. Chim. Acta,* **61,** 1086–1090 (1978); (b) M. Stern, A. Warshawsky, and M. Fridkin, *Int. J. Pept. Protein Res.*, **13,** 313–319 (1979).
(64) (a) S. Shaltiel, *Biochem. Biophys. Res. Commun.*, **29,** 178–183 (1967); (b) F. Chillemi and R. B. Merrifield, *Biochemistry,* **8,** 4344–4346 (1969); (c) S. Shaltiel and M. Fridkin, *ibid.*, **9,** 5122–5127 (1970); (d) J. M. Stewart, M. Knight, A. C. Paiva, and T. Paiva in "Progress in Peptide Research", S. Lande, Ed., Gordon and Breach, New York, 1972, pp. 59–64; (e) M. C. Lin, B. Gutte, D. G. Caldi, S. Moore, and R. B. Merrifield, *J. Biol. Chem.*, **247,** 4768–4774 (1972).
(65) Review: R. G. Hiskey, V. R. Rao, and W. G. Rhodes in "Protective Groups in Organic Chemistry", J. F. W. McOmie, Ed., Plenum Press, New York, 1973, pp 235–308.
(66) B. Iselin, *Helv. Chim. Acta,* **44,** 61–78 (1961).
(67) (a) E. D. Bergmann and A. Kaluszyner, *Recl. Trav. Chim. Pays-Bas,* **78,** 289–314 (1959); (b) J. W. Haefele and R. W. Broge, *Proc. Sci. Sect. Toilet Goods Assoc.*, **32,** 52–59 (1959); *Chem. Abstr.*, **54,** 17233 (1960); (c) R. A. Houghten and C. H. Li, *Anal. Biochem.*, **98,** 36–46 (1979).
(68) (a) B. Kamber, *Helv. Chim. Acta,* **56,** 1370–1381 (1973); (b) R. G. Hiskey, N. Muthukumaraswamy, and R. R. Vunnam, *J. Org. Chem.*, **40,** 950–952 (1975); (c) K. Nokihara and H. Berndt, *ibid.*, **43,** 4893–4895 (1978).
(69) G. L. Ellman, *Arch. Biochem. Biophys.*, **82,** 70–77 (1959).
(70) D. R. Grassetti and J. F. Murray, *Arch. Biochem. Biophys.*, **119,** 41–49 (1967).
(71) R. Kuhn and G. Quadbeck, *Chem. Ber.*, **84,** 844–847 (1951).
(72) R. B. Martin, S. Lowey, E. L. Elson, and J. T. Edsall, *J. Am. Chem. Soc.*, **81,** 5089–5095 (1959).
(73) R. E. Barnett and W. P. Jencks, *J. Am. Chem. Soc.*, **91,** 2358–2369 (1969).
(74) R. E. Basford and F. M. Huennekens, *J. Am. Chem. Soc.*, **77,** 3878–3882 (1955).
(75) J. Gerstein and W. P. Jencks, *J. Am. Chem. Soc.*, **86,** 4655–4663 (1964).

# α-Ketoketene Dithioacetal Chemistry. 1. Alternate Modes of Lithium Aluminum Hydride Reduction. Regio- and Stereospecific vs. Reduction–Alkylation–Fragmentation

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**Abstract:** Two modes of lithium aluminum hydride reduction for α-ketoketene dithioacetals have been established. One pathway proceeds in a regio- and stereospecific manner illustrating for the first time that such reductions on acyclic α,β-unsaturated ketones can in fact be stereospecific. A second pathway arises when a steric interaction in an early intermediate forces the reaction to proceed through a reduction–alkylation–fragmentation mechanism. Lithium aluminum hydride reduction of 3,3-bis(methylthio)-1-phenylpropen-1-one (**5**) gave α-[2,2-bis(methylthio)ethyl]benzenemethanol (**8**) in 93% yield. The regio- and stereospecificity of the reduction of **5** was established by reduction with lithium aluminum deuteride which gave [α*S*(*S*)]-α-[2,2-bis(methylthio)ethyl-1-*d*]-benzenemethanol-α-*d* (**9**) in 92% yield. Analysis by $^1H$ NMR established the formation of a single diastereomer. Reduction of **5** with lithium aluminum hydride followed by quenching with DCl–$D_2O$ gave α-[2,2-bis(methylthio)ethyl-2-*d*]benzenemethanol (**10**) in 90% yield thus establishing the presence of an organoaluminum intermediate in the reduction. Reduction of 3,3-bis(methylthio)-1-phenylpropen-1-one-2-*d* (**11**) with lithium aluminum hydride gave [α*S*(*R*)]-α-[2,2-bis(methylthio)ethyl-1-*d*]benzenemethanol (**12**) which had the opposite configuration at carbon-2 to that of **9.** In contrast to the above reductions, lithium aluminum hydride reduction of 1-phenyl-2-(1,3-thiolan-2-yl)ethanone (**13**) gave 2,2′-(2-phenyl-1,3-propanediylidene)bis(1,3-dithiolane) (**14**) and benzaldehyde (**15**). Reduction of **13** with lithium aluminum deuteride gave 2,2′-(2-phenyl-1,3-porpanediylidene-2-*d*)bis(1,3-dithiolane) (**21**) and deuteriobenzaldehyde (**22**). Lithium aluminum hydride reduction of 2-(1,3-dithan-2-ylidene)-1-phenylethanone (**23**) gave α-phenyl-1,3-dithane-2-ethanol (**24**) in 95% yield and reduction of **23** with lithium aluminum deuteride gave the corresponding α-phenyl-1,3-dithane-2-ethanol-2-*d* (**25**). The organoaluminum intermediate V, resulting from the reduction of **23,** was characterized by $^{13}C$ NMR and shown to be symmetrical.

Some 30 years ago, Nystrom and Brown[1] reported that lithium aluminum hydride (LAH) reduction of cinnamic acid (**1**) unexpectedly gave dihydrocinnamyl alcohol (**4**) (eq 1) rather than cinnamyl alcohol. This was to be the first of several reports[2] in which LAH reduction of acyclic α,β-unsaturated carbonyl compounds resulted in carbon–carbon double-bond

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