mode. The quantity $\Delta\bar{\nu}_{0,1/2}$ is the full width at half maximum for the individual vibronic components, and E_0 is the energy of the $0 \rightarrow 0$ vibronic transition for the mode $\hbar\omega$.

We have utilized the results of emission spectral fitting to calculate $\Delta G°$ from $\Delta G° = (E_0 + (\Delta\bar{\nu}_{0,1/2})^2/16k_BT \ln 2)_{acceptor} - (E_0 + (\Delta\bar{\nu}_{0,1/2})^2/16k_BT \ln 2)_{donor}$. In this equation the band width at half maximum is related to λ', as shown in eq 2. The quantity λ' is the sum of reorganizational energies for the solvent and the averaged, low-frequency vibrational mode treated classically.

$$\lambda' = (\Delta\bar{\nu}_{0,1/2})^2/16k_BT \ln 2 \quad (2)$$

The quenching scheme involves the series of reactions in eq 3.[12,15,16b] A kinetic analysis based on this scheme gives the

$$Os^{II}* + Q \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} Os^{II}*, Q \underset{k_{-et}}{\overset{k_{et}}{\rightleftharpoons}} Os^{II}, {}^3Q \xrightarrow{k'_{-d}} Os^{II} + {}^3Q \quad (3)$$

relationships shown in eqs 4a and 4b. In applying these equations,

$$k_q = k_d/(1 + k_{-d}/k_{et} + 1/K_{et}) \quad (4a)$$

$$k_{et} = (K_A\{1/k_q - (1 + K_{et})/k_dK_{et}\})^{-1} \quad (4b)$$

it was assumed that $k_{-d} = k'_{-d}$ and the relationships in eqs 5 and 6 were utilized.[17]

$$K_A = k_d/k_{-d} \quad (5)$$

$$k_{et}/k_{-et} = K_{et} = \exp -(\Delta G°/RT) \quad (6)$$

A quantum mechanically derived expression for k_{et} is shown in eq 7. It is based on the coupling to the energy-transfer process of averaged, medium-frequency, ring-stretching modes in the donor (m) and acceptor (n) and low-frequency and solvent modes treated classically.[8,16] In eq 7b, S_m and S_{n*} are the electron-vibrational

$$k_{et}(calcd) = (2\pi V^2/\hbar)F(calcd) \quad (7a)$$

$$F(calcd) = [1/(4\pi\lambda' k_BT)^{1/2}] \sum_{n^*=0}^{\infty} \sum_{m=0}^{\infty} [\exp(-S_{n^*})] \times [\exp(-S_m)](S_{n^*}^{n^*}/n^*!)(S_m^m/m!) \times [\exp\{-(\Delta G° + \lambda'_{12} + n^*\hbar\omega + m\hbar\omega)^2/4\lambda'_{12}k_BT\}] \quad (7b)$$

coupling constants. The sums are over the ground-state vibrational levels of the donor (m) and the excited-state vibrational levels of the acceptor (n^*). The terms in the sums past $n^* = 4$ and $m = 4$ were negligible.

In Figure 1 is shown a calculated plot of log k_q vs $\Delta G°$ for the data *with anthracene as quencher*. The line was calculated by using eq 4a with $k_d = 9.1 \times 10^9$ M^{-1} s^{-1} and $k_d/k_{-d} = 2.3$,[17] eq 6 to calculate K_{et}, and eq 7 to calculate k_{et}. In the calculation of k_{et}, average values of S (1.2) and λ' (1860 cm^{-1}) were utilized for the complexes with $\hbar\omega = 1300$ cm^{-1}.[8] The parameters for anthracene were taken from the emission spectral fitting results. The quantity λ'_{12} is the average value for the complexes (λ'_{11}) and λ' for anthracene (λ'_{22}), $\lambda'_{12} = (\lambda'_{11} + \lambda'_{22})/2 = 1090$ cm^{-1}. The magnitude of the energy exchange matrix element, V, used in eq 7 to calculate the line was $V = 4$ cm^{-1}. This is consistent with literature values found for energy transfer involving organic triplets.[18]

Several important conclusions emerge from our results. The first is that the inverted region can be reached for energy transfer as well as for electron transfer. It is more easily observed for energy transfer because, in forming the lowest triplets of anthracene and 2,3-benzanthracene, the maximum rate constant is below the diffusion-controlled limit. The second is that, as for electron transfer, higher lying, accessible excited states can interfere with the observation of inverted behavior in energy transfer. Finally, it is possible to utilize structural and energetic parameters derived from emission spectral profiles to calculate relative rate constants for energy transfer. This approach has been utilized to calculate relative rate constants for nonradiative decay for MLCT excited states including polypyridyl complexes of Os^{II} and Ru^{II}.[8,19] It may have additional applications elsewhere.

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Reaction of a Distorted Amide with Nucleophilic Thiolate-Containing Zwitterions Produced from Thiolamines. A Model for the Acylation Step in Cysteine Proteases and Transglutaminases

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Distorted amide **1** (2,3,4,5-tetrahydro-2-oxo-1,5-ethanobenzazepine)[1] has been shown to be susceptible toward bifunctional nucleophiles such as β-amino alcohols[2] and certain dicarboxylic acids[3] as simple model systems for the serine and aspartate proteases, respectively. Herein, we report the reactions of **1** with β-amino thiols which can be taken as a simple model for the third of the four classes of proteases, namely, the cysteine proteases[4] (and phenomenologically similar transglutaminases[5]).

The maximum rate of disappearance of **1** in the presence of thiolamines **2a-c** occurs at pH levels above the value of pK_{thiol} and lower than the value of pK_{NH^+} so that the zwitterionic ammonium thiolate is the active attacking species.[6] Alkyl thiols

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Table I. k_2^{max} and Kinetic pK_{thiol} Values of the Reactions of Various Thiols with Distorted Amide **1** [T = 25 °C, μ = 1.0 (KCl)]

thiol	k_2^{max},[a] M^{-1} s^{-1}	kinetic pK_{thiol}[b]	lit. pK_{thiol}	lit. pK_{amino}
2a, cysteamine	99.2	8.37	8.35[c]	10.86[c]
2b, (*N*,*N*-dimethylamino)-ethanethiol	30.5	7.73	7.74[d]	10.89[d]
2c, 4-(2-mercaptoethyl)morpholine	1.47	6.49	6.57[e]	9.55[e]
2d, ethyl 2-mercaptoacetate	0.36		8.03[f]	
2e, 3-mercaptopropionitrile	<0.02[g]		8.74[f]	
2f, ethanethiol	<0.05[g]		10.55[h]	
2g, [2-(ethylthio)ethyl]amine	nil			

[a] Relative standard error (from standard deviation of the fits) is less than 5%; determined from fits of k_{2obsd} vs $[H_3O^+]$. [b] Standard error is less than 0.05 pK_a units; determined from fits of k_{2obsd} vs $[H_3O^+]$. [c] Benesch, R. E.; Benesch, R. *J. Am. Chem. Soc.* **1955**, *77*, 5877–5881. [d] Kostyukovskii, Y. L.; Bruk, Y. A.; Pavlova, L. V.; Slavachevskaya, N. M.; Kokushkina, A. V.; Mirkin, B. S.; Belen'kaya, I. A. *Zh. Obshch. Khim.* **1972**, *42*, 662–665. [e] Bagiyan, G. A.; Koroleva, I. K.; Soroka, N. V.; *Zh. Neorg. Khim.* **1977**, *22*, 3078–3085. [f] Kostyukovskii, Y. L.; Bruk, Y. A.; Kokushkina, A. V.; Mirkin, B. S.; Slavachevskaya, N. M.; Pavlova, L. V.; Belen'kaya, I. A. *Zh. Obshch. Khim.* **1972**, *42*, 2104–2106. [g] Because the thiol kinetics were difficult to determine accurately against the background of fast alkaline hydrolysis, only a rough estimate of the small catalytic rate constant could be determined. [h] Nenasheva, T. N.; Salinkova, G. A. *Zh. Org. Khim.* **1979**, *15*, 835–839.

without a pendant amino group (**2d–f**) are not appreciably reactive toward **1**; nucleophilic attack of RS^- on **1** does not produce the thiolester unless a pendant amine is present. During these kinetic studies, no evidence for the buildup of tetrahedral intermediates (such as biphasic or saturation kinetics) was observed in any case. A product study of large-scale reactions of cysteamine (**2a**) with amide **1** and 2-(*N*,*N*-dimethylamino)ethanethiol (**2b**) with amide **1** showed that, with **2a**, the final product was the secondary acyclic amide (**4a**) (identical with that prepared by independent synthesis[7]) and, with **2b**, the corresponding thiolester (**3b**).[8]

The rate of disappearance of amide is given by eq 1,

$$-d[\mathbf{1}]/dt = k_{2obsd}[\mathbf{1}][RSH]_{tot} \quad (1)$$

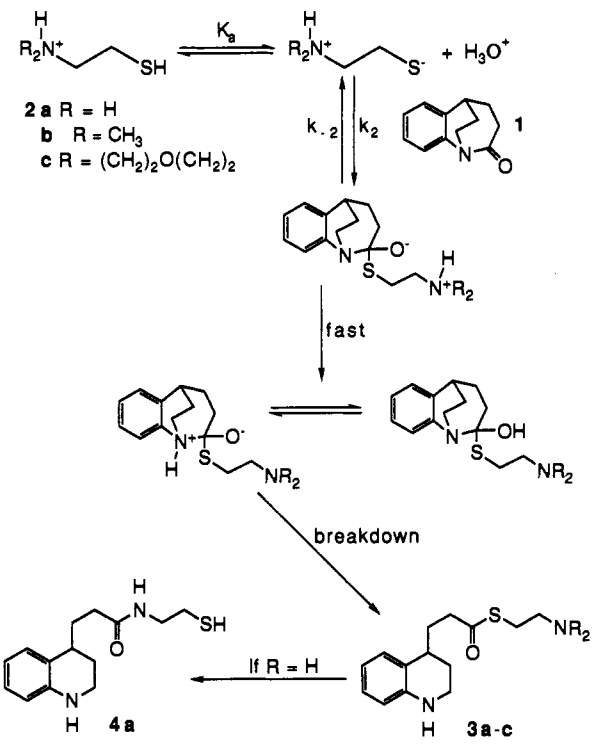
where $[RSH]_{tol} = [RS^-] + [RSH]$. Since RS^- is the attacking species of thiol, then

$$k_{2obsd} = k_2^{max}K_a/(K_a + [H_3O^+]) \quad (2)$$

where K_a refers to the RSH acid dissociation constant and k_{2obsd} is the second-order rate constant for RS^- attack on **1** at a given $[H_3O^+]$. Nonlinear least-squares fitting of the k_{2obsd} data vs $[H_3O^+]$ for each of **2a–c** to eq 2 yielded the values of k_2^{max} and the kinetic pK_{thiol} values given in Table I. A Brønsted plot for the reaction of **2a–c** with **1** (not shown) has a slope $\beta = 0.96 \pm 0.05$. Alkyl thiols without the pendant amino group, **2d–f**, lie well below the Brønsted plot.

The process that best accommodates the data is shown in Scheme I. Although the kinetic data suggest that the zwitterionic thiolate is the nucleophilic species, the amino functionality in **2a–c** clearly plays important roles in the overall reaction. Direct attack

Scheme I



of the amine portion on the amidic carbonyl is apparently unimportant since the *S*-ethyl derivative (**2g**) is unreactive toward **1**. When the amine is tertiary (**2b,c**), rapid formation of the corresponding thiolester (**3**) is observed. With cysteamine (**2a**), a similar S-acylation is envisioned to form **3a**, which subsequently undergoes rapid S → N acyl transfer yielding **4a**. Phenomenologically, the latter reaction resembles acyl transfers mediated by the transglutaminases[5] which are known to involve *S*-acyl enzyme intermediates.

The role of the amino group seems to be the trapping of the tetrahedral intermediate, preventing the reejection of thiolate. Since it exists as the ammonium form at the moment of formation of the tetrahedral intermediate, the protonated amino group can easily function as a proximal intramolecular general acid. This role is supported by the observation that the alkyl thiols that lack an amino group showed very little reactivity toward amide **1** alone, but when the concentration of buffers (intermolecular trapping agents) such as CAPS,[9] CHES,[10] and EPPS[11] was increased, the rate of thiolysis of **1** increased. This is the predicted result if the rate-limiting step in this case is not the attack by thiolate, but the actual trapping of an unstable tetrahedral intermediate. This trapping of the tetrahedral intermediate through a proton transfer is not without precedence. The microscopic reverse reaction, the intramolecular aminolysis of a thiolester, is subject to general base catalysis and has been proposed to proceed with a rate-limiting removal of a proton from the ammonium in the tetrahedral intermediate, causing the expulsion of the *thiolate* to be favored.[12]

These results have important implications for the cysteine proteases and the transglutaminases. Amino groups within the active site adjacent to the essential cysteine residues (e.g., His159 in papain) are able to act to reduce the pK_a of the thiol, thereby forming an active-site zwitterion.[4,6] While not explicitly proven for the enzyme, the ammonium group could then act as a general acid trap of the unstable tetrahedral intermediate formed after attack of the thiolate on the amidic carbonyl. Furthermore, the

(7) The respective N-tosylated acid chloride of **1** was reacted with [2-(benzylthio)ethyl]amine and triethylamine to yield the protected amide. The benzyl and tosyl groups were both removed at once with sodium in liquid ammonia. Amide **4a** spectra data: 300-MHz ^1H NMR ($CDCl_3$) δ 6.95 (m, 2 H), 6.60 (d of t, 1 H), 6.45 (d of d, 1 H), 6.34 (br s), 3.8 (br s, 1 H), 3.54 (q, 2 H), 3.28 (q, 2 H), 2.97 (t, <1 H), 2.76 (t, 2 H), 2.30 (m, 1 H), 2.25 (m, 2 H), 2.09–1.84 (br m, 2 H), 1.84–1.67 (br m, 2 H), 1.4–1.0 (br s, 1 H); 300-MHz ^{13}C NMR ($CDCl_3$) δ 173.54, 4 °C; 144.22, 4 °C; 129.13, 3 °C; 127.05, 3 °C; 124.04, 3 °C; 116.54, 3 °C; 114.16, 3 °C; 77.47, 77.04, 76.62, $CDCl_3$ triplet; 38.43, 2 °C; 38.27, 2 °C; 37.76, 2 °C; 35.08, 3 °C; 33.89, 2 °C; 31.90, 2 °C; 26.22, 2 °C; FTIR ($CHCl_3$ cast) 3307, 3002, 2936, 1647, 1605 cm^{-1}; CIMS (NH_4^+ positive ionization) 265 (M – H^+, 100), 261 (15.5), 247 (10.5), 234 (15.9), 188 (4.5).

(8) Thiolester **3b** spectral data: 200-MHz ^1H NMR (CD_3CN/D_2O) δ 6.94 (m, 2 H), 6.51 (d of t, 2 H), 3.8 (m, <1 H), 3.42–3.36 (m, 3 H), 3.26–3.10 (m, ?H under excess thiolamine), 2.37 (s, <1 H), 2.1–1.6 (m, >5 H), 1.5–1.1 (br m, 2 H); FTIR (CH_3CN solution with triethylamine and thiolamine) 3307, 3002, 2936, 1705, 1600 (weak) cm^{-1}.

(9) CAPS = 3-(*N*-cyclohexylamino)propanesulfonic acid.
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reaction of **1** with cysteamine is shown here to mimic the transglutaminase enzymes, at least phenomenologically, by proceeding through an *S*-acyl intermediate and completing the S → N acyl transfer to form subsequently an amide.

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Supplementary Material Available: Table of k_{2obsd} values vs pH for the reaction of **2a-c** with **1** (2 pages). Ordering information is given on any current masthead page.

Sequence-Specific Alkylation of DNA Activated by an Enzymatic Signal

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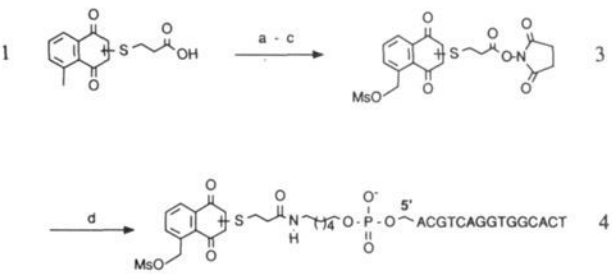
Enzymatic reduction of such drugs as mitomycin C, adriamycin, and daunomycin is thought to play a crucial role in the antitumor activity of these compounds. In each case, a highly electrophilic quinone methide or related semiquinone is likely generated under reducing conditions for the ultimate alkylation of DNA.[1,2] Numerous model studies have continued on this topic in order that bioactivation may become an integral step in the activity of newly developed therapeutics.[3] This communication now reports the successful production of a sequence-selective alkylating agent that is controlled by chemical and biological reduction. In this study, a naphthoquinone derivative[4] serves as the latent quinone methide while an attached oligonucleotide provides the site specificity for reaction.

Photolytic bromination of β-[(5-methyl-1,4-naphthoquinonyl)thio]propionic acid[5] (**1**) (Scheme I), followed by transformation of the 5-bromomethyl moiety to its corresponding (methylsulfonyl)oxy derivative by treatment with silver mesylate,[6] yielded the acid **2**. Treatment of the acid **2** with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide yielded the activated ester **3**. This in turn was used to acylate a hexamethyleneamino linking arm that was coupled to the 5′ terminus of an oligonucleotide 15 bases in length. The final product **4** was then purified by reverse-phase chromatography. A target strand of DNA, 5′-d(AGTGCCACCTGACGTGAG) (**5**), was also prepared and labeled at the 5′ terminus with ^{32}P.[7] The 5′ amino linker derivative (Clontech) and other oligonucleotides were synthesized via the standard procedures of solid-phase phosphoramidite chemistry.

Scheme I[a]



[a] (a) *N*-Bromosuccinimide, dibenzoyl peroxide, 2,2′-azobis(2-methylpropionitrile), *hν*, 90 °C, CCl_4/Ac_2O; (b) silver mesylate, CH_3CN; (c) *N*-hydroxysuccinimide, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, DMF; (d) oligo-$(CH_2)_6NH_2$, 3-(*N*-morpholino)propanesulfonic acid pH 7.5, 66% DMF.

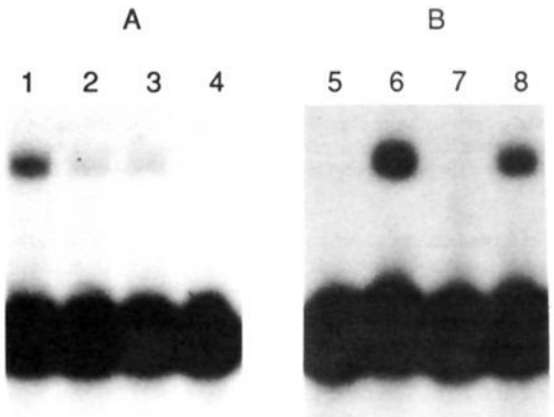


Figure 1. Autoradiograms of denaturing polyacrylamide gels (20%) used to identify the cross-link form of duplex DNA generated upon reduction. Oligonucleotides **4** and **5** (2.2 μM in each strand) were incubated in 100 mM potassium phosphate (pH 7) at room temperature (30 min) and then treated with the following reducing agents for an additional 30 min. (A) Lane 1: sodium borohydride (100 mM). Lane 2: sodium dithionite (100 mM). Lane 3: nicotinamide mononucleotide (10 mM). Lane 4: glutathione (10 mM). (B) Lane 5: cytochrome *c* reductase (1 mg/mL). Lane 6: cytochrome *c* reductase (1 mg/mL) and NADH (100 μM). Lane 7: NADH (100 μM). Lane 8: NADH (10 mM). The reaction mixtures were diluted 10-fold, dialyzed, lyophilized, and resuspended in 80% formamide for electrophoresis.

Oligonucleotide **4** was first annealed to its target strand **5** and then treated with a variety of reducing agents prior to analysis by polyacrylamide gel electrophoresis (20%, 7 M urea) and autoradiography (Figure 1). Treatment of the annealed duplex with sodium borohydride led to formation of a high molecular weight species consistent with the covalent attachment of the fully hybridized strands (lane 1, Figure 1A).[5] The yield of cross-linked material (12%, by densitometry) did not change appreciably by increasing the time of reaction from 10 min to 2 h or by changing the reaction temperature from 4 °C to 37 °C. The use of sodium dithionite (lane 2, Figure 1A) and NMNH[8] (lane 3, Figure 1A) as reductants also resulted in the formation of the cross-linked species, but these reductants appeared much less efficient at generating the desired product. Glutathione (lane 4, Figure 1A) did not induce the cross-linking reaction, perhaps because of its

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(8) Abbreviations used: β-nicotinamide adenine dinucleotide reduced form (NADH) and β-nicotinamide mononucleotide reduced form (NMNH).

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