Intramolecular Catalysis of Thiol Ester Hydrolysis by a Tertiary Amine and a Carboxylate

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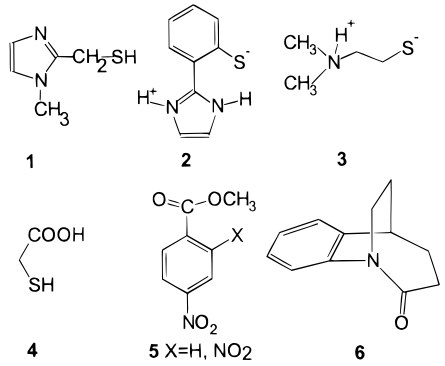
The syntheses of 4-nitro thiol benzoate esters of ethyl 2-mercaptoacetate, thioglycolic acid, 2-(dimethylamino)ethanethiol, and 2-(*N*,*N*,*N*-trimethylammono)ethanethiol iodide (**10**−**13**) have been carried out and their rates of hydrolysis at 50 °C studied as a function of pH. Thiol esters **10** and **13** have linear pH−log k_{obs} profiles indicative of an exclusive specific base attack of OH^-. Thiol esters **11** and **12** exhibit a plateau in their pH/log k_{obs} profiles due to the participation of pendant carboxylate and dimethylamino groups, respectively, most probably as intramolecular general bases. At higher pH, specific base catalysis becomes predominant for both **11** and **12**. In the plateau region, the hydrolysis of **12** is subject to a solvent deuterium kinetic isotope effect of 2.2, consistent with the operation of a general base role for the pendant dimethylamino group. The hydrolysis of **12** in the presence of Ellman's reagent produces the Ellman's anion at a rate that is identical to that for disappearance of the thioester, consistent with a general base process where the thiolate anion product of hydrolysis is produced in the rate-limiting step.

Introduction

The cysteine proteases comprise a large group of enzymes from plant, bacterial, and animal sources, and all contain an essential cysteine SH and imidazole in the active site.[1] The catalytic cycle of these enzymes is known to proceed through an intermediate thioester[2] which is subsequently hydrolyzed with general base assistance of the histidine imidazole to regenerate the native enzyme. In ongoing efforts to develop small molecule biomics of the cysteine proteases, we have synthesized **1**−**3**[3−7] which contain reasonable approximations for the histidine and cysteine active site residues, and studied their reactions with activated esters **5**[3,4] and the distorted amide **6**.[5,6] In addition, we have recently reported the reaction[8] of thioglycolic acid (**4**) with **6** as a potential model for a hypothetical enzymatic site containing SH and COOH.

In each of the above cases the main process in the neutral pH region involves attack of the zwitterionic form of **1**−**3** (monoanion of **4**) on the amide to form a tetrahedral intermediate which is subsequently intramolecularly trapped by proton transfer from the pendant ammonium ion (carboxylic acid in the case of **4**). This is shown in Scheme 1 for the reaction of **1** with **6**, the final product being the ring-opened thioester. The latter process can be taken as a model for the acylation chemistry believed to occur in the active site of the cysteine proteases.



Despite the fact that thioesters are "energy rich" (in terms of the free energy of hydrolysis) relative to the corresponding oxygen ester analogue,[9] where comparison can be made, the thio and oxy analogues have similar rates for hydrolysis.[10] It is therefore clear that in order for thioester intermediates such as **7** to be hydrolyzed rapidly to regenerate the starting catalyst, some sort of intramolecular catalysis by the pendant amine (or carboxylate in the case of attack of **4** and **6**) needs to be operative. Although the enzymatic deacylation is believed to occur via such a general base assisted delivery of H_2O by the active site His-imidazole to the thio acyl enzyme, few small molecule analogues for that process are available.[11−13] Bruice[11a] and Fife and DeMark[11b] have shown that thioesters **8** and **9** undergo rapid intramolecular attack of the imidazole units to give

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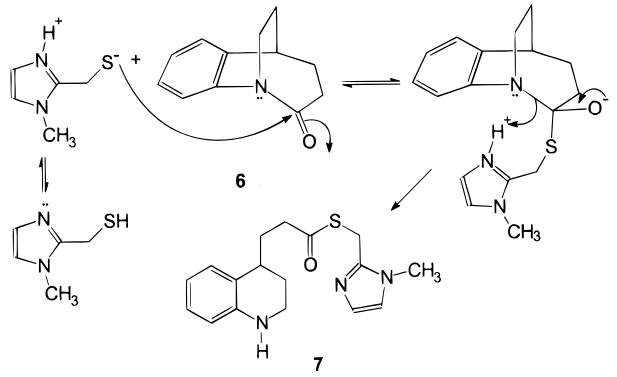
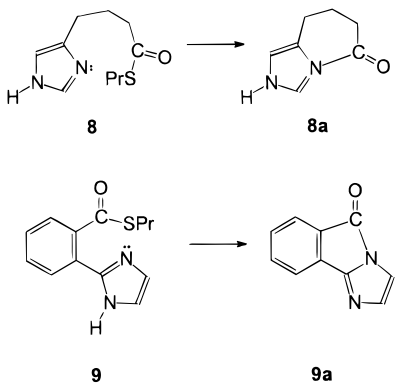
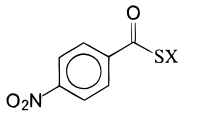
Scheme 1



8a and **9a** which subsequently undergo rapid hydrolysis. These findings fit nicely with the general rule that thioesters are not particularly susceptible to attack by oxygen nucleophiles,[10] but are rapidly attacked by nitrogen nucleophiles.[10b,14] There are few examples where intermolecular general base catalysis of thio-



ester hydrolysis is documented[11c,12,15] and, to our knowledge, even fewer where intramoleular general base catalysis is observed. Given the widely held view that general base assistance of the hydrolysis of the cysteine protease acyl enzymes is a facile process, it is surprising that so few small molecule examples of this process exist, the most notable being the hydrolyses of the acetyl thioesters of **1** and **2**[4,13] which can be taken as reasonable models for the chemistry believed to occur in the enzymatic active site. In order to investigate the propensity for intramolecular assistance by a carboxylate and tertiary amine of the hydrolysis of thioesters we have investigated the pH-dependent rate profile for the cleavage of the *p*-nitrobenzoyl derivatives **10**−**13**. The following describes our findings which indicate that **11** and **12** most probably exhibit general base mechanisms for their hydrolyses in the neutral pH domain, while the control compounds, **10** and **13**, hydrolyze with specific base catalysis throughout the accessible pH/rate range.



10 X=CH_2COOEt
11 X=CH_2COOH
12 X=$CH_2CH_2N(CH_3)_2$
13 X=$CH_2CH_2\overset{+}{N}(CH_3)_3$

Experimental Section

(a) Materials and General Methods. The following materials were obtained from commercial suppliers: 4-nitrobenzoyl chloride (Aldrich), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB; Ellman's reagent, Sigma), 2-(dimethylamino)ethanethiol:HCl (dimethylcysteamine:HCl, Aldrich), ethyl 2-mercaptoacetate (Aldrich), thoiglycolic acid (Sigma), iodomethane (BDH), glacial acetic acid (Fisher Scientific), sodium acetate (General Intermediates of Canada), and triethylamine (BDH). Buffers, MES [2-morpholinoethanesulfonic acid], MOPS [3-morpholinopropanesulfonic acid], and EPPS [*N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid], were reagent grade (Sigma) and were used as supplied. Purified deoxygenated water from an Osmonics Aries water purification system was used for buffer preparation. Acetonitrile was dried over 3 A molecular sieves and distilled from phosphorus pentoxide under argon prior to use. The pH was measured using a Radiometer Vit 90 video titrator equipped with a GK2321C combination electrode, standardized by Fisher Certified pH 2, 4, 7, and 10 buffers.

^1H and ^{13}C NMR spectra were obtained using a Bruker AC-200 or a Bruker AM-400 spectrometer. Infrared spectra were obtained using a Bomem MB-120 FTIR spectrometer. High resolution mass spectra were obtained using a Concept 2H (Kratos) spectrometer. All melting points were obtained using a Fisher-Johns melting apparatus and are uncorrected.

(b) Synthesis. The thioesters **10** and **11** were prepared by the typical proceedure described below.

***p*-Nitrobenzoate Ester of Ethyl 2-Mercaptoacetate (10).** *p*-Nitrobenzoyl chloride (0.025 mol, 4.64 g) was dissolved in 10 mL of CH_3CN and then added to a mixture of 0.025 mol (3.00 g) of $HSCH_2COOEt$ and 0.025 mol (2.52 g) of $N(Et)_3$ in 15 mL of CH_3CN. The reaction mixture was then stirred at room temperature for 1 h after which the CH_3CN was evaporated to obtain reddish yellow crystals which were dissolved in 50 mL of CH_2Cl_2. This solution was washed with 3×100 mL each of 1 N HCl, a saturated solution of $NaHCO_3$, and water. The CH_2Cl_2 layer was then dried with $MgSO_4$ and the solvent removed by rotary evaporation to obtain the crude product (85% yield, 5.73 g). A 2 g portion of this was recrystallized from acetone (10 mL)−hexane (a few drops) to yield light yellow, needle-shaped crystals: mp 51−52 °C; IR (KBr) 3113, 2982, 2935, 1730, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H), 3.89 (s, 2H), 4.20 (q, 2H), 8.07−8.30 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 13.98 (CH_3), 31.67 (CH_2), 62.00 (CH_2), 123.84 (aromat CH) 128.28 (aromat CH), 140.53 (aromat C), 150.58 (aromat C), 167.89 (C═O), 188.60 (C═O). Exact mass, *m*/*z* calcd for $C_{11}H_{11}NO_5S$: 269.03528; found: 269.03477 (4.1%). Anal. Calcd for $C_{11}H_{11}O_5NS$: C, 49.07, H, 4.09, N, 5.20, S, 11.90. Found: C, 49.26, H, 3.83, N, 5.21, S, 12.08.

***p*-Nitrobenzoyl Ester of Mercaptoacetic Acid (11).** This was prepared in 71% crude yield as above. A part (∼1.5 g) of the product was crystallized from acetone (15 mL)−hexane (few drops) to give light yellow crystals: mp 155−156 °C; IR (KBr) 2727 −3363 (s, br), 3111, 2914, 1704, 1670 cm^{-1}; ^1H NMR (CD_3CN) δ 4.06 (s, 2H), 8.12−8.49 (m, 4H); ^{13}C NMR (CD_3CN) δ 32.29 (CH_2), 125.12 (aromat CH), 129.33 (aromat CH), 141.54 (aromat C), 150.84 (aromat C), 169.67 (C═O), 189.90 (C═O). Anal. Calcd for $C_9H_7O_5NS$: C, 44.81, H, 2.90, N, 5.81, S, 13.28. Found: C, 44.81, H, 2.62, N, 5.79, S, 12.96.

***p*-Nitrobenzoyl Ester of 2-(*N*,*N*-Dimethylamino)ethanethiol (12).** *p*-Nitrobenzoyl chloride (0.025 mol, 4.64 g), $HS(CH_2)_2N(CH_3)_2$ (0.025 mol, 3.54 g) and pyridine (0.025 mol, 3.96 g) were mixed using the above procedure. After 6 h, the reaction mixture was filtered. The $CHCl_3$ solution was extracted with 1 N HCl, and the pH of the aqueous wash was adjusted to 7 with NaOH (pH paper). The mixture was extracted with $CHCl_3$, this layer being dried over $MgSO_4$, filtered, and evaporated to give a yellow solid in 93% crude yield (5.90 g). A part (∼2 g) of it was recrystallized from $CHCl_3$

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Table 1. Pseudo-First-Order Rate Canstants for Hydrolysis of Thioesters 10−13 [Buffer] = 0, T = 50 °C, μ = 0.1 (KCl)[a]

	rate constant, k_{obs} (s^{-1})			
pH	**10**	**11**	**12**	**13**
1.98		$(4.63 \pm 1.00) \times 10^{-6}$		
2.33		$(5.67 \pm 1.00) \times 10^{-6}$		
3.83		$(1.44 \pm 0.07) \times 10^{-5}$		
4.77		$(1.73 \pm 0.08) \times 10^{-5}$		
6.60	$(1.49 \pm 0.60) \times 10^{-6}$	$(2.19 \pm 0.13) \times 10^{-5}$	$(4.08 \pm 0.61) \times 10^{-4}$	$(6.80 \pm 0.38) \times 10^{-6}$
7.61	$(3.83 \pm 0.06) \times 10^{-5}$	$(3.09 \pm 0.10) \times 10^{-5}$	$(3.15 \pm 0.04) \times 10^{-3}$	
7.72				$(6.20 \pm 0.20) \times 10^{-5}$
8.04	$(8.36 \pm 0.15) \times 10^{-5}$		$(6.82 \pm 0.12) \times 10^{-3}$	$(1.33 \pm 0.02) \times 10^{-4}$
8.09			$(9.32 \pm 0.27) \times 10^{-3}$	
8.55				$(4.94 \pm 0.08) \times 10^{-4}$
8.70	$(1.73 \pm 0.03) \times 10^{-4}$	$(5.90 \pm 0.10) \times 10^{-5}$	$(1.26 \pm 0.04) \times 10^{-2}$	$(6.13 \pm 0.22) \times 10^{-4}$
9.70				$(2.91 \pm 0.11) \times 10^{-3}$
9.78	$(2.26 \pm 0.03) \times 10^{-3}$	$(4.99 \pm 0.40) \times 10^{-4}$	$(1.61 \pm 0.14) \times 10^{-2}$	
11.46	$(8.30 \pm 0.23) \times 10^{-2}$ [b]	$(1.37 \pm 0.03) \times 10^{-2}$	$(3.05 \pm 0.05) \times 10^{-2}$	
11.90	$(0.15 \pm 0.01) \times 10^{0}$ [b]		$(5.97 \pm 0.03) \times 10^{-2}$	

[a] Extrapolated to [buffer] = 0. Errors are standard deviations from fits of two to three replicates at each of two [buffer]. [b] Determined at 46 °C by stopped flow proceedure.

(7 mL)−hexane (2 mL) to give a yellow solid: mp 32−33 °C; IR (KBr) 2953, 2769, 1664 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 6H), 2.59 (t,2H), 3.25 (t, 2H), 8.08−8.29 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 27.33 (CH_2), 44.88 (CH_3), 57.75 (CH_2), 123.60 (aromat CH), 128.01 (aromat CH), 141.30 (aromat C), 150.26 (aromat C), 190.12 (C=O). Exact mass m/z calcd for $C_{11}H_{14}N_2O_3S$: 254.07114; found: 254.06976 (4.2%).

***p*-Nitrobenzoyl Ester of Thiocholine Iodide (13).** Into 10 mL of dry diethyl ether was placed 1.6 mmol (0.41 g) of **12** followed by 2 mmol (0.28 g) of CH_3I. The reaction mixture was stirred overnight at room temperature. The bright yellow precipitate was collected by filtration and recrystallized from methanol (3 mL)−acetonitrile (few drops) to give 0.61 g of solid (96% crude yield): mp 224-225 °C dec; ^1H NMR (60% $CDCl_3$, 40% CD_3OD) δ 3.12 (s, 9H), 3.25−3.51 (m, 4H), 7.86−8.22 (m, 4H). Exact mass (FAB) m/z calcd for $C_{12}H_{17}N_2O_3S$: 269.0960; found: 269.0880. Anal. Calcd for $C_{12}H_{17}N_2O_3SI$: C, 36.36, H, 4.29, N, 7.07, S, 8.08. Found: C, 36.10, H, 4.43, N, 7.07, S, 8.48.

(c) Kinetics. The pH rate profiles of hydrolysis of esters **10**−**13** were determined at 50 °C, μ = 0.1 (KCl). To control pH in the 6.6−6.9 range, tertiary amine buffers were used (MES, MOPS, EPPS, and Et_3N). Acetate buffer was used at pH 3.8−4.8. In all cases two buffer concentrations, 0.05 and 0.10 M, were used to check for buffer catalysis. At pH ≥ 11.5, NaOH was used as the buffer (μ = 0.10 (KCl)) and at pH ≤ 2.3, HCl (μ = 0.10 (KCl)) was used. All pH readings were measured before and after the reaction, the reported values being the average of the two readings.

Rates of disappearance of the starting esters were followed at 270 nm using instrumentation and methods previously described.[3−8] Reactions were initiated by injecting 20−25 μL of a 0.009−0.012 M solution of the ester in dry acetonitrile into 3 mL of buffer solution held in 1-cm quartz cuvettes which were thermally equilibrated at 50 ± 0.7 °C in the cell holder for 10 min prior to initiation of the run. The pseudo-first-order rate constant for the reaction (k_{obs}) was obtained by NLLSQ fitting of absorbance vs time data to a standard exponential model ($A_t = A_\infty + (A_0 - A_\infty)\exp(-kt)$). Values reported are the averages of at least duplicate runs.

(d) D_2O Studies. The rate of hydrolysis of **12** was determined in D_2O at pD 10.20 ± 0.04 (pD = pH + 0.4)[16] in triethylamine buffer ([buffer] = 0.05 and 0.10 M, μ = 0.10 (KCl)). pD was adjusted by additions of 3 M DCl. The kinetic data were obtained as above, also at 50 ± 0.7 °C.

(e) Hydrolysis of 12 in the Presence of DTNB (14). The rate of generation of the Ellman's anion accompanying the hydrolysis of **12** was determined in the presence of Ellman's reagent (DTNB, **14**) at pH 9.77 ± 0.04, triethylamine buffer ([buffer] = 0.05 and 0.10 M, μ = 0.10 (KCl)) at 50 ± 0.7 °C. For this series of reactions, 125−250 μL aliquots of DTNB solution (3.2×10^{-3} M in the same buffer) were injected into 3 mL of the thermally equilibrated buffer in 1-cm cuvettes (giving a final [DTNB] = $1.3-2.5 \times 10^{-4}$ M), immediately followed by 4−8 μL of the stock solution of **12** (0.012 M in dry acetonitrile). The rate of appearance of the Ellman's thiolate anion from DTNB was followed at 412 nm. Since DTNB itself decomposes under these conditions, the pseudo-first-order rate constants were obtained by nonlinear least squares fitting of the absorbance vs time data to a double exponential model ($A_t = ((A_0 - A_{1\infty})\exp(-k_1t) + (A_0 - A_{2\infty})\exp(-k_2t) + (A_{1\infty} + A_{2\infty}))$ to obtain the rate constants for the thiolate-induced and spontaneous productions of Ellman's anion. To verify the rate constants computed in this way, a second run was also performed wherein one cell containing buffer and DTNB only was used as a reference against a cell containing the identical amount of DTNB and **12**. These data were also fitted to a double exponential model equation.

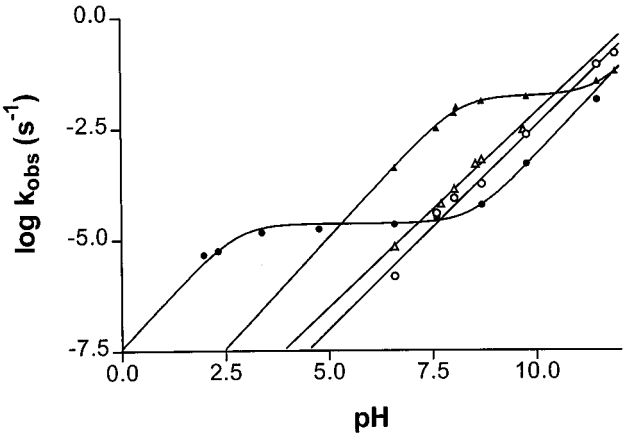

Figure 1. Plot of the pseudo-first order rate constants for hydrolysis of thioesters **10** (○), **11** (●), **12** (▲), and **13** (△) as a function of pH at 50 °C, μ = 0.1 (KCl). Lines are from fits of the data to eq 1 for **11** and **12** and from linear regression for **10** and **13**.

Results

The kinetics of hydrolysis of esters **10**−**13** were followed at 270 nm as a function of pH (1.9−11.9, μ = 0.1 (KCl), T = 50 °C). The pseudo-first-order rate constants for hydrolysis, extrapolated to zero buffer concentration, are given in Table 1. Given in Tables 1S and 2S are the peudo-first-order rate constants at each of the buffer concentrations and the second order rate constants for buffer catalysis, respectively. The pH/rate profiles (Figure 1) for the hydrolyses of compounds **10** and **13** show only a linear dependence on [OH^-], while those for compounds **11** and **12** show evidence of a plateau in the neutral pH region. In the low pH regions, the hydrolysis rates for the latter materials decrease, this being dependent on the pK_a for the COOH and dimethylammo-

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Table 2. Pseudo-First-Order Rate Constants for the Hydrolysis of Thioester 12 in D_2O, pD = 10.20, T = 50 °C, μ = 0.1 (KCl)

pD[a]	[buffer][b]	k_{obs} (s^{-1})	k (s^{-1})[c]	k_H/k_D[d]
10.20	0.05	$(9.28 \pm 0.15) \times 10^{-3}$	$(8.02 \pm 0.18) \times 10^{-3}$	2.23
10.24	0.10	$(10.55 \pm 0.08) \times 10^{-3}$		

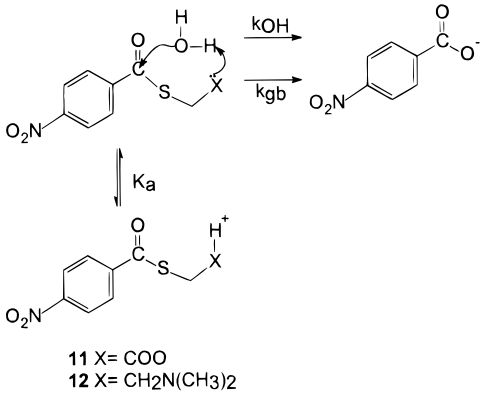
[a] pD = pH + 0.4 (ref 16). [b] Triethylamine. [c] Extrapolated to zero [buffer]. [d] Rate constant for hydrolysis of **12** at pH 10.2 is computed to be 1.78×10^{-2} s^{-1} computed from eq 1.

Table 3. Rate Constant for the Appearance of Ellman's Anion Accompanying the Hydrolysis of 12 in the Presence of Ellman's Reagent, T = 50 °C, μ = 0.1 (KCl)[a]

pH	[buffer][b]	k_{obs} (s^{-1})	k (s^{-1})[c,d]
9.77	0.05	$(1.88 \pm 0.08) \times 10^{-2}$	$(1.66 \pm 0.10) \times 10^{-2}$
9.75	0.10	$(2.10 \pm 0.03) \times 10^{-2}$	

[a][Ellman's reagent, 5,5′-dithiobis(3-nitrobenzoic acid)] = 2.46×10^{-4} M. [b]Triethylamine. [c] Extrapolated to zero [buffer]. [d] Pseudo-first order rate constant for hydrolysis of **12** at pH 9.78 extrapolated to zero [buffer] is $(1.61 \pm 0.14) \times 10^{-2}$ s^{-1} (Table 1).

Scheme 2



nium pendants, pK_a 2.8 and 8.1 respectively. Given in Table 2 are the rate constants for hydrolysis of compound **12** in D_2O in the pH independent region (pH = 10.20, triethylamine buffer). The rate constants for the appearance of Ellman's anion, tied to the hydrolysis of **12** in the presence of excess DTNB in the pH independent region (pH = 9.75, triethylamine buffer), are given in Table 3.

Discussion

The pH rate profiles for the hydrolyses of **11** and **12** shown in Figure 1 are consistent with the general process shown in Scheme 2 where X−H represents COOH or $N(CH_3)_2H^+$. For this process can be derived the kinetic expression given in eq 1 where k_{OH} represents the hydroxide term, k_{gb} represents the intramolecularly assisted term, and K_w and K_a are the autoprotolysis constant of water and acid dissociation constants of the pendants, respectively.

$$k_{obs} = (k_{OH}(K_w/[H^+])) + (k_{gb}K_a/(K_a + [H^+]))$$

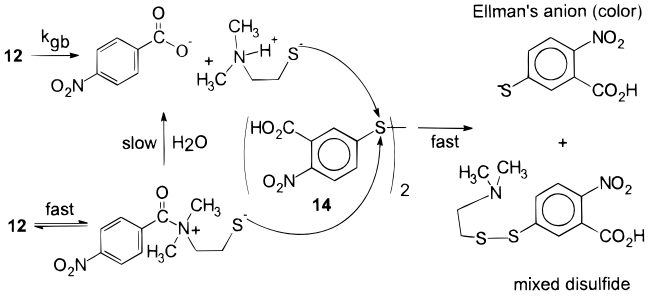
The values for k_{gb}, k_{OH}, and K_a for **11** and **12**, obtained from the NLLSQ fitting of k_{obs} values at different pHs (Table 1) to eq 1, are given in Table 4. Also given in that table are the second-order rate constants for hydroxide attack on **10** and **13** determined from linear regression of the k_{obs} vs $[OH^-]$ plots (not shown). The plateau region from pH 8 to 11 in the case of **12** and pH 2 to 11 in the case of **11** indicates that the basic or deprotonated forms of the pendant groups are involved with the hydrolysis.

Table 4. Computed Rate and Equilibrium Constants for the Hydrolyses of Thioesters 10−13[a]

thioester	k_{OH} (M^{-1} s^{-1})	k_{gb} (s^{-1})[b]	pK_a
10	28.7 ± 0.1[d,e]		
11	4.7 ± 0.1[c]	$(5.58 \pm 2.71$[c]$) \times 10^{-5}$	2.80[f]
12	5.3 ± 0.2[c]	$(1.72 \pm 0.08$[c]$) \times 10^{-2}$	8.18[f,g]
13	55.8 ± 3.5[d]		

[a] T = 50 °C, μ = 0.1 (KCl). [b] From fits of the k_{obs} vs pH data to eq 1. [c]Standard error from NLLSQ fit of the k_{obs} vs pH data to eq 1. [d] Standard error from fits of k_{obsd} at [buffer] = 0 against $[OH^-]$. [e] From fits of five data in Table 1 excluding two highest [NaOH] since the latter were at 46 °C. [f] Set value to obtain best fit to all kinetic data. [g] pH of half-neutralized solution of **12** is 7.9 at 25 °C.

Scheme 3



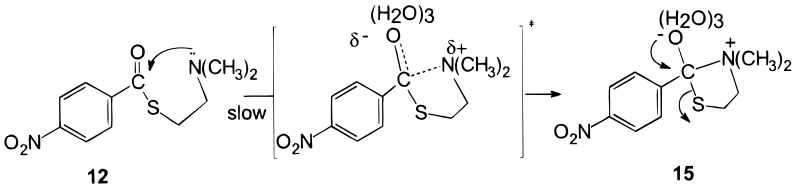
In the case of **12** we have determined the solvent deuterium kinetic isotope effect (Dkie) for this process in the plateau region at pH 10.2 where the reaction proceeds almost entirely through the intramolecularly assisted process; the value $(k_{gb})^H/(k_{gb})^D$ is 2.2. This primary Dkie would be consistent with the pendant acting as a general base as in Scheme 2, but not uniquely so since it could also be consistent with a nucleophilic role as in Scheme 3 where rapid reversible S to N (COO^-) acyl transfer occurs with subsequent slow attack of water on the intermediate.

The putative slow acyl transfer to water in Scheme 3 bears a strong analogy to water-promoted hydrolysis of activated acyls[17] which can also show large solvent Dkie's of 2.5−3.7. In order to distinguish between the two possibilities, **12** was hydrolyzed in the plateau region (pH 9.7) in the presence and absence of Ellman's reagent (**14**). This should react rapidly[18] with the transiently formed thio(ate) to generate the highly absorbing Ellman's anion, the rate of generation of which can be followed at 412 nm. Should the nucleophilic role be operative then added Ellman's reagent will trap the transient thiolate and prevent its return to **12**. The trapping process should be dependent on the [Ellman's reagent], ultimately showing a saturation phenomenon when the rate-limiting step becomes the intramolecular S to N acyl transfer. This technique has been used previously by Heller *et al.*[11c] and us[4] to test for the process of S to N acetyl transfer that could generate reversibly formed thiolates during the hydrolysis of the acetyl thioesters of **1** and **2**. We observe that the appearance of the Ellman's anion accompanying the hydrolysis of **12** does not depend on the [Ellman's reagent], and from the data in Table 3, the rate of generation of the Ellman's anion is identical to the rate of disappearance of **12**. This verifies that the

(17) (a) Fife, T. H. *Acc. Chem. Res.* **1993**, *26*, 325, and references therein. (b) Young, J. K.; Pazhanisamy, S.; Schowen, R. L. *J. Org. Chem.* **1984**, *49*, 4148. (c) Hegazi, M.; Mata-Segreda, J. F.; Schowen, R. L. *J. Org. Chem.* **1980**, *45*, 307. (d) Komiyama, M.; Bender, M. L. *Bioorg. Chem.* **1978**, *7*, 133. (e) *Idem. Ibid.* **1979**, *8*, 141.

(18) (a) Semenow-Garwood, D.; Garwood, D. C. *J. Org. Chem.* **1972**, *32*, 3804. (b) Whitesides, G. M.; Lilburn, J. E.; Szajewski, R. P. *J. Org. Chem.* **1977**, *42*, 332.

Scheme 4

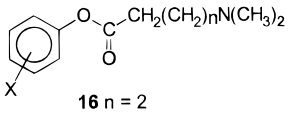


intramolecular nucleophilic process depicted in the lower portion of Scheme 3 is neither dominant nor competing during the hydrolysis of **12**.

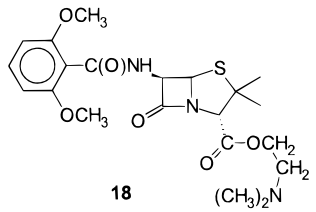
As a final point we consider the possibility that the process involves a rate limiting nucleophilic attack of the pendant as in Scheme 4, but with rapid subsequent capture of the *N*-acyl ammonium ion by water. This process could still show some isotope effect because of the solvation of the developing oxyyanion in **15**,[19] but it is rendered unlikely since it would require that after the breakdown of tetrahedral intermediate **15**, the poor intermolecular nucleophile, water, attacks the *N*-acyl ammonium C=O in preference to the intramolecular S^-. We conclude that a rate-limiting nucleophilic role for the pendant is unlikely. This, coupled to the Ellman's experiments above, strongly supports the general base role for the pendants in assisting the hydrolysis of a thioester.

This behavior contrasts the situation proposed by Bruice and Benkovic[20] for the hydrolysis of the oxygen esters **16**, **17** and also the process proposed by Ferres[21] for the hydrolysis of esters such as **18** which were considered candidates for drug delivery in the penicillin system.

The best fit rate and equilibrium constants given in Table 4 invite some comparisons. The second-order rate constants for hydroxide attack on the thioesters span a range of 10-fold with the order being **13** > **10** > **12** ≥ **11**. The ordering might be explicable on electrostatic grounds reflecting some stabilization for the transition state for OH^- attack on the positively charged thiocholine derivative, **13**, relative to attack on neutral **12** or anionic **11**. The electrostatic effect that enhances attack of [OH^-] on **13** is similar to the rate enhancement afforded by the inductively withdrawing ester group in **10**.



16 n = 2
17 n = 3



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It is instructive to determine the effective molarities of the intramolecular catalytic COO^- and $N(CH_3)_2$ pendants in **11** and **12** relative to reasonable intermolecular counterparts. From Table 4, the k_{gb} for **11** is $(5.6 \pm 2.7) \times 10^{-5}$ s^{-1} while that for **12** is 1.7×10^{-2} s^{-1}. An appropriate intermolecular general base for each of these would be a carboxylic acid and tertiary amine having similar pK_a's to the acid and amine pendants in the thioesters. Using the second-order rate constants for buffer catalysis afforded to hydrolysis of **11** by acetate (pK_a = 4.76[22]), and to **12** by EPPS (*N*-(2-hydroxyethyl)-piperazine-*N*′-3-propanesulfonic acid, pK_a = 8.00[22]), gives effective molarities of roughly 0.2−0.3 and 0.4 M for these two thioesters.[23] These are values well within the range of what is commonly accepted for general base processes (rarely greater than 10 M) but far lower than what is normally seen of intramolecular nucleophilic catalysis.[24] It may be that acetic acid, by virtue of its pK_a of 4.76, is an inappropriate comparison for the intramolecular carboxylate in **11**. Correcting the intermolecular rate by assuming a Brønsted β of 0.5 and dropping the pK_a of the intermolecular carboxylic acid to 2.8[24] would only raise the effective molarity in **11** to roughly 2 M.

Finally, it is useful to estimate the rate enhancement provided by these intramolecular pendants to the hydrolysis around neutrality. For **12** the $t_{1/2}$ for hydrolysis at pH 8, 50 °C is 100 s which is some 50 times faster than that observed for hydrolysis of **13** under the same conditions. For **11** at pH 6.6, the $t_{1/2}$ for hydrolysis is roughly 8 h, which is 16 times faster than its comparison, **10**. Due to the plateau in the pH rate profile for **11** that extends down to pH 3, and the linearity in the profile for **10**, the enhancement becomes much larger with decreasing pH, the value at 3.6 being 16000-fold.

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Supporting Information Available: Table of pseudo-first-order rate constants for the hydrolyses of **10**−**13** in various buffers, T = 50 °C (Table 1S), and table of second-order rate constants for buffer catalysis of the hydrolyses of **10**−**13** at various pHs (Table 2S) (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(20) Bruice, T. C.; Benkovic, S. J. *J. Am. Chem. Soc.*, **1963**, *85*, 1.
(21) Ferres, H. *Chem. Ind. (London)* **1980**, *11*, 435.
(22) Perrin, D. D.; Dempsey, B. In *Buffers for pH and Metal Ion Control*; Chapman and Hall: London, 1974; pp 157−163.
(23) From Table 2S, Supporting Information, the second-order rate constants for acetate catalysis and EPPS catalysis are the following: acetate with **11**, 3.34×10^{-5} M^{-1} s^{-1} (pH 3.83) and 9.98×10^{-5} M^{-1} s^{-1} (pH 4.77); EPPS with **12**, 3.74×10^{-2} M^{-1} s^{-1} (pH 8.70). The fact that the acetate catalysis increases with increasing pH indicates that the process is dependent on the anionic or basic form, as expected for a general base. When one corrects the buffer rate constants for the amount of the basic form present at the indicated pH, the true rate constants are 2×10^{-4} M^{-1} s^{-1} and 4.5×10^{-2} M^{-1} s^{-1}, respectively, for acetate with **11** and EPPS with **12.** These values can be used to compute the effective molarities for the COO^- and $N(CH_3)_2$ pendants given in the text.
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(25) The kinetic pK_a of COO^- group in **11** can be compared with that in thioglycolic acid ($HSCH_2COO$**H**, pK_a 3.55[8]) which should be reduced by *p*-nitrobenzoylation of the S. By way of comparison, the pK_a of the ammonium group in dimethylcysteamine is reduced from 10.89[8] to 7.9−8.2 on *p*-nitrobenzoylation of the S (see Table 4).