Molecular Structure of 2,3,4,5-Tetrahydro-2-oxo-1,5-ethanobenzazepine and Its Reaction with β-Amino Alcohols as a Model for the Acylation Step of the Serine Proteases

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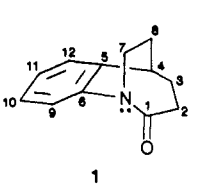
The molecular structure of strained amide 1 (2,3,4,5-tetrahydro-2-oxo-1,5-ethanobenzazepine) and the kinetics of its reaction with several β-amino alcohols including *N,N*-dimethylethanolamine, 2-(hydroxymethyl)imidazole, and *N*-methyl-2-(hydroxymethyl)imidazole are reported. The molecular structure shows the N to be nearly sp^3 pyramidalized and the N lone pair twisted some 60° from collinearity with the C=O π bond. Tertiary amines including triethylamine and *N*,2-dimethylimidazole do not react with 1, nor do they accelerate attack of H_2O or ethanol on 1 by intermolecular general-base catalysis. However, β-amino alcohols show large reactivity as their neutral forms by a process involving intramolecular N,O-cooperativity. Product studies show that O-acylation occurs. These same β-amino alcohols were investigated as to their ability to attack *p*-nitrophenyl acetate (pNPA). Although some O-acylated product occurs, it arises from initial N-acylation and subsequent intramolecular transfer. In no case could a significant N,O-cooperativity of O-acylation be demonstrated with pNPA. The relevance of these findings to the acylation of serine proteases by amides is discussed.

The active site of the most well-studied example of the serine proteases, chymotrypsin (CT), contains a catalytic triad consisting of Asp-102,His-57,Ser-195, the latter group becoming transiently acylated during the course of hydrolysis of both ester and amide substrates. Although the role of the Asp-102 residue remains unclear, it is generally believed that with naturally occurring substrates formation of the acyl-enzyme intermediate involves a His-57 general-base activation of the Ser-195 as a nucleophile as is stylized in Figure 1.[1] However, recent evidence suggests that unnatural substrates such as *p*-nitrophenyl benzoates[2a] and *p*-nitrophenyl acetate[2b] (pNPA) may be hydrolyzed by CT via a route that involves first N-acylation of the His-57 imidazole followed by a rapid acyl transfer to Ser-195.

A number of reports[3] concerning the reaction of esters such as pNPA or reactive amides[3m] with simple amino alcohols as models for the acylation step of the serine proteases have appeared. However, the N,O-cooperativity is kinetically small or nonexistent, and there remains a mechanistic ambiguity as to which of the N or OH units is initially acylated when appropriate controls are considered.[4] Aminolysis of the reactive amide acetylimidazole (AcIm) has been shown to be subject to intramolecular general-base catalysis.[5] However, the reaction of AcIm with amino alcohols such as ethanolamine and 3-quinuclidinol, while proceeding ~5-fold faster than with amines of comparable basicity but containing no OH group, was proposed to proceed via nucleophilic attack of the zwitterionic form $^-O\sim\sim NH_3^+$.[5] Thus, with small molecules, significant N,O-cooperativity during the O-acylation by amides such as is believed to occur during serine protease catalyzed hydrolysis of amides is at present unknown.

Recently we reported the synthesis and detailed kinetic study of anilide 1[6] which is activated toward nucleophilic addition by NC=O distortion. (Numbering of compound 1 is for crystallography purposes only and does not correspond to naming of compound.) That study showed



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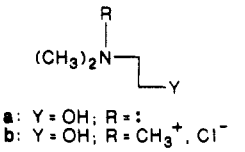
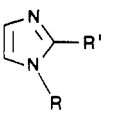


2a: Y = OH; R = :
b: Y = OH; R = CH_3^+, Cl^-



3a: R = H; R' = CH_2OH
b: R = H; R' = CH_3
4a: R = CH_3; R' = CH_2OH
b: R = R' = CH_3

that the entire pH/rate profile (pH 0-14) at [buffer] = 0, T = 25 °C consists of two terms: H_2O attack on an O-protonated form and OH^- attack on the neutral form.[6] In addition, at pH >7, buffers containing both a basic N and

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(4) When deciding whether N,OH-cooperativity in the acylation process occurs, the major considerations should be whether the amino alcohol reacts significantly faster than a blocked comparison molecule having a similar pK_a (e.g., methoxy derivative) *and* that initial O-acylation is indeed observed.

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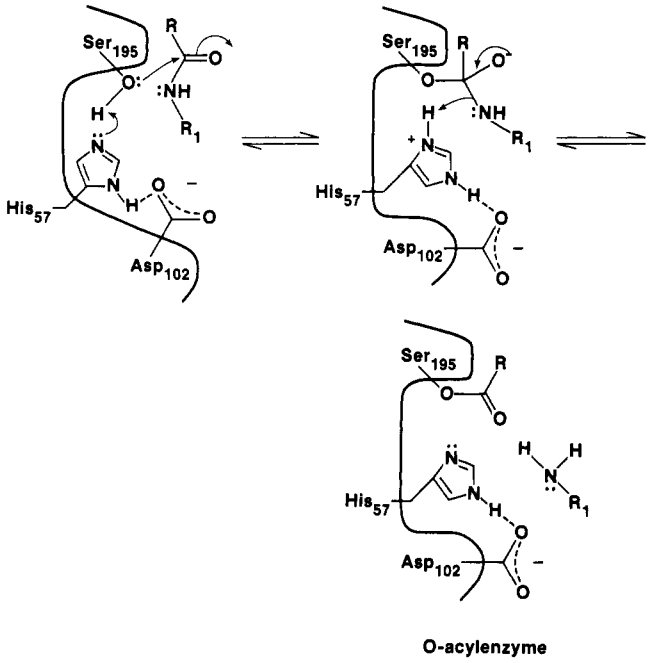

Figure 1. Stylized representation of the acylation of the active site of the serine proteases.

a β-OH group were found to be unusually active toward 1 although buffers containing only a tertiary amino group showed no activity toward 1 at all. Herein we report the crystal and molecular structure of 1, which indicates the nature of the NC=O distortion. In addition, the reaction of both 1 and pNPA with β-hydroxy amines 2–4 is presented. In parallel to the situation with chymotrypsin, the amide reacts with the neutral forms of 2–4 via N-assisted O-acylation, while pNPA reacts as an N-acylating agent.

Experimental Section

a. Amide 1 was prepared as previously described.[6] Imidazoles **3a** and **4a** were prepared via $NaBH_4$ reduction of the corresponding aldehydes.[7]

4a: 80% yield recrystallized from 95% EtOH–ether; mp 108–110 °C (lit.[8] mp 114 °C).

3a: 43% yield recrystallized from 95% EtOH; mp 112–114 °C (lit.[7] mp 114 °C).

Compounds **2a,b**, **3b**, and **4b** were commercial materials (Aldrich) and, if solid, used as supplied; liquids were distilled prior to use.

Buffers TRICENE [*N*-tris(hydroxymethyl)methylglycine, pK_a 8.1], TRIS [tris(hydroxymethyl)aminomethane, pK_a 8.1], HEPES [*N*-(hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid, pK_a 7.5], BISTRIS [bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane, pK_a 6.5], MOPS (morpholinoethanesulfonic acid, pK_a 7.2), and CAPS [(cyclohexylamino)propanesulfonic acid, pK_a 10.4] were used as supplied by Sigma. *p*-Nitrophenyl acetate was recrystallized from hexane. Water for kinetic runs was triply distilled. pH values were determined with a Radiometer TTT-2 titration apparatus using a Radiometer GK2321 C combination electrode standardized with Fisher certified buffers (pH 4.00, 7.00, 10.00). Values recorded before and after a kinetic run were identical within limits of ±0.03 unit. Deuteriated buffered solutions were made with 99.7% D_2O, adjusting pD with DCl or NaOD. pD values were determined by adding 0.4 unit to the meter reading.

b. Kinetics. Kinetic data were obtained at 25.0 °C in aqueous buffered solutions (μ = 0.2 M KCl) containing (8.0–16.0) × 10^{-5} M 1 by following the rate of production of the product anilide at 291 nm. The hydrolysis rate of 4.33 × 10^{-5} M pNPA was monitored at 25.0 or 37.0 °C under similar conditions by observing the rate of appearance of *p*-nitrophenolate at 400 nm with a Cary 210 UV–visible spectrophotometer interfaced as previously described.[9] In all cases, pseudo-first-order conditions of excess 2–4 over 1 or pNPA were maintained, the rate constants (k_{obsd}) being evaluated by fitting the absorbance vs. time curves to an exponential model[10] by nonlinear least-squares treatment. Duplicate or triplicate runs were obtained, and the k_{obsd} value is the average, with the error limits being the maximum deviation from the mean. Reactions were followed to at least three $T_{1/2}$ and displayed excellent first-order kinetics. From the slopes of the plots of k_{obsd} values vs. [2–4] or [β-hydroxy amine] buffers [[buffer$_t$] = 0.02–0.2 M] were obtained the second-order rate constants (k_2^{obsd}) for the attack of those species on 1 or pNPA.

c. Product Studies. (*N*,*N*-Dimethylamino)ethyl 1,2,3,4-Tetrahydroquinoline-4-propanoate from 1 + (*N*,*N*-Dimethylamino)ethanol. To a solution of 1 (37 mg, 0.2 mmol) in 500 μL of $CDCl_3$ in an NMR tube was added 17.7 mg (0.2 mmol) of (*N*,*N*-dimethylamino)ethanol (NNDAE). The tube was sealed and maintained at 40 °C and the progress of the reaction monitored by periodic NMR analysis of the $HOCH_2$ triplet of NNDAE (δ 3.66) over 40 h. At the completion of the reaction, 100 μL of this solution was diluted with 400 μL of $CDCl_3$ and the ^1H NMR spectrum monitored at 200 MHz. The acylated hydroxymethylene group of NNDAE appeared as a downfield-shifted triplet at δ 4.20. The remaining contents of the original NMR mixture were stripped of solvent and analyzed by IR and exact-mass spectrometry: IR 1725, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.0 (m, 2 H), 6.6 (m, 1 H), 6.45 (m, 1 H), 4.18 (t, 2 H), 3.9 (s br, 1 H), 3.3 (m, 2 H), 2.8 (m, 1 H), 2.5 (m, 3 H), 2.28 (s, 6 H), 2.2–1.7 (m, 5 H); exact mass for $C_{16}H_{24}N_2O_2$, calcd *m/e* 276.1838 (M^+), found *m/e* 276.1839, 205.1101, 191.0943, 144.0811, 132.0824, 117.0580, 58.0681.

Isolation of the product produced from the reaction of 1 with NNDAE under kinetic conditions was effected as follows. A 500-μL CH_3CN solution of 1 (0.1 M) was injected into 30 mL of buffer consisting of 0.3 M NNDAE, μ = 0.2 M KCl, pH 9.40 at 25 °C. After 15 min the solution was extracted with 3 × 10 mL of ether, and the combined extracts were dried over Na_2SO_4. After filtration and removal of the volatiles, 19 mg of residue remained, which proved to consist of ester and some recovered amine. The ^1H NMR spectrum of this residue was virtually identical with that of the authentic ester (above) with the exception of additional peaks ascriable to NNDAE: IR (film) 1725, 1605 cm^{-1}; exact mass for $C_{16}H_{24}N_2O_2$, calcd *m/e* 276.1838 (M^+), found *m/e* 276.1839, 205.1102, 191.0947, 144.0812, 132.0817, 117.0582, 58.0686.

(*N*-Methyl-2-imidazolyl)methyl 1,2,3,4-Tetrahydroquinoline-4-propanoate from 1 + *N*-Methyl-2-(hydroxymethyl)imidazole. These experiments were conducted by the same protocol as above. The authentic ester was prepared from 27 mg of amide 1 and 17 mg of **4a**, heating in 500 μL of $CDCl_3$ in an NMR tube at 40 °C for 60 h: IR (film) 3350, 1730, 1608 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.90 (m, 4 H), 6.60 (m, 1 H), 6.46 (d, 1 H), 5.8 (s, 2 H), 4.05 (br s, 1 H), 4.68 (s, 3 H), 3.28 (m, 2 H), 2.76 (m, 1 H), 2.45 (m, 2 H), 2.05 (m, 1 H), 1.88 (m, 2 H), 1.76 (m, 1 H); exact mass for $C_{17}H_{21}N_3O_2$, calcd *m/e* 299.1633 (M^+), found *m/e* 299.1630, 167.0819, 154.0742, 132.0812, 111.0557.

For isolation of product from the kinetic reaction medium, 30 mL of buffer made from **4a**, pH 7.50, 0.1 M (μ = 0.2 M KCl) was used. The isolated material (13 mg) from this medium proved to be identical with the authentic ester: IR (film) 3350, 1730, 1608 cm^{-1}; ^1H NMR ($CDCl_3$) identical with authentic product but has some peaks attributable to **4a** [δ 4.64 (s, 2 H), 3.70 (s, 3 H)]; exact mass for $C_{17}H_{21}N_3O_2$, calcd *m/e* 299.1633 (M^+), found *m/e* 299.1631, 167.0820, 154.0742, 132.0813, 111.0557.

Product Analysis for 3a and 4a with pNPA. In an NMR tube was placed 20 mg (1.2 × 10^{-4} mol) of **3a** and an equivalent amount of pNPA along with 500 μL of CD_3OD ([**3a**] = [pNPA] = 2.4 × 10^{-1} M). The reaction progress at 25 °C was monitored by periodic ^1H NMR analysis of the acetate peaks of pNPA (δ 2.45), (*N*-methyl-2-imidazolyl)methyl acetate (AcOIm) (δ 2.15), and CH_3COOCD_3 (δ 2.10). Percentages of species were judged by the relative intensities of the CH_3CO_2 peaks. After 12 h the relative amounts of pNPA, AcOIm, and $CH_3CO_2CD_3$ were 5%, 75%, and 19%, respectively. Monitoring the spectrum for more prolonged times indicated that AcOIm slowly solvolyzed to

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Table I. Experimental Details of Crystallographic Determination

A. Crystal Data

$C_{12}H_{13}NO$, FW = 187.24
cryst dimens, 0.24 × 0.19 × 0.53 mm
monoclinic space group $P2_1/n$
a = 11.716 (3), b = 6.479 (4), c = 12.425 (3) Å
β = 90.61 (2)°
V = 943 Å^3, Z = 4, D_c = 1.319 g cm^{-3}, μ = 0.78 cm^{-1}

B. Data Collection and Refinement Conditions

radiation	Mo Kα (λ = 0.71073 Å)
monochromator	incident beam, graphite crystal
take-off angle	3.0°
detector aperature	2.40 mm horiz × 4.0 mm vert
cryst-to-detector dist	205 mm
scan type	ω–2θ
scan rate	10.1–0.8° min^{-1}
scan width	(0.84 + 0.35 tan θ)°
data collectn 2θ limit	54.00°
data collectn index range	$h,k,\pm l$
reflcns measd	2231 unique, 1246 with I > 3σ(I)
observns variables ratio	1246/166
agreement factors R_1, R_2, GOF	0.040, 0.053, 1.7
corrections appl	abs correction

produce $CH_3CO_2CD_3$ (6 days, relative amounts 60/40; 13 days, relative amounts 49/51): exact mass for $C_6H_8N_2O_2$, calcd m/e 140.0586, found, m/e 140.0583; IR (MeOH/$CHCl_3$ cast) 1742 cm^{-1}; ^{1}H NMR (CD_3OD, 80 MHz) δ 2.15 (s, 3 H), 5.25 (s, 2 H), 7.0 (s, 1 H), 7.15 (s, 1 H).

A similar study conducted with 3.57 × 10^{-1} M **4a** and the equimolar pNPA in CD_3OD indicated that the O-acetylated **4a** $CH_3CO_2CD_3$ ratio was 42/58 after 6 days and remained the same at 12 days: exact mass for $C_7H_{10}N_2O_2$, calcd m/e 154.0743, found m/e 154.0743; IR (MeOH/$CHCl_3$ cast) 1742 cm^{-1}; ^{1}H NMR (CD_3OD, 80 MHz) δ 2.15 (s, 3 H), 3.80 (s, 3 H), 5.25 (s, 2 H), 7.0 (m, 2 H).

d. Crystallography. Crystals of **1** suitable for single-crystal X-ray structure determination were obtained by sublimation of crude **1** at ambient temperatures (0.025 torr).

Data Collection. A representative parallelepiped crystal was mounted, in a capillary, in a nonspecific orientation on an Enraf-Nonius CAD4 automated diffractometer. The crystal was cooled to −65 °C, by a cold air stream apparatus, and all measurements were made at this temperature.

The automatic peak search and reflection indexing programs[11] in conjunction with a cell reduction program showed the crystal to be monoclinic and from the systematic absences of $h0l$ ($h+l$ odd) and $0k0$ (k odd); the space group was determined to be $P2_1/n$, an alternative setting of $P2_1/c$ (No. 14).[12]

Cell constants were obtained from a least-squares refinement of the setting angles of 15 reflections in the range 7 < 2θ < 23°. There were two reflections that were chosen as standard reflections, and these were remeasured every 60 min of exposure time to check on crystal and electronic stability over the course of data collection. These reflections changed in intensity by 1.5% and 2.5%, respectively, over the time span of data collection which was considered negligible. The various crystal parameters are given in Table I.

Data Reduction. A total of 2337 reflections were collected, and these were corrected for Lorentz, polarization, and background effects according to I = SR(SC − RB)/Lp and $\sigma(I)$ = SR(SC + R^2B) + (pI)^2/Lp^2, where SR is the scan rate, SC is the total scan count, R is the ratio of scan time to background time, B is the total background count, p is a factor to downweight intense reflections (chosen as 0.040 in this experiment), and Lp is the Lorentz and polarization correction term.

The data were corrected for asorption effects by an empirical correction based on the absorption surface method of Walker and Stuart.[13] The maximum and minimum correction coefficients

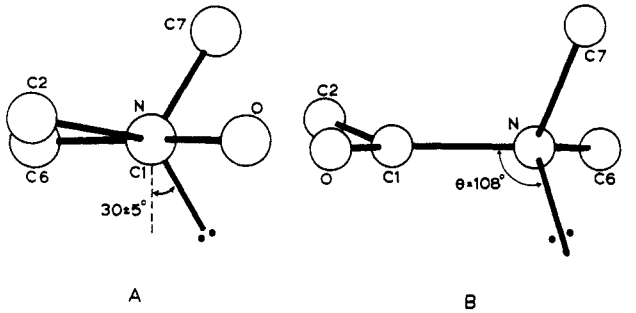


Figure 2. (a) Twist angle of amide **1** as seen from projection down the O=C1N axis. (b) Pyramidalization of N as viewed perpendicular to the O=CNC6 plane. Electron pair determined as mean position of peak of electron density observed near N in crystallographic difference Fourier map.

applied to F_o were 1.1482 and 0.8172, respectively. After equivalent forms were averaged (R factor for averaging is 0.034) and any systematically absent data were rejected, there were 2231 unique reflections of which 1246, having $I > 3\sigma(I)$, were used in the structure solution and refinement.

Structure Solution and Refinement.[14] The structure was solved by the direct methods program MULTAN[15] which gave the positional parameters for all the non-hydrogen atoms. Refinement of atomic parameters was carried out by using full-matrix least-squares techniques of F_o, minimizing the function $\sum w(|F_o| - |F_c|)^2$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively, and the weighting factor w is given by $w = 4F_o^2/\sigma(F_o^2)$.

The neutral-atom scattering factors were calculated from the analytical expression for the scattering factor curves.[16a] The f' and f'' components of anomalous dispersion[16b] were included in the calculations for all non-hydrogen atoms. All hydrogen atoms were located and included in the refinement with isotropic thermal parameters fixed at 0.75 times that of the attached C atom. This value was chosen after attempts at refining these parameters that, while not entirely successful, did indicate the range of approximate values.

In the final cycle 166 parameters were refined from 1246 observations having $I > 3\sigma(I)$. The final agreement factors were $R_1 = \sum||F_o| - |F_c||/\sum|F_o| = 0.040$ and $R_2 = (\sum w(|F_o| - |F_c|)^2/\sum wF_o^2)^{1/2} = 0.053$.

The largest shift in any parameter was 0.1 times its estimated standard deviation, and the error in an observation of unit weight was 1.79e. An analysis of R_2 in terms of F_o, $\lambda^{-1}(\sin\theta)$, and various combinations of Miller indices showed no unusual trends. The highest peak in the final difference Fourier was 0.34 (2) e Å^{-3} located near C1 and C2 at fractional coordinates 1.057, −0.084, 0.307.

After refinement was complete, a difference Fourier was examined for evidence of the nitrogen lone pair. A peak (0.103 e Å^{-3}) was located at a reasonable position (1.057, 0.207, 0.223), which maintained this position upon changing the $\lambda^{-1}(\sin\theta)$ limits of the data used to calculate the map. Thus, we are quite confident that these coordinates do represent the position of a localized lone pair of electrons on the N atom.

Using a rigid-body thermal motion analysis[17] to correct the bond distances and angles in the molecule resulted in negligible changes (less that 1.5σ) in any parameter; hence, the reported distances and angles are the uncorrected values.

An examination of intermolecular distances showed there were no close contacts, thus indicating only normal, weak van der Waals

(11) The diffractometer programs are those supplied by Enraf-Nonius for operating the CAD4F diffractometer with some local modifications and additions.
(12) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1969; Vol. 1.
(13) Walker, N.; Stuart, D. *Acta Crystallogr., Sect. A.: Found Crystallogr.* **1983**, *A39*, 158.
(14) The computer programs used in this analysis include the Enraf-Nonius Structure Determination Package by: Frenz, B. A. *Computing in Crystallography*; Delft University: Delft, Holland, 1978; pp 64–71.
(15) Main, P.; Lessinger, L.; Woolfson, M. M.; Germain, G.; Declercq, J. P. MULTAN 11/82, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.
(16) (a) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV, Table 2.2B. (b) *Ibid.* Table 2.3.1.
(17) Schomaker, V.; Trueblood, K. N. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1968**, *B24*, 63.

Table II. Selected Bond Distances (Å) and Angles (deg) for the Amide Unit in 1[a]

atom 1	atom 2	dist		angle
O	C1	1.216 (2)		
N	C1	1.401 (2)	O–C1–N	119.8 (2)
N	C6	1.452 (2)	C1–N–C6	116.5 (1)
N	C7	1.490 (2)	C1–N–C7	109.3 (1)
C1	C2	1.507 (2)	O–C1–C2	123.4 (2)
			N–C1–C2	116.1 (1)

[a] Numbers of parentheses are estimated standard deviations in the least significant digits.

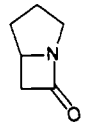
forces between molecules of a unit cell.

Available as supplementary material are tables of observed and calculated structure factors (Table 2) and atomic positional and thermal parameters (Table 1, 3, and 4).

Results and Discussion

Structure Description. As a whole, the molecule displays entirely normal bond distances and angles. (Full listings can be found in Tables 5 and 6, supplementary material.) The interest lies in the geometry of the amide group of atoms (C2, C1, O, N, C6, C7), which is depicted in two perpendicular views in Figure 2. Selected bond distances and angles are given in Table II. The N is approximately tetrahedral, which leads to a C7–N–C1–O torsional angle (defined as ω_1) of 60°. It was reasonable to expect that a large ω_1 angle would lead to a significant reduction of the N electron pair delocalization. Indeed an electron density map shows a peak lying 1.2 Å from the N atom in almost exactly the position one would predict for a lone pair based upon geometrical considerations of a tetrahedral N. That this peak can be observed at all suggests that the delocalization cannot be particularly significant, which is undoubtedly responsible for the enhanced reactivity of 1 relative to more normal anilides. It is important to note that pyramidalization of the N[18] leads to both a twisting and tilting of the lone pair from optimum conjugation with the C=O π unit, these values being 30 ± 5° and 15–20° as illustrated in Figure 2.

In order to judge the uniqueness of the distortion in 1 we extracted a set of 1285 compounds containing some 1548 amide groups from the Cambridge Crystallographic Data Base. Only 103 had torsion angles (ω_1) exceeding 20°. Of these, there were six compounds containing the moiety **5** which had ω_1 angles between 50 and 60°, and one com-



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pound containing an aziridine fragment was also in this range. There were 31 compounds (with 35 amide groups) having the N in rings of more than four members, and in only seven cases was ω_1 greater than 35°. In 1, the N atom is in rings of six and seven members and the ω_1 value is 60° which to our knowledge, is unique.[30]

Kinetics. Hydroxy amine buffers such as TRICENE, TRIS, HEPES, and BISTRIS as well as **2a** markedly accelerate the decomposition of 1. For each, only the basic form is active. The k_2^{max} values extrapolated to 100% free base are given in Table III. while the primary data for the buffers at various pH values are given in Table 7 (supplementary material). A Brønsted plot of log k_2^{max} for these materials statistically corrected for the number of

Table III. Second-Order Rate Constants (k_2^{max}) for the Reaction of Various β-Hydroxy Amines with Anilide 1[a]

compd	pK_a	no. β-OH gps	k_2^{max},[b] M^{-1} s^{-1}
(*N*,*N*-dimethylamino)ethanol	9.35 ± 0.05[c]	1	(2.56 ± 0.06) × 10^{-1}
TRICENE	8.1	3	(4.30 ± 0.06) × 10^{-2}
TRIS	8.1	3	(5.07 ± 0.13) × 10^{-2}
HEPES	7.5	1	(4.48 ● 0.25) × 10^{-3}
BISTRIS	6.5	5	(9.70 ± 0.40) × 10^{-3}

[a] k_2^{obsd} values determined from slopes of plots of pseudo-first-order k_{obsd} values vs. [buffer$_t$] (0.02–0.15 M) at T = 25 °C, μ = 0.2 M (KCl) at various pH values. [b] k_2^{max} values extrapolated to 100% free base utilizing indicated pK_a. Error limits are the standard deviations of linear least-squares plots. [c] Titrimetric value, μ = 0.2 M KCl, T = 25 °C.

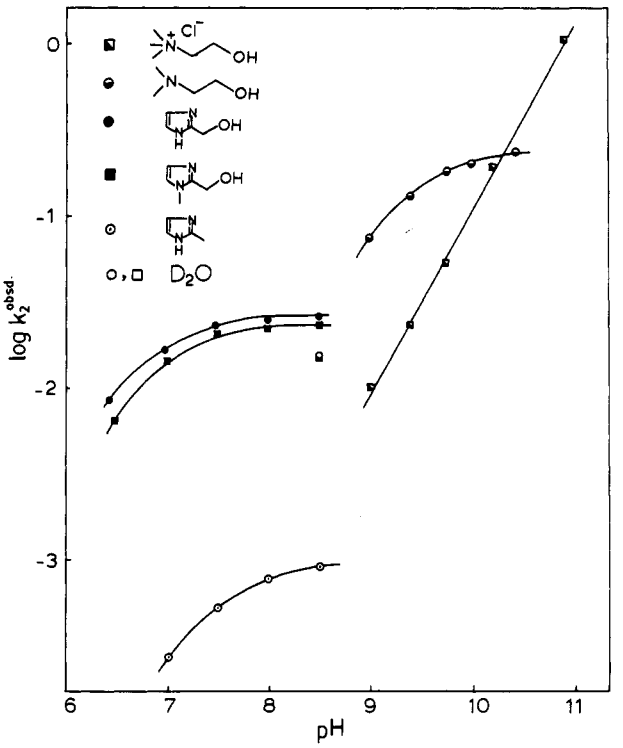
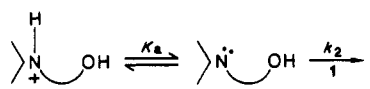


Figure 3. log k_2^{obsd} vs. pH for the attack of **2a,b**, **3a,b**, and **4a** on 1. T = 25 °C, μ = 0.2 M (KCl).

β-OH groups vs. pK_a yields a slope of β = 0.75 ± 0.1 and intercept of −7.82 ± 0.2, r = 0.950. Triethylamine, a much more basic amine (pK_a 10.75[19]), is inactive toward 1 as are MOPS and CAPS buffers. The latter buffers, with added 0.1 M ethanol produce no acceleration in rate. Also, experiments conducted with 0.1 triethylamine buffers at pH 9.75 and 10.4 (μ = 0.2 M KCl) in the presence of 0.02–0.1 M added ethanol show no enhanced rates of decomposition of 1 over the specific base-catalyzed process. These experiments indicate that the mechanism of reaction with 1 requires an intramolecular N–OH cooperativity of the β-hydroxy amines.

Shown in Figure 3 are plots of log k_2^{obsd} vs. pH for the attack of **2a,b**, **3a,b**, and **4a** on **1**. With the exception of choline (**2b**) the reactions of each of these β-hydroxy amines adhere to the generalized mechanism given in eq 1. The primary k_2^{obsd} data at various pH values (Table



(1)

(18) Mock, W. L. *Bioorg. Chem.* **1975**, *4*, 270–278.

(19) Perrin, D. D. *Dissociation Costants of Organic Bases in Aqueous Solution*; Butterworths: London, 1965.

Table IV. Second-Order Rate Constants and Kinetic pK_a Values for the Attack of 2-4 on Amide 1[a]

compd	pK_a thermodynamic[b]	pK_a kinetic[c]	k_2^{max},[c] M^{-1} s^{-1}
(*N*,*N*-dimethylamino)ethanol (**2a**)	9.35 ± 0.05	9.38 ± 0.02	(2.54 ± 0.04) × 10^{-1}
choline (**2b**)	12.8[d]		(8.50 ± 0.08) × 10^{1} [e]
2-(hydroxymethyl)imidazole (**3a**)	6.73[f]	6.77 ± 0.02	(2.66 ± 0.02) × 10^{-2}
			(1.45 × 10^{-2})[g]
2-methylimidazole (**3b**)	7.56[i]	7.44 ± 0.04	(9.53 ± 0.35) × 10^{-4}
2-(hydroxymethyl)-*N*-methylimidazole (**4a**)	6.93 ± 0.05	6.92 ± 0.08	(2.45 ± 0.12) × 10^{-2}
			(1.50 × 10^{-2})[g]

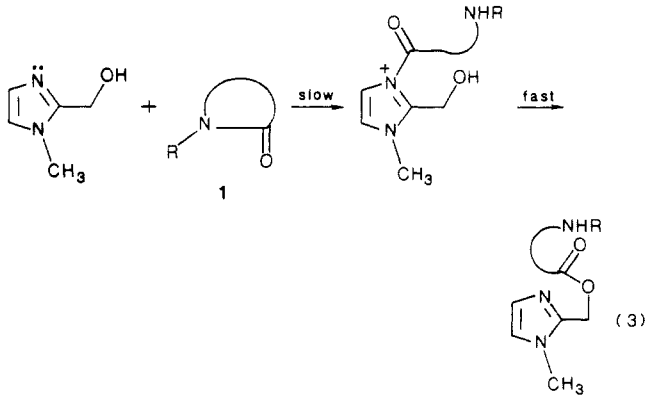
[a] T = 25.0 °C, μ = 0.2 M KCl). k_2^{obsd} values determined from plots of k_{obsd} vs. [2-4] at 0.02–0.15 M. [b] Titrated at μ = 0.2 M (KCl), T = 25 °C unless otherwise stated. [c] From fits of k_2^{obsd} vs. [H^+] according to eq 2. Errors from least-squares fit. [d] Haberfield, P.; Pessin, J. *J. Am. Chem. Soc.* **1982**, *104*, 6191. [e] Extrapolated to 100% free base using pK_a 12.8. [f] Eiki, T.; Kawada, S.; Matsushima, K.; Mori, M.; Tagaki, W. *Chem. Lett.* **1980**, 997. [g] Determined in D_2O, pD = 8.50, T = 25 °C, μ = 0.2 M (KCl). [h] Reference 19. [i] Quoted in: Grimmett, M. R. *Comprehensive Heterocyclic Chemistry*; Pergamon: Elmsford, NY, 1984; Vol. 4, p 384.

7 and 8, supplementary material) can be fit to the expression given in eq 2 by nonlinear least-squares treatment

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

(solid lines), which yields the kinetic pK_a and k_2^{max} values given in Table IV. The 2-(hydroxymethyl)imidazoles are somewhat more reactive than their pK_a values suggest since their log k_2^{max} values (Table IV) lie above the Brønsted line defined by the β-hydroxy amines in Table III. However, **3a** and **4a** are structurally different from, and have fewer degrees of rotational freedom than, the others. Perhaps this enforces a greater degree of N and OH association since rotation about the C_2–CH_2OH single bond places the OH group in H-bonding proximity to one or the other tautomerically equivalent N.

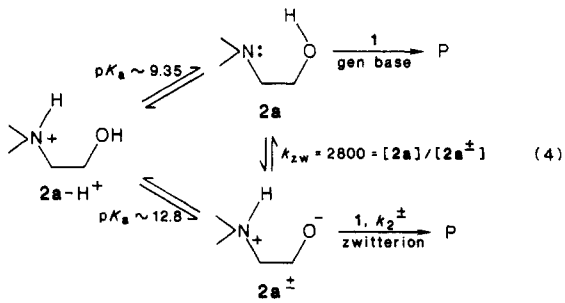
Products isolated from the kinetic media containing **1** and **2a** or **4a** are shown by spectral comparison (IR, ^1H NMR, exact mass) to be identical with the expected esters. This finding is a necessary consequence of oxygen attack but does not necessarily rule out N attack followed by rapid *N*- to *O*-acyl transfer as in eq 3.



(3)

However, that eventuality is ruled out by two pieces of evidence: First, the solvent kinetic isotope effects (k_{H_2O}/k_{D_2O}) of 1.79 and 1.53, while somewhat lower[20] than the normal values of 2–3 expected for general-base-catalyzed acyl transfer to oxygen, are significant and consistent with that process. A variety of general-base-catalyzed processes such as the aminolysis of phenyl acetate by glycine also show small solvent isotope effects.[20] Nucleophilic attack of imidazole N on such species as *p*-nitrophenyl acetate generally proceeds with kinetic solvent isotope effects of 1.0–1.1.[21] More definitively, the k_2^{max} values for attack of both **3a** and **4a** on **1** exceed that of **3b** by 30-fold and **4b** by an appreciably larger factor.[22] Since N nucleophilicity on acylating agents generally follows a Brønsted relationship (β = 0.8 for amines on *p*-NPA[3a,b,l,23]), the more basic amines **4b** and **3b** would be better N nucleophiles than **3a** or **4a** if such attack on **1** were important.

There remains a possibility that the O-acylation proceeds in part or predominantly via the zwitterionic form as shown in eq 4 for **2a** and that the relatively low solvent



(4)

isotope effect is a reflection of this. We have shown in the case of imidazolylmethyl thiols analogous to **3a** and **4a** that the dominant nucleophilic form in the attack on pNPA at neutrality is zwitterionic.[24] This possibility has been favored for the attack of ethanolamine or quinuclidinol on acetylimidazole[5] and has been considered and evaluated for the reaction of some hydroxy amines on pNPA.[3b,c,l] Given the amine pK_a in **2a**-H^+ of 9.35 and a corresponding OH pK_a equivalent to that of choline (12.8[25]), it can be calculated that an upper limit for the ratio of **2a**/**2a**$^\pm$ = 2800. Now, if the $k_2^\pm$ rate constant for the attack of the zwitterionic form of **2a** on **1** can be approximated by that for choline (85 M^{-1} s^{-1}; Table IV), its contribution to k_2^{max} would be (85 M^{-1} s^{-1} × 1/2800) = 0.03 M^{-1} s^{-1}. The value is probably inaccurate given the assumptions, but nevertheless it is not clear that the involvement of **2a**$^\pm$ can be ruled out. It seems reasonable that the reduced pK_a of the imidazolium units in **3a** and **4a** renders the involvement of zwitterionic forms less likely, and therefore we favor a general-base pathway. Nevertheless, despite the mechanistic ambiguity, it is important to note that the involvement of either neutral species (>N̈〰OH or >N^+H〰O^-) requires the intramolecular juxtaposition of the two groups.

(20) (a) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 243–281. (b) Jencks, W. P.; Carriulo, J. *J. Am. Chem. Soc.* **1960**, *82*, 675. (c) Bruice, T. C.; Benkovic, S. J. *Ibid* **1964**, *86*, 418.

(21) Bender, M. *Chem. Rev.* **1960**, *60*, 53.

(22) Since the attack of up to 0.1 M **4b** produces no appreciable acceleration of the decomposition of **1** over the spontaneous hydrolysis at pH 8.3 (k_{obsd} = 1.4 × 10^{-4} s^{-1}) and an acceleration of 10% would have been detectable, we calculate that k_2^{max} for **4b** on **1** would be no more than 1.4 × 10^{-5} s^{-1}/0.1 M = 1.4 × 10^{-4} M^{-1} s^{-1}.

(23) Bruice, T. C.; Japinski, R. *J. Am. Chem. Soc.* **1958**, *80*, 2265.

(24) Street, J. P.; Skorey, K. I.; Brown, R. S.; Ball, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 7669. The pK_a values for 2-methylimidazole and *N*,2-dimethylimidazole were inadvertently interchanged in the above report as were their k_2^{max} values for attack on pNPA. The correct pK_a and k_2^{max} values are reported in Table 8, this report (supplementary material).

Table V. Second-Order Rate Constants and Kinetic pK_a Values for the Attack of 3-4 on pNPA[a]

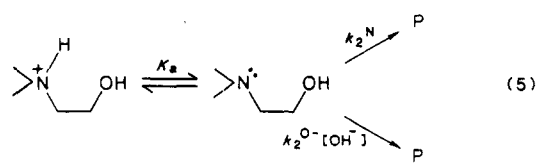
compd	pK_a[b]	k_2^{max},[b] M^{-1} s^{-1}
2-(hydroxymethyl)imidazole (**3a**)	6.76 ± 0.02	$(3.17 \times 0.1) \times 10^{-2}$ $(3.36 \pm 0.2) \times 10^{-2}$ [d]
2-methylimidazole[c] (**3b**)	7.64	$(2.2 \pm 0.2) \times 10^{-1}$ $(2.1 \pm 0.2) \times 10^{-1}$ [e]
2-(hydroxymethyl)-*N*-methylimidazole (**4a**)	7.25 ± 0.05	$(6.04 \pm 0.2) \times 10^{-3}$ $(6.38 \pm 0.1) \times 10^{-3}$ [d]
N,2-dimethylimidazole[c] (**4b**)	7.94	$(1.3 \pm 0.3) \times 10^{-1}$ $(1.2 \pm 0.2) \times 10^{-1}$ [e]

[a] T = 37.0 °C, μ = 0.3 M KCl. [b] Values from nonlinear least-squares fits of primary k_2^{obsd} data vs. [H^+] to eq 2. Errors are standard deviations of parameters. [c] From ref 24. [d] Values in D_2O, 37 °C, pD = 8.95. [e] Values in D_2O, 37 °C, pD = 10.0.

It was of interest to determine the situation with the more widely studied acylating agent pNPA. Shown in Figure 4 is the plot of log k_2^{obsd} vs. pH for **3** and **4** at 37.0 °C [μ = 0.3 M (KCl)]. These reactions also adhere to the expression given in eq 2, and the derived rate constants and pK_a values from nonlinear least-squares fitting of the data (Table 9, supplementary material) are given in Table V. Also shown in Figure 4 are the data for the attack of **2a** on pNPA at T = 25 °C, μ = 1.0 M (KCl) as well as the curve calculated for *N*,*N*-(dimethylmethoxy)ethylamine on the basis of Khan's report[31] [k_2^{max} = 7.2×10^{-3} M^{-1} s^{-1}, pK_a 9.31, μ = 1.0 M (KCl)].

The imidazole alcohols are *less* reactive toward pNPA than their comparison imidazoles. Indeed, the data are most consistent with a rate-limiting N attack, the reduced values for **3a** and **4a** relative to **3b** and **4b** being a consequence of the lower N pK_a values for the former imidazole alcohols.[25] Consistent with this are the k_{H_2O}/k_{D_2O} solvent isotope effects of 0.94 ± 0.15 and 0.95 ± 0.05 for **3a** and **4a**, respectively. Nevertheless, analysis (see the Experimental Section) shows that the products of the reaction conducted in CD_3OD consist of some ester, as well as CD_3OCOCH_3 + *p*-nitrophenolate. This is consistent with N nucleophilic attack to yield the *N*-acylimidazole, which undergoes both intra- and intermolecular N → O acyl transfer, the latter being to solvent. Controls establish that pNPA in CD_3OD does not solvolyze under the reaction conditions nor does the imidazole ester. Thus, the observation of O-acylated material in the absence of significant acceleration of the reaction cannot be used as a sufficient criterion for N,O-cooperativity.[4]

The appearance of the curve in Figure 4 for the reaction of **2a** with pNPA is also consistent with the attack of the neutral amine being through N. However, since the profile does not tend to plateau above the pK_a of 9.35, it is clear that an [OH^-]-dependent term is also important. The likely process involves the attack of alcoholate anion on pNPA as has been observed with other alcohols.[3b,26,27] Analysis of the data in terms of the process in eq 5 with



(5)

$$k_2^{obsd} = (k_2^N + k_2^{O^-}[OH^-])[K_a/(K_a + [H^+])] \quad (5a)$$

(25) Haberfield, P.; Pessin, J. *J. Am. Chem. Soc.* **1982**, *104*, 6191.
(26) (a) It is generally observed that N-nucleophiles reacting with pNPA adhere to a Brønsted relationship having β = 0.8.[1c,3] (b) For a more complete discussion of this point see: Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1968**, *90*, 2622.
(27) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1962**, *94*, 2910.

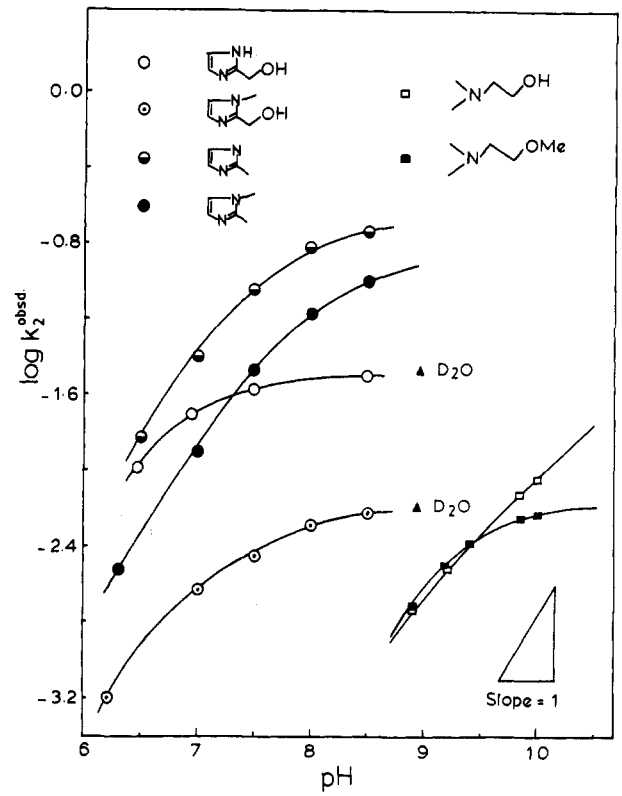


Figure 4. log k_2^{obsd} vs. pH for the attack of **2a**, **3a**,**b**, and **4a**,**b** on pNPA. For **2a** and *N*,*N*-(dimethylmethoxy)ethylamine (data from Khan's study[31]) T = 25 °C, μ = 1.0 M (KCl). For imidazoles, T = 37 °C, μ = 0.3 M (KCl); data for **3b** and **4b** from ref 24. Filled triangles indicate log k_2^{obsd} for **3a** and **4a** in D_2O, pD = 8.95.

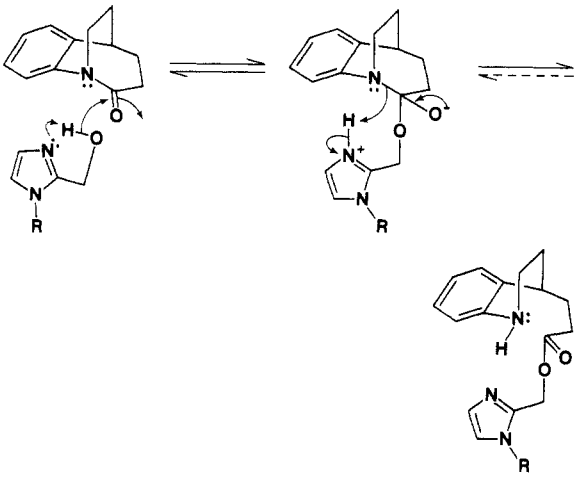
the derived expression for k_2^{obsd} in eq 5a yields k_2^N = 6.6 × 10^{-3} M^{-1} s^{-1} and $k_2^{O^-}$ = 47 M^{-2} s^{-1} (Table 9, supplementary material). The derived value for k_2^N is remarkably close to that reported for *N*,*N*-(dimethylmethoxy)-ethylamine (k_2 = 7.2×10^{-3} M^{-1} s^{-1}),[31] which has a similar pK_a (9.31)[31] and does not provide evidence for kinetically beneficial cooperativity between N and OH in **2a** in the attack on pNPA. The above conclusion is in apparent contradiction with that of Werber and Shalitin[3b] and Khan[31] who favored N,O-cooperativity in acylation of some amino alcohols by pNPA. In those studies, the difference in reactivity between the amino alcohols and tertiary amine[3b] or methoxyamine[31] controls was also small, similar to what is observed here. Apparently the strongest case for significant N,O-cooperativity in O-acetylation from esters is that presented by Hine and Khan.[3c] They observed that *o*-[[(N,N-dimethylamino)methyl]benzyl alcohol reacted with pNPA at least 50-fold faster than does the methyl ester and at least 160-fold faster than *N*,*N*-dimethylbenzylamine.

With the exception of this study, reports of N,O-cooperativity during O-acylation by amides are conspicuously sparce,[3m,5] the effects being nonexistent[3m] or small (~5-fold).[5]

The contrasting behavior of these amino alcohols with **1** and the more common acylating agent pNPA merits some comment. One might have envisioned that intramolecular cooperativity would have facilitated the O-acylation by either acylating agent, and yet with pNPA undoubtedly N-acylation is the faster process. Two factors may be considered: First, N attack on the C=O unit of **1** could be inhibited by steric compression in the transition state, which would be more severe than with pNPA. Consistent with this is the ordering of rate constants for nucleophilic attack of OH^-, imidazole, and 2-methyl-

imidazole with 1 which is 60, 1.92×10^{-2}, and 9.53×10^{-4} M^{-1} s^{-1}, respectively while for pNPA the ordering is 15,[28a] 3.4×10^{-1}, and 4.5×10^{-2} M^{-1} s^{-1},[28b,c] respectively. Thus, the more sterically encumbered nucleophile, 2-methylimidazole is 50-fold less nucleophilic toward 1 than pNPA, while the smaller nucleophile OH^- is 4-fold more nucleophilic. Presumably, O attack on 1 would not be subject to as severe steric encumbrance as would N attack. On the other hand in the case of reaction of the amino alcohols with the less sterically encumbered C=O unit in pNPA, the N when neutral is inherently more nucleophilic than OH and becomes acylated.

An alternative explanation similar to that invoked by Tonellato[3m] utilizing the relative leaving group abilities from tetrahedral intermediates produced from attack on esters such as pNPA with good leaving groups or activated amides with poorer leaving groups can be advanced. Our previous studies[6,29] have shown that whereas attack of the strongly nucleophilic OH^- on 1 proceeds irreversibly, attack of a weaker nucleophile H_2O on the neutral amide or 1-H^+ proceeds reversibly. There remains a possibility that with the amino alcohols studied here, neither nucleophilic attack nor breakdown of the tetrahedral addition intermediate is entirely rate limiting. Hence in the case of nucleophiles that are also good leaving groups (i.e., imidazole), the reversal of addition could be prominent and the overall rate of reaction by that pathway slow. It may be envisioned as in eq 6 that the breakdown of the tetrahedral intermediate formed from O attack is facilitated by intramolecular general-acid catalysis by the pendant protonated



(6)

amine. While the proposal remains speculative in this case, it is interesting that the accepted mechanism of acylation of the serine group in chymotrypsin involves an analogous process.

Acknowledgment. We gratefully acknowledge the financial support of the Natural Sciences and Engineering Research Council of Canada and University of Alberta.

Registry No. 1, 102586-88-9; **2a**, 108-01-0; **2l**, 67-48-1; **3oa**, 3724-26-3; **3a** (acetate), 45815-85-8; **3b**, 693-98-1; **4a**, 17334-08-6; **4a** (acetate), 104876-20-2; **4b**, 1739-84-0; TRICENE, 5704-04-1; TRIS, 77-86-1; HEPES, 7365-45-9; BISTRIS, 6976-37-0; *p*-NPA, 830-03-5; (*N*,*N*-dimethylamino)ethyl 1,2,3,4-tetrahydroquinoline-4-propanoate, 104876-18-8; (*N*-methyl-2-imidazolyl)methyl 1,2,3,4-tetrahydroquinoline-4-propanoate, 104876-19-9.

Supplementary Material Available: Listings of anisotropic and isotropic thermal parameters (Table 1), positional parameters for hydrogen and non-hydrogen atoms (Tables 3 and 4), bond distances (Table 5), bond angles (Table 6), and observed second-order rate constants (Tables 7-9), and ORTEP view of amide 1 (9 pages); listings of observed and calculated structure factors (12 pages). Ordering information is given on any current masthead page.

(28) (a) Menger, F. M.; Portnoy, C. E. *J. Am. Chem. Soc.* **1968**, *90*, 1875. (b) Bender, M. L.; Turnquist, B. W. *Ibid.* **1957**, *79*, 1652. Values for nucleophilic attack on pNPA at 25 °C.

(29) Šlebocka-Tilk, H.; Somayaji, V.; Brown, R. S., submitted for publication.

(30) **Noted Added in Proof**: After the acceptance of this manuscript, a paper appeared reporting torsional angles of a coordinated amide comparable to those reported here: Collins, T. J.; Coots, R. J.; Furutani, T. T.; Keech, J. T.; Peake, G. T.; Santarsiero, B. D. *J. Am. Chem. Soc.* **1986**, *108*, 5333.

Linear Solvation Energy Relationships. 39. A Double-Difference Method for Estimating Electrophilic Solvent Assistance Effects in Solvolysis Reactions

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Solvent effects on solvolysis rates may be expressed by a linear solvation energy relationship that combines terms that measure solvent dipolarity/polarizability (SDP) effects, electrophilic solvent assistance (ESA), nucleophilic solvent assistance (NSA), and solvent electrostrictive effects (CAV = a cavity term). A method of double differences is presented, by which an estimate of ESA can be obtained for systems in which there is no NSA. The method requires that the rates of reaction be measured in four solvents—methanol, ethanol, trifluoroethanol, and hexafluoro-2-propanol.

During the course of the past 30 years, many investigations in physical organic chemistry have dealt with mechanisms of solvolysis reactions, and numerous schemes have been devised for sorting out the contributions of nucleophilic solvent assistance (NSA) and "solvent ionizing power" (SIP) to solvolysis reaction rates. SIP has long been recognized[1] as including contributions of solvent

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