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Rapid Cleavage of Unactivated, Unstrained Amide Bonds at Neutral pH

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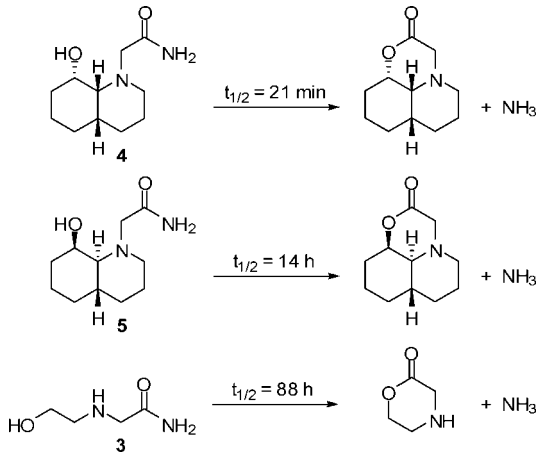
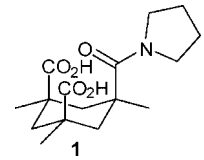
Received March 29, 2008



Building upon the discovery of Suggs and Pires that *N*-(2-hydroxyethyl)glycine amides undergo rapid amide cleavage under mild conditions [Suggs, J. W.; Pires, R. M. *Tetrahedron Lett.* **1997**, *38*, 2227–2230], we synthesized the derivatives (4aα,8*β*,8aα)-1-ethylamido-8-hydroxydecahydroquinoline (**4**) and (4aα,8α,8a*β*)-1-ethylamido-8-hydroxydecahydroquinoline (**5**). These two species are conformationally constrained, but steric compression is not introduced between the hydroxyl group and the amide functionality it attacks. At 20 °C and slightly basic pH, derivatives **4** and **5** undergo amide cleavage with half-lives of 21 min and 14 h, respectively, which correspond to rate increases of 251- and 6.3-fold relative to the acyclic analogue *N*-(2-hydroxyethyl)glycine amide (**3**).

At neutral pH and 25 °C, the half-life for peptide bond hydrolysis is approximately 500 years.[1] In the active site of a protease, however, the half-life can be well under 1 s, and the rate acceleration for carboxypeptidases A and B has been calculated to be 5×10^{11}- and 1.3×10^{13}-fold, respectively.[1a,2] We have been interested in the design of artificial proteases and have recently been exploring small-molecule model systems involving rapid amide bond hydrolysis. We were originally drawn to a 1988 report from Menger and Ladika[3] on the pyrrolidyl amide derivative of Kemp's triacid (**1**), which undergoes amide cleavage at pD 7.05 and 25 °C with a half-life of 8 min.



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This rapid rate was initially attributed to "sustained proximity" of the carboxylic acid and amide functionalites,[3] but subsequent work revealed that a large fraction of the rate acceleration is due to pseudoallylic (pseudo $A^{1,3}$) strain between the pyrrolidine ring and adjacent methyl group.[4] (In general, intramolecular systems that react with exceptionally fast rates involve relief of steric strain.[5]) An artificial protease based upon the Kemp's triacid system would therefore require that the peptide substrate be strained upon binding.

Our current focus has thus been on unstrained intramolecular systems in which simple juxtaposition of the amide bond and catalytic functionality leads to large rate accelerations. Of course, this is not a new idea. In 1962, Westheimer suggested[6] that enzymes can act as "entropy traps." In 1971, Page and Jencks[7] addressed the proposition, "We would like to know the nature and magnitude of the maximum increase in rate that may be brought about by bringing together two properly orientated reactants in the active site of an enzyme without invoking strain or desolvation." Naturally occurring enzymes utilize a combination of catalytic strategies, but an artificial protease that functions simply by binding the peptide substrate in juxtaposition with a catalytic functionality would seem to be the most straightforward to create. Although Bruice and Lightstone[8] have recently emphasized that the positioning of reacting groups can also have considerable *enthalpic* costs, once a catalyst has used its binding energy to juxtapose the reacting groups, it would then simply "watch" as the reaction proceeded.

In 1997, Suggs and Pires[9] reported that *N*-(2-hydroxyethyl)glycine amides cleave remarkably quickly. *N*-(Bis-2-hydroxyethyl)glycine amide (**2**) cleaves with a half-life of 3 h at pD

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10.1021/jo800706y CCC: $40.75 © 2008 American Chemical Society
Published on Web 07/10/2008

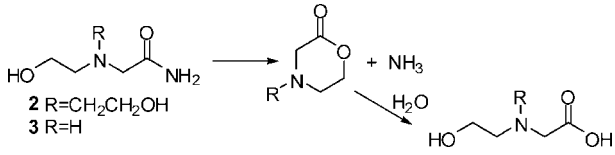


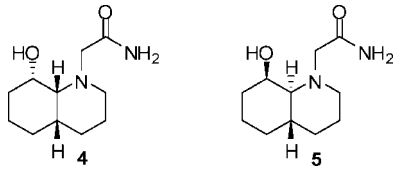
FIGURE 1. Hydrolysis of *N*-hydroxyethylamides.

7.0 at 25 °C, while derivative **3**, with just a single hydroxyethyl group, cleaves 29 times more slowly.

As far as we are aware, the 3 h half-life for glycine amide **2** is the fastest ever observed for an unstrained amide at neutral pH. [This rapid cleavage of *N*-(bis-2-hydroxyethyl)glycine amide derivatives has recently been employed in the development of amine-containing prodrugs.[10]]

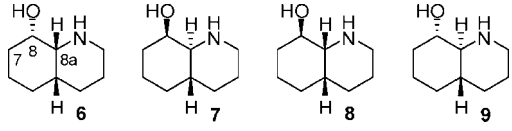
In accord with earlier work on the hydrolysis of hydroxyamides,[11] the above reactions proceed via the lactone, which then hydrolyzes to yield the corresponding glycine derivative. The glycine amine plays a key role in the mechanism (γ-hydroxybutyramide and δ-hydroxyvaleramide cleave orders of magnitude more slowly[11]), and Suggs and Pines proposed that the attacking hydroxyl group is activated through hydrogen bonding to this central nitrogen.

Given that derivatives **2** and **3** are conformationally quite flexible, we saw the opportunity to design more reactive analogues and report here the syntheses and rates of amide cleavage of (4aα,8β,8aα)-1-ethylamido-8-hydroxydecahydroquinoline (**4**) and (4aα,8α,8aβ)-1-ethylamido-8-hydroxydecahydroquinoline (**5**).



In these derivatives, two internal rotations present in the *N*-(hydroxyethyl)glycine amide parent are "frozen out"—however, the amide functionality is not strained nor is steric compression between it and the hydroxyl group introduced.

To synthesize derivatives **4** and **5**, we began with the corresponding 8-hydroxydecahydroquinoline derivatives **6** and **7**. As reported previously by one of us (F.F.),[12] the reduction of 8-hydroxyquinoline with Rh/Al_2O_3 in methanol yields a diastereomeric mixture of hydroxydecahydroquinolines **6**−**9** (each as a racemic mixture).



Isomers **6** and **7** are the predominate reduction products, and each was purified by chromatography on silica gel eluting with 78/20/2 dichloromethane/methanol/ammonia; the R_f for **6** is 0.3 and that for **7** is 0.2. The minor diastereomers **8** and **9** elute between the two predominant isomers ($R_f \approx 0.28$ for both), and a pure sample of only the later eluting isomer could be obtained; neither minor isomer was elaborated further to the corresponding 1-ethylamido derivative.

TABLE 1. Expected Multiplicities of H_8 in Diastereomers 6−9

diastereomer	dihedral angles of H_8 to the three vicinal hydrogens	hydroxyl group position	expected multiplicity
6 (principal conformation)	52° (H_{8a}), 55° (*cis*-H_7), 173° (*trans*-H_7)	equatorial	doublet of triplets
7	60° (H_{8a}), 57° (*cis*-H_7), 60° (*trans*-H_7)	axial	quartet (small *J*)
8 (major conformation)	175° (H_{8a}), 55° (*cis*-H_7), 173° (*trans*-H_7)	equatorial	triplet of doublets
8 (minor conformation)	63° (H_{8a}), 57° (*cis*-H_7), 60° (*trans*-H_7)	axial	quartet (small *J*)
9	176° (H_{8a}), 56° (*cis*-H_7), 174° (*trans*-H_7)	equatorial	triplet of doublets

TABLE 2. Observed Multiplicities of H_8 in Diastereomers 6−9

diastereomer	chemical shift of H_8 (ppm)	observed multiplicity	assignment
major ($R_f = 0.3$)	3.60	doublet of triplets ($J = 11.6$, 4.5 Hz)	diastereomer **6**
minor (first to elute)	3.33	triplet of doublets ($J = 9.8$, 4.7 Hz)	diastereomer **8** or **9**
minor (second to elute)	3.38	broad multiplet	diastereomer **8** or **9**
major ($R_f = 0.2$)	3.88	quartet ($J = 2.5$ Hz)	diastereomer **7**

Following the approach described by Solladié-Cavallo and co-workers for the analogous 1-hydroxydecalin diasteromers,[13] the structures of **6** and **7** were unambiguously assigned on the basis of the ^1H NMR multiplicity of the proton on the carbon bearing the hydroxyl group (C_8). Here, we designate this proton H_8. To begin, the energetics and geometries of the four hydroxydecahydroquinoline diastereomers were analyzed by molecular mechanics, and the dihedral angles between H_8 and the three protons vicinal to it—H_{8a}, H_7 cis to H_8, and H_7 trans to H_8—were measured (Table 1). Of the two chair−chair conformations that can be adopted by *cis*-hydroxydecahydroquinoline **6**, that with the hydroxyl group equatorial is calculated to be more stable by over 3 kcal/mol (in the minor conformer, the hydroxyl group is involved in costly synaxial interactions[14]). Based upon the dihedral angles shown in Table 1, the multiplicity expected[13,15] for proton H_8 in this principal chair−chair conformation is a doublet of triplets.

The *trans*-hydroxydecahydroquinoline derivative **7** can adopt only a single chair−chair conformation, for which the multiplicity of H_8 is predicted to be quartet with a small coupling constant. The two chair−chair conformations of cis diastereomer **8** are of similar energy, but that with the hydroxyl group equatorial is calculated to be more stable by 0.3 kcal/mol. The dihedral angles and expected multiplicities for both conformations of **8** are listed in Table 1. Finally, the multiplicity for H_8 in the chair−chair conformation of trans isomer **9** is predicted to be a triplet of doublets.

The experimentally observed ^1H NMR multiplicities for H_8 in each of the four isomers **6**−**9** are given in Table 2.

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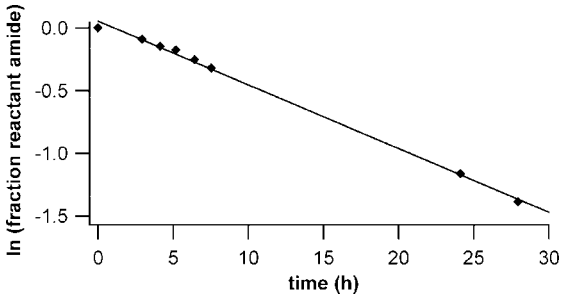

FIGURE 2. Plot of ln (fraction reactant amide) vs time for the cleavage of amide **5** at pD 7.5.

As indicated by the data in Tables 1 and 2, the structures of the major isomers **6** and **7** can be unambiguously assigned: The multiplicity of H_8 in diastereomer **6** is expected to be a doublet of triplets, as is uniquely observed for the major isomer with the R_f of 0.3; the multiplicity of H_8 is diastereomer **7** is expected to be a quartet with a small coupling constant, as is uniquely observed for the major isomer with the R_f of 0.2.

The observed multiplicities for H_8 in the two minor isomers do not allow for unambiguous assignment of these diastereomers. The expected multiplicity of H_8 in both **8** and **9** is a triplet of doublets (with the magnitudes of the coupling constants for the cis isomer **8** dependent upon the precise ratio of the two rapidly interconverting chair–chair conformations). Experimentally, the first minor isomer to elute from the silica gel column shows a triplet of doublets for H_8; the second minor isomer shows a broad multiplet, the appearance of which is also consistent with a triplet of doublets. (Expansions of the H_8 multiplets for all four isomers are provided in the Supporting Information. The multiplet shown for the earlier eluting minor isomer was obtained from a spectrum of a mixed fraction also containing major isomer **6**.)

The ethylamido derivatives **4** and **5** were synthesized using a modification of a published procedure.[16] Each of the purified 8-hydroxydecahydroquinolines **6** and **7** was treated with 2-bromoacetamide and sodium carbonate in DMSO-d_6. A slight excess of **6** or **7** was used, and the reactions were monitored by ^{1}H NMR until the 2-bromoacetamide was completely consumed. *N*-(bis-2-hydroxyethyl)glycine amide (**2**) was prepared from diethanolamine in a similar fashion.

To measure the rate of amide cleavage, a small aliquot of the above reaction mixtures was added directly to buffered D_2O, and the disappearance of starting amide was measured by ^{1}H NMR. For **2**, the reaction rate was determined at pD 3.5, 5.5, 7.8, and 10 and for **4** and **5** at pD 2.5, 3.8, 5.4, and 7.5 (the insolubility of these two derivatives under basic conditions precluded studying the reaction at pD > 8). The kinetic data obtained at pD 7.5 for derivative **5** are plotted in Figure 2.

The pD-rate data for the amide derivatives **2**, **4**, and **5** may be described by the following equation

$$k_{obs}=k_a[D_3O^+]/(K_a+[D_3O^+])+k_bK_a/(K_a+[D_3O^+]) \quad (1)$$

where k_{obs} is the experimentally measured first-order rate constant for amide cleavage, k_a the amide-cleavage rate constant when the amine functionality is in its conjugate acid form, k_b the amide-cleavage rate constant when the amine functionality is in its conjugate base form, and K_a the apparent acid dissociation constant for the amine (Figure 3).

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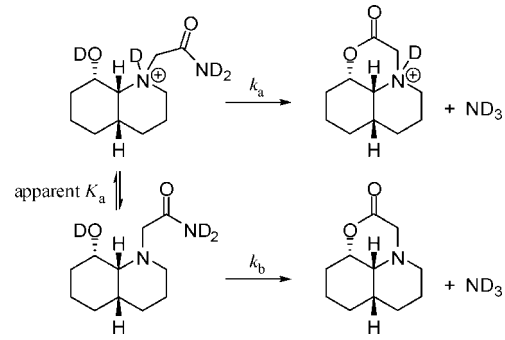

FIGURE 3. Schematic of the kinetic parameters k_a, k_b, and apparent K_a illustrated for the reaction of ethylamido derivative **4**.

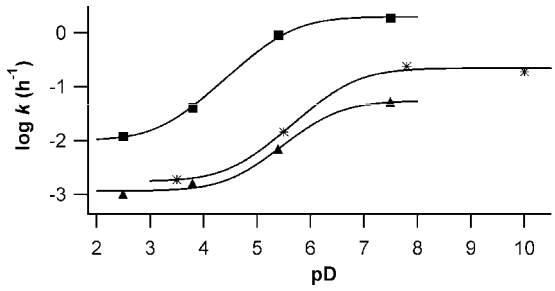

FIGURE 4. Plot of log k vs pD for amides **2** (∗), **4** (■), and **5** (▲).

TABLE 3. Kinetic Data for Amides 2, 4, and 5

compd	k_a (h^{-1})	k_b (h^{-1})	app K_a (in D_2O)	app pK_a (in D_2O)
2	1.76 (±0.41) × 10^{-3}	0.22 (±0.03)	1.99 (±0.63) × 10^{-6}	5.7
4	1.00 (±0.16) × 10^{-2}	2.00 (±0.25)	2.78 (±0.61) × 10^{-6}	5.6
5	1.14 (±0.20) × 10^{-3}	0.05 (±0.01)	6.08 (±2.62) × 10^{-7}	6.2

The k_a, k_b and K_a values determined from a nonlinear Levenberg−Marquardt fit of the observed pD-rate data are listed in Table 2. The fitted pD−rate profiles for **2**, **4**, and **5** are shown in Figure 4. As the results Table 2 and Figure 4 indicate, the amide-cleavage reaction proceeds ∼100 times more rapidly when the amine functionality is in its conjugate base form, a result confirming that this group acts as a general base,[9] even when incorporated into the hydroxydecahydroquinoline ring system. [Note, however, that a constrained *N*-(bis-2-hydroxyethyl)-2-piperidinone derivative does not undergo rapid hydrolysis.[9]] Since the apparent pK_a of the amine functionality is ∼6 in **2**, **4**, and **5**, close to the maximal rate of amide cleavage occurs at neutrality.

Unlike bis(hydroxyethyl)glycine (**2**), the 1-ethylamidohydroxydecahydroquinoline derivatives **4** and **5** have only a single hydroxyl group, and thus, *N*-(2-hydroxyethyl)glycine (**3**) provides a better benchmark for the rate increase due to freezing out rotations. As noted above, Suggs and Pires determined that this derivative cleaves 29 times more slowly than the bis(2-hydroxyethyl) derivative **2**. The relative rates of amide cleavage for derivatives **3** (at pD 7.0 and 25 °C[9]), **4**, and **5** are listed in Table 4.

TABLE 4. Relative Rates of Amide Cleavage in Derivatives 3−5

compd	k_b (h^{-1})	$k_{relative}$	$t_{1/2}$
3[9]	0.008	1	88 h
4	2.00 (±0.25)	251	21 min
5	0.05 (±0.01)	6.3	14 h

Evident from these data is the fact that derivative **4** undergoes amide cleavage far more rapidly than does **5**. Consideration of

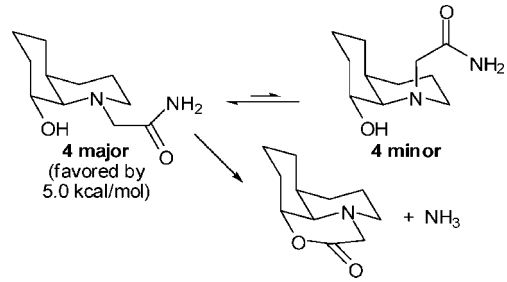

FIGURE 5. Amide cleavage in hydroxydecahydroquinoline **4**.

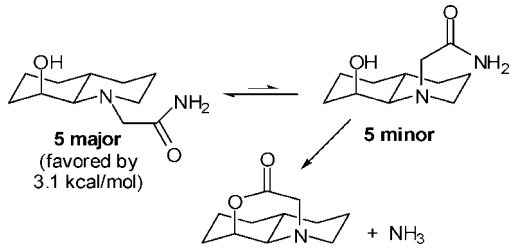

FIGURE 6. Amide cleavage in hydroxydecahydroquinoline **5**.

the conformational preferences of the ethylamido group in these two species provides an explanation. In hydroxydecahydroquinolines **4** and **5**, the ethylamido group, not surprisingly, prefers to be in the equatorial position (favored over axial by 5.0 kcal/mol in **4** and 3.1 kcal/mol in **5** as determined by molecular mechanics calculations). However, as indicated in Figures 5 and 6, when in the equatorial position, the ethylamido group can undergo intramolecular nucleophilic attack by the 8-hydroxyl group only in **4**. In derivative **5**, the ethylamido group must first, at an energetic cost, invert to the axial position.

Finally, as originally noted by Suggs and Pires,[9] the mechanism for amide cleavage in the above derivatives parallels that of the enzyme penicillin acylase, in which the hydroxyl group of N-terminal serine B1 acts serves as a nucleophile activated by the N-terminal amino group itself.[17] The enzyme active site also contains an oxyanion hole akin to that in serine proteases. The acylase has a k_{cat} of 50 s^{-1} at pH 7.5 and 25 °C for the hydrolysis of the side-chain amide bond in benzylpenicillin,[18] a rate acceleration of 10^{12}-fold relative to uncatalyzed peptide-bond hydrolysis under the same conditions.[1] However, the rate of amide cleavage in ethylamido derivative **4** is only 10^5 times slower than that achieved by the acylase, which suggests that a large fraction of the enzymic rate acceleration is due to simple juxtaposition of the catalytic serine residue with the amide bond to be cleaved (especially since the acylase·substrate complex does not have the free internal rotations still present in derivative **4**). An artificial enzyme designed to position a β-hydroxyamino group and a peptide substrate in an arrangement similar to that in penicillin acylase might thus be an excellent protease.

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Experimental Section

General Procedure for the Synthesis of Compounds 4 and 5. 2-Bromoacetamide (6.3 mg, 0.046 mmol) and sodium carbonate (10 mg, 0.092 mmol) were added to an NMR tube. The respective 8-hydroxydecahydroquinolinol **6** or **7** (10 mg, 0.064 mmol) was then added, followed by DMSO-d_6 (0.5 mL). The reaction was allowed to proceed at room temperature with periodic shaking. The 2-bromoacetamide was completely consumed in approximately 48 h to yield ethylamido derivatives **4** or **5**. The reaction mixture, which contained a small amount of starting **6** or **7**, was then used directly for the kinetic measurements.

Kinetic Measurements. To 50 μL of the above solution of **4** or **5** was added 0.1 M buffer solution in D_2O (0.5 mL). Phosphate was used for pD 2.5, 7.5, and 7.8; pyrophosphate for pD 10; and mellitic acid for pD 3.5, 3.8, 5.4, and 5.5. The ionic strength for all buffers was adjusted to 1 using sodium chloride. The samples were maintained at 20(±1) °C in a thermostated room. Narrow-range pH paper was used to measure the pD of each solution—the pD was taken as the "pH" indicated plus 0.5, the correction factor for well-behaved buffers.[19] The rate constants were determined by integrating resolved signals of the starting material relative to the product. For some reactions, the starting amide was integrated relative to the signal for the residual protons in the DMSO-d_6 solvent present. Data analysis and curve fitting were performed using IGOR Pro, Version 4.0 for Windows (WaveMetrics, Inc.).

Acknowledgment. We thank the Howard Hughes Medical Institute for support of M.R. (through an Undergraduate Biological Sciences Education Program award to Amherst College). Mass spectral data were obtained at the University of Massachusetts Mass Spectrometry Facility, which is supported in part by the National Science Foundation. Finally, we are grateful to the reviewers for their extremely helpful comments.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **4** and **5**; ^{1}H NMR spectrum of the mixture of diastereomers **6**−**9**obtained from the reduction of 8-hydroxyquinoline; expansions of the ^{1}H NMR multiplets for H_8 in compounds **6**−**9**; ^{1}H NMR, ^{13}C NMR, and IR spectra of compounds **6** and **7**; a tabulation of the spectral data, including HRMS, for compounds **4**−**7**; a table of the rate constants for amide cleavage at each pD for compounds **2**, **4**, and **5**; and the details of the molecular mechanics calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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