precipitated by the addition of 2 g of $NaClO_4 \cdot H_2O$. Yield 230 mg, a further crop of 42 mg was obtained by the addition of ethanol. NMR (Me_2SO), δ 1.87 (s, 3 H), 3.95 (br, 3 H), 4.3 (br, 12 H), 5.2 (br, 1 H); (D_2O, in basic solution) δ 2.05 (s); (0.5 M DCl) δ 2.36 (s); IR 1585 cm^{-1}. Anal. Calcd for $C_2H_{19}N_6Cl_2IrO_9$: C, 4.50; H, 3.58; N, 15.73. Found: C, 4.7; H, 3.4; N, 14.9.

$[(NH_3)_5IrOOCH](ClO_4)_2$. A suspension of 200 mg of the DMF complex in 2 mL of 2M NaOH was swirled for 10 min at ambient temperature. A solution of 1g of $NaClO_4 \cdot H_2O$ in 1 mL of water was added, the mixture cooled, and white crystals of the product were filtered: yield of 0.16 g (100%); NMR (Me_2SO) δ 4.2-4.7 (br, 15 H), 7.70 (s, 1 H); IR 1645 cm^{-1}. Anal. Calcd for $CH_{16}N_5Cl_2IrO_{10}$: C, 2.30; H, 3.09; N, 13.44. Found: C, 2.7; H, 3.2; N, 13.4.

$[(NH_3[_5RhOOCH](ClO_4)_2$. This was prepared in the same manner as the above compound in quantitative yield. NMR Me_2SO δ 3.6 (br), 3.8 (br), 7.91 (d, 2.5 Hz); IR 1630 cm^{-1}; UV λ 266 (106), 321 (142) nm. Anal. Calcd for $CH_{16}N_5Cl_2O_{10}Rh$: C, 2.78; H, 3.73; N, 16.21. Found: C, 3.2; H, 3.8; N, 16.3.

^{15}N Experiment. Acetonitrile (1 mL, 100% label) was condensed onto 100 mg of the iridium triflate complex and the solution heated for 4 h at 80 °C for 4 h. After cooling, the acetonitrile was removed in vacuo and 1 mL of 1 M NaOH added. This was then quenched by the addition of concentrated HCl to give a pH of ca. 2 and the resultant mixture evaporated and left on the vacuum line for 24 h. The 100-MHz ^1H NMR spectrum of the white powder in 0.1 M DCl in D_2O showed only a doublet at δ 2.48 (J = 2 Hz) and a broad band at δ 4.4 corresponding to the coordinated ammonias. There were no obvious satellite peaks for this signal, resulting from ^{15}N-^1H coupling which are typically of the order of 60 Hz and therefore would be clearly visible in this spectrum. In a control experiment, using acetonitrile-^{14}N, the same spectrum was observed except that the acetamide peak at δ 2.45 was a singlet.

Acknowledgment. We gratefully acknowledge the ANU Microanalytical Service for elemental analyses.

Registry No. $[(NH_3)_5IrCH_3CN](ClO_4)_2CF_3SO_3$, 88035-84-1; $[(NH_3)_5Ir(DMF)](ClO_4)_3$, 88035-85-2; $[(NH_3)_5RhCH_3CN](ClO_4)_2CF_3SO_3$, 88035-86-3; $[(NH_3)_5Rh(DMF)](ClO_4)_3$, 68851-29-6; $[(NH_3)_5RhNHCOCH_3](ClO_4)_2$, 52843-08-0; $[(NH_3)_5IrNHCOCH_3](ClO_4)_2$, 88035-87-4; $[(NH_3)_5IrOOCH](ClO_4)_2$, 15646-83-0; $[(NH_3)_5RhOOCH](ClO_4)_2$, 15612-60-9; $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$, 84254-59-1; $[(NH_3)_5RhOSO_2CF_3](CF_3SO_3)_2$, 84254-57-9; CH_3CN, 75-05-8; DMF, 68-12-2.

Supplementary Material Available: Tables of the observed rate constants for base hydrolysis of the title compounds, as a function of $[OH^-]$ and $[OD^-]$ (2 pages). Ordering information is given on any current masthead page.

Geometrical and Stereochemical Factors in Metal-Promoted Amide Hydrolysis

John T. Groves* and R. Rife Chambers, Jr.

Contribution from the Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109. *Received April 22, 1983*

Abstract: The importance of the precise geometric orientation of a metal in metal-promoted amide hydrolysis has been demonstrated. Large rate enhancements (10^3-10^6) at neutral pH were found in zinc and copper complexes in which the metal is forced to lie above the plane of an amide. For this study, lactams 1-[(6-(dimethylamino)methyl)-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one (**1**) and 1-[(6-((bis(carboxymethyl)amino)methyl)-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one (**2**) were synthesized. Titrimetrically determined formation constants indicated that both **1** and **2** readily bind divalent metals (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}). Detailed investigations of the various metal complexes were possible over a wide range of pH. At 50 °C, the Cu^{2+}-promoted hydrolysis of **1** exhibited a sigmoidal pH-rate profile. The rates increased commensurate with the ionization of a metal-bound water molecule. A similar behavior was observed with the **2**-Zn^{2+} complex at 70 °C. Both Cu^{2+} and Zn^{2+} greatly facilitate amide hydrolysis at pH 7. Compared to base hydrolysis of the lactams with no metal, a rate enhancement of 9×10^5 and 1.0×10^3 was observed with the **1**-Cu-OH_2 and **2**-Zn-OH_2 complexes, respectively. Activation parameters for the metal-promoted hydrolyses indicated that catalysis results from a substantial increase in $\Delta S^{\ddagger}$. These observations are interpreted in terms of nucleophilic catalysis by a metal-hydroxo species in basic media. Concurrent carbonyl oxygen exchange accompanied base hydrolysis of **1**. By contrast, significant oxygen-18 exchange was not observed during the Cu^{2+}-promoted hydrolysis of **1**. These results are considered in the context of the known stereoelectronic control in the cleavage of tetrahedral intermediates.

The acceleration of enzymic reactions can be attributed to specific chemical effects such as entropic advantage, transition-state binding, and chemical catalysis by neighboring groups.[1] The study of simple model systems has served to probe the relative importance of such effects and as a test of perceived insight into a particular mechanism.[2] Carboxypeptidase A (CPA), a C-terminal peptidase, has become a paradigm case of a substantiated enzymic mechanism.[3] Surprisingly, in the 16 years since the first, high-resolution X-ray structural data for CPA have been avail-

(1) Lipscomb, W. N. *Acc. Chem. Res.* **1982**, *15*, 232-238.

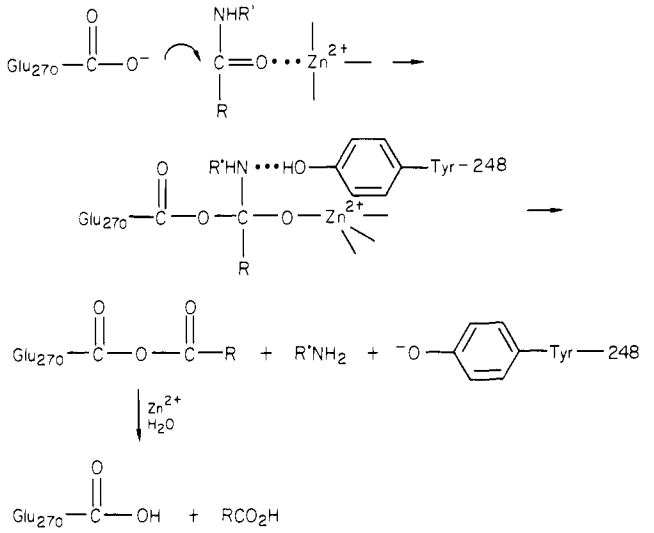
(2) Breslow, R. In "Bioinorganic Chemistry"; Gould, R. F., Ed.; American Chemical Society: Washington, DC, 1971; Adv. Chem. Ser. No. 100, Chapter 2.

(3) (a) Neurath, H.; Bradshaw, R. A. *Acc. Chem. Res.* **1970**, *3*, 249-257. (b) Quiocho, F. A.; Lipscomb, W. N. *Adv. Protein Chem.* **1971**, *25*, 1-78. (c) Hartsuck, J. A.; Lipscomb, W. N. in "The Enzymes", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, Vol. IV, p 1. (d) Kaiser, E. T.; Kaiser, B. L. *Acc. Chem. Res.* **1972**, *5*, 219-22. (e) Ludwig, M. L.; Lipscomb, W. N. In "Inorganic Biochemistry"; Eichhorn, G. L., Ed.; American Elsevier Publishing Co.: New York, 1973; Vol. I, p 438. (f) Lipscomb, W. N. *Tetrahedron* **1974**, *30*, 1725-1732. (g) Galdes, A.; Hill, H. A. O. In "Inorganic Biochemistry", Specialist Periodical Report; The Chemical Society, Burlington House: London, 1979; Vol. I, Chapter 8. (h) Rees, D. C.; Honzatho, R. B.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *77*, 3299-3291. (i) Lipscomb, W. N. *Ibid.* **1980**, *77*, 3875-3878.

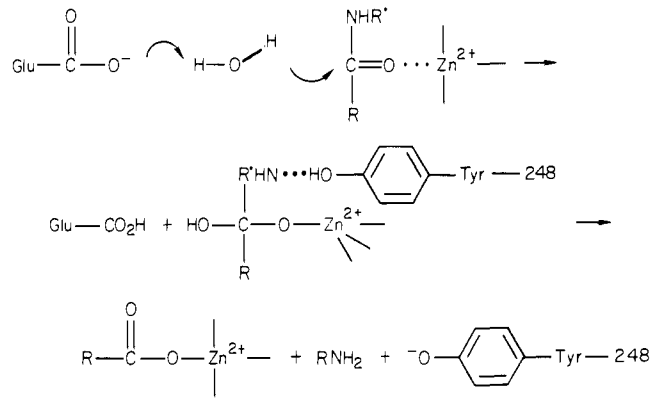
0002-7863/84/1506-0630$01.50/0

Scheme I. Proposed CPA Mechanisms

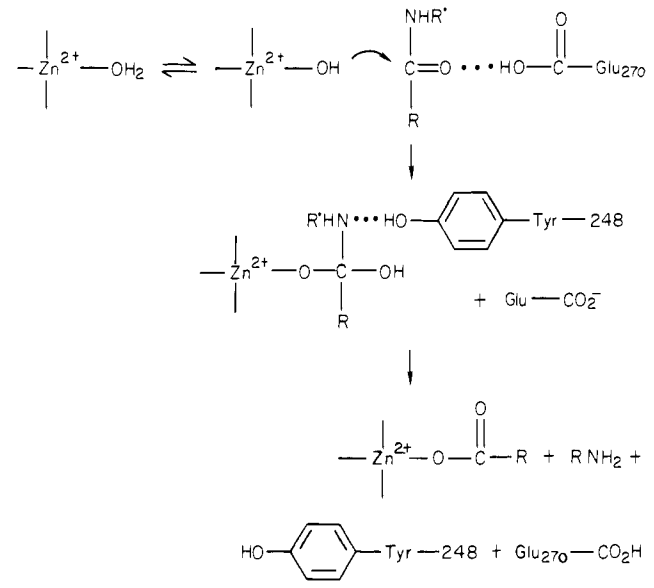
(i) Anhydride



(ii) General Base



(iii) Zinc Hydroxide



able,[4] divergent views on the molecular details of catalysis by this enzyme have persisted.

The range of considerable mechanisms for CPA may be summarized as follows (Scheme I).[5]

(i) The anhydride mechanism: The reaction of CPA with O-(*trans*-*p*-chlorocinnamoyl)-L-β-phenylacetate at low temperature has provided evidence suggestive of an acyl-enzyme intermediate.[6] The corresponding amide, however, is a poor substrate.

(ii) General base mechanism: The presence of an intervening water molecule between Glu-270 and the substrate carbonyl has been supported by the observed requirement of free amino acid in the ^{18}O exchange between water and the substrate.[5,7] In order for the anhydride mechanism to account for this result the water molecule must not exchange with the milieu during the course of the reaction.

(iii) Zinc hydroxide mechanism: Retention of a water molecule at the active site can be accommodated if it is a ligand of zinc. The role of the metal ion would then become a proximate binding site for a nucleophilic hydroxide rather than that of a Lewis acid activator of the carbonyl. Three principal objections to the zinc-bound hydroxide mechanism have been the carbonyl coordination discerned in the X-ray structure of Gly-Tyr/CPA, the unusually low pK_a that would be required for $Zn-OH_2$, and the weak nucleophilicity expected for L_nZn-OH.

There is now reason to believe that none of these are serious objections to the zinc hydroxide path for CPA or related enzymes such as thermolysin or angiotensin converting enzyme. Indeed, Gly-Tyr is a slow substrate for CPA.[4] Thus, the crystal structure may have revealed a nonproductive binding mode. Acidic zinc hydrate complexes have been prepared.[8] Further, the role of zinc in carbonic anhydrase (CA) appears to be that of providing a metal-bound hydroxide. Interestingly, the hydration of aldehydes and the hydrolysis of esters are also catalyzed by CA.[9]

Numerous model systems have been examined for the role of metal ions in hydration and hydrolysis reactions of esters,[10] amides,[11] and nitriles.[12] Significantly, though, amides have been found to be much less susceptible to enhanced hydrolysis rates than esters. An exception is the large acceleration observed by Buckingham et al. for Co^{3+} complexes.[13] Further, Zn^{2+} has been found to be a notoriously poor catalyst for acyl transfer except for anhydrides[14a] where the zinc hydroxide mechanism occurs.[14b]

(4) Lipscomb, W. N. *Acc. Chem. Res.* **1970**, *3*, 81-89.

(5) (a) Wernick, D. L. Ph.D. Thesis, Columbia University, 1977. (b) Breslow, R.; Wernick, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1303. (c) Auld, D. S.; Holmquist, B. *Biochemistry* **1974**, *13*, 4355-4361.

(6) (a) Makinen, M. W.; Yamamura, K.; Kaiser, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3882-388. (b) Makinen, M. W.; Kuo, L. C.; Dymowski, J. J.; Jaffer, S. *J. Biol. Chen.* **1975**, *254*, 356-366. (c) Kuo, L. C.; Fukuyama, J. M.; Makinen, M. W. *J. Mol. Biol.* **1983**, *163*, 63-105.

(7) Breslow, R.; Wernick, D. *J. Am. Chem. Soc.* **1976**, *98*, 259-261.

(8) (a) Wooley, P. R.; Prince, R. H. *Nature (London), Phys. Sci.* **w1972**, *240*, 117. (b) Woolley, P. *Nature (London)* **1975**, *258*, 677-682. (c) Coates, J. H.; Gentle, G. J.; Lincoln, S. F. *Ibid.* **1974**, *249*, 773-775. (d) Brown, R. S.; Salmon, D.; Curtis, N. J.; Kusuma, S. *J. Am. Chem. Soc.* **1982**, *104*, 3188-3194.

(9) (a) Pocker, Y.; Deits, T. L. *J. Am. Chem. Soc.* **1982**, *104*, 2424-2434. (b) Lindskog, S.; Hendrickson, L. E.; Kannan, K. K.; Liljas, A.; Nyman, P. O.; Standberg, B. "The Enzymes", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. V, p 687. (c) Lindskog, S. *Struct. Bonding (Berlin)* **1970**, *8*, 153. (d) Pocker, Y.; Meany, J. E. *Biochemistry* **1967**, *6*, 239-246. (e) Pocker, Y.; Dickerson, D. G. *Ibid.* **1968**, *7*, 1995-2004. (f) Tashian, R. E.; Douglas, D. P.; Yu, Y. L. *Biochem. Biophys. Res. Commun.* **1964**, *14*, 256. (g) Kaiser, E. T.; Lo, K. W. *J. Am. Chem. Soc.* **1970**, *91*, 4912-4918. (h) Pocker, Y.; Stone, J. T. *Ibid.* **1965**, *87*, 5497-5498. (i) Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668-678. (j) Umeyama, H.; Kitaura, K.; Morokuma, K. *Chem. Pharm. Bull. (Tokyo)* **1981**, *29*, 1-6.

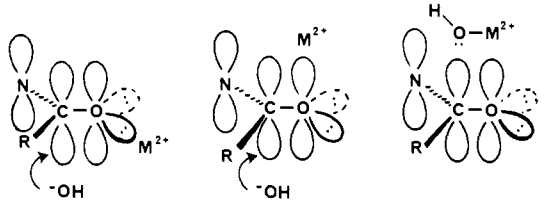
(10) (a) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2036-2039. (b) Bender, M. L.; Turnquest, B. W. *Ibid.* **1957**, *77*, 4271-4275. (c) Wells, M. A.; Rogers, G. A.; Bruice, T. C. *Ibid.* **1976**, *98*, 4336-4338. (d) Wells, M. A.; Bruice, T. C. *Ibid.* **1977**, *99*, 5342-5356. (f) Bender, M. L. "Mechanisms of Homogeneous Catalysis from Protons to Proteins"; Wiley-Interscience: New York, 1971; Chapter 8. (g) Fife, T. H.; Squillacote, V. L. *J. Am. Chem. Soc.* **1978**, *100*, 4787-4793. (h) Fife, T. H.; Przystas, T. J.; Squillacote, V. L. *Ibid.* **1979**, *101*, 3017-3026.

(11) (a) Meriwether, L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 5119-5123. (b) Fairweather, R. B. Ph.D. Thesis, Columbia University, 1967.

(12) Breslow, R.; Fairweather, R.; Keana, J. *J. Am. Chem. Soc.* **1967**, *89*, 2135-2138.

(13) (a) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1968**, *90*, 6032-6040. (b) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *Ibid.* **1969**, *91*, 4102-4112. (c) Buckingham, D. A.; Keene, F. R.; Sargeson, A. M. *Ibid.* **1974**, *96*, 4981-4983. (d) Boreham, C. J.; Buckingham, D. A.; Keene, F. R. *Ibid.* **1979**, *101*, 1409-1421.

The failure of model systems to reveal large metal-mediated rate enhancements for amide hydrolysis suggests that some criterion for catalysis has been missing from the earlier models. It has been the goal of our approach to prepare metal–amide complexes in which the modes of interaction between the metal and the amide carbonyl are strictly defined.[15] Consideration of the three families of CPA mechanisms detailed above indicates three likely modes of metal–amide interaction, A, B, and C.

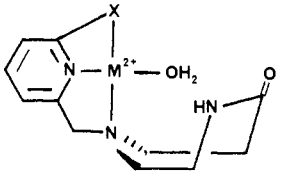


A B C

In A, prior coordination of the metal to an oxygen lone pair is depicted. Nucleophilic attack of hydroxide (or Glu-270) would then produce the tetrahedral intermediate. Several model approaches have achieved this geometry, with the result of modest acceleration or even inhibition by the metal.[10h,16] Case B is a variation of A except that the metal is interacting with the carbonyl π-system. Two factors that could favor this mode of attack over A are the antiperiplanar relationship of M^{2+} and HO^- and the conversion of a poor ligand, the amide π-system, into a strong ligand in the tetrahedral intermediate.

Nucleophilic attack of a metal-bound hydroxide is depicted in C. The role of the metal in this case is to assist the deprotonation of a coordinated water molecule and to position the resulting metal hydroxide for addition to the amide carbonyl. Simultaneously, interactions of the amide oxygen and the metal center are also possible with this geometry.[8c,9a,j] Space-filling models indicate that the complexes **1–3** can achieve either the metal–carbonyl interactions of B or the metal hydroxide-carbonyl interactions of C but not that shown in A.

Rapid amide hydrolysis has been observed in copper and zinc complexes of **3**. However, the limited solubility of these com-



1, X = $-CH_2NMe_2$
2, X = $-CH_2N(CH_2COO^-)_2$
3, X = $-COO^-$

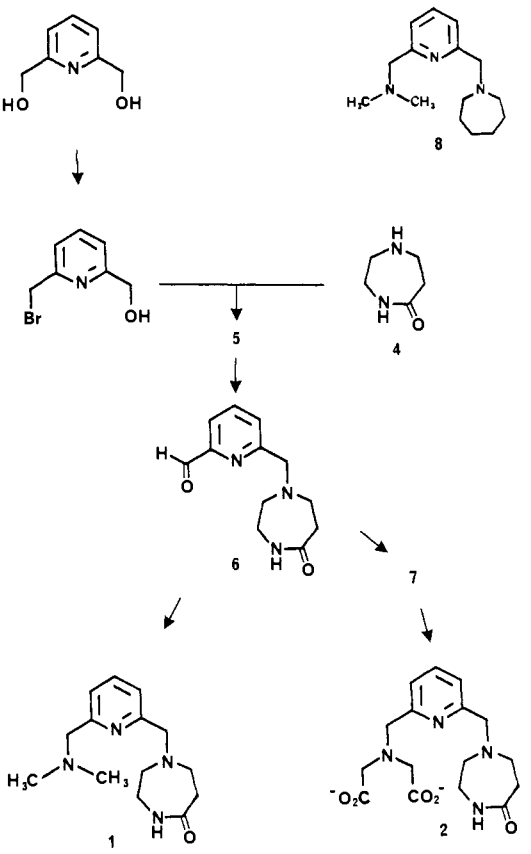
pounds above neutral pH hindered a detailed analysis.[15] We describe here the synthesis and metal-promoted hydrolysis of **1** and **2**.[17]

Results

Synthesis and Metal Complexation of the Ligands. The requisite ligands were prepared conveniently according to the procedures outlined in Scheme II. Spectral data and elemental analyses of **1**, **2**, and all intermediate compounds were fully in accord with the assigned structures.

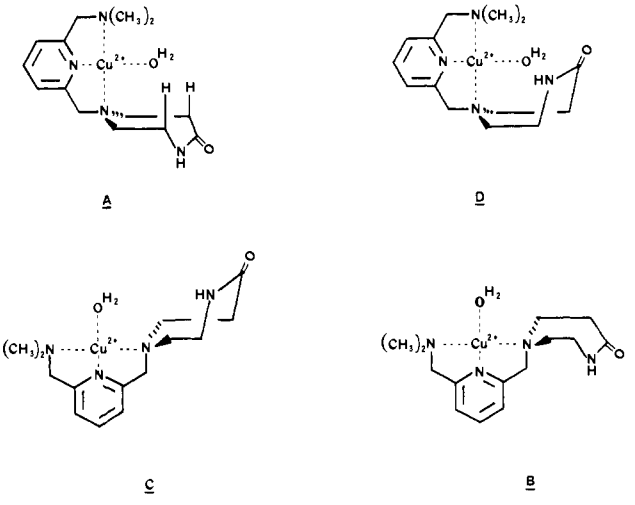
The titration of **1** revealed two equivalence points corresponding to pK_a values of 5.5 and 8.1, which were assigned to the tertiary

Scheme II



amines of the azlactam and the dimethylamine groups, respectively. In the presence of cupric ion, formation of a 1:1 complex, **1**–Cu^{2+}–OH_2, was indicated by the lowering of these two pK_a's. In addition, ionization of a copper-bound water molecule with a pK_a of 7.2 was evident. The carbocyclic analogue of **1**–Cu^{2+}–OH_2, **8**–Cu^{2+}–OH_2, was shown to have a pK_a of 7.3.

There are four reasonable conformations for the **1**–Cu^{2+}–OH_2 as depicted in A–D below. Inspection of molecular models reveals



A D C B

distinct nonbonded interactions between the pseudo-axial hydrogens in A and the metal-bound water. Thus, structure C, with a chair lactam and the copper equatorial to the ring, is expected to be the preferred conformer. The similarity of the pK_a of **1**–Cu^{2+}–OH_2 to that of **8**–Cu^{2+}–OH_2 indicates that no unusual effects of the amide carbonyl are required to assist the deprotonation of **1**–Cu^{2+}–OH_2. Further, an X-ray crystal structure of **3**–Cu^{2+}–Br·H_2O has shown that conformation C, with the bromide ion occupying the fourth equatorial site, is preferred in the solid state.[18]

(14) (a) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 1638–1642. (b) Breslow, R.; McClure, D. E.; Brown, R. S.; Eisenach, J. *Ibid.* **1975**, *98*, 194–195.
(15) (a) Dias, R. M. Ph.D. Thesis, University of Michigan, 1979. (b) Groves J. T.; Dias, R. M. *J. Am. Chem. Soc.* **1979**, *101*, 1033–1035.
(16) Tang, C. C., Ph.D. Thesis, Columbia University, 1978.
(17) Abstracted from the Ph.D. Thesis of R.R.C., The University of Michigan, 1981.

Table I. Formation Constants for Metal Complexes of **1** and **2**

metal	$K_f(1-m)$, M^{-1} [a]	$K_f(2-m)$, M^{-1} [b]	$pK_a(2-m)$
Cu^{2+}	2.8×10^9	$>10^{13}$ [c]	
Ni^{2+}	1.6×10^5	2.5×10^{11}	3.3[d]
Zn^{2+}	2.1×10^4	8.9×10^9	3.1[d]
Co^{2+}		8.5×10^9	3.3[d]

[a] Measured at 50.0 ± 0.1 °C in 0.5 M $NaClO_4$. [b] Measured at 70.0 ± 0.1 °C in 0.5 M $NaClO_4$. [c] This value was too large to be accurately determined from the titration data. [d] pK_a for 2-M(H^+) ⇄ 2-M + H^+.

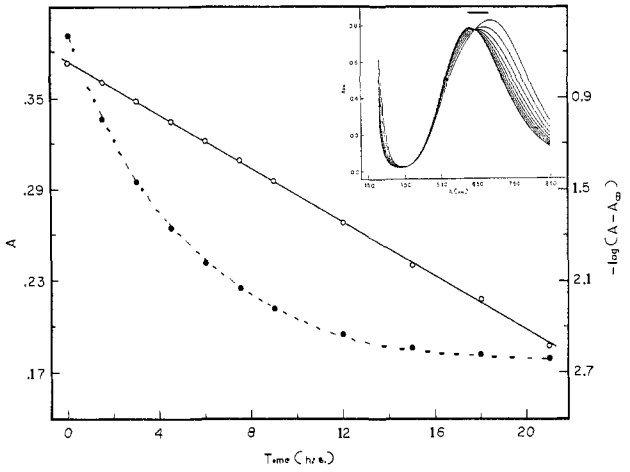


Figure 1. Absorbance changes at 780 nm vs. time (●) and $-\log(A - A_\infty)$ vs. time (○) for the hydrolysis of **1**–Cu^{2+}–OH_2 at pH 7.64 (HEPES buffer). Inset: visible spectra of **1**–Cu^{2+}–OH_2 (3.79×10^{-3} M) at 90-min intervals.

Formation constants for the 1:1 complexes of **1** and **2** with divalent metal ions were calculated according to the method of Bjerrum[19] and are presented in Table I. Titrimetric data for the complexes of **2** were complicated by apparent protonation of one of the carboxyl groups. Similar observations have been reported for complexes of EDTA with divalent metal ions.[20]

Hydrolysis of 1–Cu^{2+}–OH_2. Hydrolysis of the lactam of **1**–Cu^{2+}–OH_2 was observed to occur over a period of several hours at 50 °C and neutral pH. The kinetics of this process were conveniently measured by following changes in the visible spectrum of **1**–Cu^{2+}–OH_2 to that of the corresponding amino acid **9**–Cu^{2+} (Figure 1). That these changes corresponded to hydrolysis was confirmed by isolation of the product **9**. The rate of hydrolysis was proportional to the mole fraction of Cu^{2+}, and no changes in **1** were observed under these conditions in the absence of copper (Figure 2). This observation, the first-order kinetics, and the large binding constant of **1** indicate that a 1:1 copper ligand complex was the kinetically reactive species.

A pH–rate profile for the hydrolysis of **1**–Cu^{2+}–OH_2 in buffered aqueous solution at 50 °C over the range pH 4.6–9.2 is shown in Figure 3. Observed rates were independent of buffer concentration between 0.3 and 0.6 M. It is apparent from the sigmoidal shape of the pH–rate curve that the rate of hydrolysis increases by about 100-fold upon ionization of some group. The upward curvature above pH 8.2 suggests an additional base-catalyzed process.

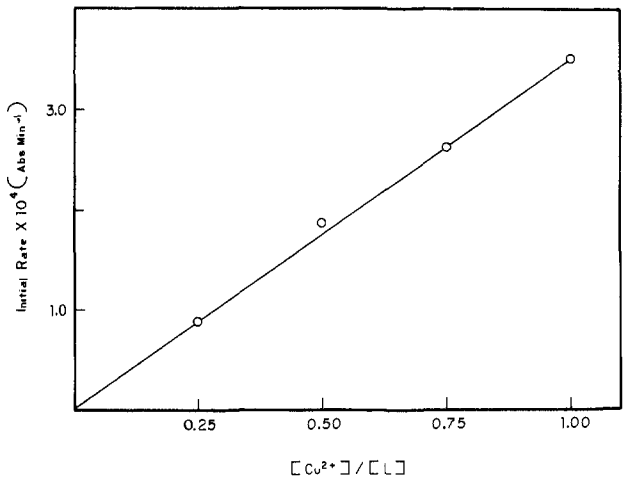


Figure 2. Changes in the initial hydrolysis rate of **1**–Cu^{2+}–OH_2 at pH 7.16 as a function of $[Cu^{2+}]$/**1**–Cu^{2+}–OH_2 ratio.

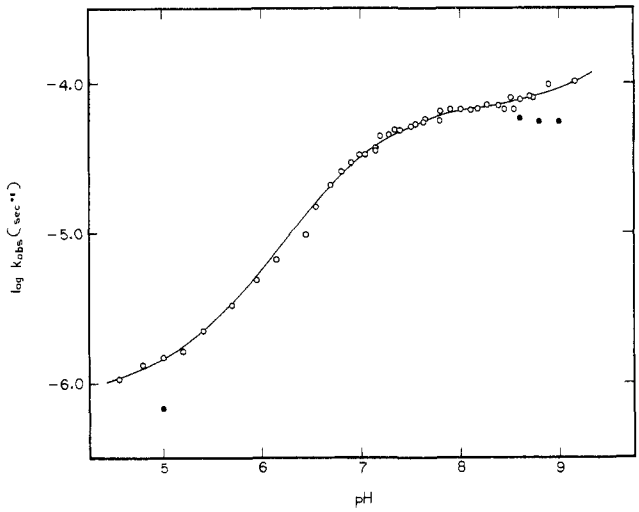
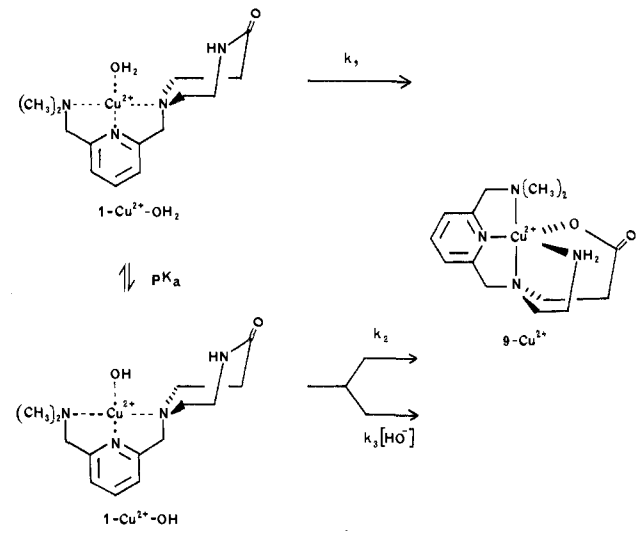


Figure 3. pH–rate profile for the hydrolysis of **1**–Cu^{2+}–OH_2 at 50 °C and μ = 0.5 ($NaClO_4$). Data obtained in D_2O (●).

Scheme III



The simplest mechanism consistent with these results is shown in Scheme III. These processes take the form of eq 1, in which

$$k_{obsd} = k_1\left(\frac{a_H}{K_{app} + a_H}\right) + \left[k_2 + k_3\frac{K_w}{a_H}\right]\left(\frac{K_{app}}{K_{app} + a_H}\right) \quad (1)$$

K_w is the autoprotolysis constant for water (pK_w = 12.97, 50 °C, 0.5 M $NaClO_4$) and K_{app} is the apparent ionization constant

(18) Olson, J. R., unpublished results.
(19) (a) Hartley, F. R.; Burgess, C.; Alcock, R. M. "Solution Equilirbia"; Ellis Horwood Limited: Chichester, West Sussex, England, 1980. (b) Martell, A. E.; Calvin, M. "Chemistry of Metal Chelate Compounds"; Prentice-Hall: New York, 1952; p 78.
(20) (a) Higginson, W. C. E. *J. Chem. Soc.* **1962**, 2761–2763. (b) Brunetti, A. P.; Nancollas, G. H.; Smith, P. N. *J. Am. Chem. Soc.* **1969**, *91*, 4680–4683. (c) Smith, G. S.; Hoard, J. L. *Ibid.* **1959**, *81*, 556–561. (d) Hoard, J. L. "Proceedings of the 8th International Conference on Coordination Chemistry"; Gutman, V., Ed., Springer-Verlag: Vienna, 1964; p 135.

Table II. Activation Parameters for the Base-Catalyzed and Metal-Mediated Hydrolysis of 1 and 2

reaction	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, eu
1 (k_{OH})	12	−42[a]
1-Cu-OH (k_2)	20	−15
2 (k_{OH})	15	−34
2-Zn-OH_2	22	−18

[a] Experimental errors in the measured rate constants were <±5%, which corresponds to errors in $\Delta H^{\ddagger}$ of <±1 kcal/mol and in $\Delta S^{\ddagger}$ of <±3 eu.

Table III. Relative Rate Constants of Hydrolysis of 1-M Complexes at pH 7.0, 50 °C

metal M	K_f	k_{obsd}, s^{-1}	k_{rel}
no metal		4.1×10^{-11} [a]	1.0
Cu^{2+}	2.8×10^9	3.3×10^{-5}	9.0×10^5
Ni^{2+}	1.6×10^5	5.6×10^{-7}	1.4×10^4
Zn^{2+}	2.1×10^4	$\approx 2.5 \times 10^{-7}$ [b]	$\approx 6.1 \times 10^3$

[a] Estimated from the second-order rate constants for the hydrolysis of 1 obtained with $[HO^-] = 0.10$–0.15 M. [b] Taken from data at pH 6.58, the highest pH at which rates could be measured.

required to fit the data. The ratios in the brackets represent the mole fractions of the metal–aquo species, 1-Cu^{2+}–OH_2, and the metal–hydroxo species, 1-Cu^{2+}–OH. A computer-assisted least-squares fit of the data to eq 1 gave $k_1 = 9.04 \times 10^{-7}$ s^{-1}, $k_2 = 7.30 \times 10^{-5}$ s^{-1}, $k_3 = 0.19$ M^{-1} s^{-1}, and $K_{app} = 10^{-7.14}$. The excellent agreement between pK_{app} and the tritrimetrically determined pK_a for 1-Cu^{2+}–OH_2 (pK_a = 7.2) indicated that the ionization responsible for the sigmoidal rate increase was the deprotonation of the copper-bound water.

Solvent Deuterium Isotope Effects. The titration of 1-Cu^{2+}–OD_2 in D_2O showed a shifted pK_a of 7.8 as expected for an oxy acid.[21] The copper-promoted hydrolysis was independent of pD in the region 8.61–9.00 and gave an average rate constant (k_2^D) of 5.58×10^{-5} s^{-1}. Since observed rates in this region are due to the reaction of 1-Cu^{2+}–OD, a solvent isotope effect, k_H/k_D = 1.3, could be estimated for the copper–hydroxo reaction. From data at pD = 5.01, at which 86.5% of the reaction was due to 1-Cu^{2+}–OD_2, k_1^D was calculated to be 5.83×10^{-7} s^{-1}. This rate corresponds to a solvent isotope effect of 1.6 for the copper–aquo reaction.

Activation Parameters for the Hydrolysis of 1-Cu^{2+}–OH_2. Activation parameters for the copper–hydroxo term (k_2) in the hydrolysis of 1-Cu^{2+}–OH_2 were determined by measurement of k_{obsd} between 40 and 60 °C in the pH-independent region (pH 8). At 25 °C the pK_a of the metal-bound water was found to be 7.5. Activation parameters for 1 and 1-Cu^{2+}-OH are compared in Table II.

Oxygen-18 Exchange. The base-catalyzed hydrolysis of 1 in $H_2{}^{18}O$ led to the reisolation of ^{18}O-enriched 1 in amounts corresponding to 41% exchange at 36% conversion. Thus, as is expected for an amide hydrolysis in basic solution, decomposition of the tetrahedral intermediate to starting amide and product amino acid are competitive.[22] By contrast, the mass spectrum of reisolated 1 from the hydrolysis of 1-Cu^{2+}–OH_2 at pH 8.2, 50 °C, indicated no more than 1% oxygen exchange at 38% conversion.

From the rate constants (k_2) measured for the hydrolysis of 1-Cu^{2+}–OH, greater than 95% of the hydrolysis proceeds through the copper–hydroxo path under these conditions. The lack of significant oxygen exchange indicates either that the hydrolysis is an ordered process in which the carbonyl oxygen and the nucleophilic oxygen are never interconverted or that formation of the tetrahedral intermediate is rate limiting.

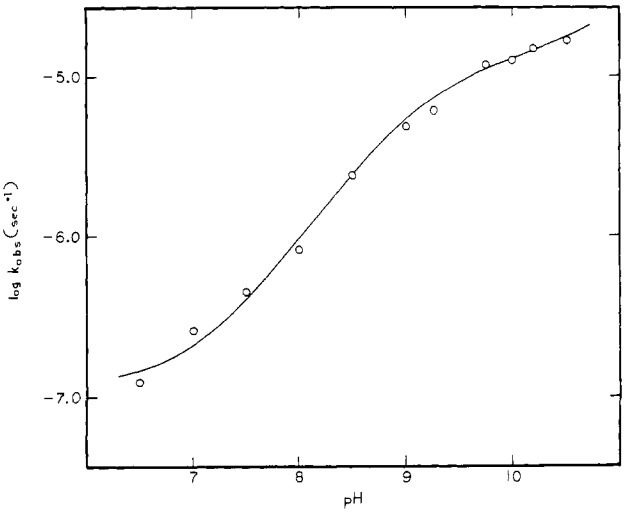
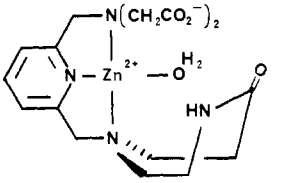


Figure 4. pH–rate profile for the hydrolysis of 2-Zn^{2+}–OH_2 at 70 °C and μ = 0.5 ($NaClO_4$).

Hydrolysis of 1-Zn^{2+} and 1-Ni^{2+}. Hydrolysis of the lactam in 1 was found to be rather slow at pH 7 in the presence of Zn^{2+} and Ni^{2+}. Since the formation constant of 1-Zn^{2+} was small (2.1×10^4), corrections were made for the presence of free 1 in solution. Approximations of the true rates were obtained by measuring initial rates. Rates for free 1, 1-Cu^{2+}–OH, 1-Ni^{2+}, and 1-Zn^{2+} are compared in Table III.

Hydrolysis of 2-Zn-OH_2. The failure of 1 to bind zinc sufficiently for an investigation over a wide range of pH prompted an examination of 2. The large binding constant of 2 with zinc



2-Zn^{2+}-OH_2

(6×10^9) allowed studies at 60–80 °C from pH 6.5 to 10.5. Hydrolysis rates were determined by quantitative analysis of primary amine with *o*-phthaldehyde–mercaptoethanol. Variations in the observed, first-order rate constant for amide hydrolysis with pH at 70 °C are shown in Figure 4. The sigmoidal shape of this plot with additional upward curvature in the high pH region indicates a mechanistic scheme for the hydrolysis of 2-Zn-OH_2, which is analogous to that of 1-Cu^{2+}–OH_2. A computer-assisted, least-squares fit for the data in Figure 4 to eq 1 gave $k_1 = 1.20 \times 10^{-7}$ s^{-1}, $k_2 = 1.29 \times 10^{-5}$ s^{-1}, $k_3 = 5.0 \times 10^{-4}$ M^{-1} s^{-1}, and $K_{app} = 10^{-9.16}$. The autoprotolysis constant for water was estimated to be 12.49 at 70 °C, with μ = 0.5 M sodium perchlorate.

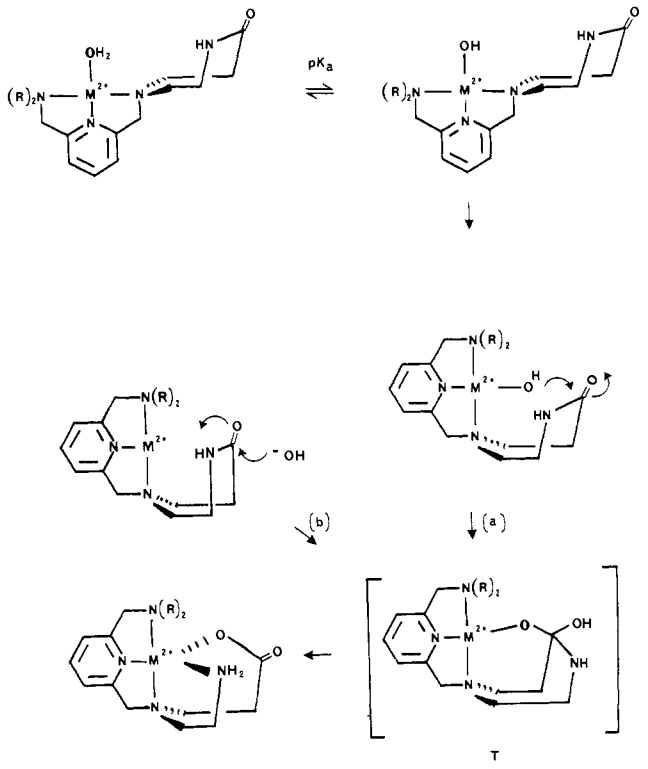
The kinetic pK_{app} was assigned to ionization of zinc-bound water in comlex 2-Zn^{2+}–OH_2. The observed pK_a for this ionization (pK_a = 9.16) is close to the expected value for such a complex.[23] As with the copper complex 1-Cu^{2+}–OH_2, the zinc–hydroxo species was approximately 100 times more reactive toward amide hydrolysis than the zinc–aquo complex.

Activation Parameters for the Hydrolysis of 2-Zn^{2+}. The temperature dependence of the hydrolysis of 2-Zn^{2+} was investigated between 60 and 80 °C at pH 10. Under these conditions the principal contributor to the reaction rate was the zinc–hydroxo process (k_2). A comparison of the activation parameters for the base-catalyzed hydrolysis of 2 and the zinc-mediated process are compared in Table II. The trends observed closely resemble those found for 1-Cu^{2+}–OH_2. The acceleration of amide hydrolysis

(21) Bunton, C. A.; Shiner, V. J., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 42–47.
(22) (a) Bender, M. L. *Chem. Rev.* **1960**, *60*, 53–113. (b) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; p 523.

(23) (a) Sillen, L. G.; Martell, A. E. "Stability Constants of Metal-Ion Complexes"; The Chemical Society; Burlington House: London, 1974; Special Publication No. 17. (b) Martell, A. E.; Smith, R. M. "Critical Stability Constants"; Plenum Press: New York and London, 1974; Vol. 1.

Scheme IV



for the zinc complex is due to the more favorable entropy of activation even though $\Delta H^{\ddagger}$ has increased by 7 kcal/mol. Comparison of the second-order rate constant for the base-catalyzed hydrolysis of **2** at 70 °C ($k_{OH} = 2.6 \times 10^{-10}$ M^{-1} s^{-1}) with the first-order hydrolysis of **2**–Zn^{2+} ($k_{obsd} = 2.6 \times 10^{-7}$ s^{-1}) indicates that the accelerating influence of Zn^{2+} (10^3) is substantially smaller than that of Cu^{2+}. However, since zinc has been reported to be ineffective[11a] in the hydrolysis of glycine amide, this 1000-fold effect is significant.

Discussion

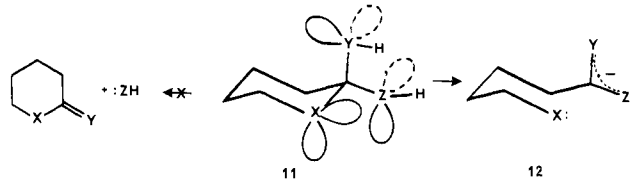
Mechanism of Amide Hydrolysis. The results described above have shown that very large rate enhancements are obtained for amide hydrolysis in a metal complex which prevents carbonyl oxygen–metal interactions. The pH–rate profile of the amide hydrolysis showed the signoidal shape expected for a prototropic equilibrium with the basic form about 100 times more reactive than the acidic form. Thus, the dominant reaction path for **1**–Cu^{2+}–OH_2 between pH 6 and 9 is a process involving catalysis by the metal–hydroxo form **1**–Cu^{2+}–OH or a kinetic equivalent. Two types of reactions may be reasonably considered for the formation of the tetrahedral intermediate, T; intramolecular addition of the metal-bound hydroxo group to the amide carbonyl (path a) and external attack of hydroxide with concomitant metal coordination of the carbonyl oxygen (path b) (Scheme IV).

Several arguments favor the intramolecular metal–hydroxo path. CPK space filling models indicate that the hydroxo oxygen of **1**–Cu^{2+}–OH can make contact with the amide acyl carbon when the lactam ring adopts the boat configuration. Further, comparison of the activation parameters for the basic hydrolysis for **1** and **2** to those of the metal complexes reveals a substantially larger $\Delta S^{\ddagger}$ for the metal-promoted hydrolyses. Such changes are characteristic of an intramolecular process and have been observed with ortho-substituted benzoic acid derivatives.[24] The small solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.3$) observed for the hydrolysis of **1**–Cu^{2+}–OH argues that the metal-bound hydroxide serves as the primary nucleophile and not as a general base for the deprotonation of water.[25] The most likely mechanism for the hydrolysis of **1**–Cu^{2+}–OH is depicted in Scheme IV.

Intramolecular attack on an amide carbonyl by a metal–hydroxo nucleophile has been unambiguously established for cobalt(III) complexes. Further, Buckingham, et al. have shown that the decrease in reactivity of a metal-bound hydroxide is not as great as expectations based on changes in pK_a. For example, the relative reactivity of $[(NH_3)_5Co–OH]^{2+}$ and hydroxide toward propionic anhydride was found to be only 300 whereas the pK_a's of these complexes differ by a factor of 10^{10}.[26]

Several factors contribute to the increase in $\Delta H^{\ddagger}$ for the metal-promoted hydrolyses of **1** and **2**. First, the lactam must adopt the less stable boat conformation for nucleophilic attack of M–OH to occur. A quantitative estimate of this energy difference may be obtained by comparing **1** and **2** to cycloheptene. The boat form of this hydrocarbon is 1.2–1.4 kcal/mol less stable than the chair.[27] In addition, the bicyclic nature of the metal-bound tetrahedral intermediate, T (Scheme IV), may bring about other conformational problems not obvious from space-filling models.

Stereoelectronic Considerations. A potentially important aspect of metal-catalyzed amide hydrolysis that has received little consideration is stereoelectronic control. The theory of stereoelectronic control as described by Deslongchamps[28] has been used to explain the kinetic preferences in the decomposition of hemiorthoesters and hemiorthoamides.[29] Specifically, the decomposition of such species is found to proceed by the expulsion of a leaving group with assistance from lone pairs on *both* of the other heteroatoms as in **11**. The trans-periplanar relationship of these orbitals to



the leaving group allows an adiabatic transformation to the allylic π-system in the product **12**.[6c,30]

Applied to the metal complexes of **1** and **2**, the concept of stereoelectronic control predicts that addition of a metal-bound hydroxide will be more facile than external hydroxide attack. As depicted in Scheme V, either external hydroxide attack on the amide carbonyl or internal addition of metal-bound hydroxide will give, ultimately, the same tetrahedral intermediate (T). Stereoelectronic considerations are most readily compared for the three possible modes of decomposition of T. Inspection of the pertinent heteroatom orbitals in T reveals that the oxygen orbital trans-periplanar to the C–O bond of the external hydroxide is involved in binding to the metal and the trans-periplanar nitrogen orbital is part of a C–N bond. Thus, stereoelectronic considerations disfavor expulsion of the external hydroxide from T. Accordingly, external addition of hydroxide to form T, the microscopic reverse, is also stereoelectronically forbidden. By contrast, orbitals are available for assistance in the expulsion of *either* the metal-bound hydroxide or the amide nitrogen. Therefore, both steps in a

(24) (a) Belke, C. J.; Su, S. C. K.; Shafer, J. A. *J. Am. Chem. Soc.* **1971**, *93*, 4552–4560. (b) Okuyama, T.; Schmir, G. L. *Ibid.* **1972**, *94*, 8805–8811. (c) Fife, T. H.; DeMark, B. R. *Ibid.* **1976**, *98*, 6978–6982. (d) Fife, T. H.; DeMark, B. R. *Ibid.* **1977**, *99*, 3075–3080. (e) Bruice, T. C.; Benkovic, S. J. *Ibid.* **1963**, *85*, 1–8. (f) Fife, T. H. "Advances in Physical Organic Chemistry"; Gold, V., Ed.; Academic Press: London, 1975; Vol. 11, p 5. (g) Fife, T. H.; Duddy, N. W. *J. Am. Chem. Soc.* **1983**, *105*, 74–79 and references therein.

(25) Bruice, T. E.; Benkovic, S. J. "Bioorganic Mechanisms"; W. A. Benjamin: New York, 1966; p 36.

(26) Buckingham, D. A.; Engelhardt, L. M. *J. Am. Chem. Soc.* **1975**, *97*, 5915–5917.

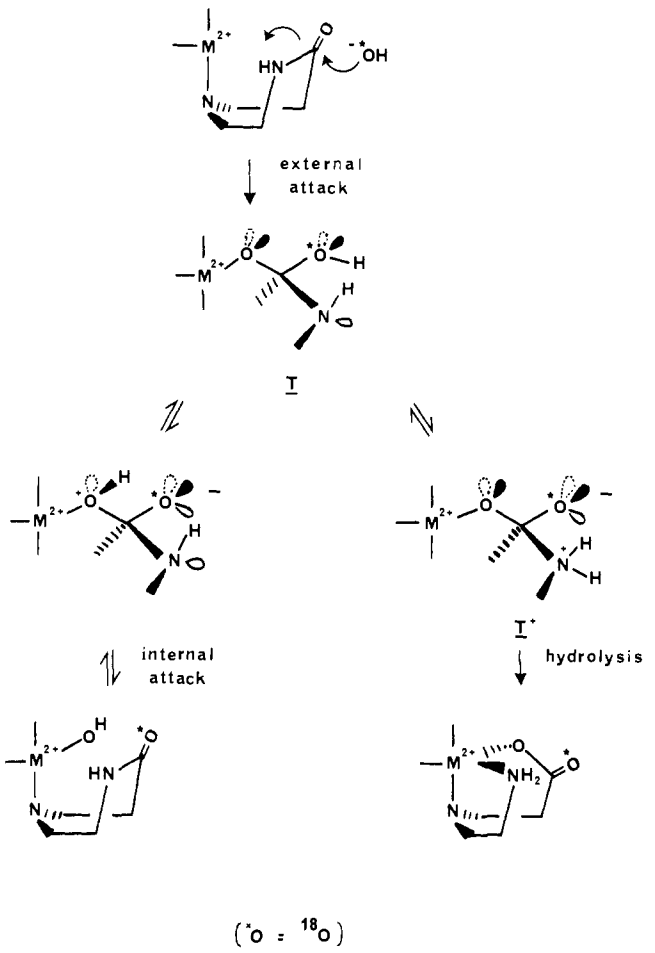
(27) Favini, G.; Buemi, G.; Raimondi, M. *J. Mol. Struct.* **1968**, *2*, 137–148.

(28) (a) Deslongchamps, P. *Tetrahedron* **1975**, *31*, 2463–2490 and references therein. (b) Deslongchamps, P.; Cheriyan, U. O.; Thaillefer, R. J. *Can. J. Chem.* **1979**, *57*, 3262–3271. (c) Deslongchamps, P.; Barlet, R.; Taillefer, R. J. *Ibid.* **1980**, *58*, 2167–2172.

(29) Perrin, C. L.; Arrhenius, G. M. L. *J. Am. Chem. Soc.* **1982**, *104*, 2839–2842.

(30) (a) Lehn, J.-M.; Wipff, G. *J. Am. Chem. Soc.* **1980**, *102*, 1347–1354. (b) *Ibid.* **1974**, *96*, 4048–4090. (c) Lehn, J.-M.; Wipff, G. *Helv. Chim. Acta* **1978**, *61*, 1274.

Scheme V



mechanism for amide hydrolysis involving addition of metal-bound hydroxide and expulsion of nitrogen are expected to be assisted by favorable orbital overlap. At pH 7 cleavage of the C–H bond in T is expected to involve protonation of the nitrogen lone pair (T^+). Calculations by Lehn and Wipff[30] have suggested that stereoelectronic preferences would be amplified for the protonated intermediates.

The lack of ^{18}O-exchange in recovered **1**–Cu^{2+}–OH requires that hydroxide addition to the amide carbonyl occur with only a single stereochemistry and that the addition process be metal catalyzed. The application of stereoelectronic control leads to the prediction that addition of a metal-bound hydroxide is preferred over external hydroxide attack.

It is expected that the same stereoelectronic considerations would apply in the low pH regime. From the rate constants k_1 and k_2 for **1**–Cu^{2+}–OH_2, the metal–aquo and metal–hydroxo reaction paths are competitive at pH 5 (relative rate 2.0:1 for M–OH_2 and M–OH, respectively). Less than 1% ^{18}O incorporation was observed in **1** after 48 h at pH 5 and 50 °C (10% conversion). Thus, either formation of the tetrahedral intermediate, T, is rate limiting at this pH or both reaction paths have the same stereochemistry for hydroxide (water) addition.

Conclusions

The results of this study have shown that (1) large rate enhancements for metal-promoted amide hydrolysis (10^3–10^6) are observed in metal–amide complexes in which the metal is forced to lie above the plane described by the amide group; (2) the kinetically reactive species is the metal–hydroxide complex; (3) cupric ion is much more effective than zinc for amide hydrolysis; (4) stereoelectronic considerations support a mechanism for hydrolysis involving nucleophilic attack of the metal-bound hydroxo group on the amide carbonyl.

Recent cryokinetic studies have indicated that two intermediates are formed prior to release of the C-terminal fragment by CPA.[31] These results have suggested that an acyl-enzyme intermediate may not be formed with either esters or amides. Glu-270 has been implicated in the stabilization of metal-bound water in Co^{2+}–CPA.[32] Taken together these results suggest that Glu-270 acts as a general base to assist the formation of a metal-bound hydroxide and that nucleophilic addition of the metal-bound hydroxide is involved in catalysis. The significant accelerations observed in the model studies presented here for a metal–hydroxide system indicate that such a path can result in substantial catalysis of amide hydrolysis.

Experimental Section

General. All chemicals used were reagent grade. Diethyl iminodiacetate (Eastman) was distilled at reduced pressure to give a colorless product (bp 82 °C (0.1 mm)). Acetonitrile was routinely distilled from phosphorus pentoxide and stored over calcium hydride. Triethylamine was freshly distilled and dried over sodium. *o*-Phthalaldehyde (OPA) obtained from Aldrich Chemical Co. was purified by two crystallizations from petroleum either (bp 38–46 °C). Activated manganese dioxide was purchased from E. M. Laboratories, Inc. (West Germany). Sodium perchlorate, copper(II) perchlorate, zinc(II) perchlorate, nickel(II) perchlorate, and cobalt(II) perchlorate were obtained from G. Frederick Smith and Co. (Columbus, OH). Stock metal solutions were standardized either gravimetrically or by complexometric (EDTA) titration, according to established procedures.[33] The buffer materials 2-(morpholino)ethenesulfonic acid (MES, pK_a = 6.15), *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES, pK_a = 7.55), *N*-(2-hydroxyethyl)piperazine-*N'*-3-propanesulfonic acid (HEPPS, pK_a = 8.00), 2-(cyclohexylamino)ethanesulfonic acid (CHES, pK_a = 9.50), and 3-(cyclohexylamino)propanesulfonic acid (CAPS, pK_a = 10.40) were purchased from Calbiochem (La Jolla, CA) or Sigma Chemical Co.

Distilled water was redistilled over potassium permanganate and used in the preparation of buffer solutions. Buffer solutions were freshly prepared in most cases, but never stored for more than 1 week. Buffers in deuterium oxide were prepared in 99.8% *d* deuterium oxide by neutralizing the acid form of the buffer with a 1 N sodium deuterioxide/deuterium oxide solution.

Measurements of pH were made with a GK 2321C (50 °C) or GK 2401 (70 °C) combination electrode attached to a Radiometer TTT-2b titrator. The titrator was also equipped with a PHA943 Titrigraph Module, an SBR3 Titrigraph, an ABU 12T Autoburette, and a TTA3 Titration Assembly. A Haake F-4291 constant temperature bath was used to control the temperature within ±0.1 °C.

Nuclear magnetic resonance spectra were recorded on a JEOL FX90Q or Bruker WM-360 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Model 457 or Beckman IR 4240 spectrophotometer. Cary Models 17 and 219 were used in obtaining UV–visible spectral data. Melting points were obtained with a Hoover Melt Temp apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratories (Eagle Harbor, MI).

Hexahydro-1*H*-1,4-diazepin-5-one (4). 4-Piperidone monohydrate hydrochloride (18.0 g, 117 mmol) was dissolved in a mixture of 90 mL of glacial acetic acid and 48 mL of concentrated sulfuric acid. The mixture was cooled to 0 °C and sodium azide (8.4 g, 129 mmol) added in small portions over 8 h. After stirring in the ice bath overnight (12 h), the mixture was cooled to 0 °C and solid sodium hydroxide added until the pH was 8–10. Water was added as necessary to keep the mixture workable. The resulting slurry was extracted with four 500-mL portions of chloroform and the organic layers were dried over sodium sulfate. Removal of the solvent under vacuum gave 10.0 g (77%) of **4** as a yellow hygroscopic solid which was pure as judged by its ^1H NMR spectrum. Anal. Calcd for $C_5H_{10}N_2O \cdot {}^1/_2H_2O$: C, 48.76; H, 9.00; N, 22.75. Found: C, 48.48; H, 8.98; N, 22.35.

1-[[6-(Hydroxymethyl)-2-pyridyl]methyl]hexahydro-1,4-diazepin-5-one (5). 2-(Bromomethyl)-6-(hydroxymethyl)pyridine (16.3 g, 80.6 mmol) was dissolved in 300 mL of anhydrous acetonitrile. A mixture of hexahydro-1,4-diazepin-5-one (9.2 g, 80.6 mmol) and triethylamine (11.23 ml, 80.6 mmol) in 40 mL of anhydrous acetonitrile was added dropwise over 3 h to the previous solution. The reaction was stirred under nitrogen for 12 h. The solvent was removed under vacuum and water (40 ml) added followed by potassium carbonate until basic (pH 9). After saturation with sodium chloride, the mixture was extracted with ten 90-mL

(31) Galdes, A.; Auld, D. S.; Vallee, B. L. *Biochemistry* **1983**, *22*, 1888–1893.
(32) Geoghegan, K. F.; Holmquist, B.; Spilburg, C. A.; Vallee, B. L. *Biochemistry* **1983**, *22*, 1847–1852.
(33) Vogel, A. E. "Quantitative Inorganic Analysis", 3rd ed., Wiley: New York, 1962.

portions of chloroform and the organic layers were dried over sodium sulfate. Removal of the solvent under vacuum gave 14.2 g (75%) of desired **5** that was of sufficient purity: mp 154–157 °C; ^{1}H NMR ($CDCl_3$) δ 6.9–7.6 (m, 3 H), 6.6 (br, 1 H), 4.7 (s, 2 H), 3.9 (br, 1 H), 3.7 (s, 2 H), 3.0–3.5 (m, 2 H), 2.6 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 178.1 (1 C), 159.1 (1 C), 157.1 (1 C), 137.2 (1 C), 121.4 (1 C), 119.1 (1 C), 64.1 (2 C), 57.0 (1 C), 50.6 (1 C), 42.4 (1 C), 37.6 (1 C); IR ν_{max} ($CHCl_3$) 3200–3500, 3420, 2810–3005, 1670, 1595, 1580, 1470, 1460, 1435, 1350, 1100–1150, 1041 cm^{-1}; MS, *m/e* (relative intensity) 236 (2.3), 218 (1.1), 192 (0.2), 177 (0.4), 149 (2.3), 123 (100.0), 105 (41.9). Anal. Calcd for $C_{12}H_{17}N_3O_2$: C, 61.25; H, 7.28; N, 17.86. Found: C, 61.13; H, 7.19; N, 17.72.

1-[(6-Formyl-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one (6). A mixture of **5** (12.0 g, 51.0 mmol) and activated manganese dioxide (120 g, 1.38 mol) in 900 mL of dichloromethane was stirred under nitrogen for 6 h. After removal of the manganese dioxide by filtration, the solvent was evaporated under reduced pressure to yield 8.92 g (75%) of desired **6**. The aldehyde product was homogeneous by TLC (Uniplate silica gel) in a mixture of ethyl acetate, methanol, chloroform, and water (67:19:9.5:4.5) (R_f 0.33): mp 169.5–172.5 °C dec; ^{1}H NMR ($CDCl_3$) δ 10.0 (s, 1 H), 7.6–8.0 (m, 3 H), 7.0 (br, 1 H), 3.9 (s, 2 H), 3.1–3.6 (m, 2 H), 2.8 (m, 6 H); IR ν_{max} ($CHCl_3$) 3420, 2800–3001, 1712, 1665, 1590, 1430–1470, 1390, 1350, 1315, 1190–1260, 1110 cm^{-1}; MS, *m/e* (relative intensity) 234 (1.5), 233 (0.1), 190 (0.2), 175 (0.8), 147 (1.9), 121 (100.0), 113 (13.9).

1-[(6-((Dimethylamino)methyl)-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one (1). To a solution of **6** (3.6 g, 13.3 mmol) in 170 mL of a saturated dimethylamine/anhydrous acetonitrile solution (ca. 0.5 M) was added glacial acetic acid until the pH to wet litmus paper was 6–7. Sodium cyanoborohydride (0.584 g, 9.29 mmol) was dissolved in 15 mL of anhydrous acetonitrile and charged over 4 h to the previous mixture. After stirring under nitrogen for 23 h, the solvent was removed under vacuum to give a brown residue. Water (40 mL) was added followed by 6 N hydrochloric acid until acidic (pH 1–2). The pH was adjusted to 9 with solid potassium carbonate and the solution saturated with sodium chloride. The mixture was extracted with four 100-mL portions of chloroform and the organic layers were dried over sodium sulfate. Removal of the solvent under vacuum gave 4.6 g of crude **1** as a yellow oil. Silica gel chromatography using a mixture of methanol, ethyl acetate, and aqueous ammonia (73.9:24.6:1.5) gave 2.05 g (59%) of **1** as a yellow solid. The product was further purified by treatment with Norite and recrystallization from ethyl acetate: mp 104–105 °C; ^{1}H NMR ($CDCl_3$) δ 7.2–7.8 (m, 3 H), 7.0 (br, 1 H), 3.8 (s, 2 H), 3.6 (s, 2 H), 3.1–3.5 (m, 2 H), 2.7 (m, 6 H), 2.3 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 178.0 (1), 158.7 (1), 157.9 (1), 136.8 (1), 121.0 (2), 65.8 (1), 64.5 (1), 57.2 (1), 50.7 (1), 45.6 (2), 42.6 (1), 38.0 (1); IR ν_{max} ($CHCl_3$) 3420, 2770–3010, 1670, 1590, 1580, 1440–1470, 1355, 1160–1170 cm^{-1}; MS, *m/e* (relative intensity) 263 (1.3), 262 (1.0), 219 (31.9), 204 (3.1), 150 (100.0), 133 (9.6), 107 (32.6), 106 (27.3), 58 (38.8). Anal. Calcd for $C_{14}H_{22}N_4O$: C, 64.09; H, 8.45; N, 21.36. Found: C, 64.04; H, 8.44; N, 21.33.

1-[(6-((Bis(carbethoxymethyl)amino)methyl)-2-pyridyl)methyl]hexahydro-1,4-diazepin-5-one (7). To a mixture of **6** (3.14 g, 13.5 mmol) and diethyl iminodiacetate (7.65 g, 40.4 mmol) in 200 mL of anhydrous acetonitrile was added glacial acetic acid until the pH to wet litmus paper was 7. Sodium cyanoborohydride (0.592 g, 9.42 mmol) was dissolved in 10 mL of acetonitrile and charged over 2 h to the previous mixture. After stirring under nitrogen for 16 h, the solvent was removed under reduced pressure. The mixture was cooled to 0 °C and water (20 mL) added followed by 6 N hydrochloric acid until acidic (pH 1–2). The pH was adjusted to 8–9 with potassium carbonate. The solution was extracted with chloroform and the organic layers were dried (Na_2SO_4). Removal of the solvent under vacuum gave an oil which was purified by silica gel chromatography using a mixture of ethyl acetate, methanol, chloroform, and water (67:19:9.5:4.5). Ultimately, 1.76 g (32%) of **7** was obtained as a yellow oil. The pure compound crystallized only after remaining in the freezer for several hours: mp 79–81 °C; ^{1}H NMR ($CDCl_3$) δ 7.3–7.9 (, 3 H), 6.8 (br, 1 H), 4.1 (q, 4 H), 4.0 (s, 2 H), 3.7 (s, 2 H), 3.6 (s, 4 H), 3.3 (m, 2 H), 2.7 (m, 6 H), 1.2 (t, 6 H); ^{13}C NMR ($CDCl_3$) δ 117.9 (1 C), 171.0 (2 C), 158.4 (1 C), 157.7 (1 C), 137.0 (1 C), 121.2 (2 C), 64.4 (1 C), 60.4 (2 C), 59.9 (1 C), 57.2 (1 C), 54.8 (2 C), 50.7 (1 C), 42.5 (1 C), 37.8 (1 C), 14.1 (2 C); IR ν_{max} ($CHCl_3$) 3425, 2820–3020, 1740, 1669, 1595, 1580, 1450, 1375, 1355, 1186, 1150, 1025 cm^{-1}; MS, *m/e* (relative intensity) 407 (1.0), 333 (11.3), 294 (32.3), 219 (28.5), 107 (100.0). Anal. Calcd for $C_{20}H_{30}N_4O_5$: C, 59.09; H, 7.44; N, 13.78. Found: C, 59.01; H, 7.45; N, 13.75.

***N*-[(6-(Hydroxymethyl)-2-pyridyl)methyl]hexamethylenimine (11).** This material was synthesized from 2-(bromomethyl)-6-(hydroxymethyl)pyridine (**13**) and hexamethylenimine (Aldrich Chemical Co.) by a procedure analogous to the synthesis of **5**. The crude product was purified by silica gel chromatography using a mixture of methanol, ethyl acetate, and aqueous ammonia (73.9:24.6:1.5) as the eluent. From 202.1 mg (1.00 mmol) of **13**, 165 mg (75%) of desired **11** was obtained as an oil: ^{1}H NMR ($CDCl_3$) δ 7.1–7.7 (m, 3 H), 5.1 (s, 1 H), 4.7 (s, 2 H), 3.8 (s, 2 H), 2.5–2.9 (m, 4 H), 1.6 (m, 8 H); ^{13}C NMR ($CDCl_3$) δ 158.7 (1 C), 156.3 (1 C), 137.2 (1 C), 121.9 (1 C), 119.1 (1 C), 64.1 (1 C), 63.1 (1 C), 55.5 (2 C), 27.0 (2 C), 26.8 (2 C); IR ν_{max} ($CHCl_3$) 3300–3500, 3001 (sh), 2930, 2860, 2820 (sh), 1598, 1580, 1460, 1438 (sh), 1415, 1340, 1150, 1135, 1050 cm^{-1}; MS, *m/e* (relative intensity) 221 (2.2), 220 (0.3), 219 (1.1), 124 (8.5), 123 (100.0), 112 (4.3), 105 (51.2), 98 (96.3). Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72. Found: C, 70.73; H, 9.33; N, 12.39.

***N*-[(6-((Dimethylamino)methyl)-2-pyridyl)methyl]hexamethyleneiminimine (8).** A mixture of **11** (1.01 g, 4.58 mmol) in 100 mL of 48% hydrobromic acid was maintained at reflux for 20 h. The solvent was removed under reduced pressure. The residue was dissolved in 10 mL of 95% ethanol and added dropwise over 2 h to 80 mL of a cold (0 °C) saturated dimethylamine/acetonitrile solution. After slowly reaching room temperature, the reaction was concentrated under vacuum. Water (10 mL) was added followed by 50% sodium hydroxide until strongly basic. The solution was extracted with chloroform and the organic layers were dried (Na_2SO_4). Removal of the solvent under vacuum gave a brown oil, which was purified by silica gel chromatography using a mixture of methanol, ethyl acetate, and aqueous ammonia (73.9:24.6:1.5). From 601 mg of crude product there was obtained 192 mg of **8** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 6.9–7.5 (m, 3 H), 3.7 (s, 2 H), 3.5 (s, 2 H), 2.5–2.8 (m, 4 H), 2.2 (s, 6 H), 1.6 (m, 8 H); ^{13}C NMR ($CDCl_3$) δ 159.7 (1 C), 158.0 (1 C), 36.5 (1 C), 120.9 (1 C), 120.8 (1 C), 65.7 (1 C), 64.2 (1 C), 55.8 (2 C), 45.5 (2 C), 28.1 (2 C), 26.9 (2 C); IR ν_{max} ($CHCl_3$) 2930, 2860, 2825, 2780, 1592, 1575, 1455, 1355, 1171, 1155, 1090, 1040 cm^{-1}; MS, *m/e* (relative intensity) 249 (11.6), 248 (65.2), 247 (2.3), 246 (11.0), 204 (12.3), 151 (22.2), 150 (100.0), 107 (39.6), 106 (30.8), 98 (18.8), 58 (76.0).

Isolation of 9 from Basic Hydrolysis of 1. Compound **1** (300 mg, 1.14 mmol) was dissolved in 6 mL of 0.50 N lithium hydroxide and the mixture maintained at reflux under nitrogen for 25 h. The pH was adjusted to 7 with perchloric acid and the solution lyophilized. The resulting solid was purified by gel filtration (Sephadex G-10, eluent water) to give **9** as the monoperchloric acid salt: NMR (D_2O) δ 4.65 (HOD), 7.78 (t, 1 H), 7.35 (t, 2 H), 4.26 (s, 2 H), 3.73 (s, 2 H), 2.99 (t, 2 H), 2.77 (s, 6 H), 2.61–2.77 (m, 4 H), 2.24 (t, 2 H). Anal. Calcd for $C_{14}H_{24}N_2O_2 \cdot HClO_4$: C, 44.15; H, 6.62; N, 14.71; Cl, 9.31. Found: C, 44.01; H, 6.59; N, 14.60; Cl, 9.22.

Isolation of 9 from Cu^{2+}-Promoted Hydrolysis of 1. Compound **1** (100 mg, 0.381 mmol) and 0.953 mL (0.381 mmol) of 0.40 M copper(II) perchlorate were added to water (3 mL) and the pH was adjusted to 8.75 with sodium hydroxide. The resulting solution was placed in a sealed ampule and maintained at 80 °C for 45.5 h. The mixture was saturated with hydrogen sulfide and the pH adjusted to 10 with sodium hydroxide. After removal of the copper(II) sulfide by filtration, perchloric acid was added (pH 7) and the solution lyophilized. As judged by its ^{1}H NMR spectrum, the recovered product was identical with that obtained by basic hydrolysis of **1**.

Isolation of 10 from Basic Hydrolysis of 2. A mixture of **2** (108 mg, 0.266 mmol) in 5 mL of 0.10 N lithium hydroxide was warmed at 50 °C for 5 min. An additional 15 mL of 0.10 N lithium hydroxide was added and the mixture maintained at 80 °C in a sealed ampule for 6 days. Thin-layer chromatography (Uniplate silica gel) in an ethanol–2-propanol–water–acetic acid (10:10:5:1) mixture indicated the presence of one ninhydrin-positive compound. The pH was adjusted to 5.3 with hydrochloric acid and the solution lyophilized. The resulting solid was purified by gel filtration (Sephadex G-10, eluent water) to give the amino acid **10**: NMR (D_2O) δ 4.78 (HOD), 7.89 (t, 1 H), 7.48 (d, 1 H), 7.42 (d, 1 H), 4.55 (s, 2 H), 3.99 (s, 2 H), 3.83 (s, 4 H), 3.22 (t, 2 H), 3.02 (t, 2 H), 2.92 (t, 2 H), 2.44 (t, 2 H).

Kinetic Measurements. *o*-Phthalaldehyde (OPA) Reagent for Monitoring Hydrolysis. Boric acid (3.092 g, 50.0 mmol) and EDTA disodium salt dihydrate (1.1633 g, 3.13 mmol) were dissolved in sufficient water to give 250-mL total volume. The pH was adjusted to 9.75 with sodium hydroxide and then recrystallized OPA (19.0 mg, 0.142 mmol) and β-mercaptoethanol (0.5 mL, 7.13 mmol) were added. This solution was always made fresh every day.

Base-Catalyzed Hydrolyses of 1 and 2. The base hydrolyses of **1** and **2** were carried out at various temperatures in 0.04–0.16 M sodium hydroxide solutions that were also 0.5 M in sodium perchlorate. The experiments were performed by using the sealed ampule technique. Typically, 0.01 mmol of **1** or **2** was dissolved in 3.0 mL of sodium hydroxide and 100 μL of this solution syringed into a number of capillary tubes (100 mm × 2 mm). The capillary tubes were sealed and placed in a constant temperature bath at the appropriate temperature. Periodically, a tube was removed, 50 μL of the solution added to 3.0 mL of

OPA reagent, and the absorbance at 333 nm (**1**) or 335 nm (**2**) measured. The infinity values, A_∞, were determined by maintaining 0.01–0.03 mmol of the appropriate lactam in 1.0 N sodium hydroxide at 80–85 °C for 24 h and then analyzing the mixture with OPA reagent. That hydrolysis was complete was confirmed by TLC (Uniplate silica gel) in a mixture of ethanol, 2-propanol, water, and acetic acid (10:10:5:1).

Rate constants were calculated from a linear least-squares regression of ln $(A_\infty - A_t)$ vs. time. Correlation coefficients (r^2) were always >0.99.

Metal Ion Promoted Hydrolyses of 1 and 2. All hydrolysis reactions were carried out in 0.4 M buffer solutions that were adjusted to an ionic strength of 0.5 M with added sodium perchlorate. The reaction temperature was controlled at 50 or 70 °C as appropriate.

The Cu^{2+}-promoted hydrolysis of **1** was accompanied by a blue shift in the visible region of the spectrum which corresponds to the d–d transitions of Cu^{2+}. This spectral change clearly showed isosbestic behavior with a shift in λ_{max} from 690 and 625 nm. Reaction progress was followed by monitoring the decrease in absorbance at 780 nm, the wavelength where the largest absorbance change occurs.

In the high pH region, where reaction rates are fast, it was possible to continuously monitor absorbance changes over a period of about 12 h. At lower pH, reaction mixtures were maintained at 50.0 °C in sealed ampules and the absorbance at 780 nm periodically measured.

Typically, samples of the complex were prepared by adding 1 equiv of copper(II) perchlorate to 0.01 mmol of **1**. The complex was lyophilized and dissolved in 4.0 mL of buffer, and the resulting mixture equilibrated at 50 °C for 10 min. The absorbance at 780 nm was then measured at appropriate time intervals.

Values of A_∞ were determined from solutions of the complex kept at 100 °C for various periods of time. That hydrolysis was complete was verified by TLC (Uniplate silica gel) in a mixture of ethanol, 2-propanol, water, and acetic acid (10:10:5:1) (1-ninhydrin-positive component).

Rate constants were calculated from a linear least-squares regression of ln $(A_\infty - A_t)$ vs. time. Correlation coefficients (r^2) were usually >0.999, but never <0.98.

Zn^{2+}- and Ni^{2+}-Promoted Hydrolysis of 1. The Zn^{2+}-promoted hydrolysis of **1** was followed by monitoring the production of primary amine with OPA reagent. In a typical experiment, 0.01 mmol of **1** and 1 equiv of zinc(II) perchlorate were combined and the solution lyophilized. The complex was dissolved in 3.0 mL of buffer and 100 μL of this solution syringed into a number of capillary tubes (100 mm × 2 mm). The capillary tubes were sealed and kept at 50 °C. At various time intervals, the reaction was analyzed by adding 50 μL of the solution to 3.0 mL of OPA reagent and the recording the absorbance at 333 nm. The A_∞ value was determined from a stock solution of the product zinc complex. Rate constants were calculated from a linear least-squares regression of ln $(A_\infty - A_t)$ vs. time. Correlation coefficients were usually >0.98.

The Ni^{2+}-promoted hydrolysis of **1** was accompanied by a blue shift of the Ni^{2+} d–d transitions in the visible spectrum. Reaction progress was monitored by following the increase in absorbance at 572 nm. To 0.09 mmol of **1** was added 1 equiv of nickel(II) perchlorate and the solution lyophilized. The complex was dissolved in 4.0 mL of a pH 7.00 buffer (HEPES) and placed in a sealed ampule. The reaction mixture was maintained at 50 °C and the absorbance at 572 nm measured periodically. The value of A_∞ was determined from a sample kept at 100 °C for 47 h. A linear least-squares regression of ln $(A_\infty - A_t)$ vs. time yielded the desired rate constant, k_{obsd}. The correlation coefficient (r^2) was >0.98.

Zn^{2+}-Promoted Hydrolysis of 2. The Zn^{2+}-promoted hydrolysis of **2** was monitored by following the production of primary amine with OPA reagent. Typically, 1 equiv of Zn(II) perchlorate was added to 0.025 mmol of **2** and the solution lyophilized. The complex was dissolved in 3.0 mL of buffer and 100 μL of this solution syringed into several capillary tubes (100 mm × 2 mm). The capillary tubes were sealed and maintained at 70 °C. Periodically, a reaction vessel was removed and the absorbance at 335 nm measured after adding 25 μL of the solution to 3.0 mL of OPA reagent. The A_∞ value was determined from a stock solution of the product zinc complex.

Rate constants were calculated from a linear least-squares regression of ln $(A_\infty - A_t)$ vs. time. Correlation coefficients (r^2) were always >0.99.

Oxygen-18 Exchange Experiments. ^{18}O Exchange under Basic Conditions. Lactam **1** (10.0 mg, 0.0381 mmol) and anhydrous sodium perchlorate (20.5 mg, 0.167 mmol) were dissolved in 0.30 mL of 99.5% atom excess ^{18}O water and then 33 μL of 1.0 N sodium hydroxide added (86.5% atom excess ^{18}O, 1.10 N NaOH). To 1.0 mL of 1.0 N NaOH was added 34 μL of the previous mixture and this solution analyzed with OPA reagent at 333 nm (50 μL in 3.0 mL of OPA reagent, $A_0 = 0.0058$). The remainder of the reaction mixture was placed in a sealed ampule and kept at 50 °C for 62.5 h. The absorbance at 333 nm ($A_t = 0.133$) was measured as before. A_∞ (0.356) was determined from a stock solution of amino acid. The reaction mixture was lyophilized and extracted with chloroform. The organic layer (ca. 20 mL) was then washed once with 5 mL of 10% sodium carbonate and dried (Na_2SO_4). TLC (Uniplte silica gel) in a mixture of methanol, ethyl acetate, and ammonium hydroxide (73.9:24.6:1.5) revealed one component whose R_f coincided with authentic **1**. Mass spectral analysis of recovered **1** gave the following ion counts:

$$I^{219} = 13\,744$$

$$I^{221} = 7456$$

$$\%\ ^{18}O = \left(\frac{I^{221}}{I^{222} + I^{219}}\right) \times 100 = 35.2\%$$

$$\%\ \text{exchange} = \left(\frac{35.2\%}{86.5\%}\right) \times 100 = 40.7\%$$

$$\%\ \text{hydrolysis} = \left(\frac{A_t - A_0}{A_\infty - A_0}\right) \times 100 = 36.3\%$$

^{18}O Incorporation of 1 in the Presence of Cu^{2+}. An ^{18}O-enriched buffer was prepared by combining HEPES sodium salt hemihydrate (85.2 mg, 0.316mmol), HEPES (10.2 mg, 0.0428 mmol), anhydrous sodium perchlorate (16.3 mg, 0.133 mmol), and 0.8 mL of 99.5% atom excess ^{18}O water (95.7% atom excess ^{18}O). The pH of this buffer at 50 °C was 8.2. The copper complex was prepared by combining **1** (9.94 mg, 0.0379 mmol) with 0.379 mL (0.0379 mmol) of 0.100 M copper(II) perchlorate. The complex was lyophilized and then dissolved in all of the buffer. To 3.0 mL of a pH 8.0 buffer (HEPES) was added 25 μL of the previous mixture and then the absorbance at 780 nm ($A_0 = 0.0535$) measured. The reaction mixture was placed in a sealed ampule and maintained at 50 °C for 3 h. The absorbance at 780 nm ($A_t = 0.0416$) was measured as before. The "A_t" solution was kept at 50 °C until hydrolysis was complete and then A_∞ (0.0222) was determined. The reaction mixture was lyophilized, dissolved in water (2 mL), and then saturated with hydrogen sulfide. After passing through Celite, the solution was made basic (pH 9–10 with sodium carbonate) and lyophilized. The resulting solid was extracted with chloroform (10 mL) and the organic layer washed once with 5 mL of 10% sodium carbonate. After drying (Na_2SO_4), the mass spectrum was recorded. The following ion counts were observed: $I^{219} = 23136$; $I^{221} = 350$. Due to the relatively small intensity at m/e 221, it was necessary to correct for the contribution to I^{221} from unlabeled **1** (I^{221}/I^{219} in unlabeled **1** = 0.0045): $I^{221}_{cor} = 246$; ^{18}O = 1.05%; exchange = 1.10%; hydrolysis = 38%.

Acknowledgment. Funds for the purchase of an NMR spectrometer and for the partial support of this work were provided by the National Science Foundation.

Registry No. 1, 87828-76-0; **2**, 87828-77-1; **4**, 34376-54-0; **5**, 87828-78-2; **6**, 87828-79-3; **7**, 87828-80-6; **8**, 87828-82-8; **9**, 87828-83-9; **10**, 87828-84-0; **11**, 87828-81-7; **13**, 40054-01-1; Cu, 7440-50-8; D_2, 7782-39-0; Zn, 7440-66-6; Ni, 7440-02-0; O_2, 7782-44-7.