Metal Ion-Catalyzed Hydrolysis of Acrylate Esters and Amides by Way of Their Conjugate Addition Adducts

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A formal catalytic cycle for the metal ion-catalyzed hydrolyses of acrylamide and acrylate esters is described. While various transition metal ions show little acceleration of hydrolysis reactions in free water, bringing them into proximity with esters and amides can yield increased hydrolysis rates. Such proximity effects can be brought about by covalent addition of donor atoms near the functional group. However, the simple conjugate adducts of butylmethylamine to methyl acrylate and to acrylamide did *not* demonstrate metal ion-catalyzed hydrolysis. Conjugate addition of a chelating amine, *N*-benzyl-*N'*,*N'*-dimethylethylenediamine, yields adducts demonstrating significant (up to 1.5×10^4-fold) hydrolysis catalysis by Cu(II) at pH 7.5 and room temperature. A model compound to test for improvement of this effect by conformational locking demonstrated no rate increase. A model compound designed to test for enhanced acceleration by two adjacent metal ions similarly showed no rate advantage as compared to the parent conjugate adduct. The conjugate reversion reaction of 3-((2-(dimethylamino)ethyl)benzylamino)propionic acid demonstrated a bell-shaped pH-rate profile with maximum rate at pH 3.

Introduction

For the past 40 years, divalent metal cations have been utilized as hydrolysis reagents and catalysts that act on carboxylic esters and amides.[1] The literature describes numerous model compounds capable of binding a metal cation proximal to an ester or amide such that the bound metal catalyzes hydrolysis.[2] Our research group has utilized reversible covalent bond formation as a novel means of transiently bringing together a metal complex and an acrylate ester or amide substrate.[3] The reported conjugate additions of secondary amines to acrylate esters and amides[4] prompted us to investigate a formal catalytic cycle for the metal cation catalyzed hydrolysis of such esters and amides by way of their reversible conjugate addition reactions. Scheme 1 shows a generic, proposed cycle for ester and amide hydrolysis based on reversible covalent bond formation *via* amine conjugate addition. In this cycle, the catalytic group (a metal complex) is brought into close proximity to the substrate by covalent bond formation. The catalyst induces hydrolysis and is then released from the product by covalent bond breaking.

In this paper, we expand upon our initial reports that the *N'*-benzyl-*N*,*N*-dimethylethylenediamine group acts to catalyze the hydrolysis of unactivated esters and amides by chelation of metal cations proximal to the functional group.[3] The formal catalytic cycle illustrated in Scheme 1 was developed with acrylate esters and amides as substrates and *N'*-benzyl-*N*,*N*-dimethylethylenediamine as the ligand for the catalyst. Rates for each of the reaction steps were measured in aqueous solution. In addition, we present the synthesis and kinetic studies of model compounds designed to increase the rates of metal cation-catalyzed hydrolysis by means of (i) the rotational immobilization of the chelating group; and (ii) the addition of a second chelating group that would allow for the possibility of binuclear metal cation catalysis.

Experimental Section

General. All reagents and solvents were obtained from the Aldrich Chemical Co. or Ohio State University stores unless otherwise noted. *N*-Benzyl-*N'*,*N'*-dimethylethylenediamine was purified by recrystallization of its HBr salt, as described below. All metal salts used for kinetics were perchlorate or trifluoroacetate salts. The following buffers (0.1 M) were used: KCl/HCl for pH 1.0–1.5, HCl/KOH for pH 2.0, H_3PO_4/KOH for pH 2.5–3.0, acetic acid/KOH for pH 3.5–5.5, 2-(*N*-morpholino)ethanesulfonic acid (MES) for pH 6.0–6.5, 4-ethylmorpholine for pH 7.0, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) for pH 7.50, *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (CHES) for pH 9.0, and 3-cyclohexylamino-1-propanesulfonic acid (CAPS) for pH 11.0. HPLC was carried out using a C-18 reversed-phase column and a 20 μL injection loop. Elution was carried out at a flow rate of 1.0 mL/min with continuous UV detection at 254 nm. Eluents for the HPLC were 7:4 (v/v) methanol/pH 2 0.2 N potassium phosphate buffer (eluent A), 1:1 methanol/pH 7.5 0.01 M EDTA buffer (eluent B), and 1.5:1 methanol/pH 7.5 0.01 M EDTA (eluent C). Thin layer chromatography was carried out on alumina plates using $CHCl_3$ with 3% ethanol as eluent and visualizing with I_2. Alumina column chromatography was carried out on neutral or basic alumina (Brockman activity I) purchased from Fisher. Ion exchange chromatography was carried out on Sephadex CM-25 resin. X-ray crystallographic data was obtained at The Ohio State University using a Rigaker AFC5s diffractometer. Elemental analysis was carried out at Atlantic Microlab Inc. (Norcross, GA).

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(1) (a) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2034. (b) Kroll, H. *Ibid.* **1952**, *74*, 2036. (c) Meriwether, L.; Westheimer, F. M. *Ibid.* **1956**, *78*, 5119. (d) Bender, M. L.; Turnquist, B. W. *Ibid.* **1957**, *79*, 1889. (e) Breslow, R.; Chipman, D. *Ibid.* **1965**, *87*, 419. (f) Sigman, D. S.; Jorgensen, C. T. *Ibid.* **1971**, *94*, 1724. (g) Groves, J. T.; Chambers, R. R. *Ibid.* **1983**, *106*, 630. (h) Fife, T. H.; Przystas, T. J. *Ibid.* **1985**, *107*, 1041. (i) Baraniak, E.; Buchingham, D. A.; Clark, C. R.; Moyihan, B. H.; Sargeson, A. M. *Inorg. Chem.* **1986**, *25*, 3466. (j) Breslow, R.; Schepartz, A. *J. Am. Chem. Soc.* **1987**, *109*, 1814. (k) Akkaya, E. U.; Czarnik, A. W. *Ibid.* **1988**, *110*, 8553. (l) Chin, J.; Banaszczyk, M. *Ibid.* **1989**, *111*, 2724. (m) Chin, J.; Jubian, V.; Mrejen, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1326. (n) Fife, T. H.; Bembi, R. *J. Am. Chem. Soc.* **1993**, *115*, 11358.

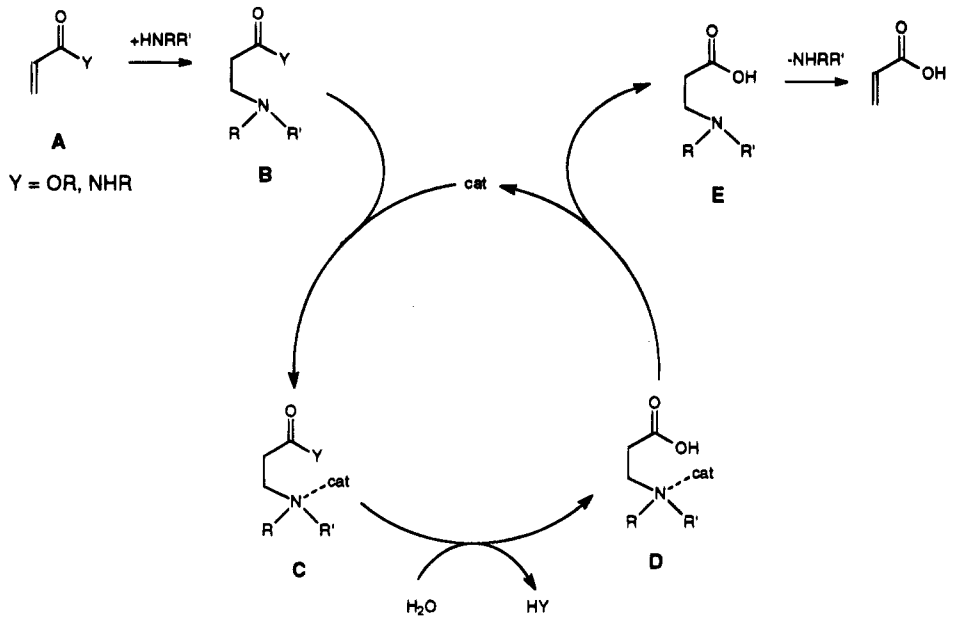
(2) For reviews of recent work, see: (a) Sutton, P. A.; Buckingham, D. A. *Acc. Chem. Res.* **1987**, *20*, 357. (b) Chin, J. *Ibid.* **1991**, *24*, 145. (c) Chin, J.; Banaszcyk, M.; Jubian, V.; Kim, J. H. *Bioorg. Chem.* **1991**, *2*, 175. (d) Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273.

(3) (a) Czarnik, A. W. *Tetrahedron Lett.* **1984**, *25*, 4875. (b) Nanjappan, P.; Czarnik, A. W. *J. Am. Chem. Soc.* **1987**, *109*, 1826. (c) Duerr, B. F.; Czarnik, A. W. *Tetrahedron Lett.* **1989**, *30*, 6951. (d) Duerr, B. F.; Czarnik, A. W. *J. Chem. Soc., Chem. Commum.* **1990**, 1707.

(4) Johnson, M. *J. Org. Chem.* **1986**, *51*, 833.

0022-3263/95/1960-7238$09.00/0

Scheme 1



Hydrolysis Kinetic Methods. All pseudo first-order rate constants were calculated using the computer program ENZFITTER (Elsevier-Biosoft, Cambridge, UK). The following general procedure was followed to monitor the hydrolyses of **2a** and **2b**. A 3.6 mM solution of ester or 4.0 mM solution of amide was prepared in the desired buffer. A second stock solution of metal perchlorate salt in buffer was prepared in such a way that 20 mL of this solution contained twice the desired concentration of metal salt. Into a 50 mL flask was placed 20 mL of the ester or amide stock solution that was vigorously stirred. A 20 mL aliquot of the metal stock solution was added with a pipet having a 23 s delivery time. Time zero was taken at the moment of delivery of the metal solution. At the desired time intervals, a 1.0 mL aliquot was removed and added to 2.0 mL of HPLC eluent A containing 3.1×10^{-3} M chlorobenzene (used as an internal standard). The solution was shaken, and a 100 μL aliquot was injected into the HPLC. Hydrolysis reactions were followed by monitoring the loss of **2a** and **2b**, or the appearance of **2c**, relative to the internal standard by electronic integration of the peak areas.

The hydrolysis of **2d** was monitored by HPLC in a manner similar to the procedure described above. A 3.6×10^{-3} M solution of **2d** was prepared in pH 7.5 HEPES buffer. A second stock solution containing Cu(triflate)$_2$ in HEPES was prepared such that the solution contained twice the desired concentration of Cu^{2+}. Aliquots (1.0 mL) of the copper stock solution were removed and placed into test tubes. The hydrolysis was initiated by the addition of 1.0 mL of the ester stock solution into the test tubes. At timed intervals, a 66 μL aliquot was removed and added to 66 μL of eluent B containing 1.0×10^{-5} M *p*-nitrophenol (used as the standard). The resulting solution was mixed with shaking and injected into the HPLC for analysis. The progress of the hydrolysis reaction was followed using separate runs for each time point by adding the ester stock solution to the copper stock solution. The appearance of acid **2c** was monitored relative to the internal standard.

The hydrolysis of **3d** was monitored by a procedure similar to the one described for **2d** but with the following changes. Elution was carried out with eluent C and nitrobenzene was used as the internal standard. The appearance of acid **3c** was monitored relative to the internal standard.

The following general procedure was followed to monitor the hydrolyses of **3b**, **4a**, and **4e**. A 1.8 mM solution of ester or 2.0 mM solution of the amide was prepared by dissolving a weighed sample of the ester or amide into 100.0 mL of pH 7.50 HEPES (0.1 M) buffer. 2,4,6-Collidine was added to the solution for use as an internal standard. The hydrolysis reaction was initiated by the addition of the appropriate metal perchlorate salt. At timed intervals, a 10 mL aliquot was removed and added to an 0.1 M EDTA solution (2.0 mL). The resulting solution was diluted to 20 mL with water, and NaOH solution was added until pH > 10 (pH paper). This solution was extracted with chloroform (2 × 20 mL), the chloroform was removed *in vacuo* and the residue was dissolved in $CDCl_3$. The ^1H NMR spectrum of the sample was obtained. The ratio of ester or amide to collidine was obtained by integration of the ester or amide peaks and the collidine peak at δ 6.8. The hydrolysis of **4a** to acid **4c** was confirmed by performing a preparative scale reaction and isolating the product.

Conjugate Addition Kinetic Method. The forward conjugate addition reaction (**5** + **7** → **2a**; Scheme 7) was followed in the following manner. A 1.8 mM solution of **7** was prepared in buffer and 80 mL was placed into a 100 mL flask. Methyl acrylate (155 μL, 10 equiv) was then added to initiate the reaction. At the desired time intervals, a 1.0 mL aliquot was withdrawn and added to 2.0 mL of eluent A containing 3.1×10^{-3} M chlorobenzene as an internal standard. The disappearance of **7** relative to the internal standard was monitored by HPLC as described above.

Conjugate Reversion Kinetic Method. The conjugate reversion reaction (**2c** → **20** + **7**; Scheme 7) was followed using 80 mL of buffer containing 1.8 mM **2c** and the appropriate concentration of metal salt. The flask was fitted with a condenser and deoxygenated by bubbling argon through the solution overnight. The condenser was fitted with a gas inlet adapter so that a steady stream of argon could constantly pass over the reaction solution. The reaction was heated in an oil bath at 100 °C and at various times an aliquot was removed and cooled to room temperature. A 1.0 mL aliquot of this solution was added to 2.0 mL of eluent A containing 3.1×10^{-3} M chlorobenzene and the formation of **7** was monitored by HPLC as described above.

Purification of *N'*-Benzyl-*N,N*-dimethylethylenediamine. A solution of 2-propanol (200 mL) and freshly distilled *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 9.22 g, 51.7 mmol) was heated to 60 °C with stirring and then concd HBr (18 mL) was added. The solution continued to stir for 3 min and was then removed from the heat. Upon cooling, a white solid formed that was collected by filtration and recrystallized by dissolving in hot methanol and then adding about 5% (v/v) diethyl ether. Upon cooling, colorless needles of the HBr salt formed (12.1 g, mp 198–200 °C). The free base of the amine was obtained by dissolving the salt (3.0 g, 8.8 mmol) into water (100 mL) and then adding KOH until solution pH > 11 (pH paper). The solution was extracted with $CHCl_3$ (3 × 30 mL), and the $CHCl_3$ layers were pooled and dried over $MgSO_4$. The $CHCl_3$ was evaporated *in vacuo* to give pure *N'*-benzyl-*N,N*-dimethylethylenediamine as a colorless oil: ^1H NMR ($CDCl_3$)

δ 7.27−7.14 (m, 5), 3.74 (s, 2), 2.63 (t, 2), 2.36 (t, 2), 2.14 (s, 6); ^{13}C NMR δ 140.2, 127.9, 127.7, 125.4, 58.8, 53.7, 46.4, 45.2.

Methyl 3-((2-(Dimethylamino)ethyl)benzylamino)propionate (2a). A solution of *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 4.2 g, 23 mmol) and methyl acrylate (**5**, 2.1 mL, 23 mmol) in methanol (30 mL) was stirred under argon for 48 h. The solution was then evaporated *in vacuo* to afford the product as a light oil in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 7.43 (m, 5), 3.78 (s, 2), 3.60 (s, 3), 2.81 (t, 2), 2.56−2.34 (m, 6), 2.32 (s, 6); ^{13}C NMR ($CDCl_3$) δ 172.8 (s), 139.1 (s), 128.5 (d), 127.9 (d), 126.7 (d), 58.7 (t), 57.4 (t), 51.6 (t), 51.2 (q), 49.6 (t), 45.7 (q), 32.3 (t). High resolution mass spectrum: calcd for $C_{15}H_{24}N_2O_2$, *m/e* 264.1837; measured, 264.1821 (M^+). Anal. Calcd for $C_{15}H_{24}N_2O_2$: C, 68.15; H, 9.15; N, 10.60. Found: C, 67.81; H, 8.94; N, 10.59.

Ethyl 3-((2-(Dimethylamino)ethyl)benzylamino)propionate (2d). A solution of *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 3.0 g, 17 mmol) and ethyl acrylate (**6**, 36 mL, 330 mmol) in ethanol (100 mL) was stirred under argon for 48 h. The solution was then evaporated *in vacuo* to afford the product as a light yellow oil in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 7.34−7.18 (m, 5), 4.11 (q, 2), 3.60 (s, 2), 2.84 (t, 2), 2.61−2.33 (m, 6), 2.18 (s, 6), 1.22 (t, 3): ^{13}C NMR ($CDCl_3$) δ 172.6 (s), 139.3 (s), 128.7 (d), 128.1 (d), 126.8 (d), 60.2 (t), 58.8 (t), 57.6 (t), 51.8 (t), 49.8 (t), 45.8 (q), 32.7 (t), 14.2 (q). High resolution mass spectrum: calcd for $C_{16}H_{26}N_2O_2$, *m/e* 278.1994; measured, 278.1999 (M^+). Anal. Calcd for $C_{16}H_{26}N_2O_2$: C, 69.02; H, 9.42; N, 10.07. Found: C, 69.14; H, 9.44; N, 9.94.

3-((2-(Dimethylamino)ethyl)benzylamino)propionic Acid (2c). A solution of *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 5.30 g, 29.8 mmol) and *tert*-butyl acrylate (**8**, 10.0 mL, 69 mmol) in methanol (150 mL) was stirred under argon for 48 h at rt. The solution was concentrated *in vacuo*, and trifluoroacetic acid (50 mL) and CH_2Cl_2 (100 mL) were added. The resulting solution was heated to reflux for 24 h, and the solution was evaporated *in vacuo* to afford a thick colorless oil. Water (150 mL) was added and the solution was evaporated *in vacuo*; this procedure was repeated until the CF_3-COOH quartets in the ^{13}C NMR did not decrease in intensity (5−10 evaporations): ^{1}H NMR (D_2O) δ 7.43−7.35 (m, 5), 4.33 (s, 2), 3.50 (bs, 4), 3.40 (t, 2), 2.79 (s, 6), 2.70 (t, 2); ^{13}C NMR (D_2O) δ 173.8 (s), 163.0 (qs), 130.9 (d), 130.6 (d), 129.9 (d), 116.2 (qs) 58.2 (t), 50.8 (t), 49.1 (t), 47.2 (t), 43.2 (q), 40.9 (s), 28.5 (t). High resolution mass spectrum: calcd for $C_{11}H_{14}NO_2$ (M − $CH_2N(CH_3)_2$)$^+$, *m/e* 192.1025; measured, 192.1033. Anal. Calcd for $C_{18}H_{24}F_6O_6N_2$: C, 45.19; H, 5.06; N, 5.86. Found: C, 45.38; H, 4.65; N, 5.77.

3-((2-(Dimethylamino)ethyl)benzylamino)propanenitrile (10). A solution of *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 5.42 g, 30.4 mmol) and acrylonitrile (20 mL) was heated in a pressure tube to 100 °C for 48 h and then allowed to cool to rt. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to afford **10** as an orange-yellow oil (5.34 g, 76%): ^{1}H NMR ($CDCl_3$) δ 7.34−7.23 (m, 5), 3.67 (s, 2), 2.85 (t, 2), 2.64 (t, 2), 2.43 (t, 4), 2.21 (s, 6); ^{13}C NMR ($CDCl_3$) δ 138.2 (s), 128.2 (d), 127.9 (d), 126.7 (d), 118.6 (s), 58.4 (t), 57.0 (t), 51.2 (t), 49.2 (t), 45.2 (d), 15.8 (t). High resolution mass spectrum: calcd for $C_{14}H_{21}N_3$, *m/e* 231.1730; measured, 231.1742.

3-((2-Dimethylamino)ethyl)benzylamino)propionamide (2b). Nitrile **10** (5.34 g, 23.1 mmol) was placed into a flask, and 3 M H_2SO_4 (10.0 mL) was added cautiously with stirring. Concentrated H_2SO_4 (30 mL) was then added slowly, the solution was stirred at rt for 2 h and then at 100 °C for 1 h. The solution was cooled to rt and then poured over crushed ice. The aqueous solution was made basic (pH > 11) with KOH and then diluted to approximately 800 mL with water. This solution was extracted with $CHCl_3$ (3 × 300 mL), and the $CHCl_3$ was dried over $MgSO_4$ and evaporated *in vacuo* to afford **2b** as a light yellow oil that crystallized upon standing (1.20 g, 21%): ^{1}H NMR ($CDCl_3$) δ 8.6 (bs, 1), 7.34−7.22 (m, 5), 5.20 (bs, 1), 3.55 (s, 2), 2.70 (t, 2), 2.49−2.41 (m, 4), 2.30 (t, 2), 2.04 (s, 6); ^{13}C NMR ($CDCl_3$) δ 175.3 (s), 138.4 (s), 129.2 (d), 128.3 (d), 127.2 (d), 58.7 (t), 56.6 (t), 51.0, 49.2 (t), 45.0 (q), 33.3 (t). High resolution mass spectrum: calcd for $C_{14}H_{23}N_3O$, *m/e* 249.1836; measured, 249.1873. Anal. Calcd for $C_{14}H_{23}N_3O$: C, 67.44; H, 9.30; N, 16.85. Found: C, 67.55; H, 9.09; N, 16.81.

***cis*- and *trans*-Ethyl 2-((2-(Dimethylamino)ethyl)amino)cyclohexane-1-carboxylate (15).** A solution of ethyl 2-oxocyclohexanecarboxylate (**12**, 8.56 g, 50 mmol), ethanol (100 mL), 5 N HCl in EtOH (20 mL), and *N,N*-dimethylethylenediamine (4.40 g, 35 mmol) was stirred at rt for 2 h. Sodium cyanoborohydride (440 mg, 7 mmol) was then added and stirring was continued at rt for 24 h. The heterogeneous reaction mixture was filtered, and the filtrate was acidified to pH < 2 with concd HCl. The solution was evaporated *in vacuo*, and the residue was dissolved in distilled water (20 mL) and washed with $CHCl_3$ (3 × 13 mL). The aqueous layer was made basic (pH > 10) with KOH and then extracted with $CHCl_3$ (3 × 13 mL). The $CHCl_3$ layers were combined, dried over anhyd $MgSO_4$, and evaporated *in vacuo* to afford a yellow oil consisting of *cis* and *trans* isomers of **15** (1.42 g, 58%): ^{1}H NMR ($CDCl_3$) δ 4.10 (q, 2), 2.91−2.48 (m, 4), 2.32 (t, 2), 2.14 (s, 6), 2.11−1.36 (m, 8), 1.20 (t, 3); ^{13}C NMR ($CDCl_3$) δ 175.6 (s), 174.6 (s), 60.1 (t), 59.9 (t), 59.6 (t), 59.3 (t), 58.2 (d), 56.1 (d), 50.5 (d), 45.6 (d), 45.5 (q), 45.3 (q), 45.1 (t), 44.4 (t), 31.9 (t), 29.2 (t), 25.3 (t), 25.1 (t), 24.7 (t), 23.7 (t), 22.2 (t), 14.3 (q). Mass spectrum 241.0 (M^+ − 1). Anal. Calcd for $C_{13}H_{26}N_2O_2$: C, 64.43; H, 10.81; N, 11.56. Found: C, 64.29; H, 10.80; N, 11.49.

***cis*- and *trans*-2-((2-(Dimethylamino)ethyl)amino)cyclohexane-1-carboxamide (16).** 2-Oxocyclohexanecarboxamide (**13**; 14.1 g, 10.0 mmol) was placed into a 250 mL flask with methanol (100 mL), and then *N,N*-dimethylethylenediamine (8.90 g, 10.0 mmol) was added. 5 N HCl in MeOH was added dropwise with stirring until the solution pH was 6−7 (pH paper). The reaction mixture was stirred at rt for 2 h, and then sodium cyanoborohydride (2.55 g, 40 mmol) was added and the resulting solution was stirred at rt for an additional 24 h. The solution was acidified to pH ~ 2 with 1 M HCl and evaporated *in vacuo*, and the residue was dissolved in water (50 mL). The aqueous solution was washed with $CHCl_3$ (3 × 50 mL) and then made basic (pH > 10) with KOH. This solution was extracted with $CHCl_3$ (3 × 50 mL) and dried over $MgSO_4$, and the $CHCl_3$ was evaporated *in vacuo* to afford **16** as a yellow oil consisting of *cis* and *trans* isomers (18.2 g, 80%). The mixture was separated into two components by column chromatography on neutral alumina using 5:1 $CHCl_3$/methanol as eluent. The major product was isolated as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 9.3 (br s, 1), 5.7 (br s, 1), 2.9−2.2 (m, 6), 2,2 (s, 6), 1.7−1.3 (m, 8); ^{13}C NMR ($CDCl_3$) δ 177.9, 59.1, 57.8, 50.2, 45.3, 43.8, 32.4, 29.2, 25.4, 25.0. The minor product was a white solid: ^{1}H NMR ($CDCl_3$) δ 7.6 (bs, 1), 5.8 (bs, 1), 2.88−2.80 (m, 1), 2.69−2.59 (m, 2), 2.49−2.34 (m, 3), 2.20 (s, 6), 2.15−2.00 (m, 3), 1.90−1.68 (m, 1), 1.65−0.90 (m, 4).

***trans*-Ethyl 2-(*N*-Benzyl-*N*-(2-(dimethylamino)ethyl)-amino)cyclohexane-1-carboxylate Hydrochloride (3d).** A solution of benzaldehyde (1.86 g, 17.6 mmol), ethanol (20 mL), 5 N HCl in EtOH (1.2 mL), and ethyl 2-((2-(dimethylamino)-ethyl)amino)cyclohexane-1-carboxylate (**15**, 1.42 g, 5.7 mmol) was heated at reflux for 24 h. The reaction was cooled to rt and then sodium cyanoborohydride (440 mg, 7 mmol) was added, and the solution was stirred at rt for 72 h. The resulting heterogeneous reaction mixture was filtered, and the filtrate was acidified to pH < 2. The solution was evaporated *in vacuo* and the residue dissolved in 1 M HCl (20 mL) and washed with CH_2Cl_2 (8 × 7 mL). The aqueous layer was adjusted to pH ~ 2 with KOH and extracted with $CHCl_3$ (3 × 7 mL). The $CHCl_3$ layers were combined, dried over anhyd $MgSO_4$, and then evaporated *in vacuo* to afford a clear oil that crystallized upon standing. The solid was recrystallized from 1:1 benzene:ether (0.27 g, 13%): ^{1}H NMR ($CDCl_3$) δ 11.80 (br s, 1), 7.40−7.20 (m, 5), 4.15 (q, 2), 3.85 (d, 1), 3.40 (d, 1), 3.10−2.70 (m, 6), 2.70−2.40 (m, 6), 2.10−1.40 (m, 8), 1.30 (t, 3); ^{13}C NMR ($CDCl_3$) δ 175.7 (s), 139.3 (s), 129.1 (d), 128.4 (d), 127.4 (d), 62.0 (d), 60.3 (t), 56.5 (t), 54.6 (t), 48.5 (d), 44.9 (t), 44.0 (broad), 42.0 (broad), 29.9 (t), 25.1 (t), 24.0 (t), 14.0 (q). High resolution mass spectrum: calcd for $C_{20}H_{32}N_2O_2$, (**3d**·HCl) *m/e* 332.2465; measured, 332.2465 (M^+). Anal. Calcd for $C_{20}H_{33}N_2O_2Cl$: C, 65.11; H, 9.02; N, 7.59. Found: C, 64.91; H, 9.06; N, 7.50.

Free Amine Form of 3d. The hydrochloride salt of **3d** (0.50 g, 1.4 mmol) was dissolved in water (20 mL), basified with KOH (pH > 11), and then extracted with $CHCl_3$ (3 × 5

mL). The $CHCl_3$ layers were combined and dried over $MgSO_4$, and the $CHCl_3$ was removed *in vacuo* to afford the free amine form of **3d** as a clear oil (0.46 g, 95%): ^1H NMR ($CDCl_3$) δ 7.30–7.15 (m, 5), 4.13 (m, 2), 2.80 (m, 1), 2.69–2.29 (m, 5), 2.10 (s, 6), 2.01–1.40 (m, 8), 1.24 (t, 3); ^{13}C NMR ($CDCl_3$) δ175.3 (s), 140.5 (s), 128.6 (d), 127.9 (d), 126.6 (d), 61.6 (d), 60.0 (t), 58.8 (t), 54.6 (t), 48.6 (d), 48.3 (t), 45.7 (q), 29.7 (t), 25.4 (t), 25.2 (t), 24.2 (t), 14.1 (q). High resolution mass spectrum: calcd for $C_{20}H_{32}N_2O_2$, *m/e* 332.2465; measured, 332.2465 (M^+).

***cis*-2-(*N*-Benzyl-*N*-(2-d(imethylamino)ethyl)amino)cyclohexane-1-carboxamide Hydrochloride (3b).** The crude 2-((2-(dimethylamino)ethyl)amino)cyclohexane-1-carboxamide (**16**, 22.5 g, 100 mmol) and benzaldehyde (16.9 g, 100 mol) were dissolved in methanol (100 mL), and 5 N HCl in methanol was added dropwise until pH 6–7 (pH paper). Sodium cyanoborohydride (2.61 g, 0.415 mmol) in methanol (10 mL) was added to the stirred solution followed by 3 Å molecular sieves (5 g), and the resulting solution was stirred at rt for 24 h. The reaction mixture was filtered, the filter cake was washed with CH_2Cl_2, and then the combined organic layers were evaporated *in vacuo*. The residue was dissolved in 1 M HCl and washed with CH_2Cl_2 (5 × 50 mL). The pH of the aqueous solution was then adjusted to 4–5 with KOH and extracted with CH_2Cl_2 (5 × 50 mL). The combined extracts were dried over $MgSO_4$, and the CH_2Cl_2 was evaporated *in vacuo* to afford *cis* and *trans* isomers of **3b** (13 g, 40%). The major component (cis isomer) was isolated as a yellow oil by column chromatography on neutral alumina using 5:1 $CHCl_3$/methanol as eluent: ^1H NMR ($CDCl_3$) δ 9.1 (br s, 1), 7.42–7.30 (5, m), 5.51 (br s, 1), 3.75 (s, 2), 2.95 (1, m), 2.78–2.68 (t, 2), 2.60–2.20 (m, 4), 2.18 (s, 6), 1.96–1.13 (m, 8); ^{13}C NMR ($CDCl_3$) δ 176.1 (s), 139.1 (s), 129.1 (d), 128.5 (d), 127.2 (d), 63.5 (d), 56.4 (t), 55.6 (t), 47.2 (t), 45.0 (q), 42.4 (d), 28.1 (t), 26.0 (t), 25.9 (t), 21.8 (t); FAB mass spectrum, *m/e* 304.15 (M^+ − HCl + 1). Anal. Calcd for $C_{18}H_{29}N_3O$·HCl·0.25 H_2O: C, 62.77; H, 8.93; N, 12.20; Cl, 10.29. Found: C, 63.06; H, 8.78; N, 12.17; Cl, 10.24.

***trans*-2-(*N*-Benzyl-*N*-(2-(dimethylamino)ethyl)amino)cyclohexane-1-carboxylic Acid (3c).** A solution of **3d** (0.10 g, 0.27 mmol) and 15 drops of saturated aqueous NaOH in ethanol (20 mL) was heated to 78 °C under argon for 20 h. The crude reaction mixture was diluted with water (75 mL) and washed with $CHCl_3$ (2 × 20 mL). The aqueous layer was neutralized to pH 7, loaded onto a CM-25 Sephadex ion exchange column, and eluted with 0.05 M aqueous NH_4HCO_3. The appropriate fractions were combined and lyophilized to afford a white solid (**3c**, 66 mg, 80%): ^1H NMR (D_2O) δ 7.45–7.20 (m, 5), 4.20 (d, 1), 3.67 (d, 1), 3.25–2.88 (m, 6), 2.50 (s, 6), 2.20–1.00 (m, 8); ^{13}C NMR (D_2O) δ 182.2 (s), 133.8 (s), 130.2 (d), 129.4 (d), 129.3 (d), 63.3 (d), 53.6 (t), 53.3 (t), 45.6 (d), 43.4 (t), 42.8 (q), 29.3 (t), 24.9 (t), 24.6 (t), 23.0 (t). High resolution mass spectrum: calcd for $C_{18}H_{29}N_2O_2$ (M^+ + H), *m/e* 305.2229; measured, 305.2217 (M^+ + H).

Methyl 2-(((2-(Dimethylamino)ethyl)benzylamino)methyl)-3-((2-(dimethylamino)ethyl)benzylamino)propionate (4a). Methyl 2-(bromomethyl)acrylate (**17**, 5.74 g, 32.1 mmol) was dissolved in methanol (25 mL) and *N'*-benzyl-*N,N*-dimethylethylenediamine (**7**, 12.5 g, 70.4 mmol) in methanol (25 mL) was added slowly with stirring. Some heat was evolved during mixing. The flask was wrapped in tin foil and stirred under argon for 6 d. Removal of methanol *in vacuo* gave a thick yellow oil, which was dissolved in $CHCl_3$ (50 mL) and washed with pH 7.0 phosphate (0.1 M) buffer (5 × 50 mL). The $CHCl_3$ was dried over Na_2SO_4, filtered, and removed *in vacuo* to afford **4a** as a yellow oil (7.54 g, 54%): R_f 0.51(alumina TLC); ^1H NMR ($CDCl_3$) δ 7.27 (m), 3.69 (d, J = 13 Hz, 2), 3.66 (s, 3), 3.47 (d, J = 13 Hz, 2), 3.05–2.15 (m, 13), 2.15 (s, 12); ^{13}C NMR ($CDCl_3$) δ 175.1 (s), 139.2 (s), 128.7 (d), 128.0 (d), 126.8 (d), 59.2 (t), 57.4 (t), 55.3 (t), 52.1 (t), 51.2 (q), 45.8 (q), 44.0 (d). High resolution mass spectrum: calcd for $C_{27}N_4H_{42}O_2$, *m/e* 454.3308; measured, 454.3345. Anal. Calcd for $C_{27}H_{42}N_4O_2$·0.3H_2O: C, 70.49; N, 12.18; H, 9.33. Found: C, 70.48; N, 12.08; H, 9.15.

***N*-Methyl-2-(((2-(dimethylamino)ethyl)benzylamino)methyl)-3-((2-(dimethylamino)ethyl)benzylamino)propionamide (4e).** A solution of **4a** (1.00 g, 2.22 mmol) in methanol (2 mL) was added to aqueous methylamine (10.0 mL, 40% wt solution) in a 50 mL flask. The flask was capped, and the cloudy yellow solution was stirred for 14 d. The solution was concentrated *in vacuo* to a yellow oil. The oil was dissolved in water (20 mL) and extracted with $CHCl_3$ (3 × 20 mL), and the $CHCl_3$ extracts were dried over Na_2SO_4. Removal of $CHCl_3$ *in vacuo* afforded a yellow oil that was eluted down a column of basic alumina with $CHCl_3$. The $CHCl_3$ fractions were pooled, and the $CHCl_3$ was removed *in vacuo* to afford **4e** as a yellow oil (336 mg, 33%): ^1H NMR ($CDCl_3$) δ 8.60 (br s, 1), 3.62 (d, 2), 3.45 (d, 2), 2.82 (m, 1), 2.70 (d, 3), 2.60–2.14 (m, 12), 2.10 (s, 12); ^{13}C NMR ($CDCl_3$) δ 174.7, 139.4, 128.9, 128.1, 126.9, 59.7, 57.2, 54.8, 52.2, 45.5, 42.0, 25.9. High resolution mass spectrum: calcd for $C_{27}H_{43}N_5O$, *m/e* 453.3467; measured, 453.3456. Anal. Calcd for $C_{27}H_{43}N_5O$·0.75 H_2O: C, 69.42; H, 9.60; N, 14.99. Found: C, 69.52; H, 9.41; N, 14.80.

2-(((2-Dimethylamino)ethyl)benzylamino)methyl)-3-((2-(dimethylamino)ethyl)benzylamino)propionic acid (4c). A solution of **4a** (308 mg, 0.674 mmol) in ethanol (2 mL) was added to water (10 mL) along with aqueous NaOH (10 N, 15 drops). The cloudy solution was refluxed under argon for 36 h, cooled to room temperature, and neutralized to pH 7 (pH paper) with concd HCl. This solution was loaded onto a CM-25 Sephadex anion exchange column and eluted with a linear gradient (0.05 M to 0.6 M) of aqueous NH_4HCO_3. The appropriate fractions were combined, and the solution volume was reduced *in vacuo* and lyophilized. The acid **4c** was isolated in virtually quantitative yield as a clear, colorless oil determined by microanalysis to be a bicarbonate salt: ^1H NMR (D_2O) δ 7.20 (s, 10), 3.72 (d, 2), 3.29 (d, 2), 3.15–2.58 (m, 8), 2.3 (s, 6), 2.23–2.52 (m, 5); ^{13}C NMR (D_2O) δ 189.2 (s), 137.9 (s), 129.9 (d), 128.6 (d), 127.6 (d), 58.0 (t), 55.0 (t), 54.0 (t), 48.7 (t), 45.3 (s), 43.1 (q); FAB mass spectrum *m/e* 441.34 (M^+ + 1). Anal. Calcd for $C_{26}H_{40}N_4O_2$·H_2CO_3: C, 64.23; N, 11.42; H, 8.72. Found: C, 64.52; N, 11.15; H, 8.42.

Hydrolysis of 4a with Cu^{2+}. Compound **4a** (197 mg, 0.434 mmol) was dissolved in 100 mL of pH 7.50 HEPES (0.1 M) buffer, and $Cu(ClO_4)_2$·$6H_2O$ (81 mg, 0.22 mmol, 0.5 eq) was added. The blue solution was stirred for 1 h, and then 4 mL of aqueous EDTA (0.1 M) was added. The solution was lyophilized to yield a blue solid and was dissolved in water (20 mL). The resulting blue solution was passed down a cation exchange column (CM-25 Sephadex) eluted with a linear gradient (0.05 M to 0.6 M) of aqueous NH_4HCO_3. The appropriate fractions were combined and the solution volume was reduced *in vacuo* and lyophilized. A colorless oil (116 mg) was obtained whose ^1H NMR corresponded to that of **4c**.

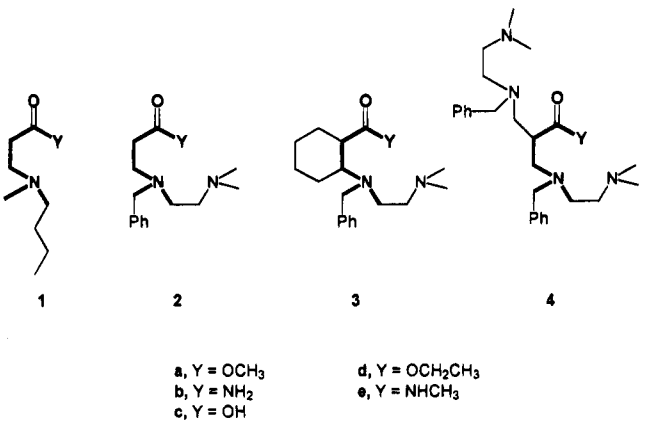


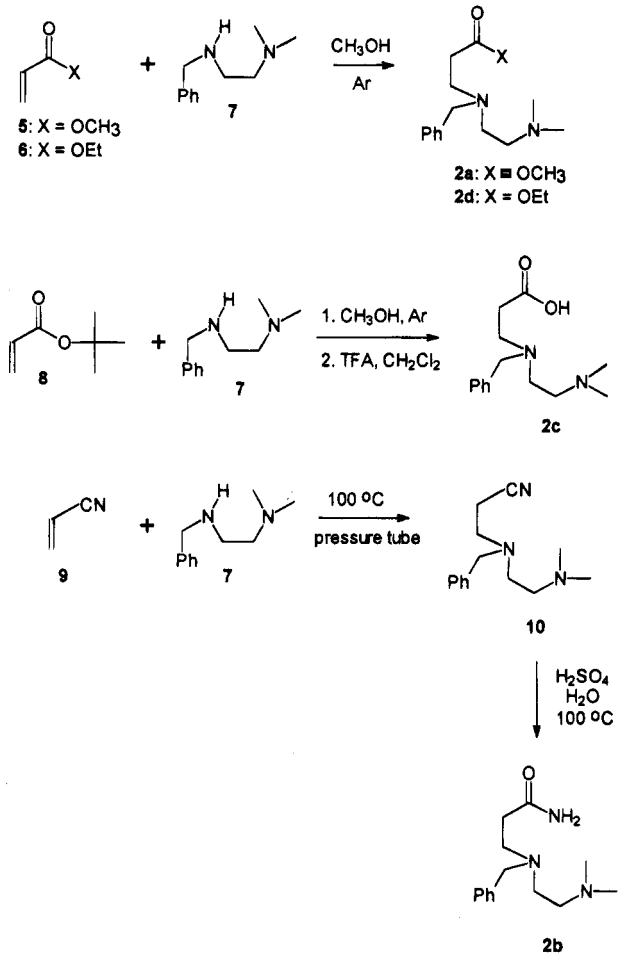
Figure 1.

Results and Discussion

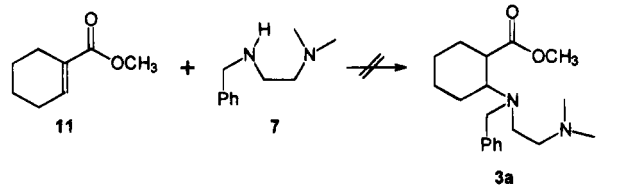
Synthesis. Ester **1a** and amide **1b** were prepared according to the reported procedures.[4] The conjugate addition reactions of butylmethylamine with methyl acrylate and acrylamide occurred under mild reaction conditions and in quantitative yield.

The addition of *N*-benzyl-*N,N*-dimethylethylenediamine (**7**) to methyl acrylate (**5**) was carried out by

Scheme 2



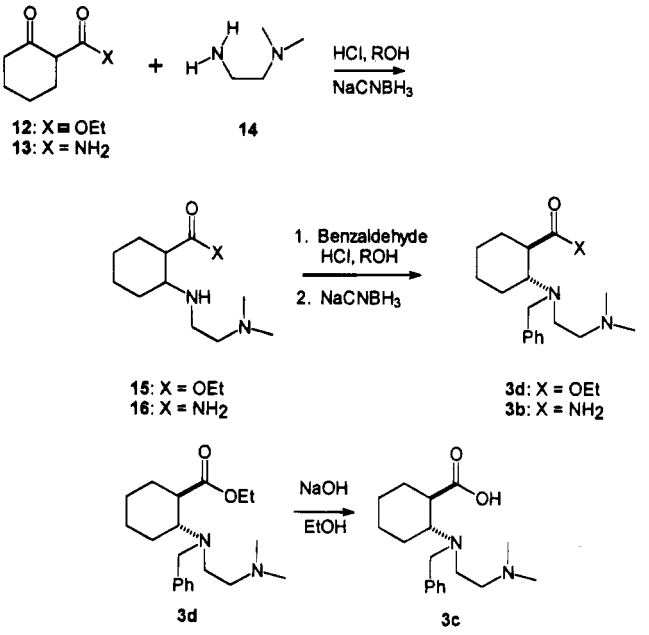
Scheme 3



stirring the reactants under argon in methanol for 48 h and afforded ester **2a** in quantitative yield (Scheme 2). Ethyl ester **2d** was prepared in the same manner using ethyl acrylate (**6**) as the Michael acceptor and ethanol as the solvent. Carboxylic acid **2c** was synthesized and isolated as its triflate salt by the reaction of *tert*-butyl acrylate (**8**) with **7** followed by deprotection of the resulting ester with trifluoroacetic acid. Amide **2b** was prepared in two steps. *N*-Benzyl-*N'*,*N'*-dimethylethylenediamine (**7**) was dissolved in neat acrylonitrile (**9**) and heated to 100 °C in a pressure tube for 48 h. Nitrile **10** was isolated by removal of unreacted **9** under high vacuum. Compound **10** was then hydrated with aqueous H_2SO_4 to give amide **2b**.

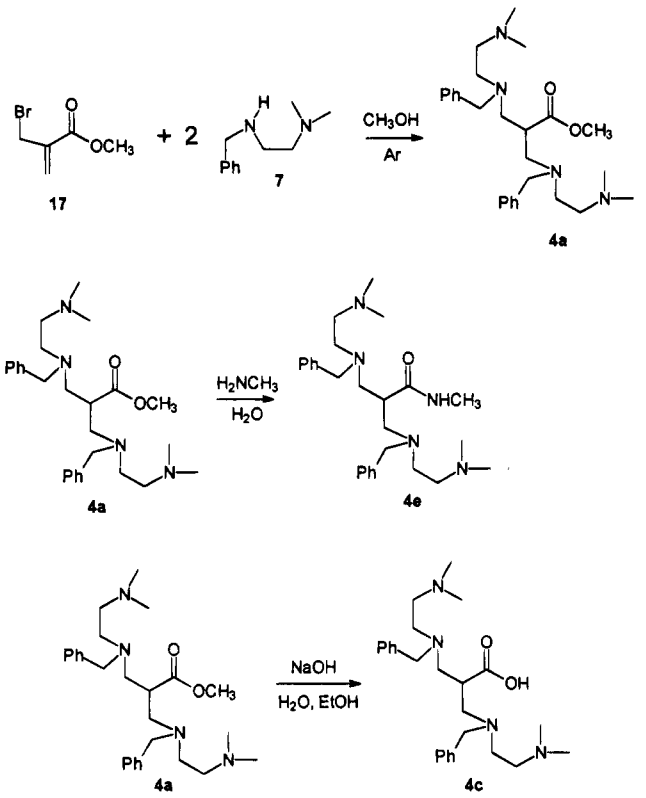
The synthesis of cyclic ester **3a** proved to be surprisingly difficult. Initially, we attempted to prepare **3a** by the reaction of **7** with methyl 1-cyclohexene-1-carboxylate (**11**) (Scheme 3). However, cyclic ester **11** proved to be unreactive toward **7** under a wide variety of reaction conditions. This was in contrast to the rapid reaction of the acrylate esters **5** and **6** with **7**. Ester **11** also proved unreactive toward *N*,*N*-dimethylethylenediamine; no 1,4 addition products were ever observed. Our attempts to add the lithium amide of **7** (generated using BuLi or LDA as base) to **11** resulted in 1,2-addition to yield an amide as product.

Scheme 4



The apparent nonreactivity of **11** as a Michael acceptor precluded its use in a catalytic cycle such as the one described in Scheme 1. Nonetheless, we felt that a cyclic ester such as **3d** appeared to be a suitable model to test for the role of enforced proximity of the catalytic group on the rate of ester hydrolysis. Consequently, we prepared cyclic ester **3d** by a reductive amination sequence that is described in Scheme 4. Equimolar amounts of ethyl 2-oxocyclohexanecarboxylate (**12**) and *N*,*N*-dimethylethylenediamine (**14**) were stirred in acidic ethanol for 2 h followed by the addition of $NaCNBH_3$ and stirring for an additional 24 h. After acidic workup and extraction into $CHCl_3$, **15** was isolated as a mixture of *cis* and *trans* isomers in 58% yield. The reaction yield was found to be highly dependent on the pH of the solution. A solution whose pH was between 6 and 7 (determined by pH paper) gave optimum yields. When the solution pH dropped below 6, reduction of the ketone to the corresponding alcohol became favored. The isomeric mixture of **15** was stirred in acidic ethanol with an excess of benzaldehyde at reflux for 24 h. Addition of $NaCNBH_3$ and stirring at room temperature for 72 h followed by an acidic workup afforded crude **3d**. To our surprise, **3d** extracted out of aqueous pH 2 solution as its HCl salt and was isolated as a clear oil that crystallized upon standing. Recrystallization from 1:1 benzene:ether afforded crystals that were suitable for X-ray crystallographic analysis (structure available as supporting information). From the X-ray structure, the stereochemistry on the cyclohexane ring of **3d** was shown to be *trans*. An insoluble solid that was isolated (by filtration) during the recrystallization of **3d** was determined to be the *cis* isomer by ^1H NMR analysis. One distinguishing feature of the ^1H NMR spectra of the *cis* and *trans* isomers of **3d** is the difference in the chemical shifts of the diastereotopic benzylic protons. The benzylic protons of the *trans* isomer appear as doublets at δ 3.40 and 3.85 ($CDCl_3$), whereas the benzylic protons of the *cis* isomer appear as a broad singlet at δ 3.76. Cyclic amide **3b** was prepared via the two-step sequence shown in Scheme 4. 2-Oxocyclohexanecarboxamide (**13**) was prepared according to the literature procedure[5] and subjected to reductive

Scheme 5

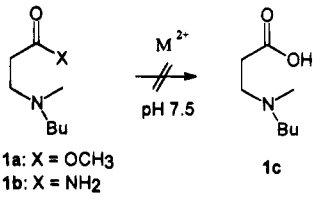


amination with *N,N*-dimethylethylenediamine and $NaCNBH_3$ in methanol. The resulting mixture of *cis* and *trans* isomers of **16** was reacted with benzaldehyde and $NaCNBH_3$ in methanol to afford a mixture of *cis* and *trans* isomers of **3b**. Microanalysis indicated that **3b** was isolated as its HCl salt. Our attempts to crystallize **3b**, and hence determine its stereochemistry by X-ray analysis, were not successful. Cyclic carboxylic acid **3c** was prepared by the saponification of **3d** in NaOEt/EtOH and purified by ion exchange chromatography.

The bis-ethylenediamine ester **4a** was prepared from methyl 2-(bromomethyl)acrylate[6] and **7** (Scheme 5). A solution of **17** and **7** in methanol was stirred, under argon, for 4 days. Removal of the methanol *in vacuo* afforded **4a** as a yellow oil contaminated with unreacted **7** as well as a minor side product. Column chromatography on basic alumina removed the side product but failed to separate unreacted **7** from **4a**. The purification of **4a** was achieved by washing a chloroform solution of the impure ester repeatedly with pH 7 phosphate buffer until all impurities were removed from the solution, as determined by ^1H NMR.[7] The homologous amide **4e** was prepared by stirring a solution of **4a** and methanol in aqueous methylamine for 14 days. Column chromatography on basic alumina afforded pure amide in 33% yield. The ester was saponified to the carboxylic acid (**4c**) in refluxing aqueous solution. Compound **4c** was purified by ion exchange chromatography and isolated as a bicarbonate salt.

Kinetics. Scheme 1 illustrates a formal catalytic cycle for the metal cation-catalyzed hydrolysis of esters and amides by coordination of a metal ("cat") by a β-amino group. The reversible conjugate addition of butylmethylamine to methyl acrylate and acrylamide allows for the possibility of metal cation catalyzed hydrolysis of **1a** and **1b** via the coordination of a metal to the β-amino group. The hydrolysis reactions of **1a** and **1b** at pH 7.5 and room temperature were monitored in the presence of Cu^{2+}, Ni^{2+}, Co^{2+} and in the absence of any metal ion by following loss of the ester or amide using ^1H NMR (Scheme 6). No rate accelerations were observed in the hydrolyses of **1a** or **1b** upon the addition of these metal ions. This result was surprising to us in view of the well known metal promoted hydrolysis reactions of α-amino acid esters and amides.[1a-c] α-Amino acid esters and amides form strong complexes with several metal ions through the formation of five-membered chelates that undergo accelerated hydrolysis. The observed nonreactivity of **1a** and **1b** toward hydrolysis using metal cations may result from weak complexation to the ion. While divalent metal cations form stable five-membered chelates to α-amino acid esters, they form more weakly bound six-membered chelates with β-amino acid esters. As a result, we chose to investigate the *N'*-benzyl-*N,N*-dimethylethylenediamine moiety as a more suitable ligand for divalent metal cations.

Scheme 6



The reactions that are described in Scheme 7 were effected entirely in pH 7.5 buffer. The conjugate addition of **7** to **5** was monitored by following the loss of **7** with reverse-phase HPLC. We have previously reported that the second-order rate constant for the reaction between **7** and **5** under these conditions is $k_1 = 2.1 \times 10^{-3}$ M^{-1} s^{-1} at 23 °C.[3c] The conjugate reversion reaction of **2c** was monitored by following the appearance of **7** by HPLC. The reaction at 23 °C was too slow to measure conveniently and as a result, first-order rate constants were measured at 100 °C. At pH 7.5, $k_2 = 0.086 \times 10^{-3}$ min^{-1} ($t_{1/2}$ = 8000 min) when [**2c**] = 1.8 mM. Figure 2 shows the effects of pH on the reaction rate. The retro-Michael reaction of **2c** shows a 10-fold increase in rate between pH 1.0–3.0. Specific acid catalysis of amine conjugate reversion reactions are well known[8] and would account for an increase in rate as the pH decreases toward the amine's pK_a, but the rate decrease at pH < 3.0 was somewhat surprising to us. The rate drop is not due to the buffer; for instance, $k_2 = 11.3 \times 10^{-3}$ min^{-1} in both pH 2.5 citric acid/KOH buffer and pH 2.5 H_3PO_4/KOH buffer. The rate change likely arises from a change in the extent of protonation of **2c**. Below a pH of about 3, both amines are likely to be protonated. As the pH is increased from 1.0 to 3.5, the concentration of the monocationic form of **2c** will increase and consequently, the probability of intramolecular general base catalysis by the dimethylamino group increases (Figure 3). As the pH becomes greater than 3.5, the acid group deprotonates, lowering the acidity of the α-proton.

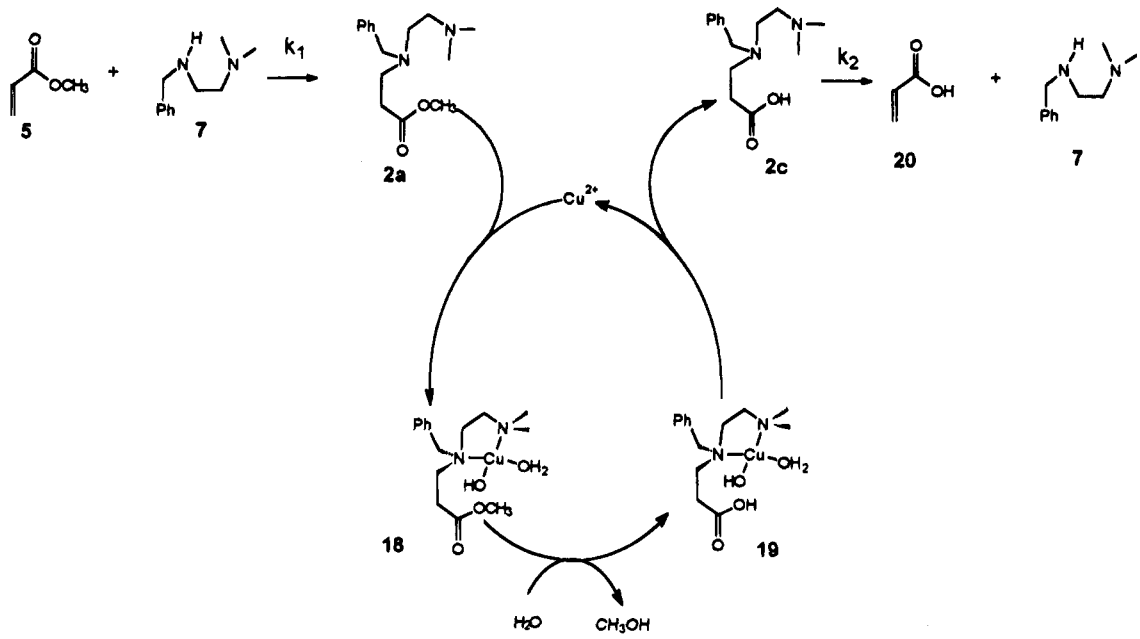
(5) U.S. Patent 4169952, 1979.

(6) Methyl 2-(bromomethyl)acrylate is commercially available from Aldrich but its high cost led us to prepare gram quantities by the literature procedures: (a) Cassady, J. M.; Howie, J.; Robinson, M.; Stamos, I. K. *Org. Synth. 61*, 77. (b) Charlton, J. L.; Sayeed, V. A.; Lypka, G. N. *Synth. Commun.* **1981**, *11*, 931.

(7) The purification of many different polyamine substituted organic molecules by selective pH extraction has worked numerous times for us, and we recommend it as an easy alternative to time-consuming chromatographic methods.

(8) (a) Andrus, A.; Heck, J. V.; Christensen, B. G.; Partridge, B. *J. Am. Chem. Soc.* **1984**, *106*, 1808. (b) Fukuyama, T.; Yang, L. *Ibid.* **1987**, *109*, 7881. (c) Guidon, Y.; Delorme, D. *Can. J. Chem.* **1987**, *65*, 1438.

Scheme 7



In order to evaluate the potential effects of metal ions on the conjugate reversion reaction, a brief study of the reaction of **2c** in the presence of 2 equiv of Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} was conducted at pH 7.5 and 100 °C. In all of the reactions, little (<15%) or no rate acceleration was observed. Intuitively, one would expect that the coordination of the β-amine to a metal would increase the propensity for the expulsion of the *N'*-benzyl-*N,N*-dimethylethylenediamine group. However, at 100 °C the binding of **2c** to a metal cation is expected to be weak and the concentration of the metal complex to be negligibly small.

First-order rate constants for the hydrolysis of **2a** were measured by following loss of the ester or the appearance of the acid **2c** by reverse-phase HPLC. Preliminary kinetic results have been presented in communication

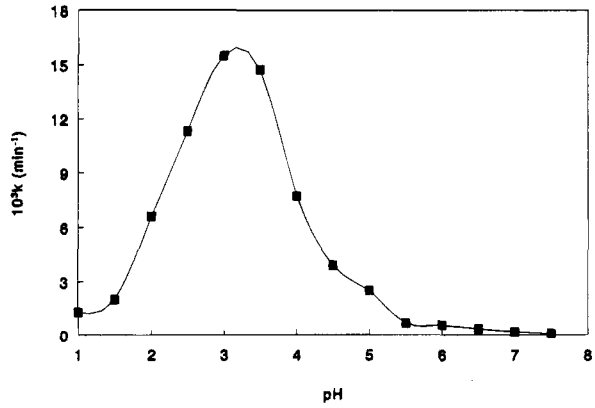


Figure 2. pH-Rate profile for the conjugate reversion reaction of **2c** at 100 °C.

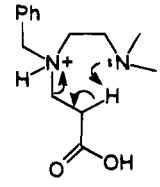


Figure 3. Proposed ionic form of compound **2c** responsible for its accelerated conjugate reversion at pH 3.

Table 1. Rate Constants for the $Cu(ClO_4)_2$-Catalyzed Hydrolysis of 1.8 mM 2a at pH 7.5 and 23 °C

equiv of metal	$k_{obs} \times 10^3$ (min^{-1})	k_{obs}/k_{uncat}
–	0.062	1.0
0.01	0.38	6.1
0.10	2.9	47
0.25	17	270
0.50	88	1400
1.0	370	6000
2.0	820	13000
3.0	770	12000
4.0	890	14000
5.0	950	15000

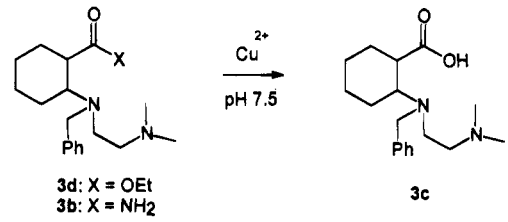
form.[3c] In the absence of any metal ion, the hydrolysis of 1.8 mM **2a** proceeded at pH 7.50 and 23 °C with $k_{obs} = 6.2 \times 10^{-5}$ min^{-1} ($t_{1/2}$ = 11 000 min). The rate constants were measured over a range of Cu^{2+} concentrations and are presented in Table 1. The hydrolysis rate for the fully complexed ester ($k_{obs} = 9.5 \times 10^{-1}$ min^{-1} at 5 equiv of Cu^{2+}) is 15 400 times faster than the same reaction without added metal. At 0.01 equiv of Cu^{2+}, $k_{obs} = 3.8 \times 10^{-4}$ min^{-1} and the reaction proceeded to >90% completion while following first-order kinetics. Hence, the hydrolysis of **2a** is catalytic with respect to Cu^{2+}.

The Cu^{2+}-catalyzed hydrolysis of amide **2b** has also been described by this group in preliminary form.[3d] Without added metals, the hydrolysis of **2b** (8 mM) is too slow to measure at pH 7.50; no (<2%) hydrolysis had occurred after 650 h at 50 °C. With 1 equiv of Cu^{2+} at pH 7.5 and 23 °C, the hydrolysis of **2b** (2 mM) occurs to at least 97% completion with $k_{obs} = 4.7 \times 10^{-5}$ min^{-1} ($t_{1/2} = 1.0 \times 10^1$ d); with 2 equivalents of Cu^{2+}, $k_{obs} = 1.3 \times 10^{-4}$ min^{-1} ($t_{1/2}$ = 3.7 d). As one basis for comparison, the hydrolysis of peptides in neutral aqueous solution at 25 °C has been measured as $k_{obs} = 3 \times 10^{-9}$ s^{-1} ($t_{1/2}$ = 7.3 yr).[9]

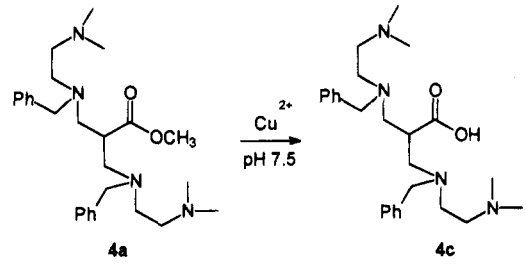
Significantly, the hydrolysis of **2b** is catalytic with respect to Cu^{2+}. With 0.25 equiv of Cu^{2+} at pH 7.5 and 50 °C, the hydrolysis of **2b** (8 mM) proceeded to 80% conversion to acid after 10 days; using 0.15 equiv of Cu^{2+} resulted in 74% conversion to acid in 15 days. While these reactions are not the fastest reported for amide

(9) Kahne, D. H.; Still, W. C. *J. Am. Chem. Soc.* **1988**, *110*, 7529.

Scheme 8



Scheme 9



hydrolysis, they represent one of the few examples of metal-*catalyzed* amide hydrolysis.[11l,m] Hence, the catalytic hydrolysis of **2b** with metal cations differs from the reported stoichiometric hydrolysis of some chelating amides.[1e,2a]

Cyclic vs Acyclic Models. The free rotation about the α,β bond of **2a** allows for the diamine group to rotate away from the ester group. This rotational freedom reduces the potential catalytic effects of the bound metal. Such free rotation is impossible in ester **3d**, in which the diamine group is forced into close proximity to the ester by the cyclohexane ring. The Cu^{2+}-catalyzed hydrolysis of **3d** (*trans*-isomer) was evaluated at pH 7.5 and 23 °C (Scheme 8) and compared to that of the acyclic ethyl ester **2d**. The formation of acids **2c** and **3c** were monitored by HPLC and found to follow first-order kinetics. The observed rate constants (k_{obs}) for the hydrolyses of **2d** and **3d** were measured at several different concentrations of $Cu(OTf)_2$ and the results are graphically represented in Figure 4. The hydrolysis of the acyclic ester **2d** appears to reach a maximum at around 3 equiv of Cu^{2+}, at which the ratio of catalyzed to uncatalyzed rate constants (k_{cat}/k_{uncat}) is 2000. This value is somewhat less than the k_{cat}/k_{uncat} = 15 400 for the fully complexed ester **2a**. It is possible that changing the leaving group from methyl to ethyl could result in this modest difference in the relative rate accelerations. The rate of hydrolysis of cyclic ester **3d** continues to increase gradually even up to 7 equiv of Cu^{2+}. Furthermore, the maximum rate acceleration measured, k_{cat}/k_{uncat} = 385, is considerably smaller than was found for **2d**. The differences in the rate accelerations between **2d** and **3d** are likely due to different binding affinities for Cu^{2+}, which we attribute to steric inhibition of chelation in cyclic ester **3d** as compared to **2d**.

The Cu^{2+}-catalyzed hydrolysis of cyclic amide **3b** (Scheme 8) was briefly examined and compared to that of acyclic amide **2b**. With 2 equiv of Cu^{2+} at pH 7.5 and 23 °C, the hydrolysis of **3b** occurs with $k_{obs} = 2.8 \times 10^{-4}$ min^{-1}. This rate constant is very similar to the one obtained for **2b** (1.3×10^{-4} min^{-1}).

Binuclear Metal Cation-Catalyzed Hydrolysis. Our interest in the observed rapid, metal-catalyzed hydrolysis of ester **2a** prompted us to investigate models that might demonstrate even larger rate accelerations. As a result, we have asked the following questions: If one bound metal cation can bring about rapid ester

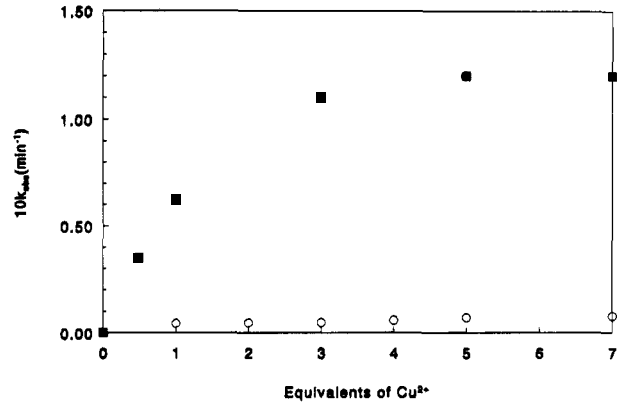


Figure 4. Rate constants for the Cu^{2+}-catalyzed hydrolysis of 1.8 mM **2d** (■) and **3d** (○) in 0.1 M HEPES, pH 7.5 at 23 °C.

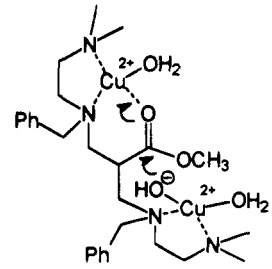


Figure 5.

hydrolysis (as was demonstrated with **2a**), how effective might two proximally bound metal cations be at catalyzing ester hydrolysis? Will the two metals work cooperatively, effecting a larger rate increase? Or, is the addition of a second bound metal cation superfluous? While cooperative promotion of phosphate hydrolysis by independent M^{3+} centers has been demonstrated,[10] there are few monomeric models that test for an advantage by a preorganized binuclear environment on carboxylate ester or amide hydrolysis.[11] Hence, we have synthesized model ester **4a**, which is capable of reversibly binding two metal cations proximal to the ester group. One can envision a binuclear metal cation hydrolysis mechanism in which one chelated metal cation acts as a Lewis acid, thereby activating the carbonyl, while one chelated metal delivers hydroxide in an intramolecular fashion (Figure 5). Such binuclear metal cation hydrolysis mechanisms have been proposed to operate in the active site of certain hydrolytic metalloenzymes including alkaline phosphatase,[12] DNA polymerase,[13] ribonuclease P,[14] and even catalytic RNA.[15] The commonplace structure of multimetal active sites suggests that binuclear metal cation hydrolysis may be a common phenomenon in biological systems.[15]

The hydrolysis of **4a** at pH 7.5 and room temperature was monitored by following the loss of the ester relative to an internal standard (2,4,6-collidine) by ^1H NMR (Scheme 9). The observed first-order rate constants (k_{obs})

(10) (a) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145. (b)Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 12165. (c) Tsubouchi, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 11614.

(11) Murthy, N. N.; Mahroof-Tahir, M.; Karlin, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 10404.

(12) Kim, E. E.; Wyckoff, W. H. *J. Mol. Biol.* **1991**, *218*, 449.

(13) (a) Freemont, P. S.; Friedman, J. M.; Beese, L. S.; Sanderson, M. R.; Steitz, T. A. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 8924. (b) Beese, L. S.; Steitz, T. A. *EMBO* **1991**, *10*, 25.

(14) Smith, D.; Pace, N. R. *Biochemistry* **1993**, *32*, 5274.

(15) Steitz, T. A.; Steitz, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6498.

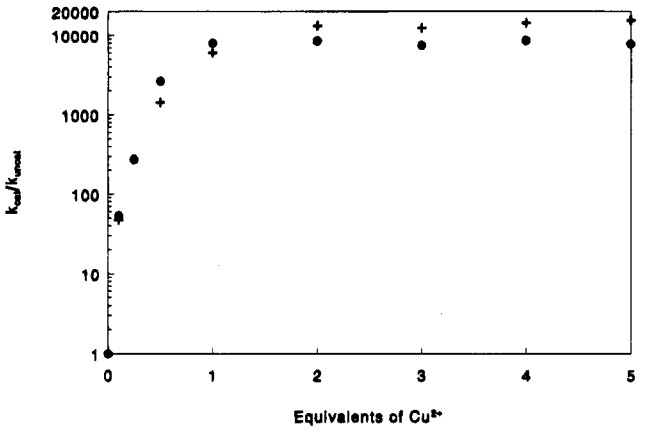


Figure 6. Cu^{2+}-catalyzed hydrolyses of 1.8 mM **2a** (+) and **4a** (●) in 0.1 M HEPES, pH 7.5 at 23 °C.

Table 2. Rate Constants for the $Cu(ClO_4)_2$-Catalyzed Hydrolysis of 1.8 mM 4a at pH 7.5 and 23 °C

equiv of metal	$k_{obs} \times 10^3$ (min^{-1})	k_{obs}/k_{uncat}
–	0.08	1
0.10	4.11	50
0.25	21.2	300
0.50	204	3000
1.0	624	8000
2.0	666	8000
3.0	589	7000
4.0	672	8000
5.0	606	8000

Table 3. k_{cat}/k_{uncat} Values for the Hydrolysis of 2a and 4a at pH 7.5 and 23 °C with 5 Equiv of Several Metals

ester	Cu^{2+}	Ni^{2+}	Co^{2+}	Zn^{2+}
2a	15400	70	8	6
4a	8000	100	9	6

were measured at several different concentrations of Cu^{2+} (Table 2). In the absence of any metal, a slow conjugate reversion reaction of **4a** is competitive with the hydrolysis reaction; thus, the rate constant measured in the absence of metal (k_{uncat}) can best be described as approximate. For this reason, we report only one significant figure for k_{uncat}. Nonetheless, its value ($k_{uncat} = 8 \times 10^{-5}$ min^{-1}) is similar to the uncatalyzed rate constant calculated for hydrolysis of ester **2a** ($k_{uncat} = 6.2 \times 10^{-5}$ min^{-1}).

The k_{cat}/k_{uncat} values for esters **2a** and **4a** were determined at several different concentrations of Cu^{2+} and are presented graphically in Figure 6. As is apparent from Figure 6, both esters demonstrate saturation kinetics. More importantly, *the k_{cat}/k_{uncat} values for both esters are of the same magnitude at any given concentration of Cu^{2+}.* At 5 equiv of Cu^{2+}, the k_{cat}/k_{uncat} values for **2a** and **4a** are 15 400 and 8000, respectively. Thus, the k_{cat}/k_{uncat} values for **4a** are not larger than for **2a** even at high Cu^{2+} concentrations and no large rate acceleration that could be ascribed to binuclear metal cation-catalyzed hydrolysis is observed. The k_{cat}/k_{uncat} values for esters **2a** and **4a** were also measured in the presence of 5 equiv of Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} (Table 3). The results confirm that Cu^{2+} is the best metal for hydrolysis. More importantly, the k_{cat}/k_{uncat} values for both esters are of similar magnitudes for all the metals studied. The lack of a binuclear metal ion catalysis in the hydrolysis of **4a** is not specific to Cu^{2+} but appears to be general.

Why is the observed rate acceleration for ester **4a** not greater than for ester **2a** at high metal concentrations? Intuitively, one would expect two proximally bound metal cations to be more effective at catalyzing hydrolysis than only one metal. We offer two explanations that could account for the similar rate accelerations observed for **2a** and **4a**. Ester **4a** may be a faulty model, one that is not structurally suited to demonstrate cooperation between two metals. It may not favor a conformation in which two metal cations can play a role in hydrolysis (as in Figure 4) or it may not even bind two metals. Alternatively, the model may not be faulty; it may be that the addition of a second bound metal cation does not open up any new mechanistic pathways toward hydrolysis. Simply stated, the second metal cation may play no significant role in hydrolysis. If, as Chin has proposed,[2] a single *cis*-diaqua Cu^{2+} can hydrolyze unactivated esters by means of a combined Lewis acid/metal hydroxide mechanism, then a second bound Cu^{2+} may have little effect on hydrolysis. However, it is important to note that a combined Lewis acid/metal hydroxide mechanism has not been demonstrated to occur in Ni^{2+}, Co^{2+}, or Zn^{2+} catalyzed hydrolysis reactions.

The preliminary kinetic results for the metal ion-catalyzed hydrolysis of model amide **4e** are unexceptional. With 2 equiv of Cu^{2+} at pH 7.5 and 23 °C, the hydrolysis of **4e** occurs with $k_{obs} = 1.8 \times 10^{-4}$ min^{-1}. Hence, amides **2b**, **3b**, and **4e** are hydrolyzed at similar rates in the presence of 2 equiv of Cu^{2+}.

Conclusions

Each step of the reaction cycle shown in Scheme 7 occurs in pH 7.5 buffer. The addition reaction of methyl acrylate with *N'*-benzyl-*N,N*-dimethylethylenediamine proceeds at a reasonable rate. The hydrolysis of the resulting ester **2a** is catalyzed by divalent metal cations, most notably by Cu^{2+}. The conjugate reversion reaction is the slowest step of the cycle and future work is needed toward accelerating this step. Cyclic ester **3d** is hydrolyzed by Cu^{2+} to less of an extent than the acyclic ester **2d**. It appears that weak metal binding limits the rate accelerations of **3d**. The bis-chelating ester **4a** did not demonstrate rate accelerations of greater magnitude than observed for **2a**. The lack of a binuclear metal cation-catalyzed effect could be a result of a design flaw in **4a** or could suggest that a second bound metal does not play a role in hydrolysis. If the former, then other similarly designed models should be prepared and studied. Amide **2b** demonstrates Cu^{2+}-catalyzed hydrolysis under mild reaction conditions. Model amides **3b** and **4e** are also hydrolyzed by Cu^{2+} at similar rates.

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Supporting Information Available: X-ray crystal structure of **3d** (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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