Synthesis of Some Copper(II)-Chelating (Dialkylamino)pyridine Amphiphiles and Evaluation of Their Esterolytic Capacities in Cationic Micellar Media

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Received May 5, 1997[⊗]

Three new (dialkylamino)pyridine (DAAP)-based ligand amphiphiles **3**−**5** have been synthesized. All of the compounds possess a metal ion binding subunit in the form of a 2,6-disubstituted DAAP moiety. In addition, at least one ortho-CH_2OH substituent is present in all the ligands. Complex formation by these ligands with various metal ions were examined under micellar conditions, but only complexes with Cu(II) ions showed kinetically potent esterolytic capacities under micellar conditions. Complexes with Cu(II) were prepared in host comicellar cetyltrimethylammonium bromide (CTABr) media at pH 7.6. Individual complexes were characterized by UV−visible absorption spectroscopy and electron paramagnetic resonance spectroscopy. These metallomicelles speed the cleavage of the substrates *p*-nitrophenyl hexanoate or *p*-nitrophenyl diphenyl phosphate. To ascertain the nature of the active esterolytic species, the stoichiometries of the respective Cu(II) complexes were determined from the kinetic version of Job's plot. In all the instances, 2:1 complex ligand/Cu(II) ion are the most kinetically competent species. The apparent pK_a values of the Cu(II)-coordinated hydroxyl groups of the ligands **3**, **4**, and **5**, in the comicellar aggregate, are 7.8, 8.0, and 8.0, respectively, as estimated from the rate constant vs pH profiles of the ester cleavage reactions. The nucleophilic metallomicellar reagents and the second-order "catalytic" rate constants toward esterolysis of the substrate *p*-nitrophenyl hexanoate (at 25 °C, pH 7.6) are 37.5 for **3**, 11.4 for **4**, and 13.8 for **5**. All catalytic systems comprising the coaggregates of **3**, **4**, or **5** and CTABr demonstrate turnover behavior in the presence of excess substrate.

Introduction

Many enzymes employ metal ions in their active site for eliciting specific catalytic activities.[1] A large number of metal ion mediated hydrolytic reactions have been examined to obtain insight into the mechanisms responsible for this kind of catalysis.[2] These include designed ligands for complexation with specific metal ions such as Cu(II),[3] Zn(II),[4] Co(II),[5] and La(III).[6] Some are highly effective in promoting hydrolytic reactions. On the basis of such studies, it has been established that metalloproteases often use water molecules or internal alcoholic side chain residues (e.g. serine or threonine) as nucleophiles. Coordination of the nucleophile or water to the metal ion enhances their acidity, facilitating deprotonation at physiological pH[7] and speeding reaction with electrophilic substrates, e.g. amides, esters, or phosphates. In addition, the electrophilicity of a substrate molecule often gets potentiated through its coordination with the metal ions present at enzyme active sites.

To achieve the ester hydrolysis at *physiological pH* (∼7.5), a whole variety of metal-complex-based hydrolase analogues have been developed.[8,9] Functional amphiphiles constructed with metal-complexing templates offer additional advantages because they aggregate in aqueous media. Such supramolecular ensembles provide hydrophobic binding sites for the substrates and induce catalytic effects through appropriate functional groups.[10,11]

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[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.

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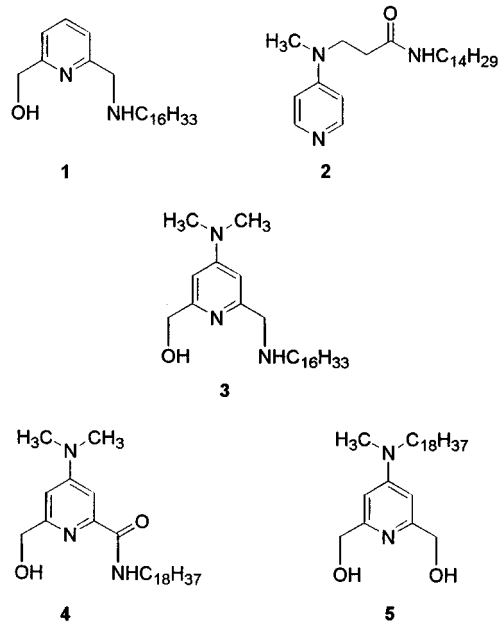
S0022-3263(97)00799-8 CCC: $15.00
Published on Web 01/09/1998

Scrimin and co-workers have extensively studied the esterolytic capacities of various Cu(II)-chelating *bidentate* ligand [(2-hydroxymethyl)pyridine] amphiphiles and bolaphiles in micellar media.[12] The corresponding metallomicelles are powerful catalysts for the cleavages of substrates, e.g. *p*-nitrophenyl picolinate or α-amino acid esters which also have the ability to participate in the ligation with the catalytic metal-chelating sites. However, these systems do not cleave esters such as *p*-nitrophenyl alkanoates which do not coordinate with the metal-complex core. Subsequent studies demonstrated that *tridentate* ligand amphiphiles such as **1**, form Cu(II)-chelating micelles that are capable of potentiating ester hydrolysis even when the esters cannot participate in the complex formation.[13] We have designed a variety of synthetic amphiphilic systems, examined the properties of their aggregates,[14] and developed several catalytic organized assemblies.[15] Efficient catalytic turnover in the hydrolysis of *p*-nitrophenyl alkanoates at mildly alkaline pH was shown by 4-(dialkylamino)pyridine (DAAP) amphiphiles in different aggregates.[15] An advantage with the DAAP systems (**2**) is that they are strongly "nucleophilic".[16] Accordingly, we thought that the design of alcohol- or amine-pendent lipophilic DAAPs would be useful for developing metal-complex-based amphiphiles. Here we introduce three new lipophilic DAAP ligands, **3**−**5**, which effectively complex different transition metal ions at ambient pH. We wanted to explore whether the increased π-electron density on the N atom of the pyridine ring in DAAP[17] relative to pyridine alone might enhance the capacity of the metal complexes toward their hydrolytic abilities. We also compared the reactivities and plausible mechanisms of the Cu(II) complexes of **2**−**5** under comicellar conditions for the cleavages of *p*-nitrophenyl hexanoate (PNPH) and *p*-nitrophenyl diphenyl phosphate (PNPDPP) at pH 7.6, 25 °C. The routes to the synthesis of the ligands, the characterization of the catalytically effective complexes, the results of the kinetic studies under pseudo-first-order conditions (excess catalysts) and in the presence of excess substrates, and potential esterolytic pathways are presented.



Results and Discussion

Synthesis of the Amphiphilic Ligands. The synthesis of the (dialkylamino)pyridine-based ligand functionalized amphiphiles, as summarized in Scheme 1, began with the conversion of chelidamic acid into dimethyl 4-chloropyridine-2,6-dicarboxylate, **6**, followed by saponification to the corresponding diacid, **7**. Part of **7** was mixed with aqueous dimethylamine in a screw-top pressure tube at 160 °C for ∼24 h. The solid product was converted to the corresponding dimethyl ester by treatment with methanolic $SOCl_2$ which afforded the 4-(dimethylamino)pyridine derivative, **8**, in ca. 70% yield. **8** was partially reduced with $NaBH_4$ to give **9** in 91% yield. Part of **9** was intimately mixed with *n*-octadecylamine, and the resulting mixture was heated at ∼90 °C to a clear melt and left in that condition for ∼12 h to give crude amide, **4**, which solidified on cooling. Column chromatographic purification over silica gel using 1:1 EtOAc and petroleum ether (bp 60−80 °C) gave pure **4** in ca. 71% yield. The remainder of **9** was oxidized with 1 equiv of MnO_2 to give the monoaldehyde **10** in ∼88% yield. Condensation of **10** with *n*-hexadecylamine gave a schiff base which was carefully reduced with $NaBH_4$ to furnish the secondary amine, **3**, in 68% yield. The second part of **7** was mixed with *N*-methyl-*N*-octadecylamine in dry methanol at ∼120 °C in a screw-top pressure tube to give the 4-(*N*-octadecyl-*N*-methylamino)pyridine derivative, which was then converted to the corresponding dimethyl ester, **11**, in ca. 49% yield. Reduction of **11** with excess $NaBH_4$ gave the 2,6-dihydroxymethyl derivative, **5**, in 72% yield. The final products, **3**−**5**, and the appropriate intermediates showed expected IR, ^{1}H NMR (200 MHz), mass spectra, and elemental analysis data, cf. Experimental Section.

Ligand Properties and Complex Formation. The lipophilic ligands **3**−**5** are not soluble in water, and their corresponding metal complexes are also only sparingly soluble in water at pH ∼7.0. However, they form stable

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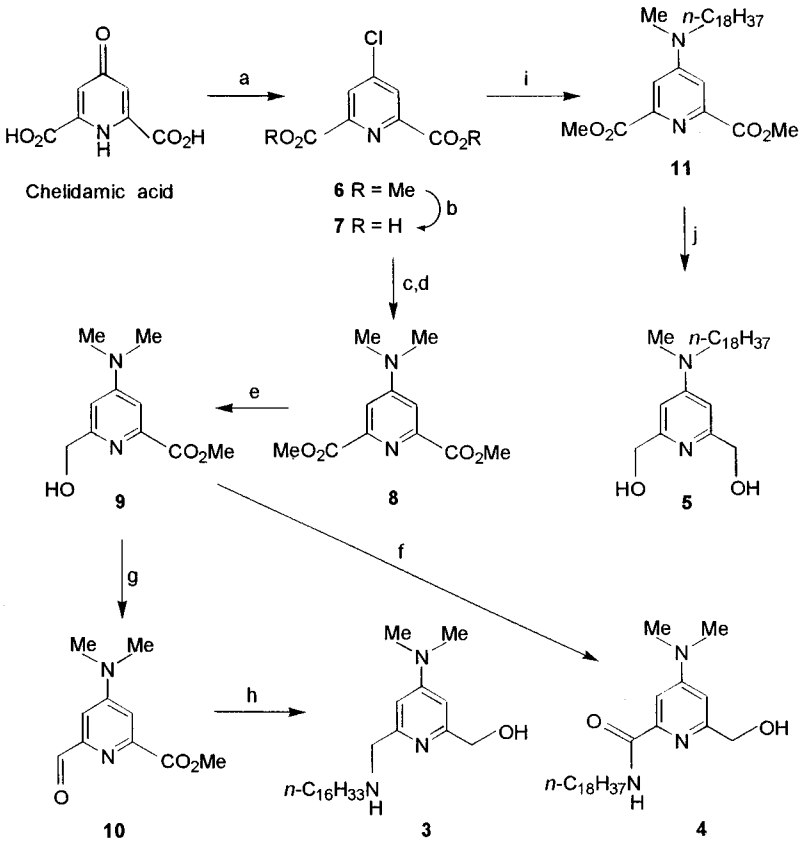
Scheme 1[a]



[a] Reagents and conditions: (a) (i) PCl_5, CCl_4, 120 °C, (ii) dry MeOH, reflux 54%; (b) 1 M NaOH, 80 °C; aqueous HCl; (c) Me_2NH, 120 °C; aqueous H_2SO_4, 43%; (d) $SOCl_2$, MeOH, −10 °C; then reflux, 83%; (e) $NaBH_4$ (1 equiv), CH_2Cl_2−MeOH, 0 °C (30 min), rt (5 h), 91%; (f) melt *n*-$C_{18}H_{37}NH_2$, 90 °C, 71%; (g) MnO_2, CH_2Cl_2, rt, 88%; (h) (i) *n*-$C_{16}H_{33}NH_2$, dry THF, rt, (ii) $NaBH_4$, MeOH, rt, 68%; (i) (i) *n*-$C_{18}H_{37}$NHMe, MeOH, 120 °C, (ii) $SOCl_2$, MeOH, reflux, 49%; (j) $NaBH_4$, MeOH, rt, 72%.

aqueous solutions when employed as comicelles in the presence of excess host, surfactant CTABr. The amphiphile bearing an amide linkage, i.e. **4**, requires at least a 20-fold molar excess of CTABr for dissolution in water. Amphiphiles **3** and **5**, however, dissolve at lower CTABr/[amphiphile] ratios. The Cu(II) complexes of ligands **3**−**5** were generated in situ under comicellar conditions of CTABr in HEPES buffer, pH 7.6, by adding the appropriate amount of stock solution of $CuCl_2$ in water (see below). Similarly the Co(II), Ni(II), and Zn(II) complexes of ligands **3**−**5** were prepared in situ in buffered comicellar media.

The lack of adequate solubility and the complex nature of comicellar aggregation prevented determination of binding constants, K_M, of various ligands with Cu(II) and other metal ions. Approximate magnitudes of the association constants were estimated from the values reported[18] for 2-(aminomethyl)pyridine, 2-[[*N*,*N*-(dihydroxyethyl)amino]methyl]pyridine and 2,6-bis(aminomethyl)pyridine. Considering possible effects of micellization, in particular coaggregation with a cationic nonligating surfactant,[12a] $K_M > 10^9$ for Cu(II) is a reasonable measure of the association constant. The binding constants for Zn(II), Co(II), and Ni(II) metal−ligand association are probably 2−3 orders of magnitude lower.[19] Only the Cu(II) complexes of ligands **3**−**5** speed ester hydrolysis reactions, and all further studies are therefore confined to the Cu(II) complexes.

UV-Absorption Studies of Cu(II) Complexes in Micelles. The formation of different Cu(II) complexes under the comicellar conditions was demonstrated by the appearance of an absorbance with a λ_{max} in the range 650−750 nm for ligand:Cu(II) ratios of 2:1 for ligands **3**−**5**. Ligand **3** in the presence of Cu(II) ion yielded a complex with λ_{max} at ∼718 nm in a comicellar CTABr solution. Under comparable conditions, **4**:Cu(II) and **5**:Cu(II) complexes gave absorption maxima at ∼670 and ∼739 nm, respectively. Thus the UV−visible absorption spectra of the Cu(II) complexes of **3**−**5** in micelles are consistent with the formation of four coordinated species, with weak axial interactions as shown by the presence of the single d−d band in each UV−vis spectra[20,21] and their EPR data (see below). Ligand **2** did not give a d−d band above 350 nm, indicating that substituents at the 2,6-positions of the pyridine moiety are essential for complex formation. The results are summarized in Table 1.

Possible Geometries of the Complexes. One of the most useful applications of the EPR is in distinguishing different geometries of a series of metal complexes for which the crystal structures are not available. The approximate geometry of the complex in solution is

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Table 1. UV−Visible Wavelength Maxima for Cu(II) Complexes of Ligands 2−5 in CTABr Comicellar Conditions[a]

		λ_{max}, nm (ϵ, M^{-1} cm^{-1})	
emtry	catalyst[b]	ligand	Cu(II) complex[c]
1	**2**	284 (3700), 262 (4050), 245 (4350)	304 (3500), 265 (3800), 240 (4200)
2	**3**	282 (6050), 247 (6650), 234 (6250)	713 (100), 296 (5300), 270 (4900)
3	**4**	308 (5800), 262 (2400)	671 (100), broad 326 (750)
4	**5**	310 (6200), 256 (6600), 240 (6850)	740 (100), 295 (4900), 239 (4650)

[a] Complexes were formed in situ by solubilizing individual ligands in CTABr micellar solutions at pH 7.6, 0.05 M HEPES buffer, 0.1 M KCl, at 25 ± 0.1 °C, [ligand] = 2.5×10^{-4} M. For the entries, 1−4, [Cu(II)] = 1.25×10^{-4} M. [b] Ratio of ligand:Cu(II) is 2:1 for all the entries. [c] The d−d bands in each absorption spectrum are underlined.

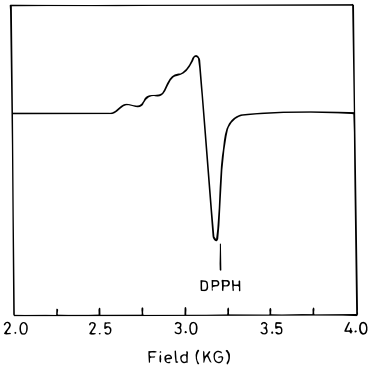

Figure 1. X-band EPR spectrum of Cu(II) complex of **3** diluted with Zn(II) complex (1:10) at 298 K. Spectrometer settings: microwave frequency, 9.05 GHz; microwave power, 2 mw; modulation frequency, 100 kHz; modulation amplitude, 4 G.

assigned by experimentally determining the anisotropic *g* values for different coordination compounds of Cu(II).[21] To extract information about the possible geometries of the Cu(II) complexes, EPR spectra were recorded for solutions of ligands **3**−**5** in water or in MeOH with Cu(II) and in micellar CTABr solutions at ambient temperature. All samples showed isotropic signals presumably because rapid tumbling led to average anisotropic *g* and *A* values. Frozen samples (−196 °C) of the complexes in CTABr solutions also failed to show any significant anisotropy.

The Cu(II) complexes of individual ligands **3** or **5** were prepared as described above in methanol and diluted with Zn(II) ions such that the ratio of Cu(II)/Zn(II) was 1:10. Evaporation of the solvent gave residues which showed anisotropic EPR spectra at ambient temperature.

The X-band EPR spectrum of Zn(II)-doped L_2Cu (L = **3**, 298 K) is shown in Figure 1. The spectrum ($g_\perp = 2.26$ and $g_\parallel = 2.06$ with $A_\parallel = 150$ G) resembles the EPR spectrum of a typical monomeric tetragonal Cu(II) complex. Although no well-resolved hyperfine coupling to first coordination sphere donor atoms is detected (Figure 1), some structural information was obtained from the EPR parameters. The *g* values of $\mathbf{3}_2$Cu indicate either a distorted square-pyramidal or an elongated rhombic octahedral geometry.[22] The Cu(II) complexes of ligand **4** or **5** when diluted (1:10) with Zn(II) ions showed similar anisotropic EPR spectra (figures not shown), but were not well-resolved and their anisotropic *g* values from **5** could not be obtained with adequate precision.

On the basis of these findings (EPR) and the kinetic studies (see below), computer-generated structures for the Cu(II) complexes of **3** were drawn using Insight II program version 2.2.0 (Biosym. Technology). Probable

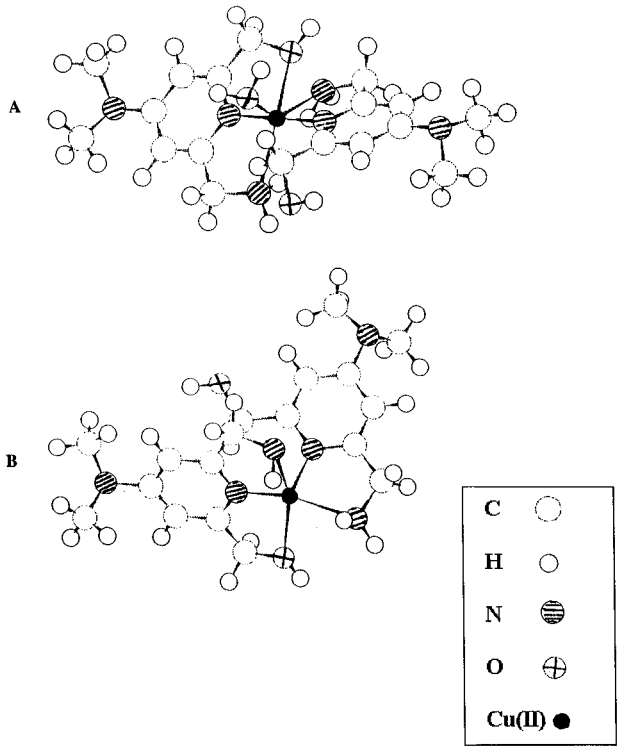

Figure 2. Computer-generated models of the Cu(II) complex of **3** showing (A) distorted octahedral geometry of Cu(II) complex of **3** (active site); (B) distorted square-pyramidal geometry of the Cu(II) complex of **3**. Note that in either case the long *n*-$C_{16}H_{33}$ chain is not shown for clarity.

alternative geometries of the active site of **3** as drawn using this program are shown in Figure 2. In a distorted square-pyramidal geometry (Figure 2B), the central Cu(II) ion utilizes one of the CH_2OH group from two of the 4-(dimethylamino)pyridine (DAAP) units in addition to the pyridine N and the $C_{16}H_{33}NCH_2$ residues at DAAPs without requiring the participation of the other CH_2OH group or any outside water molecules in coordination with the Cu(II) ion. In contrast, in a distorted rhombic octahedral geometry (Figure 2A), the Cu(II) ion utilizes one of the CH_2OH group from one of the two DAAP units in addition to the pyridine N and the $C_{16}H_{33}NCH_2$ residues at DAAPs without requiring the coordination of the other CH_2OH group. However, one outside water molecule is required in this situation to satisfy the coordination sites surrounding the Cu(II) ion for an octahedral geometry. On the basis of the data obtained experimentally on the apparent value of pK_a and due to the lack of the presence of two ionization residues, it appears that the distorted square-pyramidal geometry may be likely to be kinetically more relevant. To ascertain the nature of the active esterolytic species, the stoichiometries of the respective Cu(II) complexes were

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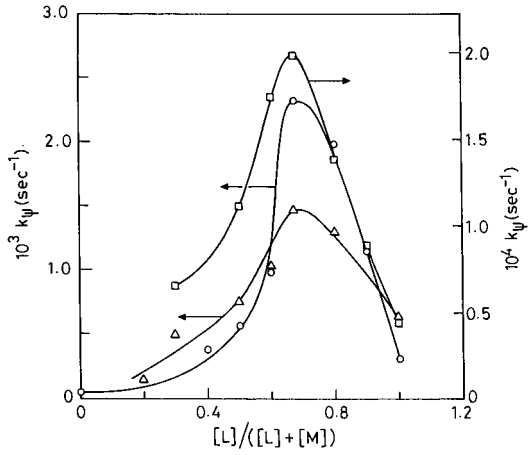

Figure 3. Kinetic Job's plot for the cleavage of *p*-nitrophenyl hexanoate (2.5×10^{-5} M) by **3** (○), **4** (□), and **5** (△). Reaction conditions: 0.05 M HEPES buffer (pH 7.6), 25 ± 0.1 °C, [ligand + Cu^{II}] = 5×10^{-4} M, [CTABr] = 1×10^{-2} M.

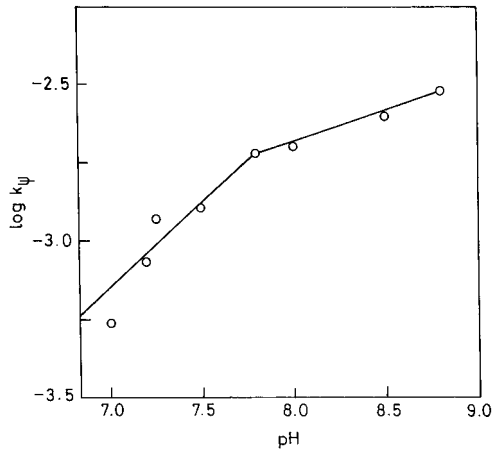

Figure 4. pH rate constant profile for the hydrolysis of *p*-nitrophenyl hexanoate (2.5×10^{-5} M) by **3** (2.5×10^{-4} M) in the presence of 1.25×10^{-4} M $CuCl_2$. Reaction conditions: 25 ± 0.1 °C and [CTABr] = 5×10^{-3} M.

further determined from the kinetic version of Job's plot (see below). Notably in all the instances, 2:1 complex ligand/Cu(II) ion were found to be the most kinetically competent species.

Kinetic Studies. Kinetic studies were performed either under the pseudo-first-order conditions or in the presence of excess substrates, monitoring the appearance of *p*-nitrophenoxide ion at 400 nm at 25 °C in 0.05 M HEPES buffer (pH 7.6). The stoichiometries of the active Cu(II) complexes were determined to establish the mechanistic aspects of the process.

Stoichiometries of the Kinetically Active Complexes. Information pertaining to the esterolytically optimal stoichiometry of the metal ion/ligand complex was obtained from the kinetic version of a Job plot analysis.[23] Briefly, the pseudo-first-order rate constants, k_ψ, for the hydrolysis of PNPH or PNPDPP were measured in solutions containing a given ligand, L, and the metal ion, Cu(II), where the ratios of [L]/[Cu(II)] were changed while keeping the total concentration of the two species constant. The results obtained with catalysts **3**−**5** under comicellar conditions are shown in Figure 3. Cleavage rates of PNPH and PNPDPP with **3**−**5** under comicellar conditions all go through maxima at [L] = ca. 2[Cu(II)]. These stoichiometries are consistent with the information obtained from the EPR spectra and also with the computer-generated structures for these complexes. To make kinetic comparisons under identical conditions, the concentration of the host surfactant CTABr was kept constant for all the kinetic runs. Further increases in [L] probably give complexes of other stoichiometries which compete with the kinetically most efficient L_2Cu stoichiometries and reduce the observed rates.

pK_a Determinations. Hydrolysis rates of PNPH in complexes **3**−**5** in cationic (CTABr) micelles are pH-dependent. A pH−rate constant profile for the cleavage of 2.5×10^{-5} M PNPH by 2.5×10^{-4} M **3** in 1×10^{-2} M CTABr in the presence of 1.25×10^{-4} M $CuCl_2$ in 0.05 M buffer (μ = 0.1 M KCl) gave an apparent pK_a of ~7.8 (Figure 4). Cu(II) complexes of **3**−**5** were generated in situ by the addition of appropriate amount of stock solution of aqueous $CuCl_2$ to the CTABr comicellar solution of the respective ligand. Reactions were initiated by the addition of an aliquot of 5 μL of stock solution of PNPH in CH_3CN. The pseudo-first-order rate constants for PNPH cleavage at 25 °C by each catalytic system were determined spectrophotometrically by following the release of the *p*-nitrophenoxide ion at 400 nm at different pH values between 5 and 9.0. We also determined the pH−rate constant profiles for PNPH hydrolysis induced by Cu(II) complexes of comicellar **4** and **5** at the concentrations of PNPH, ligand, CTABr, and $CuCl_2$ mentioned above. Plots of log k_ψ vs pH gave discontinuities at pH = ca. 8.0 for comicellar **4** and **5**, which were taken as systemic pK_a values for the ionization of hydroxyl ($CH_2OH \rightarrow CH_2O^-$) bound to Cu(II) ion in micelles (see below).

We ascribe these inflections with the systemic pK_a values of the pendent CH_2OH functions on ligands **3**−**5** as a consequence of coordination to the Cu(II) ion in micellar conditions. The small differences in the experimentally obtained pK_a values are in accord with the structural differences in the other substituents in these DAAP ligands. Therefore, such pH dependencies of the esterolytic reactions could originate from participation of Cu(II)-activated CH_2OH group in the rate-determining step.[24] A pK_a value of 7.7 has been reported for a Cu(II)-coordinated hydroxyl by Scrimin[13] in holometallomicellar aggregates. We believe that the electron-donating character Me_2N group at the 4-position of the pyridine nucleus raises the pK_a values in **3** by ca. 0.1 unit and by 0.3 unit in **4** and **5**, respectively. Similar pK_a values were obtained by Tagaki and others in the studies of Cu(II) complexes of 2-(hydroxymethyl)imidazole functionalized surfactant aggregates.[25]

Hydrolysis in the Absence of Metal Ions. The reactions of lipophilic ligands **2**−**5** in CTABr comicellar conditions with PNPH and PNPDPP in HEPES buffer, pH = 7.6, show small rate accelerations when compared to PNPH or PNPDPP cleavage reactions conducted in CTABr only (background). The observed modest but definitive rate acceleration is probably caused by the

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Table 2. Pseudo-First-Order Rate Constants for the Cleavages of *p*-Nitrophenyl Hexanoate and *p*-Nitrophenyl Diphenyl Phosphate by Ligands 2−5 in the Absence of Metal Ion in 0.01 M CTABr[a]

	PNPH			PNPDPP	
entry	catalyst	$10^3 k_\psi$	k_ψ/k_{CTABr}	$10^3 k_\psi$	k_ψ/k_{CTABr}
1	none	0.04	1	0.05	1
2	**2**	5.11	127.8	0.13	2.6
3	**3**	0.31	7.8	0.31	6.2
4	**4**	0.16	4.0	0.07	1.4
5	**5**	0.64	16.0	0.15	3.0

[a] Conditions: 0.05 M HEPES buffer; μ = 0.1 M KCl; pH = 7.6, 25 ± 0.1 °C; [cat.] = 5×10^{-4} M.

nucleophilic (dimethylamino)pyridine moiety in **3**−**5**. The relevant kinetic parameters with pertinent experimental conditions are summarized in Table 2. Comicelles of **2**/CTABr comicelles represent the best catalytic formulation for the cleavage of PNPH under these conditions. This result indicates that in the absence of metal ions, the presence of ortho substituents on the DAAP moiety mitigates the esterolytic potential of these catalysts presumably due to steric effects. However, it is not apparent why **3**/CTABr comicelles showed greater catalytic activity than their counterparts for PNPDPP hydrolysis.

Hydrolysis in the Presence of Metal Ions. The coordination complexes of the lipophilic ligands **3**−**5** with various transition metal ions such as Zn(II), Co(II), Ni(II), and Cu(II) were tested for their abilities to cleave alkanoate (PNPH) and phosphate (PNPDPP) esters. The complexes of ligands **3**−**5** with Zn(II) and Ni(II) showed insignificant rate accelerations. However, the complexes of Cu(II) and Co(II) show substantial activity. Cu(II) complexes of **3**−**5** are the most reactive, ∼2 times more reactive than Co(II) complexes (results not shown).

In Table 3, we collect the values of k_ψ^{obs} for the cleavages of both PNPH and PNPDPP by each of the Cu(II) complexes of lipophilic ligands **3**−**5** at optimized ligand and CTABr concentrations. Entries **2**−**4** in Table 3 include the kinetic parameters under the conditions where the overall ratio of each catalyst to CTABr is fixed (1:20) for all four DAAP catalysts in the presence of same [Cu(II)] (2.5×10^{-4} M). Clearly these complexes afforded much better kinetic advantage when used in the presence of Cu(II) ions. This may be caused by the greater Lewis acidic character of Cu(II), which may also explain why the Cu(II) complexes form more stable complexes in micellar solutions than the other transition metal ions. The most reactive catalyst among the metallomicellar systems in this study is the Cu(II) complex of **3**. It has a rate acceleration of ∼89-fold for the hydrolysis of PNPH and ∼22-fold rate acceleration for PNPDPP when compared to background (entry 1, Table 3, CTABr and Cu(II) without any ligand) under similar conditions. The second most reactive compound **5** has a rate acceleration of ∼24 for PNPH and ∼10 for PNPDPP. The least reactive is the Cu(II) complex of **4** which has ∼20-fold rate acceleration for PNPH and is only 2 times more reactive toward PNPDPP when compared to background.

We have also included in Table 3 the calculated second-order "catalytic" rate constants ($k_{cat} = k_\psi^{obs}$/[catalyst]. These have been corrected for the 100% ionization of the Cu(II)-coordinated OH group responsible for the nucleophilic activities. It might be noted that the comparisons of rate constants and dissociation constants are complicated by the dependence on concentrations of reactants, surfactant, and sometimes pH. Therefore, insofar as with the use of one CTABr concentration, comparisons may also depend on the choice of concentrations. Nevertheless at this given situation, a qualitative comparison of k_{cat} may be useful. On this basis the analysis of the k_{cat} values for the PNPH cleavages clearly indicates that $\mathbf{3}_2$Cu catalysts are the most effective systems for such reactions. The next best catalyst is $\mathbf{5}_2$Cu. Perhaps the electron-withdrawing character of the amide linkage in **4** mitigates its catalytic activity. Similar trends are also observed for the cleavage of PNPDPP.

Involvement of the CH_2OH unit in DAPP for Cu^{2+} coordination in **3**−**5** should be anticipated because of the following reasons: (i) the higher apparent acidity, by ca. 2 pK_a units, of the 2-(hydroxymethyl)pyridine than that of H_2O;[25c] (ii) earlier investigations[25d] with the action of metal chelates of 2-(hydroxymethyl)pyridine derivatives on *p*-nitrophenyl picolinate have clearly indicated that the second slow step indeed involves the hydrolysis of the transacylation product. We believe that a similar mechanism is also operative in the present cases. Therefore, based on geometric features of **3**−**5**, the CH_2O^- produced upon the ionization of the CH_2OH group coordinated to Cu(II) must be responsible for esterolytic activities of the alkanoates in the metallomicelles.

The presence of the CH_2OH group ortho to the pyridine nitrogen appears to be crucial for the cleavage of the alkanoate esters, but for the phosphotriester substrate, PNPDPP, the presence of an CH_2OH group is not so important. We believe that the major difference between the two types of esters lays in their ability to be involved in the Cu^{2+} coordination. Such a possibility is open only for the PNPDPP but not for the carboxylate esters. This is because the affinity of the P═O group for Cu^{2+} is much greater than that of an alkanoate C═O group.[25b] One may also speculate that the packing of ligand amphiphiles in micelles does not allow the PNPDPP substrate to take advantage of the activation of the CH_2OH group with the metal ion and the hydrophobic environment at the same time.

Table 3. Kinetic Parameters for Cu(II)-Catalyzed Esterolysis of *p*-Nitrophenyl Hexanoate and *p*-Nitrophenyl Diphenyl Phosphate[a]

			PNPH		PNPDPP	
entry	catalytic ligand	pK_a[b] [% ionization]	$10^3 k_\psi^{obs}$, s^{-1}	k_{cat}[c], M^{-1} s^{-1}	$10^3 k_\psi^{obs}$, s^{-1}	k_{cat},[c] M^{-1} s^{-1}
1	none[d]		0.04		0.06	
2	**3**	7.8 [38.7]	3.6	37.5	1.3	13.6
3	**4**	8.0 [28.5]	0.81	11.4	0.14	2.0
4	**5**	8.0 [28.5]	0.98	13.8	0.6	8.4

[a] Conditions: 0.05 M buffer, pH 7.6, μ = 0.1 M KCl, 25 ± 0.1 °C, 0.3 vol % CH_3CN. For entries 2−5 [ligand] = 5×10^{-4} M, [Cu(II)] = 2.5×10^{-4} M, [CTABr] = 1×10^{-2} M. [b] See text for discussion of pK_a values. Values in brackets are percent ionizations at pH 7.6. [c] $k_{cat} = k_\psi^{obs}/[L_nCu]$, corrected for 100% ionization of a given catalytic system at pH 7.6, where *n* represent the stoichiometry of the kinetically most efficient formulation. [d] [cat.] = 0 i.e. without lipophilic ligands, [CTABr] = 1×10^{-2} M, [$CuCl_2$] = 2.5×10^{-4} M.

Table 4. Relative Kinetic Advantages for the Cu(II) Complexes of Catalysts 1 and 3 for the Cleavage of *p*-Nitrophenyl Hexanoate[a]

entry	catalyst	$10^4 k_\psi$, s^{-1}	k_ψ/k_{CTABr}
1	none[b]	0.037	1
2	**1**Cu(II)	5.12	138
3	**3**Cu(II)	8.59	232

[a] Conditions: 0.05 M MES buffer, μ = 0.1 M KCl, pH = 6.25, 35 °C, [ligand] = [Cu(II)]= 4×10^{-4} M, [CTABr] = 4×10^{-3} M. [b] [CTABr] = 4×10^{-3} M, [Cu(II)] = 4×10^{-4} M, [ligand] = 0.

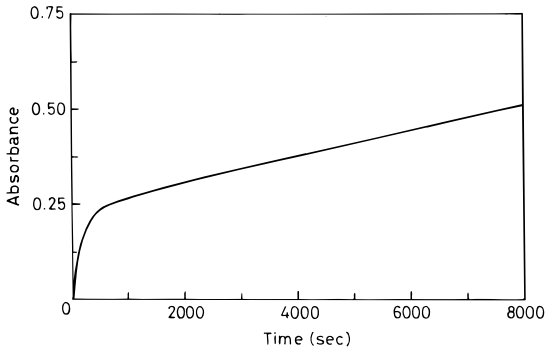

Figure 5. Kinetics under excess substrate conditions. Reaction conditions: 0.05 M HEPES buffer (pH 7.6), 25 ± 0.1 °C, [**3**] = 2.5×10^{-5} M, [$CuCl_2$] = 1.25×10^{-5} M, [PNPH] = 5×10^{-5} M, and [CTABr] = 1×10^{-3} M.

The reactivity of the catalyst **3** toward PNPH cleavage was also compared to that of the pyridine-based catalyst **1**, which was studied in detail by Scrimin et al.[13] At pH 6.3, 35 °C, under comicellar conditions of CTABr (4×10^{-3} M), 4×10^{-4} M catalyst, and 4×10^{-4} M $CuCl_2$, k_ψ values for **1** and **3** are 5.1×10^{-4} and 8.6×10^{-4} s^{-1}, respectively. When compared to the background rate (CTABr + Cu(II)), catalyst **1** is 138 times faster and compound **3** is 232 times faster, Table 4.

The reactivities of ligands **3**−**5** were also compared with **2**, which does not have any CH_2OH group at the ortho positions of the DAAP unit. Catalyst **2** alone cleaves PNPH efficiently under comicellar conditions.[15a] However, Cu(II) solutions of **2** are unstable and turn turbid immediately after the addition of $CuCl_2$. Reactions of Cu(II) complexes of **2** with either PNPH or PNPDPP deviated from strict pseudo-first-order kinetic behavior, perhaps because of light scattering during the reaction.

Turnover Experiments. To examine the true catalytic activities of different Cu(II) complexes of ligands **3**−**5** under comicellar (CTABr) conditions, experiments in the presence of excess substrates were performed with PNPH and PNPDPP. At pH 7.6 and 25 °C, using a comicellar formulation of [ligand] = 2.5×10^{-5} M, [Cu(II)] = 1.25×10^{-5} M, and [CTABr] = 1×10^{-3} M), we observed a quantitative release of *p*-nitrophenoxide ion in the presence of up to a 4-fold excess of PNPH and a 2-fold excess of PNPDPP over the catalyst, with **3**$_2$Cu(II) as catalyst. Clear evidence of *slow* turnover in this reaction was seen from the "burst" kinetic profile (Figure 5). The catalyst with an amide linkage, **4**$_2$Cu(II), also showed "burst" kinetics under similar conditions (not shown). Turnover rate constants of 1.0×10^{-4} and 6.5×10^{-5} s^{-1} for catalysts **3** and **4**, respectively, were obtained from the linear sections of the kinetic profiles. In contrast, with the Cu(II) complex of **5** under comparable comicellar (CTABr) conditions in the presence of 2-fold excess of substrate PNPH, no evidence of burst kinetics was observed (Figure 6), although *p*-nitrophenoxide is released quantitatively. Monoexponential, quantitative hydrolysis of PNPH with **5**$_2$Cu(II) indicates a *fast* turnover mechanism. The catalytic effectiveness of all three ligands remained unaltered even after the consumption of 10-fold excess of substrate. A similar observation was also reported by Scrimin and co-workers.[13]

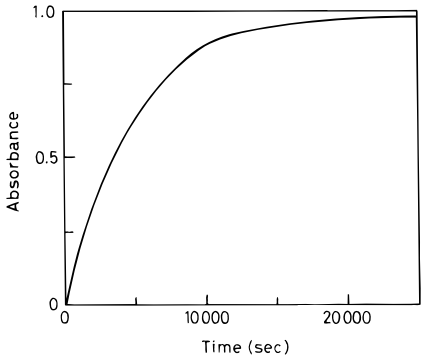

Figure 6. Kinetics under excess substrate conditions. Reaction conditions: 0.05 M HEPES buffer (pH 7.6), 25 ± 0.1 °C, [**5**] = 2.5×10^{-5} M, [$CuCl_2$] = 1.25×10^{-5} M, [PNPH] = 1×10^{-4} M, and [CTABr] = 1×10^{-3} M.

Conclusions

The reactions of the lipophilic ligands **3**−**5** in CTABr comicellar conditions with PNPH and PNPDPP in pH = 7.6 show small rate accelerations when compared to PNPH or PNPDPP cleavage reactions conducted in CTABr only (background). The observed modest but definitive rate acceleration is probably caused by the nucleophilic (dialkylamino)pyridine moiety in **3**−**5**. Comicelles of **2**/CTABr comicelles represent the best catalytic formulation for the cleavage of PNPH in the absence of Cu(II) ions. This result indicates that in the absence of metal ions, the presence of ortho substituents on the DAAP moiety mitigates the esterolytic potential of these catalysts presumably due to steric effects.

Cu(II) micellar complexes of lipophilic ligands **3**−**5** are effective catalysts for the hydrolysis of PNPH at pH 7.6. Comparison of the cleavage efficiency and catalytic behavior of the micellar **3** and **5** shows that differences in their structures do not significantly influence their reactivities in the presence or in the absence of Cu(II) ions. Ligand **3** has a lipophilic *n*-hexadecyl chain pendent on the 2-aminomethyl substituent of the DAAP moiety, but **5** has a lipophilic *n*-octadecyl chain attached at the 4-(dialkylamino)pyridine residue of DAAP. In ligand **3**, the complex-forming part is composed of CH_2OH and CH_2NHR substituents in addition to the pyridine nitrogen. In **5**, the Cu(II)-coordinating substituents are 2- and 6-CH_2OH groups in addition to the basic nitrogen on the pyridine nucleus. While comicellar **3**/CTABr is ca. 2.07-fold more reactive toward PNPDPP relative to **5**/CTABr in the absence of any Cu(II) ion, **5**/CTABr is ca. 2.09-fold more reactive toward PNPH relative to **3**/CTABr. In the presence of Cu(II) ions, however, comicellar **3**/CTABr is ca. 2.72 and 1.62 times more reactive toward the hydrolysis of PNPH and PNPDPP, respectively, relative to **5**/CTABr. It might be possible that both Br^- and Cl^- may interact with Cu(II) ion in the reaction conditions. In

that instance it is probable that the differences in k with and without ligand could be also due to differing reactant distributions between water and micelles and not entirely due to the differences in their intrinsic reactivities. Whatever may be the exact reason of the metal ion potentiation of the hydrolytic rates, all of the Cu(II) complexes of the amphiphilic ligand systems discussed here show true catalytic (either slow or fast turnover) behavior for the esterolysis of PNPH and PNPDPP in the presence of *excess* substrates. We are currently examining the effectiveness of these catalysts with phosphodiester and phosphothioate substrates.

Experimental Section

General Methods. Descriptions of analytical instruments and ^{1}H NMR, IR and UV−Vis spectrometers have been previously published.[14a,15a] Mass spectra (MS) were recorded on a JEOL Model JMS-DX 303 spectrometer equipped with JEOL JMA-DA mass data station. Mass spectra of all the samples were recorded by a direct inlet system (70 eV). The pH values of all solutions were measured using Schott pH meter CG 825. EPR spectra were measured for frozen and other solutions or for the solid samples using Varian E-series spectrometer operating at X-band frequency of 9.05 GHz. The EPR spectra were recorded as the first derivative of absorption with DPPH (α,α′-diphenyl-β-picrylhydroxyl) as internal standard. Modeling studies were done using Insight II program version 2.2.0 (Biosym Technology) on a Silicon Graphics Indigo Workstation.

Materials. All the buffers were made from Millipore water. The buffering agents MES, HEPES, and EPPS were used as supplied by Fluka. All reagents and solvents were of highest grade commercially available and used purified, dried, or freshly distilled as required by literature procedures.[26] The substrates used in the present study, viz. *p*-nitrophenyl alkanoates and *p*-nitrophenyl diphenyl phosphate were synthesized and purified by methods described.[27]

Synthesis. The synthesis of compound **2** is described in ref 15a. Compounds **8**−**12** were synthesized by appropriate modification of the literature procedures.[28]

Dimethyl 4-Chloropyridine-2,6-dicarboxylate (6). A mixture of 4-hydroxypyridine-2,6-dicarboxylic acid (chelidamic acid) (5.54 g, 30 mmol) and PCl_5 (15.43 g, 74 mmol) in CCl_4 (23 mL) was heated at 120 °C for 10 h and then cooled to room temperature. Dry methanol (23 mL) was added to this mixture dropwise over a period of 30 min, and the resulting mixture was refluxed for 3 h. The solvents were evaporated from this reaction mixture to afford a tan yellow solid which was dissolved in EtOAc and treated with activated charcoal under boiling conditions. Repeated crystallizations from EtOAc gave 3.78 g (54%) of **6** as white crystals: mp 138−140 °C (lit.[28a] mp 142 °C); IR (Nujol) 1710 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$, 90 MHz) 4.0 (s, 6H), 8.3 (s, 2H).

4-Chloropyridine-2,6-dicarboxylic Acid Hydrochloride (7). A suspension of dimethyl 4-chloropyridine-2,6-dicarboxylate, **6** (1 g, 4.36 mmol), in 1 N NaOH (11 mL) was stirred at 80 °C for 3 h. The mixture was cooled in a ice bath and acidified to pH ∼4 with ice-cold 1 N HCl. The white, solid mass precipitate was filtered and dried to give crude 4-chloropyridine-2,6-dicarboxylic acid (1.2 g) which was used without further purification for the next step: IR (Nujol) 1725 cm^{-1}.

Dimethyl 4-(Dimethylamino)pyridine-2,6-dicarboxylate (8). A suspension of 4-chloropyridine-2,6-dicarboxylic acid, **9** (1.0 g, 4.2 mmol), in 15 mL of 45% aqueous dimethylamine (10.2 g, 230 mmol) in a screw-top pressure tube was stirred for 24 h at 160 °C. The reaction mixture was cooled and treated with concentrated H_2SO_4 until it became acidic. The product was dried under vacuum to give crude 4-(dimethylamino)pyridine-2,6-dicarboxylic acid as a white solid (0.74 g, 43%). Without purification, the solid was refluxed with 3.3 g of $SOCl_2$ in 16 mL methanol for 4 h. The solvent was removed under vacuum to give a very hygroscopic material that was treated with ice-cold, aqueous saturated $NaHCO_3$ solution, and the aqueous layer was extracted with EtOAc (2 × 25 mL). The organic layer was dried (anhyd $MgSO_4$), and the solvent was removed by evaporation to give diester, **8**, as a white solid (0.83 g, 70%): mp 164 °C (lit.[28b] mp 167−168 °C); IR (Nujol) 1700 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$, 90 MHz) 3.1 (s, 6H), 4.0 (s, 6H), 7.55 (s, 2H).

Methyl 6-(Hydroxymethyl)-4-(dimethylamino)pyridine-2-carboxylate (9). Solid $NaBH_4$ (0.08 g, 2.1 mmol) was added cautiously to an ice-cold, stirred solution of dimethyl 4-(dimethylamino)pyridine-2,6-dicarboxylate, **8** (0.25 g, 1.05 mmol), in a mixture of MeOH (11 mL) and CH_2Cl_2 (1 mL). After addition of $NaBH_4$, the reaction mixture stirred for 5 h, and the resulting reaction mixture was neutralized with 1 N HCl and concentrated. The product residue was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ (20 mL), the aqueous layer was repeatedly extracted with dichloromethane (2 × 25 mL), and the CH_2Cl_2 layers were combined, washed with water followed by brine, and finally dried over anhyd $MgSO_4$. Evaporation of CH_2Cl_2 gave **11** as a solid (0.20 g, 91%): mp 120 °C; ^{1}H NMR (δ, $CDCl_3$, 90 MHz) 3.0 (s, 6H), 3.8 (s, 3H), 4.6 (s, 2H), 6.7 (d, 1H), 7.3 (d, 1H).

Methyl 6-Formyl-4-(dimethylamino)pyridine-2-carboxylate (10). A mixture of the above alcohol, **9** (0.16 g, 0.76 mmol), and MnO_2 (0.75 g, 8.6 mmol) in dry CH_2Cl_2 (15 mL) was stirred under nitrogen for 24 h, during which almost all of the alcohol was converted to aldehyde. The reaction mixture was filtered through Celite, the CH_2Cl_2 layer evaporated, and the residue chromatographed over a silica gel column using ethyl acetate as an eluent to give 0.14 g (88%) of the aldehyde, **10**, as a white powder: mp 118 °C (lit.[28b] mp 117−117.5 °C); IR (Nujol) 1710, 1735 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$, 80 MHz), 3.0 (s, 6H), 4.0 (s, 3H), 7.2 (d, 1H), 7.75 (d, 1H), 9.93 (s, 1H).

2-(Hydroxymethyl)-6-(octadecyl)carbamoyl)-4-(dimethylamino)pyridine (4). A mixture of 0.104 g (0.5 mmol) of methyl 6-(hydroxymethyl)-4-(dimethylamino)pyridine-2-carboxylate, **9**, and 0.135 g (0.5 mmol) of *n*-octadecylamine was heated to a clear melt and kept for 12 h at ∼90 °C. Upon cooling a solid was obtained which was purified by column chromatography (silica gel) using a mixture of (1:1) EtOAc and petroleum ether (bp 60−80 °C). Evaporation of the organic solvents from appropriate fractions gave **4** as a solid (0.157 g, 71%): mp 62 °C (74 °C clear melt); IR (Nujol) 3300, 1650 cm^{-1}; ^{1}H NMR (δ, $CDCl_3$, 200 MHz), 0.9 (t, 3H), 1.3−1.6 (br s and br t, 32H), 3.0 (s, 6H), 3.4 (m, 2H), 4.7 (s, 2H), 6.5 (d, 1H), 7.4 (s, 1H). Anal. Calcd for $C_{27}H_{49}N_3O_2$: C, 72.48; H, 10.96; N, 9.34. Found: C, 72.53; H, 11.02; N, 9.06.

6-[(Hexadecylamino)methyl]-4-(dimethylamino)-2-(hydroxymethyl)pyridine (3). Methyl 6-(formyl)-4-*N*,*N*(dimethylamino)pyridine-2-carboxylate, **10**, (0.2 g, 0.96 mmol), was taken up in dry THF (10 mL). A solution of *n*-hexadecylamine (0.23 g, 0.96 mmol) in THF (3 mL) containing a few 4 Å molecular sieves was added and the reaction mixture was stirred under N_2 atmosphere for 4 h. The solution was filtered, and the solvent was stripped from the filtrate to give a white solid. The unpurified solid (imine) was dissolved in dry MeOH, 0.18 g (0.48 mmol) of $NaBH_4$ was added, and the reaction mixture was stirred at ambient temperature for 15 h. Excess $NaBH_4$ was quenched by the addition of small aliquots of 1 N HCl until pH ∼7. The solvent was evaporated, and the residue was extracted thoroughly with $CHCl_3$. The $CHCl_3$ layer was washed with saturated, aqueous $NaHCO_3$ solution and dried. $CHCl_3$ was removed from the organic layer to give a crude solid which was purified by column chromatography over neutral alumina with 5% MeOH in $CHCl_3$ to give **3** as a solid (0.264 g, 68%): mp 55−56 °C; ^{1}H NMR (δ, $CDCl_3$, 200 MHz) 0.9 (t, 3H), 1.3−1.5 (br s and br t, 28H), 2.7 (t, 2H), 3.0 (s, 6H), 3.9 (s, 2H), 4.7 (s, 2H), 6.3 (s, 1H), 6.5 (s, 1H). Anal. Calcd for

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$C_{25}H_{47}N_3O$: C, 74.02; H, 11.68; N, 10.36. Found: C, 73.57; H, 11.68; N, 9.98.

Methyl 4-(*N*-Methyl-*N*-octadecylamino)pyridine-2,6-dicarboxylate (11). A mixture of 0.41 g (1.72 mmol) of 4-chloropyridine-2,6-dicarboxylic acid, **7**, and 0.53 g (1.9 mmol) of *N*-methyl-*N*-octadecylamine was taken up in 10 mL of dry MeOH in a screw-top sealed tube and heated to 120 °C for 48 h. Then the solvent was removed, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ layer was washed thrice with water and passed through anhydrous Na_2SO_4 from which the solvent was evaporated to give a crude 4-(*N*-methyl-*N*-octadecylamino)pyridine-2,6-dicarboxylic acid. Because of the difficulty in its purification possibly due to aggregation, this material was converted into the corresponding dimethyl ester as follows.

To 13 mL of ice-cold dry MeOH was added dropwise 4 mL of $SOCl_2$, and the mixture was stirred for 10 min. Then 0.84 g of crude 4-(*N*-methyl-*N*-octadecylamino)pyridine-2,6-dicarboxylic acid was added and the mixture refluxed for 8 h. The solvent was evaporated in vacuo, the residue dissolved in $CHCl_3$ (25 mL), and the solution washed with a 1% $NaHCO_3$ (2 × 10 mL) solution. The $CHCl_3$ layer was passed through anhydrous Na_2SO_4. The evaporation of organic solvent gave a white solid which was further purified by column chromatography (silica gel) using $CHCl_3$ as an eluent. Fractions corresponding to the pure compound as indicated by TLC were evaporated to give **11** a white, hygroscopic solid (0.4 g, 49%): mp 67−69 °C; IR (Nujol) 1710 cm^{-1}; ^1H NMR (δ, $CDCl_3$, 200 MHz) 0.9 (t, 3H), 1.3 (s, 32H), 3.0 (s, 3H), 3.4 (t, 2H), 4.0 (s, 6H), 7.8 (s, 1H), 8.3 (s, 1H); IR (Nujol) 1710 cm^{-1}; MS *m*/*z* 476 (M^+, 12), 237 (100). We also obtained a hygroscopic solid upon the conversion of **11** to its hydrochloride salt which was used for elemental analysis. Anal. Calcd for $C_{28}H_{48}N_2O_2 \cdot HCl \cdot 1.5H_2O$: C, 66.2; H, 9.46; N, 5.52. Found: C, 66.48; H, 9.27; N, 5.52.

2,6-Bis(hydroxymethyl)-4-(*N*-methyl-*N*-octadecylamino)pyridine (5). A 0.1 g (0.23 mmol) sample of dimethyl ester, **11**, was dissolved in dry MeOH and cooled to 0 °C. $NaBH_4$ (0.043 g, 1.13 mmol) was added, and the resulting mixture was stirred at ambient temperature for 12 h. The reaction mixture was neutralized with dilute HCl and the solvent evaporated in vacuo. The residue was extracted with 5% MeOH in $CHCl_3$. Upon evaporation of the solvent, a solid product, **5**, was obtained (0.068 g, 72%): mp 68 °C (soften), 120 °C (complete melt); IR (Nujol) 3300 cm^{-1} (broad); ^1H NMR (δ, $CDCl_3$, 200 MHz) 0.9 (t, 3H), 1.4−1.6 (br s and br t, 32H), 3.0 (s, 3H), 4.6 (s, 2H), 4.8 (s, 4H), 6.7 (s, 1H), 7.3 (s, 1H); MS *m*/*z* 420 (M^+, 20), 181 (100); HRMS mass calcd for $C_{26}H_{48}N_2O_2$ 420.3716, found 420.3697.

Kinetic Measurements. Solutions of ligands and additives (CTABr) were prepared in 0.05 M HEPES buffer (μ = 0.1 KCl). Reaction temperatures were maintained at 25 ± 0.1 °C unless otherwise mentioned. The metallomicelles were generated in situ by the addition of an appropriate amount of stock solution of a given metal salt to the cuvette. The solution was carefully stirred, and the reaction was initiated by addition of 15 μL of stock solution of a given substrate in CH_3CN. Substrate hydrolysis was followed spectrophotometrically by measuring the absorbance at 400 nm for the release of *p*-nitrophenoxide ion as a function of time. Esterolysis followed pseudo-first-order kinetics when a large excess of catalyst (5×10^{-4} M) was employed over substrate (2.5×10^{-5} M) and the rate constants were obtained by nonlinear fit of equation $(A_\infty - A_0)/(A_\infty - A_t) = e^{kt}$, where A_∞ and A_t are absorbances at infinite time and time *t*, respectively.

Acknowledgment. This research was sponsored by the Grants-in-Aid Scheme of DRDO, Government of India. K.S. thanks the UGC for a senior research fellowship.

JO9707996