Amphiphilic urocanic acid derivatives as catalysts of ester hydrolysis

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The hydrolysis of *p*-nitrophenyl acetate at pH 8 and 25 °C is demonstrated to be a good test reaction to study the influence of the structure of several long-chain derivatives of urocanic acid on the imidazole ring reactivity in organized media. Cetyltrimethylammonium bromide micelles including (*E*)-dodecyl urocanate gave an approximately 7-fold rate enhancement over (*Z*)-dodecyl urocanate, 30-fold over (*E*)-urocanic acid and 4790-fold over CTABr. The behavior of (*E*)-dodecyl urocanate in the presence of excess substrate was also been investigated. The value of k_{obs} decreased by 37% as the substrate : catalyst ratio was increased from 1 : 10 to 10 : 1. Under the same conditions, the total activity was restored when one equivalent of chloral was added to one equivalent of (*E*)-dodecyl urocanate. Chloral significantly enhanced the deacylation rate of *N*-acetyl dodecyl urocanate, giving a good reaction turnover.

(*E*)-Urocanic acid, a major metabolite of histidine, is one of the major UV light absorbers in the epidermis where it acts as a natural photoprotecting agent.[1,2] The *Z* isomer has been found to have immunosuppressive activity[3,4] although the mechanism has yet to be completely elucidated. In our laboratory several derivatives of urocanic acid[5,6] have been synthesized to (*i*) improve solubility and facilitate formulation and (*ii*) study structure-activity relationships in immunology. Intramolecular hydrogen bonding in the *Z* isomer may affect the interactions of the molecule with membranes and the nucleophilic properties of the imidazole ring. This ring is involved in many biological processes, for example in α-chymotrypsin the His-57 imidazole group is the nucleophile that attacks the substrates.[7]

Imidazole-functionalized surfactants have been reported to be remarkably effective catalysts under micellar conditions for the hydrolysis of *p*-nitrophenyl acetate[8] and phosphate esters.[9] Generally, the catalysis involves the formation and decomposition of an acyl imidazole intermediate. This reaction has been chosen to study the influence of the structure of several long-chain derivatives of urocanic acid on the imidazole ring reactivity. Some imidazole derivatives have been chosen as references. The various catalyst structures, containing a urocanic moiety and an imidazole ring, are given in Scheme 1.

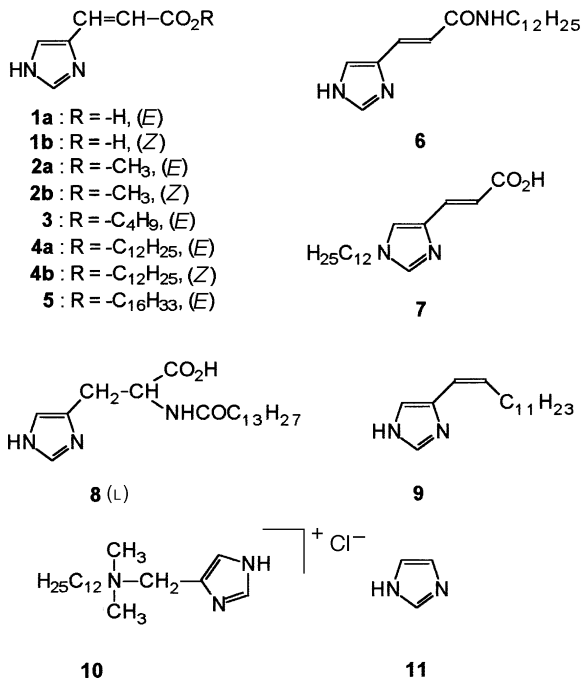
Results and discussion

The present study describes the kinetic analysis of imidazolyl catalysis during the hydrolysis of *p*-nitrophenyl acetate (PNPA) in the presence of cetyltrimethylammonium bromide (CTABr) micelles.

Hydrolysis of PNPA catalyzed by CTABr micelles alone

We studied the catalysis by CTABr micelles of the PNPA hydrolysis in 0.05 M Tris buffer, pH 8, ionic strength 0.044 (KCl), at 25.0 ± 0.1 °C. The critical micelle concentration (CMC) of CTABr in the buffer solution was determined at 25 °C. As expected, the presence of electrolytes lowered the CMC to 9.3×10^{-5} M (9.4×10^{-4} M in water).

When [CTABr] > [PNPA], pseudo-first-order rate constants (k_{obs}) were evaluated by spectrophotometrically monitoring the time-dependent concentration of released *p*-nitrophenoxide ion at 400 nm (Guggenheim's method). The reproductibility of k_{obs} was within ±3% in duplicate runs. k_{obs} was measured at various CTABr concentrations above the CMC at pH 8. The k_{obs} *vs.* [CTABr] profile (Fig. 1) shows that k_{obs} increases initially with increasing [CTABr], reaches a maximum at about 10^{-2} M and then declines. Such a bell-shaped rate profile is characteristic of reactions in surfactant solutions involving ionic species and partly dissolved substrates.[10] The pseudo-first-order rate constant increases with CTABr concentration because of an increase in the relative amount of the two reactants (OH^- and PNPA) dissolved in



Scheme 1 Catalysts containing an imidazole ring.

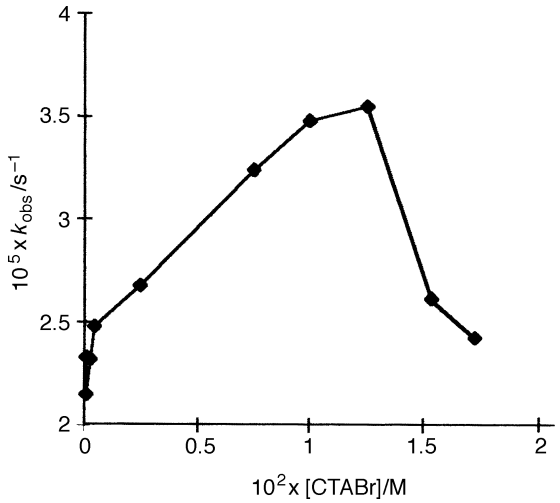


Fig. 1 Pseudo-first-order rate constants (k_{obs}/s^{-1}) for the hydrolysis of PNPA ($[PNPA]_0 = 5 \times 10^{-5}$ M) by various concentrations CTABr (10^{-4} to 1.75×10^{-2} M) at pH 8, 0.05 M Tris buffer, ionic strength 0.044, 25 ± 0.1 °C.

the micelle pseudophase in which the reaction takes place. The k_{obs} value reaches a maximum when binding is optimal and is reduced when additional CTABr dilutes the micellar pseudophase. Similar micellar catalysis profiles for different pH (various NaOH concentrations) have been reported in the literature.[11]

The maximum value of k_{obs} ($k_{obs}^{max} = 3.48 \times 10^{-5}$ s^{-1}) reached at 10^{-2} M CTABr under the described experimental conditions may be compared to $k_{obs(buffer)} = 2.6 \times 10^{-5}$ s^{-1} (measured for non-micellar cleavage of PNPA under similar buffer conditions). A catalytic factor of 1.4 for CTABr micelles relative to buffer alone in PNPA cleavage was estimated.

Hydrolysis by imidazolyl derivatives in the presence of CTABr micelles

The catalytic process for the hydrolysis of esters by a functionalized micellar system can be described by eqn. (1) where cat_{Im} designates the imidazolyl derivative catalyst, PNPA is the substrate, cat_{Im}-Ac is the acylated intermediate and k_a and k_d the rate constants for the acylation and deacylation processes, respectively.

$$\text{PNPA} + \text{cat}_{Im} \xrightarrow{k_a} \text{cat}_{Im}\text{-Ac} \xrightarrow[+\,H_2O]{k_d} \text{cat}_{Im} + CH_3COOH \quad (1)$$

p-nitrophenoxide ion
$\lambda_{max} = 400$ nm

Hydrolysis was carried out at various catalyst concentrations at a fixed CTABr concentration (10^{-2} M) such that [CTABr] > [catalyst] > [PNPA]. The kinetics of the acylation process were clearly first-order with respect to catalyst over the range of concentrations considered. The rate law is given in eqn. (2), where k_0 is the rate constant for substrate cleavage in the absence of the functionalized catalyst and k_a is the second-order rate constant for hydrolysis by a functionalized catalyst.

$$\text{rate} = (k_o + k_a[\text{cat}_{Im}])[\text{PNPA}] = k_{obs}[\text{PNPA}] \quad (2)$$

The activities of the catalysts were determined and the values of k_{obs} are given in Table 1. The second-order rate constants k_a were determined and are reported in Table 2. The reaction in CTABr at 10^{-2} M has been taken as reference and the k_a/k_{CTABr} ratios calculated (Table 3) where $k_{CTABr} = k_{obs}^{max}/10^{-2}$, that is 3.48×10^{-3} M^{-1} s^{-1}.

All the systems studied enhanced the hydrolysis rate at least 95-fold with respect to CTABr, except for compound **7**, which did not catalyze the reaction.

In the presence of an imidazole moiety, the reaction is accelerated and proceeds by nucleophilic catalysis leading to the formation of an acetylimidazole. This intermediate may be identified by its characteristic absorption at 245 nm[8] but its molecular extinction coefficient is too weak to allow the kinetics to be systematically followed at this wavelength. Such an *N*-acetyl intermediate cannot be formed from compound **7**; this explains the absence of a catalytic effect (Table 1). Indeed, one effect influencing the catalytic efficiency is the nucleophilic character of imidazoles, which is dependent on the nature of the ring substituents. In order to catalyze the reaction, imidazole must be sufficiently nucleophilic to form the cat_{Im}-Ac intermediate, which must not accumulate.

We note that the rate increased as the catalyst acidity increased, with the best catalysts having the lowest pK_a. This is in disagreement with what would be expected and is actually misleading. Other factors, including the ability of the catalyst to be incorporated into the CTABr micelles, are probably more important and the acidity may play a role in this incorporation. Table 3 gives the pK_a values of the imidazolium derivatives taken from the literature[12] or determined here spectrophotometically. The pK_a of the imidazolium itself is 7.1, so at pH 8 one can assume that the active species is the neutral ring. For compounds of lower pK_a, according to the literature[13] the rate enhancements observed might be attributed to the intervention of the anionic form of the imidazole moiety in the acylation step. This can explain the greater

Table 1 Apparent rate constants ($10^4 \times k_{obs}/s^{-1}$) for the hydrolysis of PNPA catalyzed by imidazolyl derivatives[a]

$10^4 \times$ [catalyst]/M	1	4	8	12	16	20
1a	1.18	3.32	5.97	7.85	9.64	11.5
1b	1.67	4.93	8.94	12.9	17.1	20.9
2a	3.05	11.2	25.4	31.0	41.1	49.5
2b	0.67	1.76	3.23	4.58	5.70	6.81
3	14.2	59	106	141	183	215
4a	25	82	154	229	290	336
4b	2.80	10.9	21.3	32.5	39.7	53.2
5	17.3	68.7	137	181	227	277
7	0.38	0.38	0.38	0.37	0.38	0.38
8	5.40	19.9	36.6	52.1	70.8	84.2
9	1.18	3.95	8.21	12.2	15.6	18.9
10	1.60	56.3	10.5	15.2	20.1	25.7
11	0.68	1.76	2.95	4.80	5.98	7.44
$10^4 \times$ [catalyst]/M	0.6	0.8	1	2	3.5	5
6	1.44	1.74	2.01	3.36	5.22	7.42

[a] $[CTABr]_0 = 10^{-2}$ M, 0.05 M Tris buffer, pH 8, ionic strength 0.044, 25 ± 0.1 °C. The reproductibility of k_{obs} was within ±3% in duplicate runs.

Table 2 Second-order rate constants ($k_a/M^{-1}\ s^{-1}$) and the k_a/k_{CTABr} ratios for the hydrolysis of PNPA catalyzed by various catalysts alone or by 50 : 50 mixtures with chloral (**12**) or aldehyde-hydrate surfactants (**13** or **14**)[a]

$k_a/M^{-1}\ s^{-1}$					k_a/k_{CTABr}			
Catalyst	Alone	+**12**	+**13**	+**14**	Alone	+**12**	+**13**	+**14**
CTABr	3.48×10^{-3}	0.41	0.34	0.86	1	120	100	250
1a	0.50	1.03	0.51		145	295	145	
1b	1.01	1.35			290	310		
2a	2.42	2.56	2.16		695	735	620	
2b	0.32	0.87	1.08		95	250	310	
3	10.42	9.31	10.67		2995	2675	3070	
4a	16.66	16.78	16.34	15.41	4790	4820	4700	4430
4b	2.59	3.01	3.12	2.43	745	865	900	700
5	13.42	13.47	13.99		3860	3870	4020	
6	1.34	2.58	1.96		385	745	565	
8	4.16	4.04	4.58	4.08	1195	1160	1320	1175
9	0.94	1.43	1.35	2.22	270	415	390	640
10	1.25	2.77	1.97	2.80	360	800	570	805
11	0.36	0.71	0.60	1.37	105	205	175	395

[a] $[CTABr]_0 = 10^{-2}$ M, 0.05 M Tris buffer, pH 8, ionic strength 0.044, 25 ± 0.1 °C.

activity of urocanic acid compared to that of imidazole but not the difference observed between the two isomers **1a** and **1b**. According to the pK_a effect, the reverse order of reactivity between the two isomers would have been expected. However, due to intramolecular hydrogen bonding in the *Z* isomer, the approach of the two urocanic molecules towards the surface of the micelle might be different and the interaction between micellar substrate and (*Z*)-urocanic acid might be more efficient.

In co-micelles of CTABr and of (*E*)- or (*Z*)-urocanate derivatives (**2**–**5**), the catalytic efficiencies depended on the lipophilic character of the urocanates. A very large rate enhancement was observed in the presence of **4a** (~30-fold more than **1a)** and therefore, the most suitable chain length seems to be twelve carbon atoms. Other imidazolic catalysts were then studied to compare this chain length effect. Compounds **9** and **10** catalyzed the hydrolysis of PNPA 270 and 360 times faster than the unfunctionalized surfactant CTABr. Imidazole surfactants **9** and **10** in co-micellar systems were more effective (3 and 4-fold, respectively) than the imidazole ring (**11**) in a micellar system. Thus, the presence of a long chain permits the incorporation of the catalyst into the CTABr micelles and so increases the catalytic effect by favoring the approach of the substrate toward the imidazolyl moiety.

The histidine derivative **(8)** is a better catalyst than **9** or **10** so, it is reasonable to assume that because of its negative charge, it is differently incorporated into the cationic micelles, the imidazole moiety being anchored in an efficient orientation. The carboxylate group can also act as a base catalyst or fix the tautomeric form of the imidazole. These results show that the lipophilic character of the molecule has a similar effect on the catalytic efficiency of simple imidazole derivatives and of (*Z*)-urocanates, but is of great importance for (*E*)-urocanates in which the orientation of the carboxylate function seems to position the imidazole ring efficiently at the micellar surface.

In the *Z*-urocanate derivatives, which are less efficient, intramolecular hydrogen bonding blocks one tautomeric form of the imidazole. This could explain the variation of pK_a and also induce a modification of the orientation of the imidazole moiety. Less favorable anchoring of the catalyst in the micelle could also be involved in the lower catalytic effect observed for **6** compared to that of compound **4a**.

These results show that PNPA hydrolysis is a good test reaction to evaluate the interaction of various urocanic derivatives with organized systems and by extrapolation with biological membranes. They also show that (*E*)-urocanates should be included among the compounds used to design enzyme models.

In the literature, micellar α-chymotrypsin models containing hydroxyl-,[14] imidazole-[15] and carboxylate[16]-functionalized surfactants have been studied. Imidazole-functionalized surfactants have been reported to be remarkably effective under micellar conditions for the hydrolysis of *p*-nitrophenyl acetate[8] and phosphate esters only if an excess of catalyst is used. In order to accelerate the deacylation of the catalyst, synthetic bifunctional surfactants or co-micellar systems having a hydroxyl group and an imidazole ring have been developed.[17] For bifunctional micelles, the mechanism involves acylation of the imidazole ring followed by relatively rapid acyl transfer to the hydroxyl in the micellar phase. However, these systems do not involve true turnover. Menger and Persichetti[18] described an aldehyde-hydrate-functionalized surfactant, with a catalytic turnover, that cleaved *p*-nitrophenyl phosphate at pH 9 with $k_{obs} = 2 \times 10^{-2}\ s^{-1}$ (for a catalyst concentration of 8×10^{-3} M), a rate enhancement of 1800-fold over pure buffer at pH 9.0 and 210-fold over the unfunctionalized surfactant, dodecyltrimethylammonium bromide (DTABr).

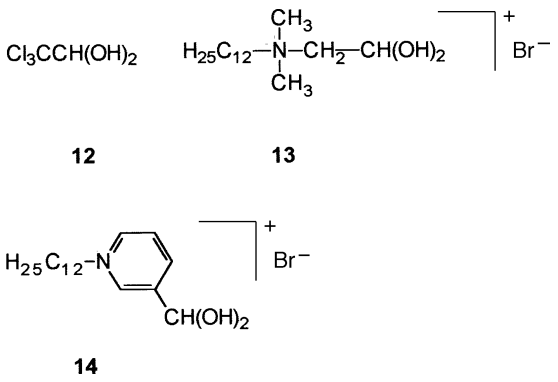
We decided to compare the efficiencies of imidazolyl and aldehyde-hydrate micellar systems in PNPA hydrolysis. In all cases, the functionalized compounds were examined in micellar or co-micellar CTABr solutions. The various catalyst structures containing an aldehyde-hydrate function are given in Scheme 2. The values of the apparent rate constants (k_{obs}) for the hydrolysis of PNPA catalyzed by aldehyde-hydrate derivatives are given in Table 4. The second-order rate constants (k_a) are reported in Table 2.

The aldehyde-hydrates **12**, **13** and **14** cleaved PNPA 120, 100 and 250 times more rapidly than micellar CTABr. The results obtained with **13**, to our knowledge the best aldehyde-hydrate catalyst described up to now, agree with those of the

Table 3 pK_a values of the various studied catalysts

Catalyst	pK_a[a]
1a	5.89 ± 0.03 [b]
1b	6.78 ± 0.03 [b]
2a	4.98 ± 0.02 [b]
2b	5.58 ± 0.03 [b]
4a	3.95 ± 0.02
4b	4.05 ± 0.02
8	6.2 [b]
11	7.10 [b]

[a] First dissociation constant. [b] Ref. 12



12 **13**

14

Scheme 2 Catalysts containing an aldehyde-hydrate function.

literature.[19] The efficiency obtained for **13** and for chloral are similar. Chloral is a cheap and easy-to-use catalyst that has an efficiency similar to that of imidazole (105 times the catalytic effect of CTABr). An NMR study showed that **12**, **13** and **14** are totally hydrated in water [absence of ^{1}H NMR (D_2O) signal of the aldehyde proton and of the ^{13}C NMR (D_2O) signal of the carbonyl]. The pK_a values of **12** and **13** given in the literature[19] are 10 and 10.9, respectively. This could explain why these compounds are better catalysts than alcohol surfactants with higher pK_as, for example *N*-hexadecyl-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl)ammonium chloride has a pK_a of 12.4[20] and an activity only 12 times that of CTABr. Under the mildly basic conditions used (pH 8), the formation of the nucleophilic oxyanion may be favored in the case of aldehyde-hydrates and enhance the rate of *O*-acylation. The new aldehyde-hydrate **14** described in this work was revealed to be more effective than **12** and **13**, which could be explained by the influence of the pyridinium ring on the nucleophilicity of the catalyst and/or on the orientation of the active part due to stacking effects.

The catalytic abilities of bifunctional micellar systems were also determined. Table 5 gives the apparent rate constants k_{obs} for the hydrolysis of PNPA catalyzed by mixtures (50 : 50 molar ratio) of aldehyde-hydrate compounds and imidazolyl derivatives in micelles of CTABr. The corresponding second-order rate constants (k_a), given in Table 2, show that the catalytic activities of bifunctional micelles are not related in any clear way to the sum of the activities of the single catalysts. Also, no clear relationship appears between these observations and the structures of the different catalysts.

Alone, or mixed with another catalyst, (*E*)-dodecyl urocanate proved to be the most efficient compound for PNPA cleavage. In order to know if it can be used as a hydrolysis catalyst, its turnover capacity had to be determined.

Turnover experiments (kinetic studies with excess substrate)

The behavior of chloral (**12**) alone, of (*E*)-dodecyl urocanate (**4a**) alone and of a mixture of the two catalysts in the presence of an excess of substrate was studied to determine their turnover capacity (with [CTABr] > [PNPA] > [catalyst]). Experiments were carried out at pH 8 and 25 °C with 10^{-2} M CTABr, the ratios of catalyst-to-PNPA concentrations used were 10 : 1, 1 : 1, 1 : 2, 1 : 5 and 1 : 10. In each run, the initial concentration of catalyst was fixed at 7×10^{-5} M for compound **4a** and at 5.8×10^{-5} M for compound **12**. For the two catalysts: (*i*) the total quantity of PNPA in each run was hydrolyzed; (*ii*) the kinetics were pseudo-first-order so the catalyst concentration remained constant during the study; (*iii*) "burst kinetics"[21] were not observed under these conditions. Similar results have already been reported in the literature.[22]

The data in Table 6 show the apparent rate constants k_{obs} obtained from the kinetics of *p*-phenoxide anion release. For **12**, whatever the substrate : catalyst ratio, the values determined are similar; chloral must therefore be quickly regenerated. For **4a**, the k_{obs} value decreased by 37% as the substrate : catalyst ratio increased from 1 : 10 to 10 : 1. The catalytic behavior of the **12**, **4a** and CTABr mixture in the presence of excess PNPA was studied to determine whether addition of chloral could accelerate the regeneration of **4a** (Table 7). Total activity was restored when one equivalent of chloral was added to one equivalent of (*E*)-dodecyl urocanate. For example, the k_{obs} ratio increased from 63% for a PNPA : **12** : **4a** ratio of 10 : 0 : 1 to 100% for 10 : 1 : 1. As seen above, with excess catalyst the addition of **12** to **4a** did not greatly improve the catalytic effect of **4a** alone, so we can assume that the chloral significantly enhances the deacylation rate of *N*-acetyldodecyl urocanate, giving a high reaction turnover.

Conclusion

This work showed that PNPA hydrolysis can be used to test differences in the interaction of configurational isomers of urocanic derivatives with organized systems. It also permitted the elaboration of a catalytic system, composed of an equimolar mixture of (*E*)-dodecyl urocanate and chloral in CTABr micelles, which presents a remarkable efficiency in PNPA hydrolysis and high turnover. Applications of this system to chemical decontamination processes are being considered.

Experimental

General

Commercial imidazole (Aldrich Chemical Co.) was used after purification by recrystallization in petroleum ether. Commercial (*E*)-urocanic acid (Aldrich Chemical Co.) and commercial *n*-dodecyltriphenylphosphonium bromide (Lancaster) were used without further purification. Compounds **2a**,[23] **3**,[24] **4a**[24] and **5**[24] were prepared by esterification of (*E*)-urocanic acid. Compounds **2b** and **4b** were prepared by photoisomerization[25] of **2a** and **4a** in CH_3CN at 254 nm. Compound **7** was prepared by N^{τ}-alkylation of compound **4a** and acid hydrolysis of the methyl ester function.[23] Dimethyldodecyl[(4-imidazol)methyl]ammonium chloride (**10**),[16a] N^{α}-myristoyl-L-histidine (**8**)[26] and **13**[27] were prepared according to literature methods. PNPA was purchased from Aldrich Chemical Co. and was purified by recrystallization from dry hexane. Acetonitrile (Prolabo) was used after drying and distillation over P_2O_5.

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 spectrometers. The DCI (NH_3) mass spectra were recorded on

Table 4 Apparent rate constants ($10^4 \times k_{obs}/s^{-1}$) for the hydrolysis of PNPA catalyzed by aldehyde-hydrate derivatives[a]

$10^4 \times$ [catalyst]/M	1	4	8	12	16	20
12	0.68	1.91	3.65	5.16	6.41	8.53
13	0.38	1.58	2.60	3.99	5.53	6.90
14	1.17	3.03	5.50	9.91	13.4	17.3

[a] [CTABr] = 10^{-2} M, 0.05 M Tris buffer, pH 8, ionic strength 0.044, 25 ± 0.1 °C. The reproductibility of k_{obs} was within ±3% in duplicate runs.

Table 5 Apparent rate constants ($10^4 \times k_{obs}/s^{-1}$) for the hydrolysis of PNPA catalyzed by 50 : 50 mixtures of aldehyde-hydrates and imidazolyl derivatives[a]

$10^4 \times$ [catalyst]/M	1			4			8			12			16			20		
	+ **12**	+ **13**	+ **14**	+ **12**	+ **13**	+ **14**	+ **12**	+ **13**	+ **14**	+ **12**	+ **13**	+ **14**	+ **12**	+ **13**	+ **14**	+ **12**	+ **13**	+ **14**
1a	2.02	1.15		6.19	3.56		10.8	5.80		14.8	7.36		18.3	9.40		21.9	11.3	
1b	2.25			7.36			13.4			18.4			23.8			27.9		
2a	3.77	3.16		13.4	11.4		24.3	21		34.9	28.8		45.1	37.6		52.1	44.2	
2b	1.36	1.39		4.24	4.46		7.79	8.48		11.4	12.6		14.8	17.2		17.9	22.0	
3	16.2	32.3		57.8	63.7		104	110		137	146		168	194		196	235	
4a	33.3	20.9	22.2	110	78.7	82.4	163	149	156	241	200	210	289	271	253	365	336	325
4b	3.86	3.46	3.37	14.0	11.9	10.8	26.8	24.8	20.1	38.7	36.0	32.9	50.2	47.6	40.4	61.2	63.9	48.6
5	18.8	17.3		72.7	49.2		135	118		192	177		233	235		276	272	
8	5.93	5.36	5.02	21.5	19.8	18.6	39.5	38.6	37.3	62.3	59.2	53.9	76.5	72.8	68.7	88.5	96.2	81.9
9	1.74	1.75	2.38	59	5.95	8.71	12.0	9.85	18.4	17.4	16.4	23.9	23.5	21.4	31.9	28.8	27.7	47.1
10	3.56	3.09	3.11	12.8	10.7	10.4	23.9	19.4	20.3	32.1	27.6	34.0	42.6	33.8	44.6	59.0	41.0	55.3
11	1.31	0.58	1.51	2.54	2.56	3.77	5.31	4.84	9.52	9.02	7.12	14.8	10.9	8.80	20.2	14.7	12.6	27.4
$10^4 \times$ [catalyst]/M	0.6			0.8			1			2			3.5			5		
6	2.28	1.75	—	2.70	2.18	—	3.29	2.62	—	5.82	4.70	—	9.67	7.52	—	13.60	10.40	—

[a] $[CTABr]_0 = 10^{-2}$ M, 0.05 M Tris buffer, pH 8, ionic strength 0.044, 25 ± 0.1 °C. The reproductibility of k_{obs} was within ±3% in duplicate runs.

Table 6 Cleavage of excess PNPA by **4a** and by **12**[a]

	4a		**12**	
[PNPA]:[catalyst]	$10^4 \times [PNPA]_0$/M	$10^3 \times k_{obs}/s^{-1}$	$10^4 \times [PNPA]_0$/M	$10^5 \times k_{obs}/s^{-1}$
1 : 10	0.07	1.55	0.058	7.0
1 : 1	0.7	1.46	0.58	6.90
2 : 1	1.4	1.26	1.16	6.86
5 : 1	3.5	1.06	2.9	6.94
10 : 1	7	0.98	5.8	6.89

[a] $[CTABr]_0 = 10^{-2}$ M, 0.05 m Tris buffer, pH 8, ionic strength 0.044, 25 °C. The initial concentrations of catalysts are: $[\mathbf{12}]_0 = 5.8 \times 10^{-5}$ M and $[\mathbf{4a}]_0 = 7 \times 10^{-5}$ M.

a Nermag R10-10 apparatus. The electrospray mass spectra were recorded on a Perkin Elmer-SCIEX API 100 spectrometer (samples were infused at 5 μL min^{-1} in MeOH). Melting points were determined on an Electrothermal apparatus (capillary tubes). The microanalyses were carried out on a Carlo Erba 1106 at the ENSCT (Toulouse, France).

Syntheses

(*E*)-4-(Undec-1,2-enyl)imidazole (9). To a stirred solution of *n*-dodecyltriphenylphosphonium bromide (2.27 g, 4.44 mmol) in 30 mL of dry THF, at −78 °C, under N_2, was added dropwise 1.6 M *n*-butyllithium in hexane (2.77 mL, 4.44 mmol). The resulting yellow suspension was stirred for an additional 15 min, after which a solution of 4-(*N*-triphenylmethyl)-imidazolecarbaldehyde[28] (1.5 g, 4.44 mmol) in 20 mL of dry THF was added dropwise. After stirring at −78 °C for 3 h, the mixture was allowed to reach room temperature overnight. The solvent was evaporated. Water was added to the residue and the mixture extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting solid was chromatographed over silica gel (eluent: CH_2Cl_2–EtOH 99 : 1) to obtain N^{τ}-triphenylmethyl-4-(undec-1,2-enyl)imidazole (0.60 g, 27%) as a white solid.

N^{τ}-Triphenylmethyl-4-(undec-1,2-enyl)imidazole (400 mg, 0.81 mmol) was dissolved in 10 mL of acetone with 0.15 mL of concentrated aqueous HCl. The solution was stirred under reflux for 5 h. After removal of the solvent, the residue was extracted with Et_2O–H_2O. The organic layer was washed with water, dried over $MgSO_4$ and concentrated. The resulting product was purified by filtration through silica gel with CH_2Cl_2 and then EtOH. The ethanolic layer was concentrated to obtain **9** (150 mg, 74%) as a white solid, mp 58 °C; ^{1}H NMR (250 MHz, DMSO-d_6): δ 0.84 (t, 3H, $J = 6.4$ Hz, CH_3), 1.23 (m, 16H, $(CH_2)_8$), 1.37 (m, 2H, $CH_2\beta$), 2.35 (td, 2H, $J_1 = J_{BX} = 7$ Hz, $CH_2\alpha$), 3.44 (s, 1H, NH), 5.42 (dt, 1H, $J_{AB} = 11.5$ Hz, $J_{BX} = 7$ Hz, =C*H*CH_2), 6.19 (d, 1H, $J_{AB} = 11.5$ Hz, ImC*H*=), 7.01 (s, 1H, H5Im), 7.61 (s, 1H, H2Im); ^{13}C NMR (250 MHz, DMSO-d_6): δ 13.86, 22.0, 28.64, 28.77, 31.21, 82.07, 120.25, 121.33, 128.9, 133.31, 134.83; CI-MS: *m/z* 249 ($M + H^+$), 266 ($M + NH_4^+$); Anal. calcd. for $C_{16}H_{28}N_2$; 0.5 H_2O: C, 74.65; H, 11.36; N, 10.88; found: C, 74.32; H, 11.35; N, 10.73%.

Table 7 Cleavage of excess PNPA by a mixture of **4a** and **12**[a]

[PNPA] : [**12**] : [**4a**]	$10^4 \times [PNPA]_0$/M	$10^3 \times k_{obs}/s^{-1}$
1 : 10 : 10	0.07	1.55
10 : 1 : 1	7	1.56

[a] $[CTABr]_0 = 10^{-2}$ M, 0.05 M Tris buffer, pH 8, ionic strength 0.044, 25 °C, $[\mathbf{4a}]_0 = [\mathbf{12}]_0 = 7 \times 10^{-5}$ M.

(*E*)-Dodecyl urocanamide (6). (*E*)-Urocanic acid (1 g, 7.24 mmol) was added to a solution of *n*-dodecylamine (1.34 g, 7.24 mmol) in 15 mL of dimethylacetamide cooled to 0 °C. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (1.14 g, 7.24 mmol) was added to the stirred solution. The mixture was stirred at 0 °C for 2 h and for an additional 5 days at room temperature. The solvent was removed by lyophilization. The residue was purified by flash chromatography on silica gel (eluent: CH_2Cl_2–EtOH 91 : 9) to obtain **6** (817 mg, 37%) as a white solid, mp 150 °C; ^{1}H NMR (80 MHz, DMSO-d_6): δ 0.85 (t, 3H, $J = 6.3$ Hz, CH_3), 1.25 (m, 20H, $(CH_2)_{10}$), 3.16 (td, 2H, $J_1 = 5.7$ Hz, $J_2 = 6.5$ Hz, CONHCH_2), 6.37 (d, 1H, $J_{AB} = 15.6$ Hz, =C*H*CONH), 7.33 (s, 1H, H5Im), 7.36 (d, 1H, $J_{AB} = 15.6$ Hz, ImCH=), 7.66 (s, 1H, H2Im), 7.90 (t, 1H, $J_2 = 6.5$ Hz, CONH), 12.23 (s, 1H, NH); ^{13}C NMR (250 MHz, DMSO-d_6): δ 13.84, 21.99, 26.38, 28.62, 28.92, 29.13, 31.20, 118.47, 165.39; CI-MS: *m/z* 306 ($M + H^+$), 323 ($M + NH_4^+$); Anal. calcd. for $C_{18}H_{31}N_3O$: C, 70.78; H, 10.23; N, 13.76; found: C, 70.99; H, 10.44; N, 13.59%.

***N*-Dodecylpyridinium-3-carbaldehyde bromide (14).** 1-Bromododecane (2.22 mL, 9.23 mmol) was added dropwise to a solution of 3-pyridine carbaldehyde (1.76 mL, 18.7 mmol) in 10 mL dry THF. The mixture was refluxed under N_2 for 48 h. The solution was concentrated and the pyridinium salt was precipitated by adding anhydrous Et_2O. The solid was isolated and recrystallized from dry CH_2Cl_2–Et_2O to obtain **14** (519 mg, 15%), mp 65 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.83 (t, 3H, $J = 6.30$ Hz, CH_3), 1.20 (m, 18H, $(CH_2)_9$), 1.31 (m, 2H, $CH_2\beta$), 2.02 (m, 2H, CH_2N^+), 3.01 (s, OH), 6.52 (m, 0.683 H, 68.3% C*H*$(OH)_2$), 7–10 (4H, pyridinium), 10.43 (s, 0.317 H, 31.7% CHO); ^{13}C NMR (250 MHz, $CDCl_3$): δ 14.13, 22.68, 26.219, 29.12, 29.34, 29.57, 31.75, 31.90, 62.47, 86.78, 90.16, 127.98, 129.28, 134.68, 142.98, 144.49, 144.72, 146.91, 148.58, 187.17; MS (electrospray positive): *m/z* 276 (aldehyde), 294 (aldehyde-hydrate); Anal. calcd. for 68.3% $C_{18}H_{32}NO_2Br$ (aldehyde-hydrate), 31.7% $C_{18}H_{30}NOBr$ (aldehyde): C, 58.81; H, 8.33; N, 3.81; found: C, 58.58; H, 8.76; N, 3.64%.

Measurements

pK_a measurements. An ethanol solution of **4a** or **4b** catalyst (5×10^{-3} M, 20 μL) was added to 1980 μL of buffer solution (0.1 M citric acid, 0.2 M Na_2HPO_4) at various pH values (between pH 2 and pH 8). The absorbance at 272 nm and the pH of the solution prepared were determined at 25 °C. A plot of the pH *versus* the absorbance gave a pH-metric titration curve from which the pK_a was determined.[29]

Kinetic studies. The hydrolysis of *p*-nitrophenyl acetate (PNPA) was followed spectrophotometrically using a Perkin-Elmer HP 8452A Diode Array spectrophotometer equipped with a thermostated water bath ($T = 25 \pm 0.1$ °C). Pseudo-first-order rate constants (k_{obs}) were evaluated by spectrophotometrically monitoring the time-dependent concentration of released *p*-nitrophenoxide anion at 400 nm (Guggenheim's method). The reactions in different aggregates were followed

to >90% completion and showed good first-order kinetics ($r > 0.99$). The reactions were initiated by injecting 20 μL of a PNPA solution in CH_3CN into 1980 μL of a micellar solution, pre-equilibrated at 25 °C, containing the catalyst and the surfactant (CTABr) at the desired concentration in the buffer.

0.05 M Tris buffer, pH 8, ionic strength 0.044 (KCl) was prepared from distilled water. The stock solutions of CTABr and of catalyst were prepared in the buffer. Specific conditions for all the kinetics runs are described under Results and Discussion.

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