Zn^{2+} inclusion complexes of endodentate tripodands as carbonic anhydrase-inspired artificial esterases. Part 2.[1] Micellar systems

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Facile syntheses of two lipophilic, endo-tridentate tris-imidazole podands (**3** and **4**) are reported. These were designed for micellar media, where pre-organization for metal binding was anticipated to better reproduce the active site of *carbonic anhydrase* (CA). pH-Metric titrations and *p*-nitrophenyl acetate (pNPOAc) hydrolyses were carried out in the presence of Zn^{2+} and the results compared with those obtained with hydrophilic tripodand varieties. The results confirm stronger metal binding with the micelle-bound ligands and show reasonably low pK_a values near 8 for the deprotonation of metal-bound H_2O, as well as evidence for a proton shuttling analogous to that seen in the enzyme. The second-order rate constant for pNPOAc hydrolysis by $[Zn(\mathbf{3})OH]^+$ was estimated at 0.19(1) M^{-1} s^{-1}, the second-highest value measured for a biomimetic CA model.

Introduction

Carbonic anhydrase (CA) (EC 4.2.1.1) is an extremely efficient Zn^{2+} enzyme catalyzing the reversible hydration of CO_2 and possessing some esterase activity.[2] Its success seems to hinge on its ready ability to cause the ionization of a metal-bound H_2O at moderate pH.[3] In turn, that ability seems to arise from the imposition of a fairly hydrophobic micro-environment, a lower coordination number than would be adopted in bulk water, and from an efficient proton shuttling mechanism.[4]

Many biomimetic Zn^{2+} complexes have been made from nitrogenous tridentate ligands that imitate the histidine residues of CA,[5–8] as well as from less biomimetic bidentates[9] or a di-nucleating hexaazamacrocycle.[10] We have recently designed and prepared artificial esterases from tripodands **1** and **2** (Scheme 1).[1] In binding Zn^{2+} with their imidazole groups, these can completely encapsulate the ion, bind H_2O as the fourth ligand, and thereby form a relatively organic cavity that prevents ready access to bulk H_2O. The complexes showed Zn^{2+}–OH_2 ionization at relatively low pK_a values and we measured higher second-order rate constants for the hydrolysis of *p*-nitrophenyl acetate (pNPOAc) than had previously been measured in other CA mimics. As well, the complex of **2** showed cooperative protonations–deprotonations, which was evidence of H^+ shuttling between Zn^{2+}-bound H_2O and the amino vertex.

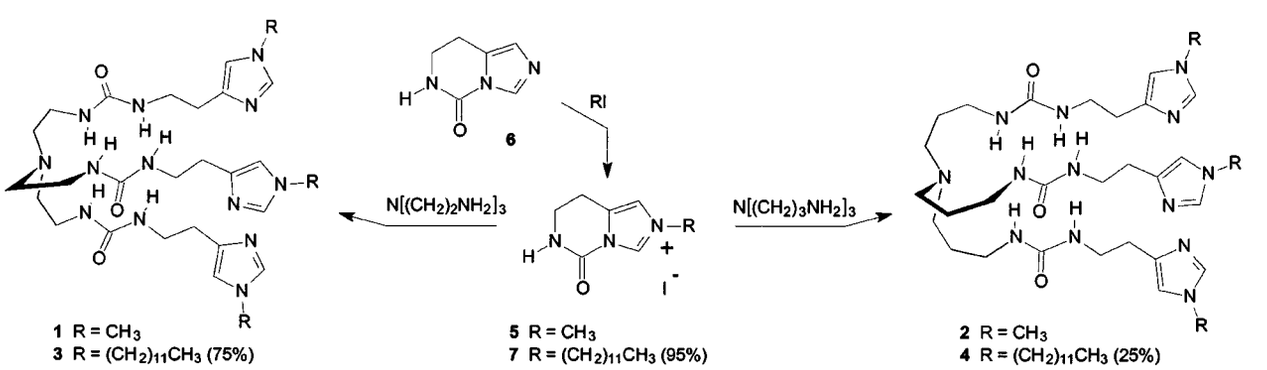
One drawback with **1** and **2** was that the binding of Zn^{2+} was only moderately strong and much free ligand and free Zn^{2+} remained at a 1:1 mixing ratio. Lower concentrations of the active catalytic forms resulted and a more complicated analysis of the kinetic data was required. An immediate improvement should result if the imidazole groups could be spatially pre-organized for binding. Unfortunately, attempts to synthesize macrobicyclic analogues of **1** or **2** were unsuccessful or produced unstable materials.[11]

We report herein the preparation of the liposoluble analogues **3** and **4**, and their Zn^{2+} binding and esterase properties in micellar media. We expected stronger binding in a micellar medium[12] owing to the preferential solubilization of the lipophilic end-chains into the micelle, thereby enforcing a reduced mobility of the imidazole groups. As well, micellar interfaces can approximate the micro-environment of reduced H_2O content near the CA active site. In previous examples of micellar systems employing Zn^{2+},[13,14] the ester substrates tested mostly bore a metal binding site, so that the hydrolysis rates benefited from pre-association and often involved acyl transfer to pendant hydroxys. This work compares the hydrolyses of the same, non-coordinating ester by both micellar and homogeneous systems.

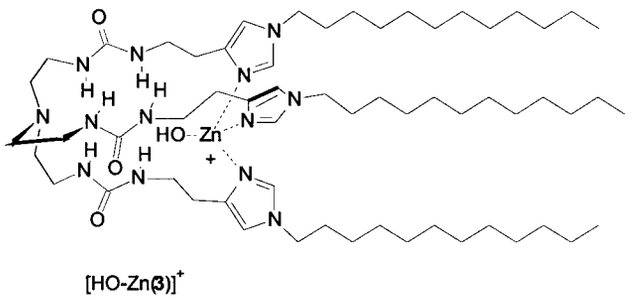
Results and discussion

Synthesis

In a variation of previous podand preparations using salt **5**,[1,15] the reaction of the imidazopyrimidine **6**[1] with 1-iododododecane in CH_3CN proceeded cleanly to produce crystalline salt **7** in



Scheme 1



[HO-Zn(3)]$^+$

95% yield. In the presence of Et_3N, heating a 3:1 mixture of **7** and tris(2-aminoethyl)amine (tren) at 50 °C for 0.5 h in CH_3CN, followed by neutralization with an excess of OH^--bearing ion-exchange resin, produced **3** in 75% yield. In contrast, reaction with the more basic tris(3-aminopropyl)amine (trpn) was slower, requiring overnight heating at 80 °C. This led to an oily, CH_3CN-insoluble deposit. After neutralization and chromatography, we obtained **4** as a semi-solid, albeit in only 25% final yield. This material was insoluble in CH_3CN and it darkened over time. Elemental analysis revealed it to be carbonated material, but this was of no consequence to our experiments.

Complexation

Ligands **3** and **4** are not soluble in H_2O, so that complexation necessitated an organic solvent. When ligand solutions in EtOH were treated with aqueous Zn^{2+} solutions, cloudy suspensions resulted. We expected the Zn^{2+} complexes of **3** and **4** to be amphiphilic but, unlike triple chain amphiphiles that form vesicles and bilayers upon sonication,[16] our 1:1 ligand–Zn^{2+} mixtures remained cloudy even after prolonged sonication. Lowering the pH to *ca.* 5 afforded clarification but this probably caused decomplexation. Adding several more volumes of EtOH (up to a final EtOH content of 85%) also resulted in clarification, but we expected this to unduly increase the pK_w and the pK_a for Zn–OH_2 deprotonation, and to thereby retard esterase activity at moderate pH. Previous metallomicellar work had used the cationic detergent CTAB or the non-ionic detergents Brij 35,[13] Triton X-100[14] or PGLE.[17] Free **3** and **4** were solubilized with CTAB or with anionic detergent (SDS), but this required high detergent levels and these preparations were easily disrupted by additions of pNPOAc solutions in CH_3CN, again producing cloudy suspensions. Because ionic detergents may hinder the complexation and because cationic detergents are esterolytic,[18] we opted for the non-ionic Nonidet P-40 (NP-40), which was able to solubilize the complexes at concentrations above the critical micelle concentration (0.29 mM), producing clear solutions that were stable for hours after treating with pNPOAc–CH_3CN solutions. Triton X-100 could also be used.

Titrimetry

In analogy to earlier experiments,[1] acidified solutions of **3** and $ZnCl_2$ containing NP-40, CH_3CN (5.6%), EtOH (6.4%) and Me_4NCl (background electrolyte) were titrated with 0.1 M NaOH. The EtOH was required for the preparation of ligand solutions and the CH_3CN was added to achieve the same solvent mixture as for the hydrolysis experiments (see below). These were deemed to have negligible effects on ligand complexation or protonation. Control experiments lacking $ZnCl_2$, **3** or both were also performed. During the titration of **3** in the presence of Zn^{2+}, the solution became milky at pH 6.4 but clarified at pH ≥ 8.9. This did not occur in the control experiments, nor during the ester hydrolyses which lacked background electrolyte. It was likely an effect of the ionic strength and was without apparent consequence to the titration. The titration curves were similar to those obtained with the hydrophilic version of **3** (*i.e.* **1**) in entirely aqueous media and the data were therefore treated as before,[1] with no apparent effects on the uncertainties in the calculated formation constants. These are recorded in Table 1 as formation constants for individual chemical species, along with equilibrium constants derived therefrom. Since the inhomogeneity evidently did not prevent material from engaging in proton transfers, any effects on the positions of equilibria must be small. Nevertheless, the calculated equilibrium constants must be regarded as less reliably determined than would otherwise be the case.

Table 1 Log formation constants for the indicated species and log derived constants for the indicated processes

Species/reaction	**1**[a]	**2**[a]	**3**	**4**
HL^+	7.63(0)	8.99(1)	6.85(3)	7.86(7)
H_2L^{2+}	14.45(0)	16.39(2)	12.68(3)	14.23(5)
H_3L^{3+}	20.56(2)	23.23(2)	17.49(4)	19.51(3)
H_4L^{4+}	—[b]	29.49(2)	22.46(4)	23.71(5)
LZn^{2+}	3.71(1)	—[b]	4.44(3)	—[b]
$[HLZn]^{3+}$	10.63(1)	12.77(1)	10.93(6)	11.68(1)
$[LZnOH]^+$	−3.92(4)	−3.13(3)	−3.35(3)	−4.65(3)
$[LZnOH]^+ + H^+$	7.63	≤7.95	7.79(6)	≥8.15
$LZn^{2+} + H^+$	6.93	≥7.95	6.58(9)	≤8.15
$L + Zn^{2+}$	3.71	≤4.82	4.44(3)	≥3.53
$HL^+ + Zn^{2+}$	3.01	3.78	4.08(9)	3.82(8)
$L + ZnOH^+$	5.26	6.05	6.22(7)	4.91(7)
$HL^+ + ZnOH^+$	5.26	≤5.39	7.16(7)	≥5.23

[a] Data from ref. 1. [b] Undetected.

The protonation of free **3** was more difficult than with **1** but all four possible ligand protonation processes could be measured. Further, the last two protonations have similar pK_a values, suggesting some cooperativity. Since $H_3\mathbf{3}^{3+}$ would be protonated at the imidazole groups for maximal charge separation, whence the alkyl chains could not well associate with each other, both $H_3\mathbf{3}^{3+}$ and $H_4\mathbf{3}^{4+}$ would be expected to be completely soluble in H_2O, whereas $H_2\mathbf{3}^{2+}$ would not. A partial extraction of $H_3\mathbf{3}^{3+}$ into the aqueous phase would facilitate the fourth protonation (or, conversely, a partial solubilization of $H_3\mathbf{3}^{3+}$ in the micelle would retard deprotonation, relative to $H_3\mathbf{1}^{3+}$). This phenomenon was not seen with **4** but we would expect it to be protonated at the amino vertex in all four protonated forms, so that $H_3\mathbf{4}^{3+}$ would remain well micellized.

As expected, the affinity for Zn^{2+} was significantly higher with **3** than with **1**, supporting our hypothesis of greater pre-organization of micelle-bound **3**. The protonation and deprotonation of $[H_2O–Zn(\mathbf{3})]^{2+}$ were both more difficult, also consistent with the hydrophobicity of the medium. The formation constant for $[HO–Zn]^+$ can be used to also calculate the binding of this ion by both free base **3** and by $H\mathbf{3}^+$.

The situation with **4** was analogous to that with **3** in that the micellar medium rendered the ligand somewhat less basic than the *N*-methyl analogue **2** in homogeneous aqueous solution[1] but, judging by the binding ability of $H\mathbf{4}^+$, the metal binding was only slightly stronger than with the *N*-methyl analogue **2** and weaker than with **3**.

As with the hydrophilic analogue **2**, but unlike **3**, titrimetry detected no $[H_2O–Zn(\mathbf{4})]^{2+}$ species but only its protonated ($[H_2O–Zn(H\mathbf{4})]^{3+}$) and deprotonated forms $[HO–Zn(\mathbf{4})]^+$. This two-proton jump is associated with an overall pK_a value of about 16.3, corresponding to two one-proton steps, each with pK_a near 8.15. The deprotonations of the Zn^{2+}-bound H_2O and the amino vertex are overlapped, *i.e.* they occur virtually simultaneously and indistinguishably, in complete analogy to the case of **2**[1] but distinct from the behaviour of the less basic **3**, which showed discrete steps. With **2**, the lower limiting pK_a value for $[H_2O–Zn(H\mathbf{2})]^{2+}$ was estimated at 7.95.[1] Given the basicity of free **4**, the single-step pK_a estimate of 8.15 must be considered an upper limit for the protonation of the amino vertex and, correspondingly, a lower limit for the deprotonation of the Zn-bound H_2O. The log formation constant of the

Table 2 Observed pseudo-first-order rate constants ($\times 10^6$ s^{-1}) with and without added Zn^{2+} and **3**, net k_1 owing to complex species ($\times 10^6$ s^{-1}), and uncertainties in the least significant digits

pH	None	Zn^{2+}	**3**	Zn^{2+} + **3**	k_1
5.77	0.59(4)	0.78(3)	3.38(6)	3.34(5)	1.56(9)
6.90	1.80(2)	2.22(3)	3.91(6)	7.47(7)	4.99(8)
7.27	4.34(9)	4.43(10)	5.95(11)	11.7(2)	6.9(2)
7.60	11.2(5)	15.4(1)	17.5(2)	38.59(8)	24.6(1)
7.88	17.6(1)	24.65(6)	23.6(1)	68.7(1)	47.8(2)
8.24	45.1(2)	58.3(5)	50.9(5)	128.0(5)	78.6(7)
8.57	51.5(4)	60.3(2)	58.7(5)	129.7(6)	75.2(7)

undetected $[H_2O–Zn(\mathbf{4})]^{2+}$ can be estimated as the half-way point between the values for the protonated and deprotonated forms, *i.e.* at 3.53, but, since we would expect the binding of Zn^{2+} by **4** to be stronger than by $H\mathbf{4}^+$, this 3.53 value must be considered a lower limit.†

Ester hydrolysis

In experiments parallelling previous work in homogeneous solution,[1] solutions were prepared as 1 : 1 mixtures of either **3** or **4** and Zn^{2+} in the presence of NP-40 in buffers at pH values ranging from 5.77 to 8.57. Reactions were launched by additions of pNPA in CH_3CN. For those reactions at pH ≤ 7.27, a ten-fold excess of ester was used to obtain measurable rates in the control experiments. As before,[1] the initial rates of hydrolysis were monitored spectrophotometrically and the data provided estimates of the pseudo-first-order rate constants. Control experiments were also conducted without metal, without ligand and in the absence of both. No cloudiness was witnessed during the course of these experiments.

Unfortunately, hydrolyses using **4** were disappointingly slow. Although the effect of free **4** improved in the presence of Zn^{2+}, this was still less efficient than free Zn^{2+} itself and was pursued no further. Ligand **3**, however, caused substantial reaction rates. The observed pseudo-first-order rate constants appear in Table 2. In analogy to earlier findings with **2**,[1] the pseudo-first-order rate constants for ligand–Zn^{2+} mixtures showed a sigmoidal pH dependence, indicating that the activity is due to deprotonated forms, possibly $[HO–Zn]^+$ species.

The amount of hydrolysis owing to complexes was calculated as before [1,6e] by subtracting the effects of the medium, of free **3** and of free Zn^{2+}, using the concentrations of free **3** and free Zn^{2+} calculated at each pH value with the help of the equilibrium constants of Table 1. The residual effect (k_1) owing to the suite of ligand–metal complexes is plotted as a function of pH in Fig. 1, overlaid with plots of the concentrations of the individual complex species. Clearly, the increase in k_1 with pH is associated with an increasing concentration of $[HO–Zn(\mathbf{3})]^+$. The slope of a plot of k_1 against the calculated concentration of $[HO–Zn(\mathbf{3})]^+$ ($r = 0.944$) provided an estimate of the second-order rate constant (k_2) for this species of 0.185 ± 0.012 M^{-1} s^{-1}. This analysis also demonstrated turnover for those runs in the presence of excess ester.

This k_2 value exceeds values found with the *N*-methyl analogues **1** and **2** in homogeneous solution, which themselves exceeded those reported for earlier CA models,[1] of which the best value was with a triazacyclododecane Zn^{2+} complex [8d,e] (0.041 M^{-1} s^{-1} in 10% CH_3CN at pH 8.2 and 25 °C). Only one recent, non-micellar model also reported pNPOAc hydrolysis, that by a di-zinc complex of a hexaazamacrocycle [10] exhibiting a k_2 value of 0.062 M^{-1} s^{-1} (pH 8.18/25 °C/10% CH_3CN), but some have been shown to react (stoichiometrically) with CO_2 (ref. 7*a*,*b*), esters or amides.[7c] A macrocyclic di-nickel complex [19] was much weaker ($k_2 = 8.5 \times 10^{-5}$ M^{-1} s^{-1} in 1 : 1 EtOH–H_2O at pH 8.4/25 °C). Only one previous metallomicellar CA model was used in pNPOAc hydrolysis: a hexadecyltetraazacyclododecane Zn^{2+} complex [14] exhibited the highest such activity ($k_2 = 5.0$ M^{-1} s^{-1} at pH 10.5 in 10 mM Triton X-100 and 10% CH_3CN). Cyclodextrin-anchored Ni^{2+} complex [20] and a Cu^{2+} analogue [21] also present a hydrophobic micro-environment and showed high levels of esterase activity with pNPOAc. Such levels nevertheless remain far short of the enzyme's.[22]

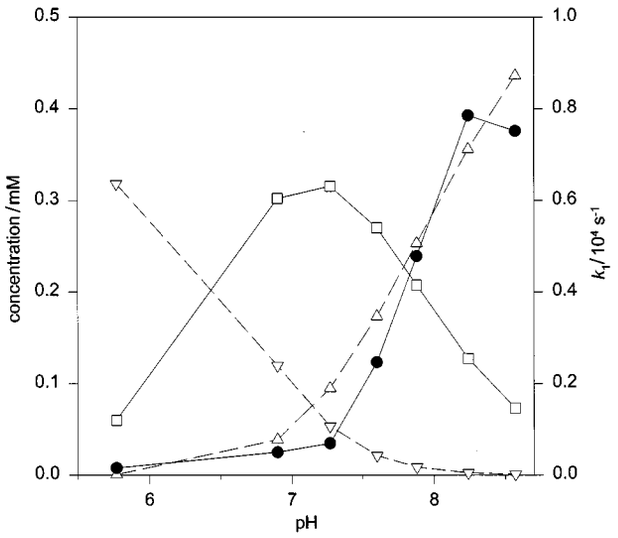


Fig. 1 Plots of the residual k_1 (●) and of the concentrations of $[HLZn]^{3+}$ (▽), LZn^{2+} (□), and $[LZnOH]^+$ (△) for the hydrolysis of 5.67×10^{-4} M pNPOAc by 6.3×10^{-4} M **3** and Zn^{2+} as a function of pH.

Conclusions

Our results demonstrate the utility of micellar inclusion in effecting a pre-organization of our podands. The pNPOAc hydrolysis witnessed here is associated with a deprotonation of the metal-bound H_2O, and we have an example showing the second highest second-order rate constants yet measured for a Zn^{2+}-based CA model.

Experimental

General

NMR spectra were recorded on a 300 MHz instrument in $CDCl_3$ with TMS as reference, bm and bs indicate broad multiplet and broad singlet, respectively. Tris-(2-aminoethyl)amine (tren) was used as received (Aldrich) and tris-(3-aminopropyl)-amine (trpn) was prepared by the method of Chin *et al.*[23] Nonidet P-40 is a Sigma product. Elemental analyses were performed by Canadian Microanalytical Services (Burnaby, BC, Canada) or Guelph Chemical Laboratories, Ltd (Guelph, ON, Canada).

2-(1-Dodecanyl)-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-*c*]pyrimidinium iodide 7

5-Oxo-5,6,7,8-tetrahydroimidazo[1,5-*c*]pyrimidine **6**[1] (4.4 mmol) was suspended in CH_3CN (30 mL) and treated with 1.2 equiv. of 1-iododododecane (5.25 mmol). The reaction mixture was protected from light and allowed to reflux for 3 d. After twice extracting with hexane, evaporation gave a brown solid that was recrystallized from EtOAc to afford a pale yellow solid (95% yield), mp 116–118 °C. Found: C, 50.03; H, 7.79; N, 9.32.

† A small but not insignificant value for the log formation constant of $[H_2O–Zn(\mathbf{4})]^{2+}$ can be obtained when treating the data by the Difference Method (P. G. Potvin, *Anal. Chim. Acta*, 1994, **299**, 43). The associated uncertainty is very large, the pK_a values calculated therefrom are unreasonable, and it cannot be considered more reliable than the lower limit estimate given here. In any case, the calculated concentrations of this species remain very low over the entire pH range.

$C_{18}H_{32}N_3OI$ requires C, 49.89; H, 7.44; N, 9.69%. ^{1}H-NMR: δ 9.77 (s, 1H, H-2′), 8.04 (s, 1H, NH), 7.35 (s, 1H, H-4′), 4.47 (t, 2H, N^+CH_2), 3.77 (t, 2H, $HNCH_2$), 3.20 (t, 2H, $HNCH_2CH_2$), 1.92 (bm, 2H), 1.32, 1.24 (bm, 18H), 0.88 (t, 3H, CH_3) ppm. ^{13}C-NMR: δ 144.5 (C=O), 134.3 (C-2′), 131.1 (C-4′), 118.4 (C-5′), 51.1 (N^+CH_2), 39.0 ($HNCH_2$), 31.9 ($HNCH_2CH_2$), 30.0, 29.6, 29.5, 29.4, 29.3, 29.1, 26.3, 22.6, 19.2, 14.1 (CH_3) ppm.

1-(2-{Bis-[2-(3-{2-[1-(1-dodecanyl)-1*H*-imidazol-4-yl]ethyl}-ureido)ethyl]amino}ethyl)-3-{2-[1-(1-dodecanyl)-1*H*-imidazol-4-yl]ethyl}urea 3

Salt **7** (1.5 mmol) was dissolved in CH_3CN (10 mL) and added to tren (0.5 mmol) with stirring. The gum that formed immediately was treated with Et_3N (3.0 mmol) and 25 mL CH_3CN, then heated at 50 °C for 30 min to produce a clear solution. Cooling first to room temperature, then to −78 °C produced crystals that were carefully decanted through a glass frit. The solid was redissolved in EtOAc, washed twice with H_2O and liberated from EtOAc to produce an oil. This was redissolved in MeOH (20 mL) and neutralized with an excess of DOWEX IRA-400 resin (OH^- form) suspended in H_2O (5 mL). Removal of the solvents produced an oil that was crystallized from CH_3CN solution at −78 °C and collected as before. Drying under high vacuum at 35–40 °C produced a waxy solid, mp 66–68 °C, amounting to a 75% yield. Found: C, 67.51; H, 10.89; N, 16.76. $C_{60}H_{111}N_{13}O_3$ requires C, 67.82; H, 10.53; N, 17.13%. ^{1}H-NMR: δ 7.38 (s, 3H, H-2′), 6.73 (s, 3H, H-5′), 6.13, 6.08 (2s, 6H, NH), 3.83 [t, 6H, $NCH_2(CH_2)_{10}$], 3.37 (t, 6H, $HNCH_2CH_2C$), 3.14 (t, 6H, $HNCH_2CH_2N$), 2.68 (t, 6H, $HNCH_2CH_2C$), 2.47 (t, 6H, $HNCH_2CH_2N$), 1.72 [m, 6H, $NCH_2CH_2(CH_2)_9$], 1.25 (m, 54H), 0.87 (t, 9H, CH_3) ppm. ^{13}C-NMR: δ 159.1 (C=O), 139.8 (C-2′), 135.9 (C-4′), 115.7 (C-5′), 46.9 ($HNCH_2CH_2N$), 40.0 ($HNCH_2CH_2C$), 38.2 ($HNCH_2CH_2N$), 31.7 [$NCH_2(CH_2)_{10}$], 30.7, 29.4, 29.3, 29.2, 29.1, 26.4, 22.4 [$NCH_2(CH_2)_{10}$], 28.9 ($HNCH_2CH_2C$), 13.9 (CH_3) ppm.

1-(2-{Bis-[2-(3-{2-[1-(1-dodecanyl)-1*H*-imidazol-4-yl]ethyl}-ureido)propyl]amino}propyl)-3-{2-[1-(1-dodecanyl)-1*H*-imidazol-4-yl]ethyl}urea 4

As with **3**, salt **7** (2.2 mmol), trpn (0.7 mmol) and Et_3N (1.5 mmol) were heated to reflux overnight in CH_3CN (40 mL). A yellow oil deposited upon cooling in an ice bath. Decanting the solvent, concentrating it until turbidity and re-cooling produced more oil. After decanting again, the combined oils were neutralized as for **3**, then chromatographed on silica gel 60, using 1 : 9 MeOH : CH_2Cl_2 saturated with concentrated NH_4OH as eluent, to afford a thick, yellow oil in 25% yield. Found: C, 61.51; H, 9.79; N, 14.03. $C_{63}H_{117}N_{13}O_3\cdot 3H_2CO_3$ requires C, 61.41; H, 9.60; N, 14.11%. ^{1}H-NMR: δ 7.59 (s, 3H, H-2′), 6.79 (s, 3H, H-5′), 6.59, 6.36 (2 bs, 6H, NH), 3.89 [t, 6H, $NCH_2(CH_2)_{10}$], 3.40 (bm, 6H, $HNCH_2CH_2CH_2N$), 3.22 (bm, 6H, $HNCH_2CH_2C$), 2.94 (t, 6H, $HNCH_2CH_2CH_2N$), 2.73 (bm, 6H, $HNCH_2CH_2C$), 1.82, 1.80, 1.77, 1.75 (m, 9H), 1.25 (m, 57H), 0.90 (t, 9H, CH_3) ppm. ^{13}C-NMR: δ 159.6 (C=O), 138.2 (C-2′), 136.0 (C-4′), 116.3 (C-5′), 50.8 ($HNCH_2CH_2CH_2N$), 40.1 ($HNCH_2CH_2C$), 37.1 ($HNCH_2CH_2CH_2N$), 31.9 [$NCH_2(CH_2)_{10}$], 30.9, 29.6, 29.5, 29.4, 29.3, 29.1, 28.8, 26.6 ($HNCH_2CH_2C$), 25.4 ($HNCH_2CH_2CH_2N$), 14.0 (CH_3) ppm.

Titrimetry

Using equipment and data treatment described earlier,[24] NaOH solutions were standardized by triplicate titrations of 10 mL aliquots of 0.1 M potassium hydrogenphthalate in the presence of 1 mL of 1 M $Me_4N^+Cl^-$. HCl solutions were standardized by titration of 3 mL aliquots with the standardized NaOH solution. $ZnCl_2$ solutions were standardized by EDTA titration with Eriochrome Black-T indicator. $Zn(NO_3)_2\cdot 6H_2O$ was used with **4**. Using a stock solution of ligand in EtOH, 50 mL stocks of solution for titrations were prepared in which [HCl] = 5 mM, [**3**] or [**4**] = $[Zn^{2+}]$ = 1 mM, $[Me_4N^+Cl^-]$ = 0.1 M, [NP-40] = 0.029 g mL^{-1}, $[CH_3CN]$ = 5.6% and [EtOH] = 6.4%. The CH_3CN was added to maintain the same solvent composition as was used in hydrolysis experiments (see below). Similar stocks were prepared while omitting the ligand or the Zn^{2+} or while omitting both. Triplicate titrations of 15 mL aliquots were carried out with the standard NaOH solution and the volume–pH data were treated as described earlier.[1,24] The control experiments provided estimates for this medium of the pK_w (14.16 ± 0.03, *cf.* Gran plot estimate 14.23), of the log formation constant of $[HOZn]^+$ (−9.56 ± 0.04) and of the ligand protonation constants (see Table 1). No corrections for possible carbonate contamination were applied since we lacked values for the formation constants of HCO_3^- and CO_3^{2-} in this medium.

Ester hydrolyses

Solutions were prepared in cuvettes by adding, in order, 0.2 mL of 10 mM solutions of **3** or **4** in EtOH, 0.6 mL of 0.1 g mL^{-1} NP-40, 0.2 mL of 0.0103 M $ZnCl_2$ then 2.0 mL of 0.05 M TRIS, HEPES or MES buffers previously adjusted to the desired pH by addition of HCl or NaOH. The reactions were initiated by the addition of 0.18–0.20 mL of either 0.01 M pNPOAc in CH_3CN for pH > 7.27 or 0.1 M pNPOAc in CH_3CN for pH ≤ 7.27. Absorbances were monitored at 348 nm (ε 5400 M^{-1} cm^{-1}), the isosbestic point for *p*-nitrophenol and its anion, over 1800 s. Using data taken 50 or 100 s apart, the data were treated as before[1] to provide estimates of the pseudo-first-order rate constants, which are reported as averages of duplicate or triplicate runs, with errors ≤2%. Similar experiments were run without ligand or without metal or in the absence of both.

There is no analytical expression of error propagation in calculating species concentrations from formation constants, which is needed to assess the errors in k_1. The uncertainties in concentrations were therefore numerically simulated and found to be typically small [≤2% for σ(log *K*) of 0.05 log units]. The computation of uncertainties in the k_1 values therefore allowed 4% errors in the concentrations and these constituted small contributions to the overall uncertainties. The computation of k_2 absorbs the errors in k_1 in any case. Errors in the independent variable (the concentration of the presumed active complex) were neglected for the same reason.

References

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