Syntheses of Macrocyclic Enzyme Models. Part 4.[1] Preparation and Characterization of Cationic Octopus Azaparacyclophanes

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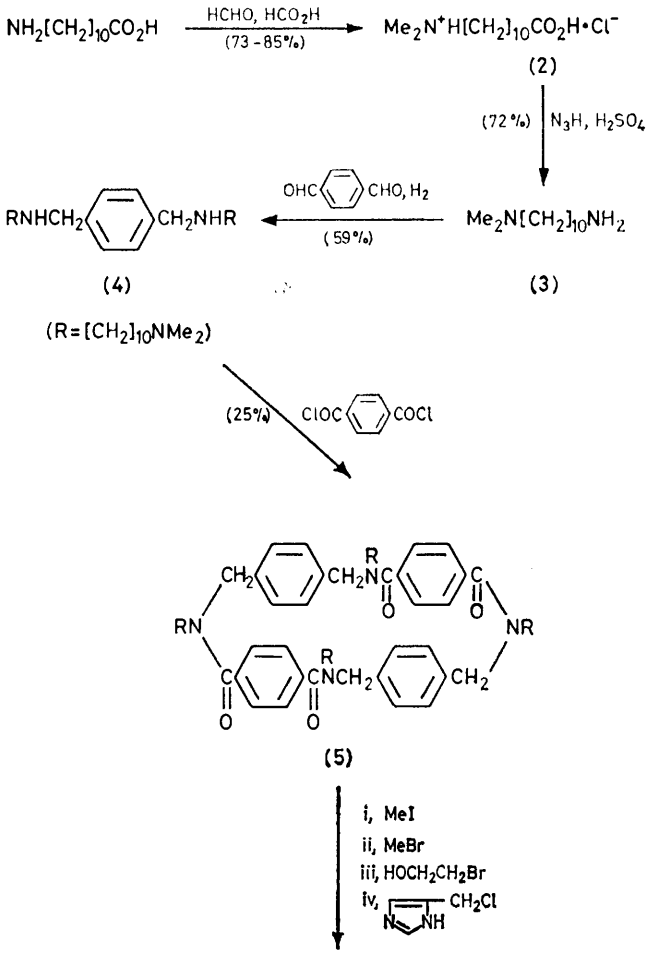
Azaparacyclophanes bearing alkyl chains as branches of the skeleton were prepared in order to investigate their ability as macrocyclic enzyme models to incorporate various hydrophobic substrates. A water-soluble azaparacyclophane, *NN'N''N'''*-tetrakis-(10-trimethylammoniodecyl)-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza-[3.3.3.3]paracyclophane tetrabromide (7), incorporates not only non-charged, hydrophobic spin-labelled and fluorescence probes but also anionic and bulky neutral dyes. The substrate specificity exhibited by (7) is primarily due to electrostatic and hydrophobic interaction with the guest molecules. The catalytic behaviour of the azaparacyclophanes *NN'N''N'''*-tetrakis-{10-[dimethyl(imidazolylmethyl)ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetrachloride (9) and *NN'*(*N''*)-bis-{10-[dimethyl(imidazolylmethyl)-ammonio]decyl}-*N''*(*N'*)*N'''*-bis-{10-[dimethyl(hydrogen)ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetrachloride (10) was investigated in aqueous media containing a small amount of organic co-solvents. The azaparacyclophane (10) may provide an efficient hydrophobic field by co-operation of the macrocyclic skeleton with the four alkyl chains forming an octopus-like structure, particularly at pHs above the pK_a of the tertiary amino-group (9.2), and may selectively catalyse the hydrolysis of *p*-nitrophenyl carboxylates having long alkyl chains. The pH-dependency of the binding ability of (10) toward substrates was examined by means of a fluorescence technique as well as by kinetic methods.

It would be useful to search for relatively simple model systems, which exhibit some of the critical features of enzymatic reactions, as an aid to understanding the mechanisms of enzyme actions. To this end, we have recently prepared the azaparacyclophane (1) and other related macrocycles with deeper hydrophobic cavities provided by substitution of long alkyl branches on the macrocyclic skeleton (octopus-like structure), and have investigated their substrate-binding behaviour.[1] The azaparacyclophane (1), which has an anionic charge at the end of each long alkyl chain, provides an efficient hydrophobic field by co-operation of the macrocyclic skeleton with the four alkyl chains causing an octopus-like structure. This can then incorporate substrates of an appropriate molecular size with positive or neutral charges. These results tempted us to prepare further functionalized octopus azacyclophanes. We now report the synthesis of azaparacyclophanes having a cationic charge (quaternary ammonium group) and/or an active functional group at the end of each long alkyl chain and present the results of our investigation of their substrate-binding ability and esterase-like activity.

RESULTS AND DISCUSSION

Preparation.—The azaparacyclophane derivatives obtained are summarized in Scheme 1. *NN'*-Bis-(10-dimethylaminodecyl)-*p*-xylylenediamine (4) was obtained from 11-aminoundecanoic acid in good yield. *NN'N''N'''*-Tetrakis-(10-dimethylaminodecyl)-2,11,20,-29-tetra-aza[3.3.3.3]paracyclophane-3,10,21,28-tetraone (5) was prepared by condensation of terephthaloyl dichloride with (4) under dilute conditions. Gel-filtration chromatography, used for the purification of (5), showed the presence of the corresponding hexaone derivative, but only (5) was isolated. The tertiary amino-groups of (5) were quaternized using methyl iodide and bromide to give *NN'N''N'''*-tetrakis-(10-trimethylammoniodecyl)-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetraiodide (6) and *NN'N''N'''*-tetrakis-(10-trimethylammoniodecyl)-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetrabromide (7), respectively. *NN'N''N'''*-Tetrakis-{10-[dimethyl-(2-hydroxyethyl)ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetrabromide (8) was prepared in a manner similar to that employed for the synthesis of (6) and (7), by using 2-bromoethanol. *NN'N''N'''*-Tetrakis-{10-[dimethyl-(imidazolylmethyl)ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane tetrachloride (9) and *NN'*(*N''*)-bis-{10-[dimethyl(imidazolylmethyl)ammonio]decyl}-*N''*(*N'*)*N'''*-bis-{10-[dimethyl-(hydrogen)ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,-29-tetra-aza[3.3.3.3]paracyclophane tetrachloride (10) were also prepared with chloromethylimidazole hydrochloride as a counter component in the presence and absence of sodium carbonate, respectively. The azaparacyclophane (10) would involve three possible isomers even though all the separation procedures employed here, such as thin-layer and gel-filtration chromatography, show the presence of only one component; two isomers have two imidazolyl groups on adjacent alkyl chains (*cis*-configuration) while the third has them on opposing alkyl chains (*trans*-configuration). The physical and analytical data for the azaparacyclophanes are listed in Table 1.

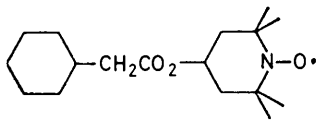
Substrate-binding Ability of the Azaparacyclophane (7).—The substrate-binding ability of (7) was studied by e.s.r. and electronic spectroscopy in a manner similar to that reported previously.[1] 4-(Cyclohexylacetoxy)-2,2,6,6-tetramethylpiperidine-1-oxyl (11), 2-(8-carboxyoctyl)-5,5-dimethyl-2-octyl-3-oxazolidine-1-oxyl (12), and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (13) were used as moderately hydrophobic, hydrophobic, and



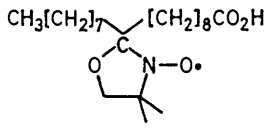
(1) R=$[CH_2]_{10}CO_2H$ (ref.1)
(6) R=$[CH_2]_{10}N^+Me_3 \cdot I^-$ (82%)
(7) R=$[CH_2]_{10}N^+Me_3 \cdot Br^-$ (60%)
(8) R=$[CH_2]_{10}N^+Me_2(CH_2CH_2OH) \cdot Br^-$ (54%)
(9) R=$[CH_2]_{10}N^+Me_2(CH_2Im) \cdot Cl^-$ (66%)
(10) 2/4:$[CH_2]_{10}N^+HMe_2 \cdot Cl^-$
2/4:$[CH_2]_{10}N^+Me_2(CH_2Im) \cdot Cl$ (79%)

SCHEME 1 (Percentage numerals refer to yields)

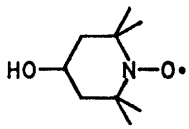
hydrophilic spin probes, respectively, and the e.s.r. parameters are summarized in Table 2. The rotational correlation times * indicate that Michaelis-type complexes [2] are definitely formed between the azaparacyclophane (7) and the hydrophobic spin probes but not between (7) and the hydrophilic one. The data also show that the hydrophobic interaction is most favourable between the long alkyl chains of substrate (12) and those of (7) since the rotational mobility of (12) is much more restricted than that of (11) in the hydrophobic cavity of (7). On the other hand, the isotropic nitrogen hyperfine splitting constants (A_N) in the presence of the azaparacyclophane do not change to any meaningful



(11)



(12)



(13)

extent, relative to those observed in its absence. Thus, the octopus cyclophane (7) may incorporate only the hydrophobic portion of the substrates into its deep cavity while the nitroxide moiety remains exposed to the bulk phase, as reported for the azaparacyclophane (1).[1] The result suggests that the substrate (12) is incorporated into (7) as shown in Scheme 2, so that (12) would be bent at the 2-position of the oxazolidine ring and the nitroxide group would be exposed to the bulk phase. The binding ability of (7) was examined with several dyes and the resulting data are listed in Table 3. An anionic dye (Orange G) and a suitably bulky neutral dye [1-(2-pyridylazo)-2-naphthol (PAN)] undergo complex formation with (7) in 1 : 1 stoicheiometry, the electronic spectra changing with changing concentration of (7) and

* A correlation time for the rotational motion of a paramagnetic molecule was obtained by equation (a) where $\Delta H_{(m=+1)}$ is the

$$\tau_c = A \cdot \Delta H_{(m=+1)}[(I_{(m=+1)}/I_{(m=-1)})^{\frac{1}{2}} - 1] \qquad (a)$$

peak-to-peak line width in gauss of the derivative of the low-field absorption line; $I_{(m=+1)}$ and $I_{(m=-1)}$ are the corresponding peak-to-peak heights for the low- and high-field lines, respectively. For the calculation of τ_c, $A = 6.6 \times 10^{-10}$, which was obtained for di-t-butyl nitroxide (O. H. Griffith, D. W. Cornell, and H. M. McConnell, *J. Chem. Phys.*, 1965, **43**, 2909), was used because it does not change much among various radicals. Refer to: (*a*) D. Kivelson, *J. Chem. Phys.*, 1960, **33**, 1094; (*b*) T. J. Stone, T. Buckman, P. L. Nordio, and H. M. McConnell, *Proc. Natl. Acad. Sci. USA*, 1965, **54**, 1010.

TABLE 1

Physical and analytical data for azaparacyclophane derivatives

Cyclophane	Formula	Calc. (%) C	Calc. (%) H	Calc. (%) N	Found (%) C	Found (%) H	Found (%) N	M.p./°C
(5)	$C_{80}H_{128}N_8O_4$ [a]	75.9	10.2	8.85	75.7	10.15	8.75	215—216
(6)	$C_{84}H_{140}I_4N_8O_4 \cdot 2H_2O$	53.95	7.75	6.0	53.7	7.7	5.95	250—254 (decomp.)
(7)	$C_{84}H_{140}Br_4N_8O_4 \cdot 3H_2O$	59.35	8.65	6.6	59.3	8.5	6.55	260 (decomp.)
(8)	$C_{88}H_{148}Br_4N_8O_8$	59.85	8.45	6.35	59.75	8.8	6.4	250 (decomp.)
(9)	$C_{96}H_{148}Cl_4N_{16}O_4 \cdot 3H_2O$	64.55	8.7	12.55	64.3	8.6	12.5	117—120
(10)	$C_{88}H_{138}Cl_2N_{12}O_4 \cdot 2HCl \cdot 2H_2O$	65.7	9.0	10.45	65.7	9.0	10.5	150 (decomp.)

[a] *m/e* 1 266 (M^+); mol. wt. 1 265.95.

TABLE 2

Isotropic nitrogen hyperfine splitting constants (A_N) and rotational correlation times (τ_c) for nitroxide radicals at room temperature [a]

E.s.r. probe [b]	Host	A_N/G	$10^{11}\tau_c$/s
(11), 5.25×10^{-4} mol dm^{-3}	None	17.08	6.5
(11), 5.25×10^{-4} mol dm^{-3}	(7), 5.05×10^{-3} mol dm^{-3}	16.99	10.74
(12), 5.66×10^{-4} mol dm^{-3}	None	15.80	21.5
(12), 5.66×10^{-4} mol dm^{-3}	(7), 5.05×10^{-3} mol dm^{-3}	15.78	68.3
(13), 5.40×10^{-4} mol dm^{-3}	None	17.11	3.3
(13), 5.40×10^{-4} mol dm^{-3}	(7), 5.05×10^{-3} mol dm^{-3}	17.05	4.4

[a] In ethanol–methanol–water (5 : 2 : 95 v/v) at pH 8.34 and μ 0.10 (KCl). [b] Alkaline hydrolysis of (11) and (12) was not detected throughout the measurements.

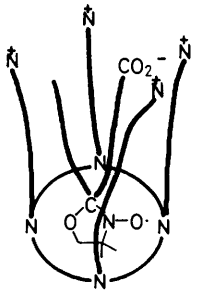
TABLE 3

Dissociation constants for the inclusion complexes of (7) [a]

Substrate [b]	K_d/mol dm^{-3}	λ_{iso} [c]/nm	λ_{meas} [d]/nm	10^4[(7)]/mol dm^{-3}
Orange G, 1.13×10^{-5} mol dm^{-3}	$(1.92 \pm 0.20) \times 10^{-3}$	512	480	0.89—8.85
PAN, 1.15×10^{-5} mol dm^{-3}	$(2.84 \pm 0.06) \times 10^{-3}$	498	470	0.89—5.34

[a] All measurements were carried out in ethanol–methanol–water (5 : 2 : 95 v/v) at pH 8.70, μ 0.10 (KCl), and 30.0 ± 0.1 °C. [b] Rhodamine 6G (1.01×10^{-5} mol dm^{-3}) and Quinaldine Red (0.98×10^{-5} mol dm^{-3}) were also used but no spectral changes due to complex formation were detected. [c] Observed isosbestic point. [d] Wavelength used for measurements.

showing isosbestic points. The dissociation constants for the 1 : 1 complexes formed between (7) and Orange G or PAN were evaluated according to the Benesi–Hildebrand equation [1,3] (Table 3). On the other hand,



SCHEME 2

cationic dyes such as Rhodamine 6G and Quinaldine Red did not show any spectral change upon addition of (7). These results again indicate that both electrostatic and hydrophobic interactions play major roles in the substrate-binding process of these octopus cyclophanes.

Substrate-selectivity of (9) *and* (10) *in the Hydrolysis of* p-*Nitrophenyl Carboxylates.*—The catalytic efficiency of (9) and (10) in the hydrolysis of *p*-nitrophenyl carboxylates was investigated by the kinetic method in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at 30.0 ± 0.1 °C and μ 0.10 (KCl). Apparent pseudo-first-order rate constants (k_{obs}) were obtained by measuring spectrophotometrically the amount of liberated *p*-nitrophenol. First-order kinetics were found to hold to at least 50% conversion of the ester substrates for all runs when [S] < [C] (where S and C stand for substrate and azaparacyclophane, respectively). Eight *p*-nitrophenyl carboxylates, which have n-alkyl groups of various lengths in their respective acyl moieties, were used as substrates. Figures 1 and 2 show the observed rate constants for the hydrolysis of these esters in the presence of (9) and (10), respectively, along with the catalytic efficiency of both cyclophanes: k_{hyd} is the rate constant for the alkaline hydrolysis; $k_c = k_{obs} - k_{hyd}$. The Figures indicate that the catalytic activity of (10) for the hydrolysis of the esters is larger than that of (9) since conditions such as higher concentration and higher pH are required for (9) to attain rate values comparable to those observed with (10). In practice it should be possible to estimate the substrate selectivities exhibited by these azaparacyclophanes from the observed rate constants for ester substrates bearing various alkyl chains. The trend of substrate selectivity does not coincide with that of catalytic efficiency. This must be caused by the fact that the spontaneous rate constant for the hydrolysis under the present conditions decreases as the alkyl-chain length of an ester increases, owing to the self-coiling property of alkyl chains [4] in addition to the molecular aggregation behaviour.[4,5] One of the roles of octopus cyclophanes in catalysis, therefore, must in addition to the binding ability itself, be unfolding of the long alkyl

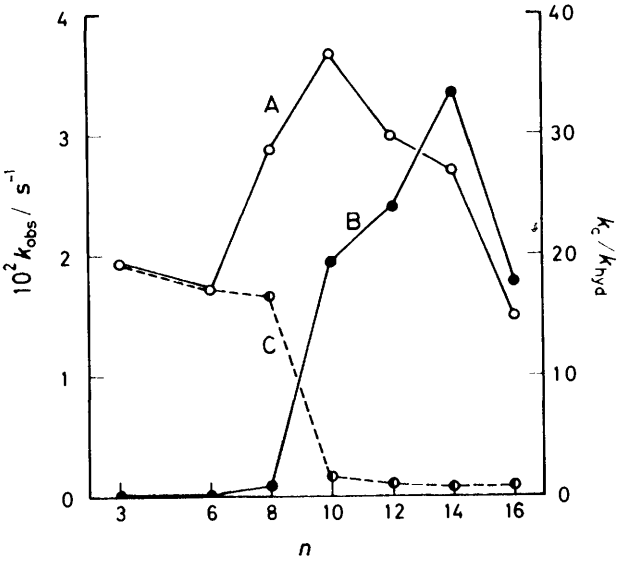


FIGURE 1 Correlations of pseudo-first-order rate constants in the presence (k_{obs}, A) and absence (k_{hyd}, C) of (9), and catalytic efficiency (B) with alkyl-chain length of substrates (n, a number of carbon atoms in an acyl moiety) for the hydrolysis of p-nitrophenyl carboxylates ($NO_2C_6H_4OCO[CH_2]_{n-2}CH_3$) in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at pH 10.11, μ 0.10 (KCl), 30.0 ± 0.1 °C, and initial concentrations: (9), 2.99×10^{-4} mol dm^{-3}; substrate, 5.0×10^{-6} mol dm^{-3}

chain of the ester substrates so that the ester bond is exposed to the catalytic group.

From a theoretical viewpoint, the activation free-energy change for the catalysis relative to the corresponding spontaneous reaction needs to be examined. On these grounds, the value of k_c/k_{hyd} must be referred to rather than the apparent rate constant (k_{obs}) for the evaluation

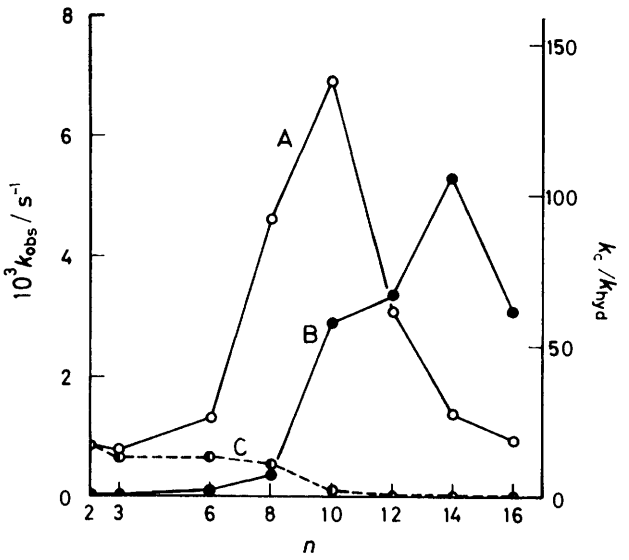


FIGURE 2 Correlations of pseudo-first-order rate constants in the presence (k_{obs}, A) and absence (k_{hyd}, C) of (10), and catalytic efficiency (B) with alkyl-chain length of substrates (n, a number of carbon atoms in an acyl moiety) for the hydrolysis of p-nitrophenyl carboxylates ($NO_2C_6H_4OCO[CH_2]_{n-2}CH_3$) in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at pH 8.88, μ 0.10 (KCl), 30.0 ± 0.1 °C, and initial concentrations: (10), 5.55×10^{-5} mol dm^{-3}; substrate, 5.0×10^{-6} mol dm^{-3}

of catalytic and selectivity efficiency. The largest catalytic efficiency of (9) and (10) was observed with p-nitrophenyl tetradecanoate. In the catalysis of octopus azaparacyclophanes, the hydrophobic interaction between the long alkyl chain of the substrate and that of the azaparacyclophane plays an important role in holding the reactive sites of both species in juxtaposition ready for nucleophilic attack of the imidazolyl group. There must be a certain threshold for the alkyl-chain length of the substrate in order to attain the most favourable mutual arrangement of both the ester bond of an incorporated substrate and the catalytic group of an octopus cyclophane; this was attained for (9) and (10) with the substrate p-nitrophenyl tetradecanoate. On the other hand, N-(imidazol-4-ylethyl)-10(11)-oxo[20]paracyclophane-22-carboxamide,[6] bearing no alkyl chain, and N-decylimidazole [7] did not show a catalytic efficiency maximum in the deacylation of the ester substrate.

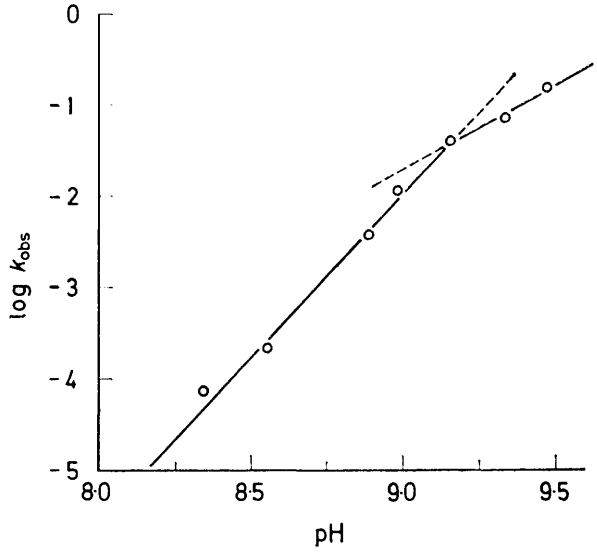


FIGURE 3 pH–Rate profile for the hydrolysis of p-nitrophenyl dodecanoate (PNPL) as catalysed by (10) in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at μ 0.10 (KCl), 30.0 ± 0.1 °C, and initial concentrations: (10), 5.55×10^{-5} mol dm^{-3}; PNPL, 4.90×10^{-6} mol dm^{-3}

This again indicates that the relative geometrical configuration of the active functional group of the azaparacyclophane to the reactive site of the ester substrate in the Michaelis-type complex is one of the most important factors for exertion of effective catalysis, as is generally suggested for enzymic reactions.[8]

Dependency of Catalytic Activity on pH.—Figure 3 shows the pH–rate profile for the hydrolysis of p-nitrophenyl dodecanoate (PNPL) in the presence of (10). The azaparacyclophane (10) catalysed the hydrolysis of PNPL to a detectable extent only at pH values greater than 8. Above pH 9.5, however, the hydrolysis occurred too fast for the reliable determination of rate constants by the usual methods. In the pH region of our present concern, a break point appeared to be present at around pH 9.2. However this result is qualitative owing to the lack of sufficient kinetic data in the higher pH range. A

potentiometric titration of (10) indicated the presence of two functional groups with pK_a values of 3.6 and 9.1 in the pH range 1.5—11.5. The first and second acid dissociation constants of the imidazolyl group have been reported for functionalized micelles which have a molecular structure for the catalytic site similar to that of (10). These are: dimethyl(imidazolylethyl)octadecylammonium chloride,[9] pK_{a1} 4.5—6.1, pK_{a2} 13; dimethyl(imidazolylmethyl)hexadecylammonium chloride,[10] pK_{a1} 3.5. pK_a Values for tertiary amino-groups have been reported previously:[11] *viz.* trimethylamine, 9.8; *NNN'N'*-tetramethylethylenediamine, 9.1. From these data, the pK_a value of 9.2 obtained from the break point in Figure 3 may be due to acid dissociation of the tertiary amino-group of (10). More convincing evidence for the presence of such a break point has been provided by fluorescence spectroscopy (see later). This was further confirmed by the pH–rate profile for the hydrolysis of *p*-nitrophenyl decanoate (PNPD) in the presence of the azaparacyclophane (9) (Figure 4); no break point

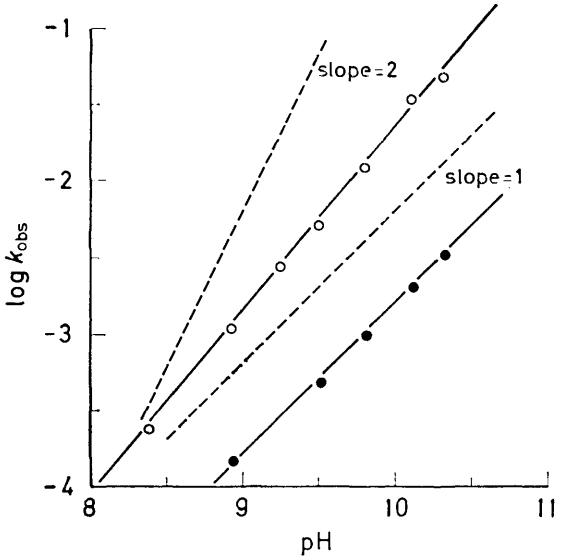


FIGURE 4 pH–Rate profiles for the hydrolysis of *p*-nitrophenyl decanoate (PNPD) in the presence (○) and absence (●) of (9) in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at μ 0.10 (KCl), 30.0 ± 0.1 °C, and initial concentrations: (9), 2.66×10^{-4} mol dm^{-3}; PNPD, 4.83×10^{-6} mol dm^{-3}

was observed over the pH range 8.3—10.5, and the potentiometric titration of (9) showed only a pK_{a1} of 3.5 for the imidazolyl group. The first pK_a value for the imidazolyl group of (9) is quite similar in magnitude to that for dimethyl(imidazolylmethyl)hexadecylammonium chloride in its micellar state.[10] This indicates that the microenvironment of the reaction site (*i.e.*, location of the imidazolyl group) provided by the present azaparacyclophanes is quite similar to that provided by functionalized micelles.

As seen from Figure 3, the slopes are 3.6 ± 0.2 and 1.8 ± 0.5 below and above pH 9.2, respectively. On the other hand, the slope in Figure 4 is 1.2 ± 0.06 over the whole pH region employed for the kinetic runs (8.3—10.5). Over such a pH region (Figures 3 and 4), the reactive functional group is deduced to be the anionic imidazolyl group in the light of the following experimental facts.

(*i*) The catalytic activities of (9) and (10) were much enhanced above pH 8.5 as is observed for functionalized micellar systems.[9,10,12]

(*ii*) The azaparacyclophane (7) did not catalyse the hydrolysis of *p*-nitrophenyl carboxylates under the kinetic conditions used in this work owing to the lack of an imidazolyl group.

(*iii*) The microenvironment of the reaction site provided by the present azaparacyclophanes is similar to that provided by functionalized micelles as mentioned above. In a microenvironment such as that provided by cationic micelles, the reactivities of anionic imidazolyl groups are much larger than those of neutral ones by a factor of 10^6—10^7.[13] We were unable to determine kinetically the second pK_a value for the imidazolyl group owing to the extremely rapid reaction rate in such a high pH region. The second pK_a values for the imidazolyl groups of (9) and (10) would be between 11.5 and 13.0, as judged from the reported values for cationic micelles and a macrocyclic compound[9,10,14] [triethylimidazolylmethylammonium chloride, pK_{a1} 4.3 ± 0.1 pK_{a2} 11.2 ± 0.3; *SS'*-bis(cycloglycyl-L-hemicystylglycyl-L-histidyl-6-aminohexanoyl-ω-aminoundecanoyl), pK_{a2} 12.3].

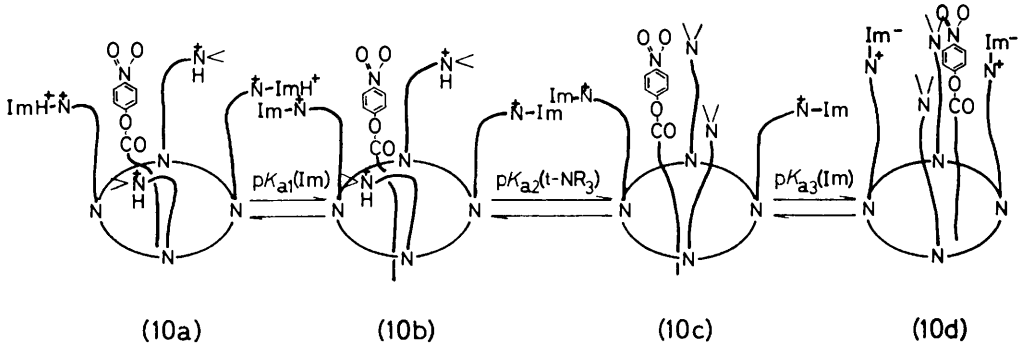
(*iv*) The pH–rate correlations in Figures 3 and 4 for the azaparacyclophane-catalysed hydrolysis of *p*-nitrophenyl esters seem to be related to the pH dependency of the substrate-binding ability of the cyclophanes; *i.e.* the apparent rate acceleration with increasing pH (slope of 3.6 ± 0.2 in Figure 3) may be attributed to an enhancement in the binding ability of (10), caused by an increase in the proportion of unprotonated tertiary amino-groups along with the conformational changes illustrated in Scheme 3. To prove this, we have evaluated the binding constants of (10) towards the ester substrate PNPL by kinetic methods.† The binding constants, however, could be determined only at pH 8.34 and 8.50 as can be seen from Figure 5; $K_b = 470 \pm 40$ mol^{-1} dm^3, $k_m = (3.9 \pm 2.2) \times 10^{-2}$ s^{-1} at pH 8.34; $K_b = 1\,710 \pm 380$ mol^{-1} dm^3, $k_m = (3.7 \pm 0.8) \times 10^{-2}$ s^{-1} at pH 8.50. At other pH values, saturation-type kinetics were not observed over the concentration range used here. Above pH 8.50, the concentration of (10) could not be increased

† The kinetic data were analysed in terms of the Michaelis–Menten treatment based on the reaction pathway given by equation (b). Here, E, S, and P stand for catalyst, substrate, and

$$\begin{array}{ccccc} E + S & \overset{K_b}{\rightleftharpoons} & (ES) & \xrightarrow{k_m} & P \\ & & \downarrow{\scriptstyle k_{hyd}} & & \\ & & P & & \end{array} \qquad \text{(b)}$$

hydrolysis products, respectively; (ES) is a Michaelis-type inclusion complex; k_{hyd} and k_m refer to the rate constants for alkaline hydrolysis and for the pseudo-intramolecular reaction of a substrate bound to an azaparacyclophane, respectively; and K_b is the binding constant for the formation of (ES). Equation (c) is consistent with the reaction scheme given above.

$$\frac{1}{k_{obs} - k_{hyd}} = \frac{1}{(k_m - k_{hyd})K_b[E]} + \frac{1}{k_m - k_{hyd}} \qquad \text{(c)}$$



SCHEME 3

beyond 5×10^{-4} mol dm^{-3} since the reaction rate was too fast to follow by the present experimental method. Below pH 8.34, however, the catalysed reaction rate was extremely slow and much higher concentrations of (10) were required to observe saturation-type kinetics. These high concentrations were not employed in order to avoid any kinetic ambiguities which might be caused by molecular aggregation. The difficulty in determining the binding constants is apparently due to the large pH-dependency of the catalytic activity. Complete dissociation of the protons of the tertiary amino-groups in

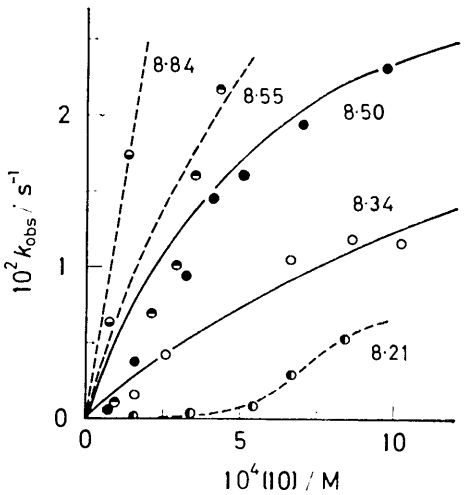


FIGURE 5 Rate–concentration profiles for the hydrolysis of *p*-nitrophenyl dodecanoate (PNPL) as catalysed by (10) in ethanol–methanol–dioxan–water (5 : 1 : 1 : 95 v/v) at μ 0.10 (KCl), 30.0 ± 0.1 °C, and initial PNPL concentration of 4.90×10^{-6} mol dm^{-3}; numerals refer to pH values. Solid lines at pH 8.34 and 8.50 are calculated curves by using K_b and k_m values given in the text

(10) may cause loss of two positive charges and the electrostatic repulsion between the long alkyl chains of (10) may be weakened consequently.

The sole reaction mechanism for the hydrolysis of PNPL catalysed by (10) must therefore involve initial attack of the anionic imidazolyl group. The unprotonated tertiary amino-group may assist removal of the imidazolyl NH proton and enhance the substrate binding during the acylation process. The acylated imidazolyl moiety is not accumulated, since the acyl–imidazolyl moiety ($\lambda_{max.}$ 245 nm) was not detected spectrophotometrically in any of the kinetic runs, but is subsequently cleaved to give a carboxylic acid and the regenerated catalyst. Such an apparent turnover behaviour was also confirmed by the rate of conversion of the substrate. Release of *p*-nitrophenol occurred continuously beyond the equimolar conversion point with respect to the active imidazolyl group in the presence of an excess of PNPL: the observed rate followed first-order kinetics up to 60% conversion of the substrate under the following conditions: concentrations of (10) and PNPL 1.00×10^{-5} and 9.57×10^{-5} mol dm^{-3}, respectively, at pH 10.06, 30.0 ± 0.1 °C, and μ 0.10 (KCl).

Orientational Behaviour of Octopus Azaparacyclophane (10).—In order to confirm the orientational behaviour of the azaparacyclophane (10) shown in Scheme 3, the effect of pH on the substrate-binding behaviour of (7) and (10) was investigated by the fluorescence technique. 1,6-Diphenylhexa-1,3,5-triene (DPH), which has no proton-dissociative group and is hydrophobic, was used as a probe at a concentration of 1.08×10^{-6} mol dm^{-3}. DPH shows excitation and emission maxima at 317 and 441 nm, respectively, in the absence of azaparacyclophane derivatives in ethanol–methanol–tetrahydrofuran–water (5 : 1 : 1 : 95 v/v). The excitation and emission maxima were shifted to 365 and 430 nm, respectively, upon addition of the octopus azaparacyclophanes. DPH was therefore excited at 365 nm and the fluorescence intensity was measured at 430 nm with a cut-off filter for radiation below 390 nm. The relative fluorescence intensity of DPH remained the same over the whole pH region in the absence of azaparacyclophanes. The fluorescence intensity increased five-fold upon addition of (7) (4.99×10^{-4} mol dm^{-3}), although it remained constant throughout the whole pH region examined owing to the lack of a proton-dissociative group in (7). On the other hand, in the presence of (10) (5.33×10^{-4} mol dm^{-3}) the fluorescence intensity increased above pH 8 and levelled off beyond pH 9.3 (Figure 6). The saturated fluorescence intensity was 83-fold larger than that observed in the absence of (10). This intensity is comparable to that observed in benzene. As a result, the pH value of 9.2 is referred to the pK_a for the tertiary amino-group of (10), consistent with the foregoing conclusion, and a tight hydrophobic interaction is apparently exercised upon deprotonation of the tertiary amino-group as shown in

Scheme 3. The binding constant for incorporation of DPH into the cavity of (10c) was determined at pH 9.32 according to the Benesi–Hildebrand treatment [3] (Figure 7); a good linear correlation of $[(10)][DPH]/\Delta I_f$ *vs.* $[(10)] + [DPH]$ was obtained for the whole concentration range examined, $1/K_b = (1.5 \pm 0.3) \times 10^{-5}$ mol

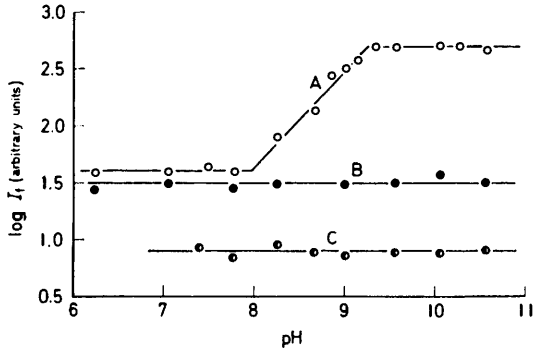


FIGURE 6 Correlations of fluorescence intensity of DPH with pH in the presence of (10) (A) and (7) (B), and in the absence of azaparacyclophane derivative (C) in ethanol–methanol–tetrahydrofuran–water (5 : 1 : 1 : 95 v/v) at initial concentrations: (10), 5.33×10^{-4} mol dm^{-3}; (7), 4.99×10^{-4} mol dm^{-3}; DPH, 1.08×10^{-6} mol dm^{-3} [μ 0.10 (KCl)].

dm^{-3}. The binding constants with (10a) and (10b), species which are present at the lower pH region, were not obtained owing to the weak binding ability of these compounds. Tabushi and his co-workers have examined

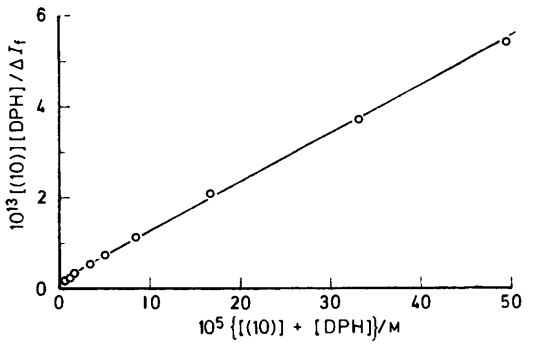


FIGURE 7 Analysis of fluorescence spectral data for the interaction of (10) with DPH (1.08×10^{-6} mol dm^{-3}) in ethanol–methanol–tetrahydrofuran–water (5 : 1 : 1 : 95 v/v) at pH 9.32, μ 0.10 (KCl), and 25.0 $\pm$ 0.1 °C by means of the Benesi–Hildebrand equation (i). Here, ΔI_f is the extent of fluorescence intensity change upon addition of (10); ΔI_a stands for the difference in fluorescence intensity between bound and free DPH; and K_d ($= 1/K_b$) is the dissociation constant

$$\frac{[(10)][DPH]}{\Delta I_f} = \frac{K_d}{\Delta I_a} + \frac{[(10)] + [DPH]}{\Delta I_a} \quad \text{(i)}$$

the binding interaction of *NN'N''N'''*-tetramethyl-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane, which has no long alkyl chain, with sodium 1-anilinonaphthalene-8-sulphonate in water at lower pH values.[15] In their case, however, electrostatic interactions between the catalyst and the substrate seem to play a major role in the incorporation, along with some minor contribution from the ' tight-fit ' property [1,7] of the naphthyl moiety into the cavity formed by a wall of benzene rings. Tight incorporation of the naphthyl moiety into (7) was examined using a non-charged dye substrate, PAN, as summarized in Table 3, the hydrophobic interaction being a major driving force for the incorporation.

The present fluorescence study indicates two possibilities for the aggregation behaviour of the present azaparacyclophanes: (*i*) there is no aggregation in the concentration range employed for the kinetic and fluorescence studies; or (*ii*) the binding ability is not dependent on aggregation, even though the critical micelle concentrations of the azaparacyclophanes in question occur in the present concentration range, as is observed for (1).[1] Of these we favour the former, in the light of surface tension measurements for (7) and (9). The surface tension was reduced simply without any clear break-point as the concentrations of (7) and (9) were increased up to 3.7×10^{-3} and 1.20×10^{-3} mol dm^{-3}, respectively. This must be attributed to the higher solubility of these cationic azaparacyclophanes having quaternary ammonium groups in aqueous media, compared to the anionic one (1). Judging from the data given in Figure 6, acid dissociation of the neutral imidazolyl group seems to make little contribution to the substrate-binding ability of the present cationic azaparacyclophanes.

In conclusion, octopus cyclophanes which have cationic charges at the end of long alkyl chains incorporate suitably bulky substrates into their macrocyclic cavities by effective hydrophobic interaction and also undergo host–guest electrostatic interaction with bulky anionic substrates, as expected from previous results.[1] Moreover, azaparacyclophanes having imidazolyl groups at the end of some or all of the long alkyl chains catalyse the hydrolysis of hydrophobic esters along with turnover behaviour. For compound (10) such catalytic activity was subject to change owing to the large pH-dependence of its substrate-binding ability.

EXPERIMENTAL

I.r. spectra were taken with a JASCO DS-403G grating spectrophotometer. ^{1}H N.m.r. spectra were obtained with a Hitachi Perkin-Elmer R-20 spectrometer with tetramethylsilane (in deuteriochloroform or deuteriomethanol) and 3-(trimethylsilyl)propanesulphonic acid (in deuterium oxide) as internal references. Melting points were measured using capillary tubes with a Yamato MP-1 apparatus (oil-bath type). E.s.r. spectra were recorded at room temperature on a JEOL JES-ME-3 X-band spectrometer equipped with a 100 kHz field modulation unit; a standard MgO–Mn^{II} sample calibrated with a n.m.r. magnetometer was employed for calibration of the magnetic field. Electronic spectra were recorded on a Union Giken SM-401 high-sensitivity spectrophotometer. Fluorescence spectra were taken with a Hitachi 650-60 fluorescence spectrophotometer. Surface tension measurements were performed at room temperature with a Kyowa DIGI-O-MATIC ESB-IV electrosurface balance assembled by the Wilhelmy principle. The platinum blade was repeatedly washed with distilled water and heated to incandescence in the flame of an alcohol

lamp after each measurement. Gel-filtration chromatography was carried out on a column of Sephadex LH-20. Methanol was used as an eluant and components eluted were detected by u.v absorption at 265 nm for chromatographic separation.

Terephthalaldehyde was purchased from Nakarai Chemicals and recrystallized from methanol–water (1 : 9 v/v); m.p. 119—120 °C. Terephthaloyl dichloride was obtained from Nakarai Chemicals and distilled before use, b.p. 115 °C at 2 mmHg, m.p. 83 °C. Palladium chloride (Ishizu Pharmaceutical Co.), 1-aminoundecanoic acid (Nakarai Chemicals), and methyl iodide and bromide (Wako Pure Chemical Industries) were obtained from commercial sources. Synthetic procedures and physical properties of spin probes [4-(cyclohexylacetoxy)-2,2,6,6-tetramethylpiperidine-1-oxyl, 2-(8-carboxyoctyl)-5,5-dimethyl-2-octyl-3-oxazolidine-1-oxyl, and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl] have been described elsewhere.[16] Rhodamine 6G (Kishida Chemical Co.), Quinaldine Red (Nakarai Chemicals), 1-(2-pyridylazo)-2-naphthol (Dojin Pharmaceutical Institute), Orange G (Wako Pure Chemical Industries), and 1,6-diphenylhexa-1,3,5-triene (Tokyo Chemical Industry Co.) were obtained from commercial sources as extra pure grades and were used without further purification. *p*-Nitrophenyl carboxylates were prepared by the reaction of the corresponding carbonyl chlorides with *p*-nitrophenol and identified by spectroscopic means and elemental analyses.

11-Dimethylaminoundecanoic Acid Hydrochloride (2).[17]—11-Aminoundecanoic acid (25 g) was added to the mixture of formaldehyde (35%; 24.5 g) and formic acid (99%; 85.8 g), and the mixture was refluxed until negative to ninhydrin. Concentrated hydrochloric acid (0.8 g) was then added to the solution and the solvent was removed *in vacuo*. The white *acid hydrochloride* was recrystallized from acetone (23.9 g, 73%), m.p 147—148 °C; $\nu_{max.}$ (KBr) 2 680 (NCH_3) and 1 730 cm^{-1} (C=O); δ (D_2O) 1.39br (16 H, s, $[CH_2]_8$), 2.33 (2 H, t, CH_2CO_2H), 2.81 (6 H, s, $N^+H[CH_3]_2$), and 3.09br [2 H, t, $CH_2N^+H(CH_3)_2$] (Found: C, 58.6; H, 10.7; N, 5.1. $C_{13}H_{27}NO_2 \cdot HCl$ requires C, 58.75; H, 10.6; N, 5.3%).

10-Dimethylaminodecylamine (3).—Concentrated sulphuric acid (31.5 ml) was added at 40 °C to a solution of 11-dimethylaminoundecanoic acid hydrochloride (12 g) in benzene (300 ml), and the mixture was stirred vigorously at the same temperature. Freshly prepared hydrazoic acid [18] (4% in benzene; 150 ml) was then added during 30 min, and the mixture was stirred for 6 h at 40 °C. After the mixture had been stirred overnight at room temperature, the acidic layer was poured into water (100 ml) and the resulting solution was made alkaline by addition of 10% aqueous sodium hydroxide. The alkaline solution was extracted with benzene (100 ml × 3), the benzene was removed *in vacuo*, and the residual oil was distilled *in vacuo* (6.5 g, 72%), b.p. 128 °C at 6 mmHg (lit.,[19] 119—120 °C at 4 mmHg); $\nu_{max.}$ (KBr) 3 240 cm^{-1} (NH); δ ($CDCl_3$) 1.28br (16 H, s, $[CH_2]_8$), 1.50 (2 H, s, NH_2), 2.20 [6 H, s, $N(CH_3)_2$], and 2.56 (4 H, t, $NCH_2[CH_2]_8CH_2NH_2$).

NN′-Bis-(10-dimethylaminodecyl)-p-xylylenediamine (4).—A mixture of terephthalaldehyde (3.4 g), 10-dimethylaminodecylamine (10 g), palladium chloride (0.14 g), and Norit SX-II (1.3 g) in methanol (145 ml) was placed in a 300-ml autoclave and agitated at room temperature with an initial hydrogen pressure of 20 kg cm^{-2} for 8 h. The filtrate was evaporated *in vacuo* to obtain a *solid* which was recrystallized from ether (7.5 g, 59%), m.p. 62.5—63.0 °C; $\nu_{max.}$ (KBr) 2 890 (CH) and 2 760 cm^{-1} (NCH_3); δ (CD_3OD) 1.30br (32 H, s, $CH_2[CH_2]_8CH_2$), 2.21 [12 H, s, $N(CH_3)_2$], 2.42 {4 H, t, $CH_2[CH_2]_8CH_2N(CH_3)_2$}, 2.52 {4 H, t, $CH_2[CH_2]_8CH_2N(CH_3)_2$}, 3.70 (4 H, s, benzyl), and 7.24 (4 H, s, aromatic) (Found: C, 76.25; H, 12.3; N, 11.05. $C_{32}H_{62}N_4$ requires C, 76.45; H, 12.4; N, 11.15%).

NN′N″N‴-Tetrakis-(10-dimethylaminodecyl)-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane-3,10,21,28-tetraone (5).—Solutions of terephthaloyl dichloride (4.1 g) and (4) (10.0 g) in dry benzene (200 ml each) were added dropwise to a refluxing solution of triethylamine (48.5 g) in dry benzene (1 000 ml) at the same rate with vigorous stirring under nitrogen in the period of 9.5 h, and the mixture was refluxed with stirring for another 1 h. The filtrate was evaporated to give a white *solid* which was subsequently recrystallized twice from acetone (3.1 g, 25%), m.p. 215—216 °C (decomp.); $\nu_{max.}$ (KBr) 2 890 (CH), 1 615 (C=O), and 2 760 cm^{-1} (NCH_3); δ ($CDCl_3$) 1.28br {64 H, s, $CH_2[CH_2]_8CH_2N(CH_3)_2$}, 2.23 [24 H, s, $N(CH_3)_2$], 2.37 {8 H, t, $CH_2[CH_2]_8CH_2N(CH_3)_2$}, 3.52 {8 H, t, $CH_2[CH_2]_8CH_2N(CH_3)_2$}, 4.52 (8 H, s, benzyl), and 7.31 (16 H, s, aromatic).

NN′N″N‴-Tetrakis-(10-trimethylammoniodecyl)-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane Tetraiodide (6).—A mixture of (5) (350 mg) and methyl iodide (600 mg) in dry methanol (25 ml) was stirred for 5 h under reflux. The solution was concentrated to 5 ml and then ether was added. The resulting pale yellow solid was washed with acetone to give a white *powder* (410 mg, 82%), m.p. 250—254 °C (decomp.); $\nu_{max.}$ (KBr) 3 400 (OH) and 1 620 cm^{-1} (C=O) [2 760 cm^{-1} (NCH_3) disappeared]; δ (D_2O) 1.32br (64 H, s, $CH_2[CH_2]_8CH_2N^+$), 3.07 [36 H, s, $N^+(CH_3)_3$], 2.82—3.50 (16 H, m, $NCH_2[CH_2]_8CH_2N^+$), 4.55 (8 H, s, benzyl), and 7.05br (16 H, s, aromatic).

NN′N″N‴-Tetrakis-(10-trimethylammoniodecyl)-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza[3.3.3.3]paracyclophane Tetrabromide (7).—A mixture of (5) (85 mg) and methyl bromide (130 mg) in dry methanol (10 ml) was placed and sealed in a glass tube. After the reaction mixture had been agitated for 96 h at room temperature, methanol was evaporated off and ether was added to the residue. The precipitated white solid was washed with acetone to give a white *powder* (68 mg, 60%), m.p. 260 °C (decomp.); $\nu_{max.}$ (KBr) 1 620 cm^{-1} (C=O) [2 760 cm^{-1} (NCH_3) disappeared]; δ (D_2O) 1.34br (64 H, s, $CH_2[CH_2]_8CH_2N^+$), 3.06 [36 H, s, $N^+(CH_3)_3$], *ca.* 3—3.50 (16 H, m, $NCH_2[CH_2]_8CH_2N^+$), and 7.06br (16 H, s, aromatic) [a proton signal of the benzyl group was overlapped with that of HOD].

NN′N″N‴-Tetrakis-{10-[dimethyl-(2-hydroxyethyl)-ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza-[3.3.3.3]paracyclophane Tetrabromide (8).—A mixture of (5) (200 mg) and an excess of 2-bromoethanol in dry methanol (5 ml) was sealed in a glass tube which was agitated for 5 days at room temperature. Ether was added to the mixture to give a white *solid*, which was then purified by gel-filtration chromatography (150 mg, 54%), m.p. 250 °C (decomp.); $\nu_{max.}$ (KBr) 3 440 (OH), 2 890 (CH), and 1 620 cm^{-1} (C=O); δ (CD_3OD) 1.34br (64 H, s, $NCH_2[CH_2]_8CH_2N^+$), 2.81 [24 H, s, $N^+(CH_3)_2$], *ca.* 2.6—3.90 {24 H, m, $NCH_2[CH_2]_8CH_2N^+(CH_3)_2CH_2CH_2OH$}, 4.20—4.90 (8 H, m, CH_2CH_2OH, benzyl overlapped with CD_3OH), and 7.02br (16 H, s, aromatic).

NN′N″N‴-Tetrakis-{10-[dimethyl(imidazolylmethyl)-ammonio]decyl}-3,10,21,28-tetraoxo-2,11,20,29-tetra-aza-[3.3.3.3]paracyclophane Tetrachloride (9).—Chloromethylimidazole hydrochloride (96 mg) was added to a solution of

(5) (200 mg) in dry methanol (5 ml) at room temperature, and sodium carbonate (67 mg) was added to the mixture in one portion. After the mixture had been stirred for 30 min at room temperature, chloromethylimidazole hydrochloride (48 mg) and sodium carbonate (33 mg) were again added, and the mixture was stirred for a further 2 h at the same temperature. The addition of both reagents was repeated twice until the quaternization was complete (n.m.r.). After the removal of sodium carbonate by filtration, the filtrate was purified by gel-filtration chromatography to afford the *tetrachloride* (180 mg, 66%), m.p. 117—120 °C, Pauly positive; $\nu_{max.}$ (Nujol) 3 200 (NH) and 1 615 cm^{-1} (C=O); δ(CD_3OD) 1.38br (64 H, s, $CH_2[CH_2]_8CH_2N^+$), 3.05 [24 H, s, $N^+(CH_3)_2$], *ca.* 2.6—3.40 {16 H, m, $NCH_2[CH_2]_8CH_2N^+(CH_3)_2$}, 4.49br (16 H, s, benzyl and NCH_2Im), 7.14br (16 H, s, aromatic), 7.50 (4 H, s, Im 5 H), and 7.80 (4 H, s, Im 2 H).

NN′(N″)-*Bis*-{10-[*dimethyl*(*imidazolylmethyl*)*ammonio*]-*decyl*}-N″(N′)N‴-*bis*-{10-[*dimethyl*(*hydrogen*)*ammonio*]-*decyl*}-3,10,21,28-*tetraoxo*-2,11,20,29-*tetra-aza*[3.3.3.3]*paracyclophane Tetrachloride* (10).—Chloromethylimidazole hydrochloride (192 mg) was added to a solution of (5) (200 mg) in dry methanol (5 ml) at room temperature. After having been stirred for 43 h at room temperature, the reaction mixture was purified by gel-filtration chromatography to afford the *tetrachloride* (196 mg, 79%), m.p. 150 °C (decomp.), Pauly positive; $\nu_{max.}$ (Nujol) 3 200 (NH) and 1 605 cm^{-1} (C=O); δ (CD_3OD) 1.35br (64 H, s, $-CH_2[CH_2]_8CH_2N^+$), 2.68 [12 H, s, $NH^+(CH_3)_2$], 3.01 [12 H, s, $N^+(CH_3)_2CH_2Im$], *ca.* 2.7—3.40 {16 H, m, $NCH_2[CH_2]_8CH_2N^+(CH_3)_2$}, 4.42br (12 H, s, benzyl and NCH_2Im), 7.10br (16 H, s, aromatic), 7.48 (2 H, s, Im 5 H), and 7.76 (2 H, s, Im 2 H).

Kinetic Measurements.—Rates of liberation of *p*-nitrophenol from *p*-nitrophenyl esters were measured at 400 nm with a Union Giken SM-401 high-sensitivity spectrophotometer. Each run was initiated by adding a solution of a substrate ester in dry dioxan (30 μl) to a mixture of a reaction medium (3.0 ml) and a solution of a catalyst in dry methanol which was pre-equilibrated at 30.0 ± 0.1 °C in a thermostatted cell in the spectrophotometer. All the aqueous buffers were prepared by using deionized and distilled water and the ionic strength of sample solutions was adjusted at μ 0.10 (KCl). Aqueous buffer solutions used are as follows: 0.1 mol dm^{-3} potassium dihydrogenphosphate: 0.05 mol dm^{-3} sodium borate for pH 6—9, and 0.05 mol dm^{-3} sodium borate: 0.05 mol dm^{-3} sodium carbonate for pH 9—11.

pH *Measurements.*—pH Measurements were carried out with a Beckman expandomatic SS-2 pH meter equipped with a Metrohm EA-125 combined electrode after calibration with a combination of appropriate standard buffers. The pH values of kinetic solutions were converted into hydroxide ion concentrations by reference to those of solutions containing known amounts of sodium hydroxide under conditions identical with the kinetic runs.[20]

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