A calixresorcinarene provides the framework for an artificial esterase

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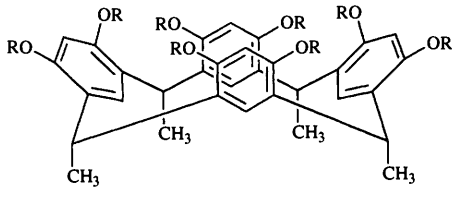
PERKIN 2 COMMUNICATION

An octa(dimethylaminopropyl)calixresorcin[4]arene (1c) is a primitive artificial esterase for 4-nitrophenyl esters.

The synthesis of chemically robust artificial enzymes which have selectivity and activity comparable to those of a natural enzyme is an important goal.[1] Rational design has not achieved this aim despite some 30 or so years of mechanistic studies and detailed knowledge of enzyme structure. One route to achieve artificial enzymes is by an empirical process of structural variation from a primitive catalyst derived from concave hosts which provide complexation. The now substantial synthetic knowledge of concave molecules makes this an attractive method;[2] it is important that the synthesis of the initial host and its subsequent structure variation involve cheap and efficient synthetic routes so as not to set an impossible limit on the structure–reactivity investigation.

Calixresorcinarenes† possess several criteria as molecules to employ as frameworks for artificial enzymes: they can be prepared with relatively stable cone shapes[3] in excellent yield from cheap materials and possess functions which can be easily derivatised selectively.[4] Complexation occurs with substrates with a wide variety of structural types[5] and 'upper-rim' bridging is easily carried out to form cages.[6] The synthetic chemistry of calixresorcinarenes is particularly efficient[3] and does not require specialist synthetic skills or equipment. There have been no reports of calixresorcinarenes acting as complexation catalysts although a few have appeared for the analogous, but more synthetically challenging, calixarene series.[7] It is the purpose of this study to indicate that a calixresorcinarene could provide a framework for an artificial enzyme.

The calixresorcinarene **1a** was prepared by the standard



1a R = H
1b R = CH_2COOEt
1c R = $CH_2CONH(CH_2)_3N(CH_3)_2$

Högberg method adjusted to maximise the yield of the cone conformation;[3] it was derivatised with ethyl bromoacetate in the presence of K_2CO_3 and the octa(carboxymethylated) product **1b** converted to the octa(dimethylaminopropyl) derivative **1c** by warming with 3-(*N*,*N*-dimethylamino)propylamine. The compounds **1a**, **1b** and **1c** were pure by TLC and were characterised by elemental analysis and by NMR spectroscopy; **1c** was also characterised by potentiometric titration. The pattern of the aromatic signals in the NMR spectra indicated that the isolated molecules were of the cone conformation.[3a] The protonation of **1c** with dilute HCl follows the simple Henderson–Hasselbalch equation [eqn. (1)] consistent with there being no interaction between the protonating dimethylamino functions.

$$[HA] = \frac{[HA_{tot}]}{1 + K_a/[H^+]} \quad (1)$$

The calixresorcinarene **1c** catalyses the fission of 4-nitrophenyl esters of a variety of carboxylic acids and the observed pseudo-first-order rate constants ($k_{obs.}$) obey equation (2) (Table 1). The pH dependence of k_{cat} for 4-nitrophenyl

$$k_{obs} = (k_{background} + k_{cat}[\mathbf{1c}]/K_s)/(1 + [\mathbf{1c}]/K_s) \quad (2)$$

acetate is sigmoid (Fig. 1) with a pK_a (determined kinetically) of 8.50 and a value for the maximal k_{cat} (k_{cat}^{max}) of 0.0109 s^{-1}. The identity of the kinetically determined pK_a with that determined titrimetrically (8.49 ± 0.02) is consistent with the neutral dimethylamino function being involved in the catalysis. This involvement is expected from model studies[8] and moreover it is reasonable to assume that the amine acts as a nucleophile to form a reactive *N*-acyl–ammonium species which then breaks down rapidly to form acid and regenerate the amine.

The overall mechanism probably involves complexation of substrate with host **1c** in its neutral form followed by intracomplex reaction of a dimethylamino function with the ester [eqn. (3), see Scheme 1].

$$\mathbf{1c} + \text{Ester} \overset{K_s}{\rightleftharpoons} \mathbf{1c}\cdot\text{Ester} \xrightarrow{k_{cat}} \text{Products} \quad (3)$$

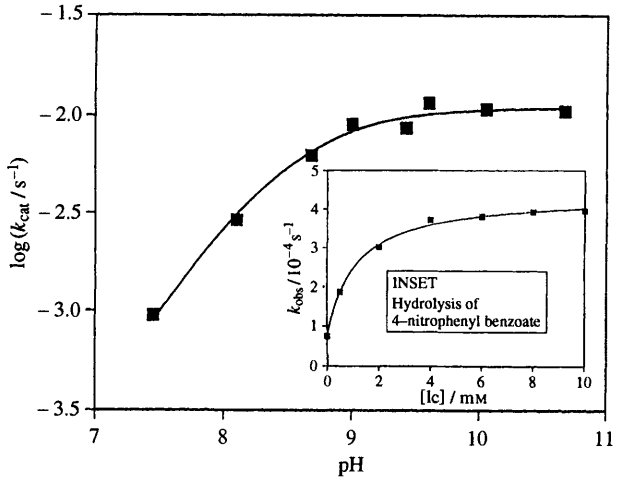


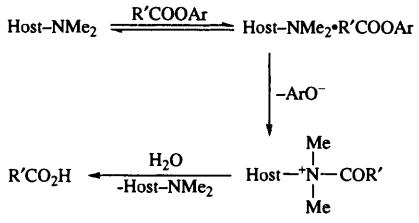
Fig. 1 Dependence on pH of the parameter k_{cat} for the fission of 4-nitrophenyl acetate catalysed by **1c**; conditions: 25 °C, 0.1 M KCl, buffers at 0.01 M concentration. Inset is the hydrolysis of 4-nitrophenyl benzoate as a function of the concentration of **1c**; conditions as above and pH set at 9.60. The lines are calculated from equations in the text using the recorded parameters.

† Calixresorcinarenes described in this paper are derived from $1^{4,6},3^{4,6},5^{4,6},7^{4,6}$-octahydroxy-2,4,6,8-tetramethyl-1,3,5,7(1,3)tetrabenzenacyclooctaphane.

Table 1 Fission of the 4-nitrophenyl ester link (R'–CO–O–C_6H_4–4-NO_2) in the presence of calixresorcinarene **1c**[a,b,c]

R'	Δk_{obs}[d]/10^{-3} s^{-1}	K_s/mM	$k_{cat}/10^{-3}$ s^{-1}	(k_{cat}/k_s)[e]/M^{-1} s^{-1}
CH_3–	0.5–4	28.3 ± 7.2	9.30 ± 1.4[f]	0.329
C_3H_7–[g]	0.22–1.0	7.74 ± 0.57	1.36 ± 0.33	0.176
C_5H_{11}–[g]	0.25–2	7.45 ± 1.2	2.80 ± 0.16	0.376
C_7H_{15}–[g]	0.2–1.2	4.33 ± 0.92	1.50 ± 0.09	0.346
$C_{11}H_{23}$–[g]	0–0.52	36.5 ± 0.16	1.55 ± 0.0045	0.0425
Ph–	0.08–0.4	1.09 ± 0.13	0.44 ± 0.008	0.404
$PhOCH_2CH_2$–	1–4	3.54 ± 0.73	4.66 ± 0.22	1.32
$C_{10}H_7$–OCH_2–[h]	5–58	3.32 ± 1.27	76.7 ± 10.0	23.1

[a] pH 9.60, 25 °C, 0.1 M KCl, ester concentration *ca.* 10 μM. [b] Range of concentrations of calixresorcinarene (**1c**) varied between 0 and 20 mM. [c] Number of data points not including duplicates varied between 6 and 7. [d] Range of observed rate constants. [e] Not corrected for the number of dimethylamino functions in one molecule of the catalyst. [f] This value is, within error limits, the same as that derived from the pH dependence of k_{cat} [eqn. (1)]. [g] The acyl function is derived from the linear alkane. [h] 2-Naphthoyl species.



Scheme 1

The kinetically equivalent mechanism can be excluded where the reaction flux is taken by a simple bimolecular reaction between uncomplexed substrate and free host.[8] The table indicates that at pH 9.6 the calixresorcinarene has a catalytic efficiency towards 4-nitrophenyl acetate more than 180 times that for the second-order rate constant for attack of a dimethylamino group of similar pK_a.

The molar values of K_s observed in this study are broadly in agreement with those expected from previous binding studies with calixresorcinarene.[6a,e–h] The relative values of k_{cat} in Table 1 are in the reactivity order expected for the acyl functions in question and this probably indicates that the conformation of the substrate is not constrained in the complex.

The value of k_{cat}^{max} (0.0109 s^{-1}) for 4-nitrophenyl acetate may be compared with the rate constants for intramolecular-catalysed fission of the 4-nitrophenyl esters of 4-*N*,*N*-dimethylaminobutyric (358 s^{-1}) and 5-*N*,*N*-dimethylaminovaleric (1.67 s^{-1}) acids.[9] The large difference is expected because in the intramolecular reactions the ester function is held against the amino group with relatively few degrees of torsional freedom of the bridging methylene groups. The substrate complexed within the host [eqn. (2)] undoubtedly does not have a constricted conformation and the ester function cannot therefore spend a substantial proportion of the time against an amino group of the host. For this reason the effective molarity of the catalyst is very much lower than that for a regular nucleophilic reaction.[10]

The system presented here provides both concave binding site and catalytic function within the same molecule. The structural features have not been optimised and moreover the binding site and catalytic functions are relatively remote from each other so that substantial catalysis would not be expected. The results indicate that calixresorcinarenes have all the prerequisites for providing the framework for potential artificial enzymes. While rational design is expected to play a part in the optimisation of catalysis the empirical structure variation approach starting with calixresorcinarenes as primitive artificial enzymes is likely to be a very good candidate and this is being pursued.

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8 This ambiguity is not unlikely and is often disregarded in studies of catalysis through complexation. The amine is completely in its neutral form at pH 11.34 where the value of the apparent bimolecular rate constant is 1.84 M^{-1} s^{-1}. Allowing for a factor of eight the individual amines have a reactivity of 0.230 M^{-1} s^{-1}. A dimethylamino group with a pK_a of 8.5 may be calculated to have a rate constant of $<1.83 \times 10^{-3}$ M^{-1} s^{-1} (T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, 1963, **85**, 1) for reaction with 4-nitrophenyl acetate thus indicating that the reaction flux taken by the intramolecular route has a lower limit of some 99% of the total. There is no direct model for the intermolecular reaction between 4-nitrophenyl acetate and a single dimethylamino group with pK_a 8.5. A rate constant may be calculated from that for trimethylamine assuming a β_{nuc} of 0.9 (A. C. Satterthwait and W. P. Jencks, *J. Am. Chem. Soc.*, 1974, **96**, 7018) and the respective pK_a values. The calculated value is an upper limit as it neglects the steric effect of replacing methyl by the calixresorcinarene group.

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