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The Ba(II) Complex of a Crown Ether Bearing a Sulphydryl Side-Arm as Turnover Catalyst of Ester Cleavage

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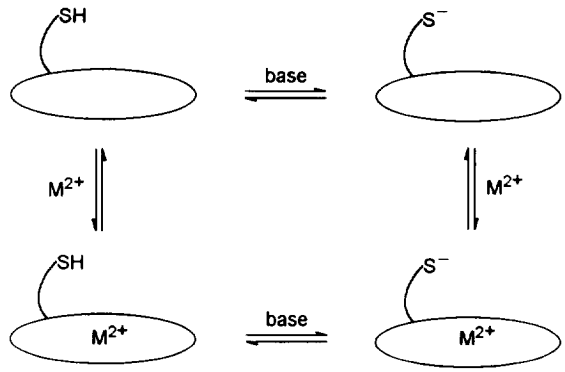
Abstract: When activated by barium(II), 18-mercaptomethyl-1,4,7,10,13,16-hexaoxacyclononadecane acts as turnover catalyst in the methanolysis of *p*-nitrophenyl acetate (pNPOAc) under slightly basic conditions in CH_3CN-CH_3OH 9:1 (v/v). The function of the complexed barium ion is to enhance the nucleophilicity of the pendant SH group towards the acetyl group of the ester, and to assist as a built-in electrophile the subsequent transfer of the acetyl group from the acetylated intermediate to the solvent. Besides the above double displacement mechanism of ester cleavage, a direct metal-ion assisted methanolysis involving reaction of pNPOAc with the ternary complex $(\mathbf{5}\cdot BaOMe)^+$ has been detected.

Partial transacylase mimics based on host structures which, like papain and ficin, bear a sulphydryl group to act as nucleophile, have been described by several workers.[1,2] The designed host structures contain substrate binding sites proximal to the sulphydryl group, so that rates of acyl transfer from suitably functionalized ester substrates are greatly enhanced. However, no provision is made to activate the cleavage of the acylated host (thiol ester), with the result that turnover catalysis is generally not observed.

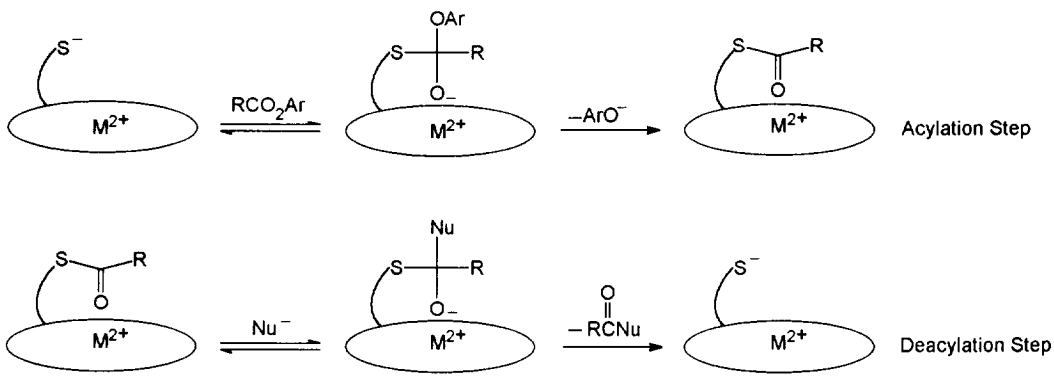
Alkaline-earth metal ions are efficient promoters of acyl transfer reactions from esters to anionic nucleophiles.[3-5] Rate enhancements are particularly large when the ester substrate is covalently linked to a crown ether moiety capable of complexing the metal ion electrophile. We reasoned therefore that a mixture of an alkaline-earth metal ion and a crown ether bearing a sulphydryl side-arm should cleave esters with catalytic turnover under moderately basic conditions, provided that the crown ether is an efficient binder for the metal ion. The rationale for our expectation is illustrated in Schemes 1 and 2. By virtue of the acidity enhancing effect of the complexed metal ion, the concentration of the thiolate nucleophile increases considerably (Scheme 1). The complexed metal ion then acts as a built-in electrophile catalyst both in the acylation of the thiolate, and in the subsequent transfer of the acyl group to an external nucleophile (Scheme 2).

The above strategy is well precedented for systems where either a phenolic[6] or alcoholic hydroxyl[7-8] serves as acyl-receiving and acyl-releasing unit. The Ba^{2+}-complex of *p-tert*-butylcalix[4]arene-crown-5[6] (**1**) in CH_3CN-CH_3OH (9:1, v/v) and the Zn^{2+}-complexes of 1-(2-hydroxyethyl)-1,5,9-triazacyclododecane[7] (**2**) and of 1-(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane[8] (**3**) in CH_3CN-H_2O (1:9, v/v) catalyse the cleavage of pNPOAc according to a double displacement mechanism proceeding *via* an acetylated catalyst intermediate.

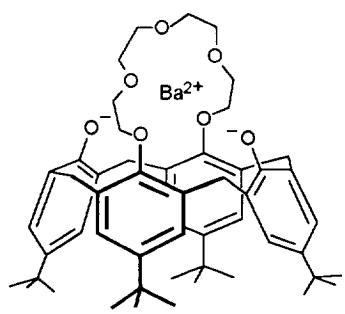
In this work, we have investigated the effect of $Ba(ClO_4)_2$ on rates of acetylation of the mercaptomethyl crown-ether **4** by pNPOAc and of deacetylation of the thiol acetate **5** in CH_3CN-CH_3OH (9:1, v/v) containing 90 mM diisopropylethylamine and 30 mM diisopropylethylammonium perchlorate at 25.0 °C.[9]



Scheme 1. The complexed metal ion increases the acidity of the thiol.



Scheme 2. The complexed metal ion acts as an electrophile catalyst both in the acylation and deacylation step.



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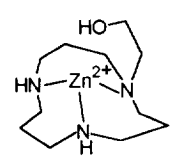

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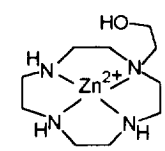

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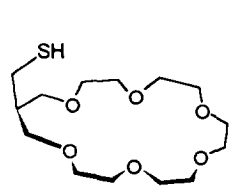

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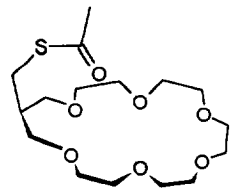


5

Acylation Step

In a first series of experiments the effect of additives on rate of liberation of pNPOH from 1.73×10^{-2} M solutions of pNPOAc was investigated spectrophotometrically. The results collected in Table 1 show that: (i) in the absence of additives liberation of pNPOH due to background methanolysis is very slow ($t_{1/2} \approx 90$ h); (ii) the modest rate enhancement of $(3.1\text{-}1.3)\times10^{-4}$ $min^{-1} = 1.8\times10^{-4}$ min^{-1} caused by addition of **4** is ascribable to slow thiolysis of pNPOAc in the weakly basic solution; (iii) addition of 2.7 mM $Ba(ClO_4)_2$ causes a sizable acceleration which, in line with previous findings,[3-5] is most likely due to the formation of a more reactive cation-paired methoxide species (eq. 1); and (iv) an equimolar mixture of **4** and Ba^{2+} is much more effective than either **4** or Ba^{2+} alone.

$$MeO^- + Ba^{2+} \rightleftharpoons (MeOBa)^+ \qquad (1)$$

Table 1. Effect of Additives on the Rate of Production of pNPOH from 17.3 mM pNPOAc in CH_3CN/CH_3OH (9:1, v/v) in the Presence of 90 mM Diisopropylethylamine - 30 mM Perchlorate Salt Buffer.

Run no.	[**4**], mM	$[Ba(ClO_4)_2]$, mM	v_o, M min^{-1}	k_{obs}, min^{-1}	k_{rel}
1	nil	nil	2.3×10^{-6}	1.3×10^{-4}	1.0
2	2.7	nil	5.3×10^{-6}	3.1×10^{-4}	2.4
3	nil	2.7	3.9×10^{-5}	2.2×10^{-3}	17
4	2.7	2.7		8.1×10^{-2} *a)*	620

a) From the exponential phase of the profile plotted in Fig. 1. The rate of production of pNPOH in the linear phase is 9.7×10^{-6} M min^{-1} (see text).

Additional information was required, however, for a correct interpretation of the last experiment. A plot of absorbance at 468 nm *vs.* time is shown in Fig. 1. The profile consists of a fast exponential phase with $k_{obs} = 8.1\times10^{-2}$ min^{-1} followed by a zeroth-order linear phase which extrapolates back to an initial burst of 0.70 absorbance units and has a slope of 2.52×10^{-3} (absorbance units) min^{-1}. In an identical run, reaction samples were withdrawn at time intervals and analysed by HPLC for the thiol acetate **5**. The results (Fig. 2) show that **5** quantitatively accumulates with a first-order rate constant of 9.7×10^{-2} min^{-1}, in reasonable agreement with the rate constant calculated from the exponential phase of the spectrophotometric run.

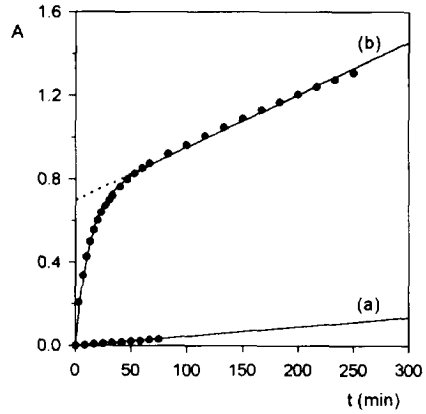


Figure 1. Absorbance increase at 468 nm as a function of time for methanolysis of pNPOAc. Curve a): background reaction measured in the presence of buffer alone. Curve b): buffer plus 2.7 mM **4** and 2.7 mM $Ba(ClO_4)_2$. The curve is a plot of eq. 4, with $k_1' = 8.1\times10^{-2}$ min^{-1}, $A_{inf} = 0.70$ absorbance units and $s = 2.52\times10^{-3}$ (absorbance units) min^{-1} (see Experimental).

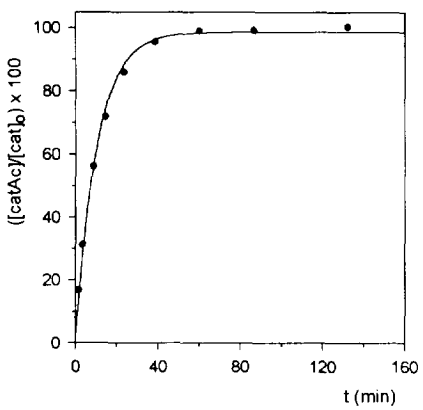


Figure 2. Mole fraction (×100) of the acetylated form of the catalyst as a function of time (from HPLC measurements). The curve is calculated from the 1st order rate equation with $k = 9.7\times10^{-2}$ min^{-1}.

It appears therefore that the initial burst of pNPOH release amounts exactly to 2.7 mM, which translates into an apparent molar absorptivity of 260 for pNPOH at 468 nm under the reaction conditions. This value was used to calibrate the absorbance profile of Fig. 1 and to calculate a rate of production of pNPOH of 9.7×10^{-6} M min^{-1} in the zeroth-order phase.

It is worth noting that the apparent molar absorptivity of 260 is significantly higher than the value of 195 measured in the same buffer solution in the absence of barium ion, and very close to the value of 277 measured in the presence of 2.7 mM $Ba(ClO_4)_2$. These findings might suggest that only a minor fraction of the metal ion is bound to **5**, and that the remaining uncomplexed fraction is available in solution for binding to the $pNPO^-$ anion. That this is not the case, however, is shown by the ^1H NMR titration experiment plotted in Fig. 3, which clearly reveals strong binding ($K>10^4$ M^{-1}) between Ba^{2+} and **5**. It seems therefore more likely that $pNPO^-$ binds to the crown-complexed Ba^{2+} ion almost as effectively as it binds to the free ion.

Deacylation Step

Since the rate of production of pNPOH in the linear portion of profile b) reported in Fig. 1 ($v = 9.7\times10^{-6}$ M min^{-1}) is more than four times higher than that due to background methanolysis

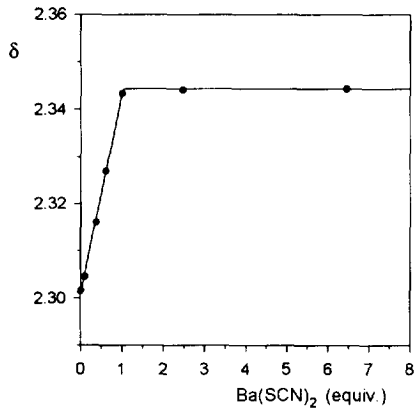


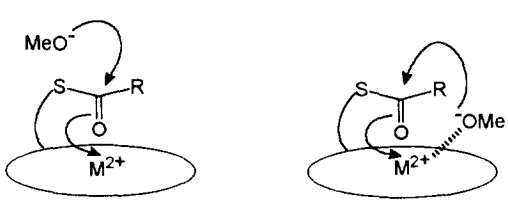
Figure 3. ^{1}H-NMR titration of **5** with $Ba(SCN)_2$ in CD_3CN-CD_3OD (9:1, v/v) at 25° C. The chemical shift of the methyl group of thiol acetate **5** is plotted against molar equivalents of added salt.

($v_0 = 2.3\times10^{-6}$ M min^{-1}), the question arises as to the role played by the thiol acetate **5** in the catalytic mechanism(s) responsible for a rate of pNPOH production of $(9.7\text{-}2.3)\times10^{-6}$ M min^{-1} = 7.4×10^{-6} M min^{-1}. We have therefore investigated the effect of Ba^{2+} ion on the methanolysis of **5** under the same experimental conditions. It is apparent from Table 2 that, whereas **5** does not undergo methanolysis to an appreciable extent over a prolonged time, its Ba^{2+} complex solvolyses much more rapidly. Since the inherent precision of the HPLC measurements is 5% at the worst, lower limits of v_0 of 3×10^{-8} M min^{-1} could have been detected for the background methanolysis. Hence the rate-enhancement factor due to complexation to Ba^{2+} ion amounts to no less than 70-fold. We visualise the mechanism of the metal-ion promoted reaction as a rate-determining nucleophilic attack at the thiol acetate - metal ion complex (Scheme 3, left), but the involvement of a ternary complex which has the same composition as the rate-limiting transition state and decomposes into products in a monomolecular step (Scheme 3, right) represents a reasonable alternative.[5b]

Table 2. Effect of $Ba(ClO_4)_2$ on the Rate of Methanolysis of **5**.[a)]

$[Ba(ClO_4)_2]$, mM	v_0, M min^{-1}	k_{obs}, min^{-1}
nil	$\leq 3\times10^{-8}$ [b)]	$\leq 1\times10^{-5}$
2.7	1.9×10^{-6}	7.0×10^{-4}

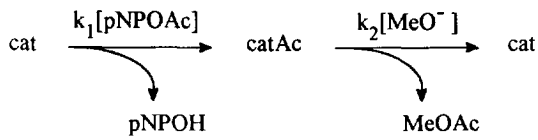
[a] Reaction conditions as in the experiments reported in Table 1.
[b] Based on the observation of no reaction after 75 h.



Scheme 3. Possible mechanisms for the metal ion assisted methanolysis of the thiol acetate **5**.

The Catalytic Mechanisms

The results reported in the preceding sections clearly show that by virtue of the beneficial influence of the complexed Ba^{2+} ion, the sulphydryl group of **4** takes the acetyl group from pNPOAc and transfers it to methoxide ion, thus restoring its active form and turning over (Scheme 4). These two steps constitute a

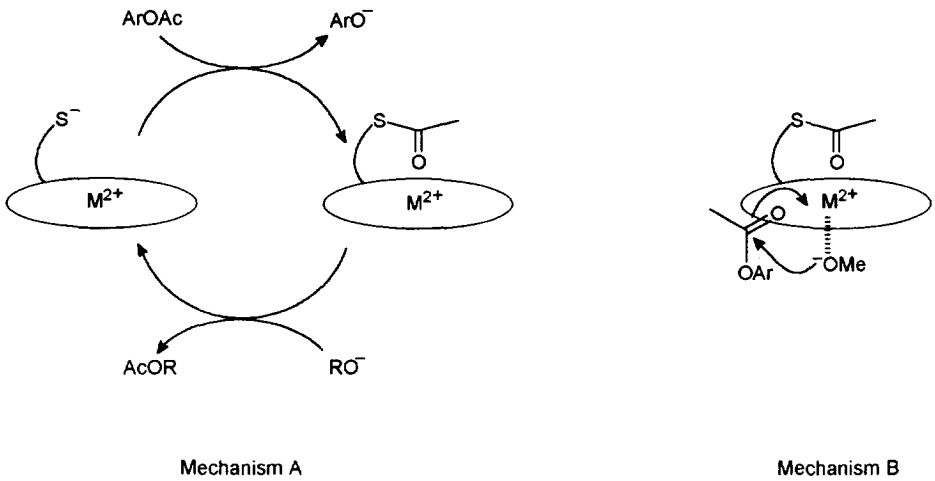


Scheme 4. Ping-pong mechanism in the methanolysis of pNPOAc catalysed by **4**·Ba^{2+} (cat).

nucleophilic catalysis of ester methanolysis (ping-pong mechanism).[10] Clearly, numerical values of the quantities k_1[pNPOAc] and k_2[MeO^-] under the conditions of the catalytic experiment are 8.1×10^{-2} min^{-1} and 7.0×10^{-4} min^{-1} from Table 1 and Table 2, respectively. Although deacylation of the acetylated catalyst is accelerated by Ba^{2+} ion to a significant extent, it is still two orders of magnitude slower than acetylation, and constitutes the slow step of the catalytic cycle. Consistently, the initial burst of pNPOH release coincides with the concentration of the catalyst in what is tantamount to an active-site titration experiment,[10,11] but the rate of production of pNPOH of 1.9×10^{-6} M min^{-1} accounts for a minor fraction of the overall catalytic rate of 7.4×10^{-6} M min^{-1} observed in the zeroth-order phase. Since the above discrepancy is obviously too large to be ascribable to experimental errors, we suggest that besides the thiol-mediated methanolysis, proceeding via an acylation-deacylation cycle (mechanism A in Scheme 5) a direct metal-ion assisted methanolysis involving reaction of pNPOAc with the ternary complex (**5**·BaOMe)$^+$ takes place as schematically depicted in mechanism B, with a rate of $(7.4\text{-}1.9)\times10^{-6}$ M min^{-1}= 5.5×10^{-6} M min^{-1}.

This interpretation is supported by our recent report[12] that in ethanol solution the ternary complex formed from equimolar amounts of Ba^{2+}, 18-crown-6 (18C6) and EtO^- is much more reactive than the ion pair $(EtOBa)^+$ in the cleavage of esters. It is further corroborated by a control experiment, which differs from run no. 4 in Table 1 for the replacement of **4** with an identical concentration of 18C6. The initial rate (corrected for background) of pNPOH production was 1.0×10^{-5} M min^{-1}. Since complexation of Ba^{2+} ion to 18C6 can be reasonably assumed to be complete,[13] the above production of pNPOH can be entirely ascribed to reaction of pNPOAc with the ternary complex $(18C6\cdot BaOMe)^+$, which is formed in minute amounts in the slightly alkaline medium according to eq 2. It appears therefore that the suggestion that the catalytic contribution of 5.5×10^{-6} M min^{-1} is due to direct cleavage of pNPOAc by the ternary complex (**5**·BaOMe)$^+$, as shown in Mechanism B (Scheme 5) is quite reasonable in the light of a comparable contribution of 1.0×10^{-5} M min^{-1} from the structurally similar ternary complex formed by 18C6.

$$(18C6\cdot Ba^{2+}) + MeO^- \rightleftharpoons (18C6\cdot BaOMe)^+ \qquad (2)$$



Scheme 5. Competing catalytic mechanisms of ester cleavage: mechanism A, thiol mediated methanolysis; mechanism B, metal ion assisted direct methanolysis.

CONCLUSIONS

We have shown that methanolysis of pNPOAc under slightly basic conditions is catalysed by the barium salt of **4**. Liberation of pNPOH displays biphasic kinetics. An initial burst of pNPOH release, corresponding to a virtually quantitative accumulation of the acetylated form of the catalyst, is followed by a zeroth-order phase. Two competing mechanisms contribute to the observed catalysis at steady state, namely, an expected double displacement mechanism in which the SH group of the catalyst acts as acetyl-receiving and acetyl-releasing unit under the influence of the complexed metal electrophile, and a less expected direct metal ion assisted methanolysis involving reaction of pNPOAc with the ternary complex (**5**·BaOMe)$^+$. The sluggish methanolysis of the thiol acetate intermediate makes the former process a relatively inefficient one.

Variations in the crown ether ring size, side arm length and alkaline-earth metal ion identity are currently being considered with the aim at improving the efficiency of the deacylation step. The results of such investigations will be reported in due course.

EXPERIMENTAL

Instruments and general methods

^{1}H-NMR spectra were recorded in $CDCl_3$ or CD_3CN-CD_3OD (9:1, v/v) with either a Bruker WP 80SY or AC 300 spectrometer, using Me_4Si as internal standard (J values in Hz). Spectrophotometric measurements were carried out on a Hewlett Packard 8452A diode array spectrometer. HPLC analyses were performed on a Hewlett Packard 1050 liquid chromatograph fitted with a UV-VIS detector operating at 230 nm. Nonlinear

least-squares calculations were carried out using the programme SigmaPlot for Windows, 1.02 (Jandel Scientific).

Materials

Dry methanol was prepared and handled as previously reported.[4a] Acetonitrile (Erba RP) was fractionally distilled over phosphorus pentoxide and subsequently over anhydrous potassium carbonate. *p*-Nitrophenyl acetate (Fluka) was crystallised from diethyl ether. Solutions of *p*-nitrophenyl acetate in CH_3CN-CH_3OH (9:1, v/v) were used after less than a week of storage at 5 °C; during this time there was no appreciable hydrolysis of the compound. Benzo-30-crown-10 was available from previous investigations.[14] Diisopropylethylamine (Aldrich) was fractionally distilled over tosyl chloride and subsequently over sodium. Other chemicals were used as received. Diisopropylethylammonium perchlorate was prepared according to a literature procedure.[15]

CAUTION! Care was taken when handling diisopropylethylammonium perchlorate because it is potentially explosive.[16] No accident occurred in the course of the present work.

18-Mercaptomethyl-1,4,7,10,13,16-hexaoxacyclononadecane (4)

This compound was prepared according to a literature procedure.[17] ^{1}H-NMR (80 MHz, $CDCl_3$) δ 1.3 (t, *J* 8.4, 1H, SH), 2.0 (m, 1H, CH), 2.6 (dd, *J* 6.3, 8.4, 2H, CH_2S), 3.5-3.7 (m, 24 H, CH_2O).

18-Acetylthiomethyl-1,4,7,10,13,16-hexaoxacyclononadecane (5)

This compound was prepared according to a literature procedure.[17] ^{1}H-NMR (80 MHz, $CDCl_3$) δ 2.0 (m, 1H, CH), 2.3 (s, 3H, CH_3), 2.95 (d, *J* 7.0, 2H, CH_2S), 3.5-3.7 (m, 24 H, CH_2O).

Equilibrium measurement

^{1}H-NMR (300 MHz) titration of **5** with $Ba(SCN)_2 \cdot 3H_2O$ was carried out in CD_3CN-CD_3OD (9:1, v/v) at 25.0 °C using both tetramethylsilane and methylene chloride as internal standards. Calculated amounts of titrating solution of $Ba(SCN)_2 \cdot 3H_2O$ were dispensed by a microsyringe to a 1.30 mM substrate solution. After each addition and thermal equilibration, the chemical shift value of the methyl group of the ligand **5** was recorded. A small but reproducible and regular downfield shift of the signal of the methyl group was observed. Strict linearity of the titration curve (Fig. 3), together with invariance of the chemical shift after the equivalence point, indicated that the association equilibrium (eq. 3) is quantitatively shifted to the right, i. e. $K > 10^4$ M^{-1}.

$$5 + Ba^{2+} \underset{}{\overset{K}{\rightleftharpoons}} 5 \cdot Ba^{2+} \qquad (3)$$

Kinetics

Kinetic experiments were carried out under argon in order to minimise air oxidation of the thiolate nucleophile. Quantitative conversion of **4** into **5** by reaction with pNPOAc, as determined by HPLC analysis, provides a good indication of the stability of the thiolate anion under the reaction conditions.

Spectrophotometric rate measurements. Methanolysis of *p*-nitrophenyl acetate was followed by monitoring the increase of absorption at 468 nm, due to the accumulation of the anionic form of the *p*-nitrophenol product. The rate profile of the methanolysis of *p*-nitrophenyl acetate carried out in the presence of equimolar amounts of **4** and Ba^{2+} was analysed as the sum of an exponential and a linear phase, according to eq. 4 where k_1' and A_{inf} are respectively the rate constant and the infinite value of the exponential phase and *s* is the slope of the linear phase. In all other cases the absorbance data were converted into pNPOH

$$A = A_{inf}\left(1 - e^{-k_1' t}\right) + st \qquad (4)$$

concentration data using the value of the apparent molar absorptivity measured under identical conditions. Rate constants were in these cases obtained by an initial rate method.

HPLC rate measurements. Samples of the reaction mixture were withdrawn at time intervals, quenched with dilute trifluoroacetic acid, and subjected to HPLC analysis after addition of a known amount of the internal standard. Samples were analysed on a Supelcosil LC8 column (25 cm. × 4.6 mm. ID; particle size 5 μm) with H_2O-CH_3CN-CH_3OH 65:20:15 (v/v/v) as eluent, at a flow rate of 0.9 ml/min. Benzo-30-crown-10 and benzo-15-crown-5 were used as internal standards in the acetylation of **4** with pNPOAc and in the methanolysis of **5**, respectively.

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

1. a) Chao, Y.; Cram, D.J. *J. Am. Chem. Soc.* **1976**, *98*, 1015-1017; b) Chao, Y.; Weisman, G.R.; Sogah, G.D.Y.; Cram, D.J. *J. Am. Chem. Soc.* **1979**, *101*, 4948-4958.
2. a) Lehn, J.M.; Sirling, C. *J. Chem. Soc. Chem. Comm.* **1978**, 949-951; b) Lehn, J.M.; Sirling, C. *New J. Chem.* **1987**, *11*, 693-702.
3. Ercolani, G.; Mandolini, L. *J. Am. Chem. Soc.* **1990**, *112*, 423-427.
4. a) Cacciapaglia, R; Lucente, S.; Mandolini, L.; van Doorn, A.R.; Reinhoudt, D.N.; Verboom, W. *Tetrahedron* **1989**, *45*, 5293-5304; b) Cacciapaglia R.; van Doorn, A.R.; Mandolini, L.; Reinhoudt, D.N.; Verboom, W. *J. Am. Chem. Soc.* **1992**, *114*, 2611-2617.
5. a) Kraft, D.; Cacciapaglia, R; Böhmer, V.; El-Fadl, A.A.; Harkema, S.; Mandolini, L.; Reinhoudt, D.N.; Verboom, W.; Vogt, W. *J. Org. Chem.* **1992**, *57*, 826-834; b) Cacciapaglia R.; Mandolini, L.; Reinhoudt, D.N.; Verboom, W. *J. Phys. Org. Chem.* **1992**, *5*, 663-669.
6. Cacciapaglia, R.; Casnati, A.; Mandolini, L.; Ungaro, R. *J. Am. Chem. Soc.* **1992**, *114*, 10956-10958.

7. Kimura, E.; Nakamura, I.; Koike, T.; Shionoya, M.; Kodama, Y.; Ikeda, T.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 4764-4771.
8. Koike, T.; Kajitani, S.; Nakamura, I.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 1210-1219.
9. The finding that pNPOH is 74% ionised in this medium indicates that the basicity toward neutral acids is the same as that of an aqueous solution having pH 7.6.
10. Fersht, A. *Enzyme Structure and Mechanism*; W.H. Freeman and Co.: New York, 1985; Chapter 4.
11. Bender, M.L. *Mechanisms of Homogeneous Catalysis from Protons to Proteins*; Wiley Interscience: New York, 1971; pp.443-446.
12. Cacciapaglia, R.; Mandolini, L.; Van Axel Castelli, V.: The Effect of Cation Complexing Agents in the Ba(II) Promoted Basic Ethanolysis of Phenyl Acetate, *Proceedings of 2° Congresso Nazionale di Chimica Supramolecolare*, Perugia, 7-9 September, 1994.
13. Izatt, R.M.; Pawlak, K.; Bradshaw, J.S.; Bruening, R.L. *Chem. Rev.* **1991**, *91*, 1721-2085.
14. Illuminati, G.; Mandolini, L.; Masci, B. *J. Am. Chem. Soc.* **1981**, *103*, 4142-4145.
15. Wilcox, C.S.; Babston, R.E. *J. Org. Chem.* **1984**, *49*, 1451-1453.
16. S.G. Luxon (ed.), *Hazards in the Chemical Laboratory*, 5th edn.; The Royal Society of Chemistry: Cambridge, 1992; p. 524.
17. Rastetter, W.H.; Phillion, D.P. *J. Org. Chem.* **1981**, *46*, 3204-3208.

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