Alkaline-earth metal complexes of thiol-pendant crown ethers as turnover catalysts of ester cleavage

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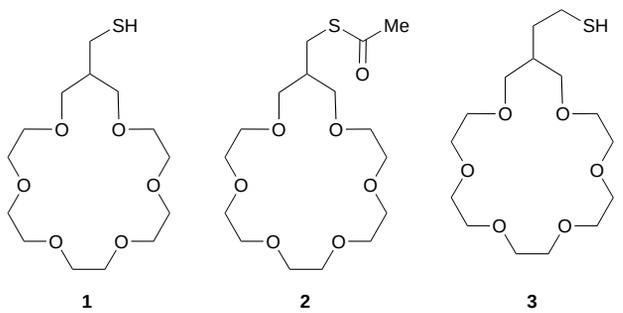
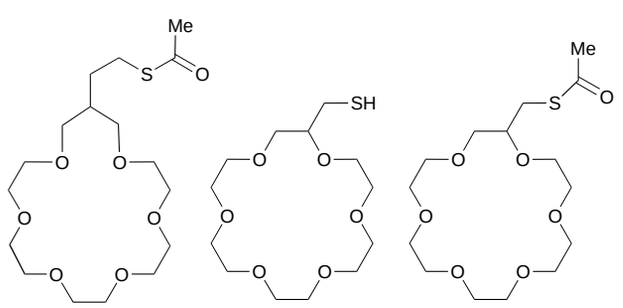
Methanolysis of *p*NPOAc under moderately alkaline conditions is catalysed by the alkaline-earth metal ion (Ca, Sr, Ba) complexes of 2-(mercaptomethyl)-18-crown-6 according to biphasic kinetics in which an initial burst of *p*NPOH release is followed by a zeroth-order phase. Two competing catalytic mechanisms contribute to the overall process, (i) a double displacement mechanism in which the complexed metal ion enables the SH group of the catalyst to undergo an acylation–deacylation cycle, and (ii) a bypass mechanism in which a catalytically active complex-bound methoxide ion is involved and no covalently bound intermediate is formed. Calcium ion is much more efficient than its larger analogues both in the acylation–deacylation cycle of the double displacement mechanism, and in the direct delivery of methoxide ion to the ester substrate.

Introduction

Metal ion catalysis of ester cleavage has received considerable attention in recent years.[1] We have shown[2] that methanolysis of *p*-nitrophenyl acetate (*p*NPOAc) under slightly basic conditions was catalysed by the barium complex of **1** *via* a double displacement mechanism (Scheme 1) in which the thiol group acted as an acyl-receiving and acyl-releasing unit with the participation of the complexed metal electrophile. The turnover efficiency of the overall process was modest because of the sluggish methanolysis of the thiol acetate intermediate **2**.

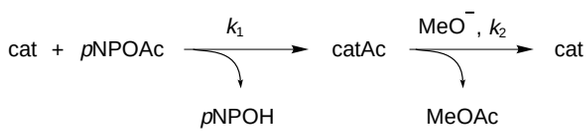


1 **2** **3**



4 **5** **6**

Since it was felt that variations in the crown ether ring size, side arm length, and alkaline-earth metal ion identity could possibly increase the rate of the deacylation step and, consequently, improve the efficiency of the overall catalytic process, we have synthesized the thiol-pendant crown ethers **3** and **5**, together with their corresponding thiol acetates **4** and **6**, with the purpose of carrying out an evaluation of catalytic efficiencies of metal complexes by means of separate investigations of the effect of alkaline-earth metal ions (Ca, Sr, Ba) on rates of deacylation of the thiol acetates **4** and **6**, and of acylation of the parent thiols **3** and **5** by *p*NPOAc. The results of this investigation are reported herein.



Scheme 1 Double displacement catalytic mechanism in the basic methanolysis of *p*NPOAc; (cat = **1**·Ba^{2+})

Results and discussion

The β-mercaptoethyl crown ether **3** and its acetylated derivative **4** were prepared according to a literature procedure,[3] whereas the analogous 18-crown-6 compounds **5** and **6**† were obtained from 2-(hydroxymethyl)-18-crown-6 according to Scheme 2.

All rate measurements were carried out at 25.0 °C in MeCN–MeOH (9:1, v/v) containing 90 mmol dm^{-3} diisopropylethylamine and 30 mmol dm^{-3} diisopropylethylammonium perchlorate. In aqueous solution[4] the pH of this buffer would be 11.3, but in MeCN–MeOH the basicity towards neutral acids is much lower. Based on the observation that *p*-nitrophenol (*p*NPOH) is 74% ionised in this medium, it is calculated that the basicity is comparable to that of an aqueous solution with pH 7.6.

Deacylation step

Because the critical step in the double displacement catalytic mechanism is deacylation of the covalent intermediate (thiol ester), our study started from an investigation of the influence of adding one molar equivalent of $Ba(ClO_4)_2$, $SrBr_2$ and $CaBr_2$ on rates of methanolysis of 2.7 mmol dm^{-3} thiol acetates **4** and **6**.

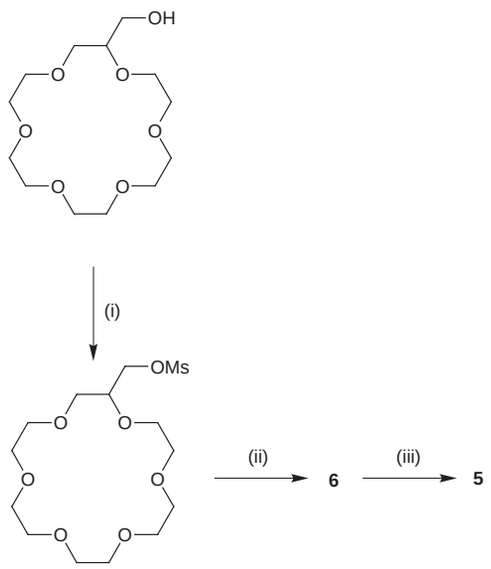
The kinetics were followed by monitoring the disappearance of the thiol acetate reactant by HPLC analysis of reaction samples taken at time intervals. It is apparent from Fig. 1, where the results are shown graphically, that the thiol acetates are extremely reluctant to undergo methanolysis in the weakly

† 18-Crown-6 is 1,4,7,10,13,16-hexaoxacyclooctadecane, and 19-crown-6 is 1,4,7,10,13,16-hexaoxacyclononadecane.

Table 1 Methanolysis of thiol esters **4** and **6** in MeCN–MeOH (9:1, v/v) in the presence of 90 mmol dm^{-3} diisopropylethylamine–30 mmol dm^{-3} perchlorate salt buffer at 25 °C. Influence of added alkaline-earth metal salts.

Substrate[a]	Metal salt[a]	$k_2/10^{-3}$ min^{-1}	$k_2/k_{background}$[b]
4	$Ba(ClO_4)_2$	0.80[c]	1 100
	$SrBr_2$	2.97	4 200
	$CaBr_2$	3.98	5 700
6	$Ba(ClO_4)_2$	1.86[c]	2 700
	$SrBr_2$	3.76	5 400
	$CaBr_2$	53.3	76 000

[a] Initial substrate and metal salt concentration 2.70 mmol dm^{-3}. [b] Lower limits of rate enhancements relative to background based on $k_{background} \leqslant 7 \times 10^{-7}$ min^{-1} (see text). [c] Under identical conditions **2** solvolyses with a k_2 value of 0.70×10^{-3} min^{-1}; ref. 2.



Scheme 2 Preparation of thiol ester **6** and of thiol **5**. *Reagents and conditions*: (i) MsCl, Et_3N in CH_2Cl_2; (ii) $CH_3COS^-K^+$ in acetone; (iii) $LiAlH_4$ in THF.

basic solution, no reaction being observed in both cases after 50 days. Based on a conservative estimate of ±5% in the experimental error of our HPLC measurements, an upper limit of $v_o = 2 \times 10^{-9}$ mol dm^{-3} min^{-1}, corresponding to a first-order rate constant of 7×10^{-7} min^{-1} was calculated. In marked contrast to the sluggishness of the background reactions, in the presence of one molar equivalent of divalent metal ion cleavage of the thiol acetates took place smoothly and proceeded to completion within reasonably short times with clean first-order time dependence.

Table 1, where the kinetic data are summarized, clearly shows that the size of the acceleration depends on the substrate–metal ion combination. The reactivity order Ca ⩾ Sr > Ba observed in both cases bears an inverse relationship to cation size. This is not really surprising for reactions in which a major component of the driving force for catalysis is electrostatic in nature.[1b]

The data listed in Table 1 provide additional evidence that very large metal ion effects on rates can be obtained when the ester function is covalently linked to a crown ether ring capable of holding the metal electrophile in close proximity to the carbonyl undergoing nucleophilic addition.[5]

^{1}H NMR titration experiments showed that downfield shifts are experienced by the methyl group of thiol acetates **4** and **6** (1.3 mmol dm^{-3} in CD_3CN–CD_3OD 9:1, v/v) upon addition of $CaBr_2$ (Fig. 2). Analysis of the titration curve afforded a K value of 1.5×10^4 dm^3 mol^{-1} for the complex between Ca^{2+} and **4**, but in the case of **6** the binding constant is too large to measure ($K > 3 \times 10^5$ dm^3 mol^{-1} assuming that upon addition of 1 mol equiv. of $CaBr_2$ the percent bound is no less than

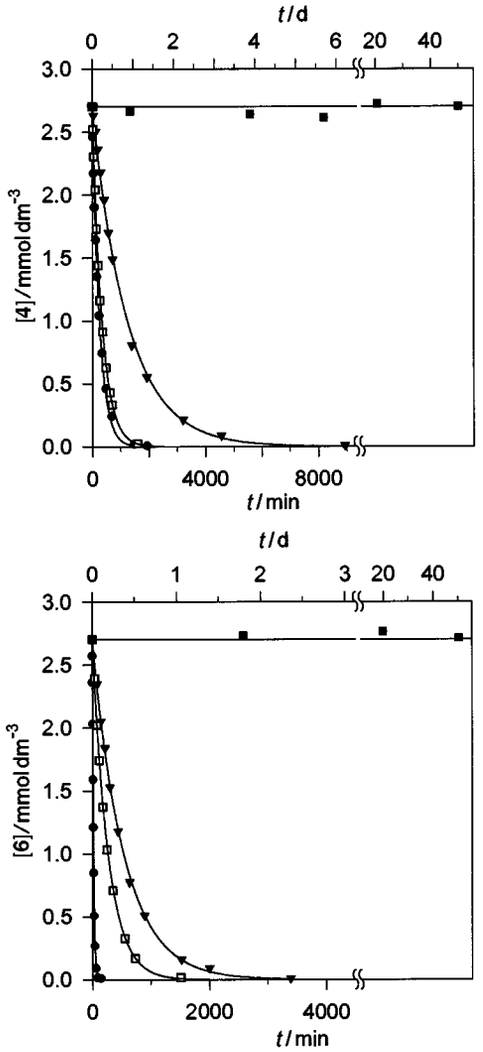


Fig. 1 Methanolysis of 2.7 mmol dm^{-3} thiol acetate **4** (top) and 2.7 mmol dm^{-3} thiol acetate **6** (bottom) in the presence of buffer alone (■), and in the presence of buffer plus 2.7 mmol dm^{-3} $CaBr_2$ (●), 2.7 mmol dm^{-3} $SrBr_2$ (□) and 2.7 mmol dm^{-3} $Ba(ClO_4)_2$ (▼) [MeCN–MeOH 9:1 (v/v), 25 °C, HPLC data]

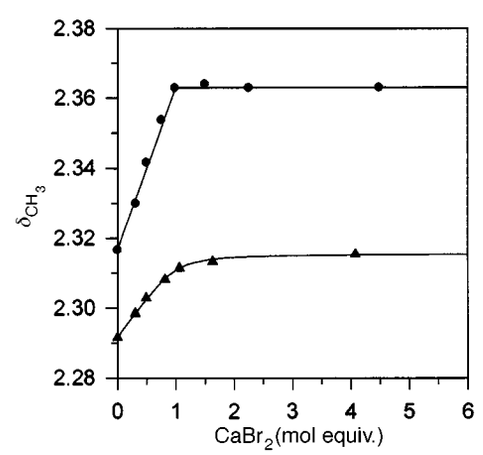


Fig. 2 ^{1}H NMR titration of 1.3 mmol dm^{-3} thiol acetate **4** (▲), and 1.3 mmol dm^{-3} thiol acetate **6** (●), with $CaBr_2$ in CD_3CN–CD_3OD (9:1, v/v) at 25 °C. The curve connecting the points (▲) is a plot of eqn. (5) with $K = 1.5 \times 10^4$ dm^3 mol^{-1} and $\delta_\infty = 2.315$.

95%). Since calcium ion is known to form in general much weaker complexes with crown ethers than barium and strontium ions,[6] we conclude that our rate measurements refer to conditions in which binding of the metal ions to the crown ether substrates is virtually, or very nearly, complete.

Table 2 Effect of additives on the rate of liberation of *p*NPOH from *p*NPOAc[a]

Entry	Additives[b]	k_{obs}/min^{-1} [c]	k_{rel}	k_1/dm^3 mol^{-1} min^{-1}
1	none	1.85×10^{-4} [d]	1.0	
2	**5**	8.64×10^{-4} [e]	4.7	0.32
3	$Ba(ClO_4)_2$	4.06×10^{-3}	21.9	
4	$SrBr_2$	1.10×10^{-2}	59.5	
5	$CaBr_2$	0.160	865	
6	**5** + $Ba(ClO_4)_2$	0.116	627	43.0[f]
7	**5** + $SrBr_2$	0.201	1 090	74.4
8	**5** + $CaBr_2$	2.57	13 900	952

[a] Reaction conditions as in the experiments in Table 1. The initial concentration of *p*NPOAc was 0.050 mmol dm^{-3} unless otherwise stated. [b] The concentration of all additives was 2.70 mmol dm^{-3}. [c] Data in entries 2–4 are corrected for background. [d] Calculated from an initial rate $v_o = 3.13 \times 10^{-6}$ mol dm^{-3} min^{-1} at $[p\text{NPOAc}]_o = 16.9$ mmol dm^{-3}. [e] Calculated from an initial rate $v_o = 1.46 \times 10^{-5}$ mol dm^{-3} min^{-1} (corrected for background methanolysis) at $[p\text{NPOAc}]_o = 16.9$ mmol dm^{-3}. [f] The k_1 value for the Ba^{2+} complex of **1** under identical conditions is 4.7 dm^3 mol^{-1} min^{-1}; ref. 2.

Acylation step

Rates of acetylation of the metal complexes of thiols **3** and **5** were investigated under single turnover conditions. Reaction mixtures containing 2.7 mmol dm^{-3} thiol and 2.7 mmol dm^{-3} metal salt were reacted with 0.050 mmol dm^{-3} *p*NPOAc. In the case of thiol **5**, solutions were homogeneous at time zero and remained this way throughout the reaction. The liberation of *p*NPOH was monitored spectrophotometrically at 388 nm and found to strictly adhere to first-order time dependence. But in the case of the metal complexes of thiol **3**, precipitation of solid material during the time course of the reactions caused non-reproducible and irregular behaviour. Reduction of the metal complex concentration to 0.5 mmol dm^{-3} caused a decrease in, but not a complete elimination of, the above complications. Our investigations were therefore restricted to the metal complexes of **5**. The results are reported in Table 2, entries 6 to 8. A number of control experiments showed that (i) in the absence of metal ions thiolysis of *p*NPOAc (entry 2) is only four times faster than the very slow background methanolysis (entry 1, $t_{\frac{1}{2}} \approx 3$ days), which is most likely due to the presumably low concentration of the reactive thiolate ion in the weakly basic solution, and (ii) remarkable accelerations are brought about by the addition of metal salts (entries 3 to 5), that are ascribed as before[2] to the formation of reactive metal bound methoxide species in equilibrium with free methoxide ion [equilibrium (1)]

$$MeO^- + M^{2+} \rightleftharpoons (MeOM)^+ \qquad (1)$$

in the buffered solution. Whereas these remarkable rate enhancements, notably the 860-fold acceleration brought about by $CaBr_2$, are interesting *per se*, in the context of the present work it is sufficient to note that mixtures of **5** and M^{2+} are much more effective than either thiol or metal ion alone. The reactivity order Ca ≫ Sr > Ba found in the acylation step closely parallels that found in deacylation.

Catalytic cycle

The data reported in the preceding sections show that by virtue of the metal ion rate enhancing effects on both acylation and deacylation processes, the alkaline-earth metal ion complexes of **5** are suitable to catalyse with turnover the methanolysis of *p*NPOAc *via* the double displacement mechanism of Scheme 1. The analytical solution for the case in which no Michaelis–Menten complex of definite stability is formed between catalyst and substrate is given in eqn. (2),[7] in which $k_1' = k_1[p\text{NPOAc}]$. Comparison of the rate constants for deacylation (k_2 in Table 1) with those for acylation (k_1 in Table 2) shows that for any *p*NPOAc concentration higher than 10 mmol dm^{-3} acylation is much faster than deacylation ($k_1' \gg k_2$). When this is the case, eqn. (2) reduces to the simple form of eqn. (3), which clearly

$$[p\text{NPOH}] = [\text{cat}]_o\left(\frac{k'_1}{k'_1 + k_2}\right)\left(\frac{k'_1}{k'_1 + k_2}\{1 - \exp[-(k'_1 + k_2)t]\} + k_2 t\right) \qquad (2)$$

shows that rate limiting deacylation leads to biphasic kinetics. After an initial transient in the pre-steady-state phase, the release of *p*NPOH is linear with time in the steady-state phase. The linear portion extrapolates back to an initial burst, which coincides with the catalyst concentration in what corresponds to an active site titration experiment.[7] The time–concentration profiles plotted in Fig. 3 as solid lines, calculated from eqn. (3)

$$[p\text{NPOH}] = [\text{cat}]_o[1 - \exp(-k'_1 t) + k_2 t] \qquad (3)$$

and the pertinent data in Tables 1 and 2 for an initial *p*NPOAc concentration of 16.9 mmol dm^{-3}, are compared with experimental data from catalytic experiments carried out under identical conditions. It is seen that the experimental data reproduce well some of the features of the simulated profiles, but not others. There is a good agreement of data points with the exponential portions of the profiles, and the magnitudes of experimental bursts closely correspond to the catalyst concentration. Consistently, HPLC analyses of reaction samples taken at times corresponding to extinction of the exponential phases (Table 3) confirmed a virtually complete accumulation of the acylated catalyst **6**.

But in the steady-state portions of the profiles the rates of production of *p*NPOH are in all cases much higher than those allowed by the relatively low deacylation rates of the metal complexes of **6**. The discrepancies between calculated and

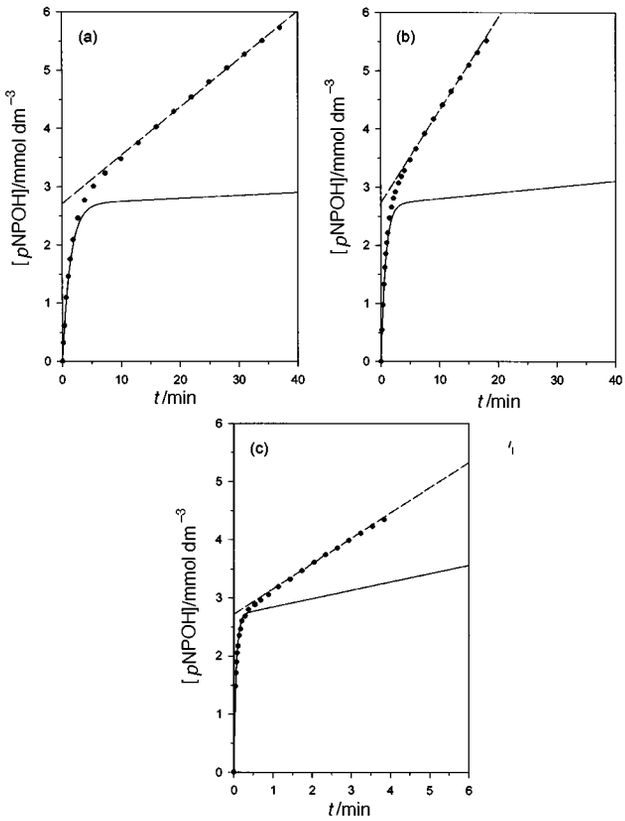


Fig. 3 *p*NPOH liberation in the methanolysis of 16.9 mmol dm^{-3} *p*NPOAc in the diisopropylethylamine buffer plus 2.7 mmol dm^{-3} **5** and (a) 2.7 mmol dm^{-3} $Ba(ClO_4)_2$, (b) 2.7 mmol dm^{-3} $SrBr_2$ and (c) 2.7 mmol dm^{-3} $CaBr_2$. The curves are plots of eqn. (3) with the pertinent k'_1 and k_2 values from Tables 1 and 2.

Table 3 Accumulation of the acetylated intermediate **6** in the catalytic experiments[a]

Metal ion	*t*/s	[**6**][b]/mmol dm^{-3}
Ba^{2+}	390	2.71
Sr^{2+}	220	2.72
Ca^{2+}	23	2.65

[a] Reaction conditions as in the experiments reported in Fig. 3. [b] Data from HPLC analyses of reaction samples taken at time *t*.

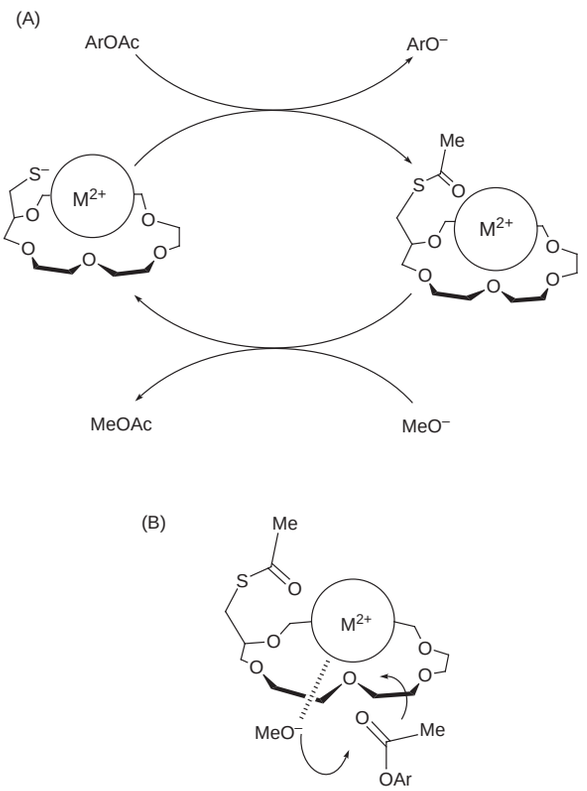


Fig. 4 Competing catalytic mechanisms of methanolysis of *p*NPOAc. Mechanism (A): thiol-mediated methanolysis *via* an acylation–deacylation cycle; mechanism (B): direct delivery of complex-bound methoxide ion.

experimental rates in the zeroth-order phases are clearly too large to be accounted for on grounds of experimental uncertainties. Therefore, besides the thiol-mediated methanolysis proceeding *via* an acylation–deacylation cycle [mechanism (A) in Fig. 4], we suggest as before[2] the coexistence of a bypass pathway where no covalently bound intermediate is involved [mechanism (B)]. In the latter mechanism, the active nucleophile is a methoxide ion that is a part of a 1:1:1 methoxide–metal ion–ligand complex, formed in minute amounts in the weakly basic solution according to eqn. (4). This interpretation

$$(\mathbf{6}\cdot\mathrm{M})^{2+} + \mathrm{MeO}^- \rightleftharpoons (\mathbf{6}\cdot\mathrm{MOMe})^+ \qquad (4)$$

is supported by our recent findings[8] that ternary complexes formed from 18C6 and alkoxide (alkaline earth metal ion) pairs are more reactive than the ion pairs themselves in the cleavage of esters.

We assume that the differences between the actual rates of production of *p*NPOH at steady-state and those calculated from eqn. (3) represent the contributions of mechanism (B) to the overall catalytic processes. Dissection of the overall turnover frequencies into separate contributions from the two competing mechanisms (Table 4) shows that the bypass mechanism (B) accounts for about two-thirds of the overall reaction in the case of the reaction catalysed by **5**·Ca^{2+}, and for even larger fractions with the corresponding Ba^{2+} and Sr^{2+} complexes.

Table 4 Methanolysis of *p*NPOAc catalyzed by alkaline-earth metal complexes of **5**. Turnover frequencies (h^{-1}).[a,b]

	Ba^{2+}	Sr^{2+}	Ca^{2+}
Overall	1.8	3.5	9.6
Mechanism (A)	0.11	0.22	3.2
Mechanism (B)	1.7	3.3	6.4

[a] The data refer to the catalytic experiments plotted in Fig. 3. [b] The corresponding data (h^{-1}) for the methanolysis of *p*NPOAc catalyzed by the Ba^{2+} complex of **1** under identical conditions are: overall 0.16; mechanism (A) 0.042; mechanism (B) 0.12 (calculated from data reported in ref. 2).

The data listed in Tables 1 and 2 clearly show that acylation of the metal complexes of thiol-pendant crown ethers, as well as deacylation of their acetates, are markedly influenced by both cation nature and ligand identity. Although the set of available data for a meaningful comparison is unfortunately limited by the solubility problems met with the metal complexes of **3**, it seems nevertheless clear that the Ca^{2+} ion turns out to be the most efficient promoter of acyl transfer processes, including the direct methanolysis of *p*NPOAc occurring *via* mechanism (B). Combination of the Ca^{2+} ion with the thiol-pendant crown ether **5** provides the most efficient catalyst with transacylase activity. As shown in Table 4, one molecule of this catalyst cleaves very nearly ten molecules of *p*NPOAc in one hour.

Experimental

Instruments and general methods

All operations involving air or moisture sensitive materials were performed under argon. ^{1}H NMR spectra were recorded in $CDCl_3$ or CD_3CN–CD_3OD (9:1, v/v) with a Bruker AC 300 spectrometer, using Me_4Si as internal standard (*J* values are given in Hertz). Spectrophotometric measurements were carried out on a Varian Cary 219 instrument or on a Hewlett Packard 8452A diode array spectrometer. HPLC analyses were performed on a Hewlett Packard 1050 liquid chromatograph fitted with a Hewlett Packard HP 1047A refractive index detector or on a Bruker LC 22 liquid chromatograph fitted with a Knauer 87.00 UV–VIS detector operating at 230 nm. Samples were analysed on a Supelcosil LC-8 column (25 cm × 4.6 mm id; particle size 5 μm). HPLC–MS experiments were carried out on a Fisons Instruments VG-Platform benchtop mass spectrometer equipped with a pneumatically assisted electrospray LC–MS interface and a single quadrupole. The mass spectrometer was operated in the positive-ion mode by applying to the capillary a voltage of 3.8 kV, while the skimmer cone voltage was set at 20 V. The mass spectrometry data handling system used was the Mass Lynx software from Fisons Instruments. GC–MS analyses were carried out on a Hewlett Packard HP 5890 gas chromatograph coupled with a HP 5970 MSD and equipped with a 15 m × 0.25 mm silica capillary column HP-1 (cross-linked methyl silicon gum; 0.25 μm film thickness). Non-linear least-squares calculations were carried out using the programme Sigma Plot for Windows, version 1.02 (Jandel Scientific).

Materials

THF was dried by distillation from sodium benzophenoneketyl. CH_2Cl_2 was distilled over P_2O_5. 4-Methylanisole (Fluka) and 1,4-dimethoxybenzene (Janssen) were used as received. $SrBr_2$·H_2O (Carlo Erba) and $CaBr_2$·$2H_2O$ (Carlo Erba) were used without further purification and their stock solutions in methanol titrated by means of potentiometric argentometry with a Mettler Toledo Ag 4805-S7/120 Silver Combination Electrode. The concentration of bromide ions was found in all cases to be within 1% of the concentration calculated on the basis of the formula weight. Other materials were as reported previously.[2]

WARNING: Care was taken when handling diisopropylethylammonium perchlorate because it is potentially explosive.[9] No accident occurred in the course of the present work.

18-(2-Mercaptoethyl)-19-crown-6 (**3**) and the corresponding acetate (**4**) were synthesized according to a literature procedure.[3]

2-(Mercaptomethyl)-18-crown-6 (**5**) and **2-(acetylthiomethyl)-18-crown-6 (6)**. **5** and **6** were prepared according to Scheme 2 starting from the commercially available 2-(hydroxymethyl)-18-crown-6 (Aldrich). A solution of CH_3SO_2Cl (Aldrich, 0.072 cm^3, 0.93 mmol) in CH_2Cl_2 (2 cm^3) was added dropwise to a cooled (−10 °C) and stirred solution of 2-(hydroxymethyl)-18-crown-6 (0.226 g, 0.77 mmol) and Et_3N (0.175 cm^3, 1.25 mmol) in CH_2Cl_2 (3 cm^3). The resulting mixture was stirred for 20 min, diluted with cold CH_2Cl_2 and washed sequentially with cold 5% HCl (aq.), cold saturated $NaHCO_3$ (aq.) and cold H_2O. The organic layer was dried over $MgSO_4$ and removal of solvent afforded the mesylate as an oil (0.245 g, 86% yield). $\delta_H(CDCl_3)$ 3.06 (3H, s, OSO_2CH_3), 3.55–4.00 (23H, m, CHO and CH_2O), 4.30–4.50 (2H, m, $CH_2OSO_2CH_3$); *m*/*z* (ES) 395 $(M + Na)^+$, 411 $(M + K)^+$.

A mixture of the mesylate (0.245 g, 0.66 mmol) and potassium thioacetate (Aldrich, 0.113 g, 0.99 mmol) in acetone (3 cm^3) was refluxed overnight. The mixture was cooled, filtered and the solvent evaporated under reduced pressure. Distillation of the crude material on a Kugelrohr apparatus [170 °C (10^{-3} mmHg)] afforded thiol acetate **6** as an oil (0.196 g, 85% yield). An analytically pure sample of thiol acetate **6** was obtained by flash chromatography on acid washed silica gel (Merck, 230–400 mesh) (1:2 $CHCl_3$–hexane, 1:1 $CHCl_3$–hexane, $CHCl_3$, 25:1 $CHCl_3$–MeOH and 10:1 $CHCl_3$-MeOH). $\delta_H(CDCl_3)$ (3H, s, $COCH_3$), 3.01–3.15 (2H, m, CH_2SCOCH_3), 3.58–3.82 (23H, m, CHO and CH_2O); $\delta_C(CDCl_3)$ 30.6, 69.9, 70.7, 70.7, 70.8, 70.8, 71.0, 72.7, 78.2, 195.5; *m*/*z* (GC–MS) 352 (M^+). Found: C, 51.0; H, 8.1. Calc. for $C_{15}H_{28}O_7S$: C, 51.1; H, 8.0%.

Thiol acetate **6** (0.544 g, 1.54 mmol) in THF (2 cm^3) was added dropwise to a stirred suspension of $LiAlH_4$ (0.112 g, 2.95 mmol) in THF (1.5 cm^3). The mixture was stirred overnight at ambient temperature, carefully quenched with 10% HCl (aq.), diluted with CH_2Cl_2 and water, and subjected to extractive workup, drying ($MgSO_4$) and removal of the solvent. Flash chromatography on acid washed silica gel (1:1 Et_2O–pentane, EtOAc) followed by Kugelrohr distillation [220 °C (2×10^{-2} mmHg)] afforded thiol **5** as an oil (0.308 g, 64% yield). HPLC analysis of this material (refractive index detector; 70.5:29:0.5 H_2O (0.05% CF_3CO_2H)–MeOH–MeCN; 0.8 cm^3 min^{-1}] revealed the presence of the corresponding disulfide [*m*/*z* (ES) 642 $(M + Na)^+$, 658 $(M + K)^+$] as the only detectable impurity in *ca.* 4% amount. Further purification by flash chromatography on acid washed silica gel (1:1 CH_2Cl_2–hexane, CH_2Cl_2, 75:1 CH_2Cl_2–MeOH and 50:1 CH_2Cl_2–MeOH) afforded thiol **5** still contaminated by trace amounts (*ca.* 2%) of the disulfide. This sample was used in the kinetic experiments without further purification. $\delta_H(CDCl_3)$ (1H, t, *J* 9, SH), 2.55–2.70 (2H, m, CH_2SH), 3.50–3.90 (23H, m, CHO and CH_2O); *m*/*z* (ES) 333 $(M + Na)^+$, 349 $(M + K)^+$.

Equilibrium and kinetic measurements

^1H NMR titrations of **4** and of **6** with $CaBr_2$ were carried out in CD_3CN–CD_3OD (9:1, v/v) at 25.0 °C according to a previously reported procedure.[2] The association constant *K* and the chemical shift of the monitored signal of the ligand in the complex (δ_∞) were obtained as best fit parameters in a non-linear least-square fitting treatment of data points to eqn. (5) where δ is the observed chemical shift at a given titrant concentration and δ_o is the chemical shift of the free ligand. The analytical concentration of the titrant $CaBr_2$ was corrected for the fraction sequestered by the ligand.

$$\delta = \delta_o + \frac{(\delta_\infty - \delta_o)K[Ca^{2+}]}{1 + K[Ca^{2+}]} \quad (5)$$

Spectrophotometric and HPLC rate measurements were carried out as before.[2] Internal standard, eluent and flow rate are indicated in the given order for HPLC monitoring of thiol ester disappearance in the methanolysis of thiol acetates **4** and **6** and for thiol ester accumulation in the acylation of thiols **3** and **5** by *p*NPOAc. Methanolysis of **4** [1,4-dimethoxybenzene, 62.5:27.5:10 H_2O (0.08% CF_3CO_2H)–MeOH–MeCN, 0.8 cm^3 min^{-1}]; methanolysis of **6** and acylation of **5** [4-methylanisole, 70.5:29:0.5 H_2O (0.05% CF_3CO_2H)–MeOH–MeCN, 0.8 cm^3 min^{-1}]; and acylation of **3** [1,4-dimethoxybenzene, 70:24:6 H_2O (0.08% CF_3CO_2H)–MeOH–MeCN, 0.8 cm^3 min^{-1}].

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