# Acyl Transfer Mediated by Complexation. The Effect of Cyclodextrins on the Reaction of Nucleophiles with *p*-Nitrophenyl Acetate and Hexanoate

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The kinetics of the cleavage of *p*-nitrophenyl acetate (*p*NPA) and *p*-nitrophenyl hexanoate (*p*NPH) by trifluoroethanol (TFE), mercaptoethanol, hydroxylamine or imidazole in the presence of α-cyclodextrin, β-cyclodextrin, or hydroxypropyl-β-cyclodextrin (CDs) have been measured in basic aqueous solution. Detailed studies established that TFE reacts with *p*NPH bound to the CDs and that the bound ester is almost as reactive as the free ester. Similar behaviour has been observed for *p*NPA and for reaction with the other nucleophiles, with slight retardations or accelerations. There are only minor differences between the effects of the three cyclodextrins and the four nucleophiles. It seems clear that *p*NPA and *p*NPH are bound to the CDs in such a way that their carbonyl groups are exposed to the medium and that transition-state binding differs little from substrate binding, even though the orientation may be different for the two esters.

There have been many studies of the cleavage of carboxylic esters by cyclodextrins[1] (CDs) in basic aqueous solution so that the general features of the reaction are now established.[1-7] In studies of the reaction of aryl alkanoate esters, carried out in our laboratory, evidence was obtained of processes involving *two* molecules of the CD.[8,9] In some cases, the process is *non*-productive, with 2:1 (CD:ester) binding retarding or inhibiting ester cleavage.[8] In other cases, the two molecules of the CD accelerate ester cleavage in either of two ways: (*i*) attack of a molecule of a CD (as its anion) on the ester bound to another molecule of CD [eqn. (1)]; (*ii*) reaction within a discrete ternary complex [eqn. (2)]. The latter pathway (2) is operative in at

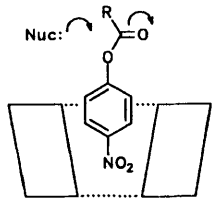
$$S + CD \rightleftharpoons S{\cdot}CD \xrightarrow{CD} \text{products} \quad (1)$$

$$S{\cdot}CD + CD \rightleftharpoons S{\cdot}CD_2 \longrightarrow \text{products} \quad (2)$$

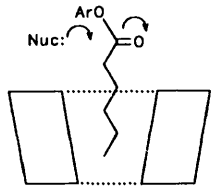
least a few cases, but the former (1) is probably more widespread. The importance of both processes increases with the acyl chain length of the alkanoate ester.[8,9]

Our discovery of the process in eqn. (1) has prompted us to study the attack of other nucleophiles on alkanoate esters, many of which bind to CDs through their acyl chains.[4,7,9] We were interested to see if CD-bound esters are more or less reactive to 'external' nucleophiles and to find out how reactivity varies with the structure of the ester, the CD and of the nucleophile. Presumably, if the ester sits deeply in the CD cavity, such that its carbonyl group is relatively buried, it will be less accessible and less reactive towards the nucleophile. On the other hand, if the ester is bound so that the carbonyl group is situated outside the CD cavity, either as in structure **1** or in structure **2**, then its reactivity might not be greatly affected. Conceivably, there might be catalysis by some means, involving the hydroxy groups on the rim of the CD cavity, *e.g.* hydrogen bonding, general base catalysis (*vide infra*). In this regard, we note that Barra and de Rossi[10] have reported catalysis of the reaction of *p*-nitrophenyl acetate with α-amino acids by CD which they suggested arises from reaction within a ternary complex in which the amino acid is hydrogen-bonded to the rim of the CD-ester complex.

This paper reports our findings for the cleavage of the two *p*-nitrophenyl esters by four different nucleophiles and three CDs in basic solution. In due course, we will report on related studies in which ester structure is varied systematically.




**1**




**2**

## Results

We have measured the kinetics of the reaction of *p*-nitrophenyl acetate (*p*NPA) and hexanoate (*p*NPH) with trifluoroethanol (TFE), mercaptoethanol, hydroxylamine, or imidazole, in the presence of α-cyclodextrin (α-CD),† β-cyclodextrin (β-CD),‡ or 'hydroxypropyl-β-cyclodextrin' (Hp-β-CD). The reaction media were basic aqueous buffers whose pHs were chosen with regard to the p$K_a$s of the nucleophiles and their known reactivities towards *p*NPA.[11]

*pNPH and TFE.*—Initial studies with *p*NPH and TFE were carried out in detail to establish firmly the kinetic behaviour in the presence of CDs. We chose to begin with the anion of TFE as the 'external' nucleophile for several reasons. First, its reactivities towards *p*NPA[11,12] and *p*NPH[12] were known. Second, the p$K_a$ of TFE (12.4)[11] is very close to that of CDs (12.2, 12.3),[13] so that the TFE anion can compete reasonably, as a nucleophile, with CD anions.[11,12] Third, the closeness of the p$K_a$s of TFE and CDs allows for the possibility of general-base catalysis: a CD anion assisting the attack of TFE (or TFE anion assisting attack by a CD hydroxy group). Fourth, the binding of TFE to CDs should be very weak, judging by that of ethanol,[5,14] meaning that at low [TFE] the complication of TFE binding to the CD should be absent, making for easier data analysis. The reaction medium was an aqueous phosphate buffer of pH 11.6, as used in other recent studies.[9,12,15]

Fig. 1 shows pseudo-first-order rate constants for the cleavage of *p*NPH at various concentrations of α-CD and TFE. Relative to esterolysis by α-CD alone,[4,7] addition of TFE

† Cyclomaltohexaose.
‡ Cyclomaltoheptaose.

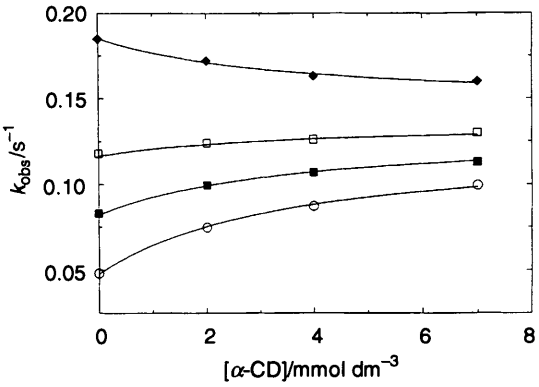



**Fig. 1** Effect of trifluoroethanol (TFE) and α-CD on the cleavage of *p*-nitrophenyl hexanoate (*p*NPH) in basic solution. The symbols for the TFE concentrations are as follows: ○, 0; ■, 5; □, 10; ◆, 20 mmol dm$^{-3}$. The curves were calculated from eqn. (7), with the parameters in Table 1.

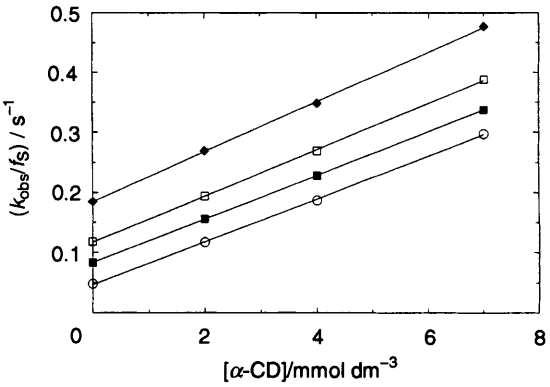



**Fig. 2** Effect of TFE and α-CD on the cleavage of *p*NPH for the data in Fig. 1, plotted according to eqn. (8). The symbols for the TFE concentrations are: ○,0; ■, 5; □, 10; ◆, 20 mmol dm$^{-3}$. The straightness of the plots, and of those in Figs. 3 and 4, precludes significant binding of TFE to the CD at the concentrations used.

increases the rate of reaction at all levels of the CD. However, the rate increases are less at high [α-CD] (Fig. 1), implying that *p*NPH bound to α-CD is less reactive than free, unbound ester. This proposal is justified by the following analysis which assumes that the relevant processes are those in eqns. (3)–(6), below. Based on earlier work,[1-9] there should be hydrolysis in the basic medium (3), reaction through an ester•CD complex (4), and reaction of the ester with TFE (5), reacting as its anion.[11] Since reaction with TFE is evident at high [CD], where most of the ester is bound to α-CD,[4,7] reaction of the ester•CD complex with TFE (6) is postulated, as well.

$$S \xrightarrow{k_u} \text{products} \quad (3)$$

$$S + CD \underset{K_s}{\rightleftharpoons} S{\cdot}CD \xrightarrow{k_c} \text{products} \quad (4)$$

$$S + TFE \xrightarrow{k_N} \text{products} \quad (5)$$

$$S{\cdot}CD + TFE \xrightarrow{k_{cN}} \text{products} \quad (6)$$

The combination of the four processes in eqns. (3)–(6) leads to the expected dependence of $k_{obs}$ on [TFE] and [CD] given in eqn. (7). As shown below, this equation fits the data in Fig. 1 for α-CD very well, and those for the two other CDs. For easier analysis of the data, we linearize eqn. (7) by dividing it by $f_s = K_s/(K_s + [CD])$, leading to eqn. (8). In accordance with this equation, the data for α-CD give strictly linear plots of $k_{obs}/f_s$ against [α-CD] for all four levels of [TFE], as shown in Fig. 2.

$$k_{obs} = \frac{(k_u + k_N[TFE])K_s + (k_c + k_{cN}[TFE])[CD]}{(K_s + [CD])} \quad (7)$$

$$k_{obs}/f_s = k_u + k_N[TFE] + k_c[CD]/K_s + k_{cN}[TFE][CD]/K_s \quad (8)$$

Using $K_s$ = 3.52 mmol dm$^{-3}$ for the binding of *p*NPH to α-CD,[12b] *all of the data* in Fig. 2 were analysed in terms of eqn. (8) by *multiple* linear regression, with [TFE], [CD], and [TFE][CD] as the three 'X' variables. An excellent fit ($r$ = 0.9999) was found that yielded values of $k_u$, $k_N$, $k_c/K_s$ and $k_{cN}/K_s$, and from which $k_u$ = 0.0476 ± 0.0019 s$^{-1}$, $k_c$ = 0.124 ± 0.001 s$^{-1}$, $k_N$ = 6.89 ± 0.10 dm$^3$ mol$^{-1}$ s$^{-1}$ and $k_{cN}$ = 1.09 ± 0.09 dm$^3$ mol$^{-1}$ s$^{-1}$. The first three constants agree well with earlier values,[12b] giving credence to the analysis. Also, using the four fitted constants in eqn. (7) one can faithfully reproduce the original data (Fig. 1).

**Table 1** Constants for the cleavage of *p*-nitrophenyl hexanoate and acetate in the presence of trifluoroethanol (TFE) and cyclodextrins[a]

| CD | $K_s$/mmol dm$^{-3}$ | $k_N$/dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{cN}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | $k_{cN}/k_N$ |
|---|---|---|---|---|
| *p*-nitrophenyl hexanoate[b] | | | | |
| α-CD | 3.52 | 6.89 ± 0.10 | 1.09 ± 0.09 | 0.16 |
| β-CD | 1.60 | 5.87 ± 0.26 | 1.75 ± 0.06 | 0.30 |
| Hp-β-CD | 1.59 | 6.50 ± 0.29 | 0.878 ± 0.078 | 0.14 |
| *p*-nitrophenyl acetate[c] | | | | |
| α-CD | 10.1 | 12.8 ± 0.1 | 8.06 ± 0.09 | 0.63 |
| β-CD | 7.92 | 12.7 ± 0.1 | 10.4 ± 0.01 | 0.82 |
| Hp-β-CD | 8.20 | 12.8 ± 0.1[d] | 3.88 ± 0.10 | 0.30 |

[a] At 25 °C, in a 0.2 mol dm$^{-3}$ phosphate buffer of pH 11.60. Values of $K_s$ are taken from earlier work.[9,12] [b] The constants $k_N$ and $k_{cN}$ were obtained by fitting eqn. (8) to the primary data, such as those in Fig. 1, using multiple linear regression. [c] The constant $k_N$ was obtained from measurements of $k_{obs}$ in the absence of CD while values of $k_{cN}$ were obtained from the slope of $k_{obs}$ *vs.* [TFE] at high [CD] (= 10 mmol dm$^{-3}$), as described in the text. [d] Same experiment as for α-CD.

Similar kinetic behaviour was found for the reaction of *p*NPH with TFE and either β-CD or Hp-β-CD. As shown by the linearity of the plots in Figs. 3 and 4, analysis of the data in terms of eqn. (8) works well for these CDs, also. The values of $k_N$ and $k_{cN}$ obtained from the analysis for all three CDs are collected in Table 1.

*pNPA and TFE.*—Since the above analysis was very successful for *p*NPH and TFE, but the measurements were time-consuming, we adopted a simpler approach for *p*NPA and TFE, one requiring fewer measurements. In essence, we carried out two series of experiments with varying [TFE]: one at zero CD, to determine $k_N$, the other with a fixed, high [CD] to estimate $k_{cN}$. In the absence of CD, $k_{obs} = k_u + k_N[TFE]$, from which $k_N$ is obtainable as the slope. With 10 mmol dm$^{-3}$ CD present, eqn. (7) must be used and the slope of $k_{obs}$ *vs.* [TFE] is $(k_N K_s + k_{cN}[CD])/(K_s + [CD])$. From this slope, and knowing $k_N$ and $K_s$, one can estimate $k_{cN}$. Fig. 5 shows linear plots of $k_{obs}$ *vs.* [TFE] for the cleavage of *p*NPA in the absence of CD, and in the presence of 10 mmol dm$^{-3}$ solutions of α-CD, β-CD, and Hp-β-CD. Values of $k_N$ and $k_{cN}$ derived from these experiments are also given in Table 1.

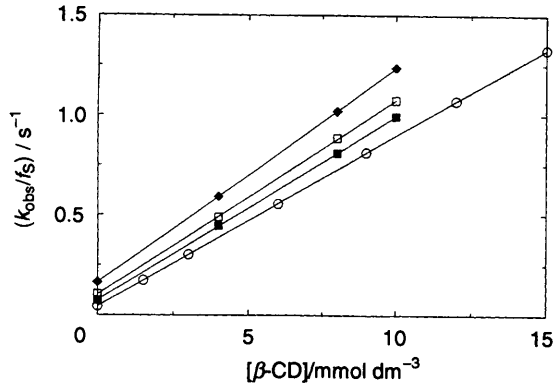



**Fig. 3** Effect of TFE and β-CD on the cleavage of *p*NPH, with the data plotted according to eqn. (8). The symbols for the TFE concentrations are: ○, 0; ■, 5; □, 10; ◆, 20 mmol $dm^{-3}$.

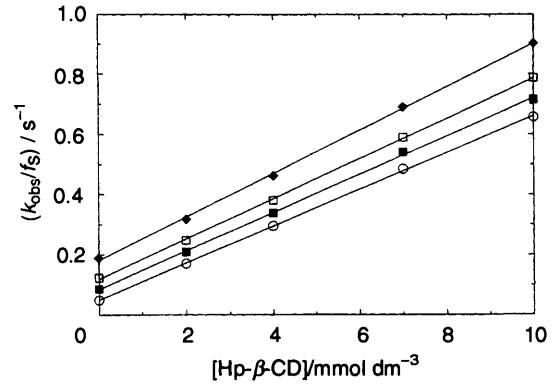



**Fig. 4** Effect of TFE and Hp-β-CD on the cleavage of *p*NPH, with the data plotted according to eqn. (8). The symbols for the TFE concentrations are: ○, 0; ■, 5; □, 10; ◆, 20 mmol $dm^{-3}$.

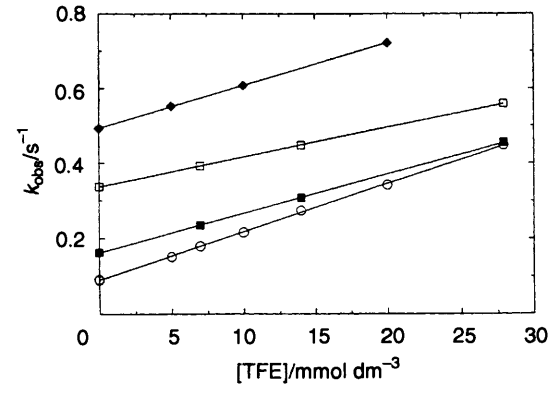



**Fig. 5** Effect of TFE on the cleavage of *p*-nitrophenyl acetate in the absence and presence of 10 mmol $dm^{-3}$ cyclodextrins. The symbols for the CDs are as follows: ○, none; ■, α-CD; □, Hp-β-CD; ◆, β-CD. The data for zero CD are composed of two replicate sets. Similar data were obtained for the other nucleophiles and for the cleavage of *p*NPH.

*p*NPA *and* *p*NPH *with Other Nucleophiles.*—Using the simplified approach we have also determined $k_N$ and $k_{cN}$ for $HSCH_2CH_2OH$, $NH_2OH$, and imidazole reacting with the two esters in the presence of α-CD and β-CD. These three nucleophiles were chosen, in part, because it appeared unlikely that they would bind strongly to CDs. Experiments were carried out at pHs high enough to ensure that the three nucleophiles were *ca.* 99% in their reactive forms. The resulting second-order rate constants for *p*NPA and *p*NPH are collected in Tables 2 and 3, respectively. These tables also contain rate constants for TFE, obtained using the simpler approach for consistency.

## Discussion

The present results conform closely to eqn. (7), in accordance with the processes in eqns. (3)–(6). Thus, we conclude that all four nucleophiles can react with *p*NPA and *p*NPH bound to CDs [eqn. (6)]. The kinetically equivalent reaction of free ester reacting with CD-bound nucleophile is unlikely since none of the nucleophiles appear to bind to the CDs. This assertion is based on the linearity of the plots in Figs. 2–5, and analogous ones, which would be curved if significant binding of the nucleophiles to the CDs occurred at the concentrations used.

A large part of the commercial interest in CDs arises from their ability to retard or inhibit the oxidation or decomposition of flavours, essences, drugs, *etc.* by the formation of inclusion complexes of the relevant molecules.[1,16] In contrast, we have found that binding of *p*NPA and *p*NPH to CDs has little effect on their reactivities towards simple nucleophiles *in aqueous solution.** For these two esters and four nucleophiles the respective values of $k_{cN}$ are close to $k_N$ and the ratios $k_{cN}/k_N$ vary only from 0.14 to 1.6 (Tables 1–3). Thus, the binding of the esters to CDs occurs in such a way that their carbonyl groups are accessible to external reagents and their reactivities towards nucleophiles are not greatly affected. Nevertheless, there may be differences between *p*NPA and *p*NPH because they bind differently to CDs: *p*NPA binds with its *p*-nitrophenyl group in the CD cavity and its acetoxy group outside (as in **1**);[17] *p*NPH, and similar aryl alkanoates, bind with their acyl groups included (as in **2**).[4,7–9,15]

*Substrate Reactivity* ($k_N$).—The values of $k_N$ for the four nucleophiles reacting with *p*NPA are in reasonable agreement with literature values,[11,12] when allowance is made for the pHs of the experiments. The analogous values of $k_N$ for *p*NPH are about half those for *p*NPA (*cf.*, Tables 2 and 3), as expected from the reactivities of the esters towards hydroxide ion.[4,7,18] †

*Reactivities of Bound Substrates* ($k_{cN}$).—As remarked already, values of $k_{cN}$ differ little from $k_N$, meaning that the CD-bound and free esters have similar reactivities towards the nucleophiles studied. This is particularly true for *p*NPA bound to α-CD or β-CD, where $k_{cN}$ values correspond to slight accelerations or retardations: $k_{cN}/k_N$ = 0.6–1.6 (for α-CD), 0.7–1.4 (for β-CD) (Table 2). For *p*NPA bound to Hp-β-CD, reacting with TFE, the retardation is larger ($k_{cN}/k_N$ = 0.30, Table 1), suggesting that CD-bound *p*NPA sits slightly deeper in the cavity of Hp-β-CD than it does in that of β-CD, even though the $K_s$ values for these two CDs are essentially identical.[9,15]

The effect of binding *p*NPH to CDs on its reactivity is marginally greater than that for *p*NPA, and more consistently in the direction of retardation: $k_{cN}/k_N$ = 0.14–1.0. This distinction, albeit small, may reflect the difference in the mode of binding of *p*NPA and *p*NPH, mentioned above. The ratios $k_{cN}/k_N$ also show little variation with the nucleophile, although they are generally lower for the two anions ($CF_3CH_2O^-$ and $HOCH_2CH_2S^-$) than for the two neutrals (Tables 2 and 3), and for *p*NPH reacting with the TFE anion $k_{cN}/k_N$ is decidedly less than one for all three CDs (Table 1). In a few cases $k_{cN}/k_N > 1.0$, but in no case is the ratio large enough to warrant speculations about 'catalysis'. Overall, our results contrast with those for the reaction of α-amino acids with *p*NPA in the

* We emphasize that our results are 'in solution' because most of the applications of CDs in stabilizing commercial products involve the use of solid complexes.[1,16]

† Generally speaking, acetate and propanoate esters have quite similar reactivities but the butanoate reacts more slowly for steric reasons. Beyond that, longer alkanoate esters have virtually the same reactivity, as long as the retarding effects of aggregation are avoided.[18]

**Table 2** Constants for the cleavage of *p*-nitrophenyl acetate by nucleophiles in the absence and presence of cyclodextrins[a]

| Constant | TFE[b] | $HSCH_2CH_2OH$[b] | $NH_2OH$[c] | Imidazole[d] |
|---|---|---|---|---|
| $k_N$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 13 | 13 | 2.1 | 0.55 |
| α-CD ($K_s$ = 10.1 mmol dm$^{-3}$) | | | | |
| $k_{cN}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 8.1 | 14 | 3.3 | 0.78 |
| $k_{cN}/k_N$ | 0.63 | 1.1 | 1.6 | 1.4 |
| $K_{TS}$/mmol dm$^{-3}$ | 16 | 9.1 | 6.5 | 7.2 |
| β-CD ($K_s$ = 7.92 mmol dm$^{-3}$) | | | | |
| $k_{cN}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 10 | 9.8 | 1.6 | 0.78 |
| $k_{cN}/k_N$ | 0.82 | 0.78 | 0.73 | 1.4 |
| $K_{TS}$/mmol dm$^{-1}$ | 9.5 | 10 | 11 | 5.5 |

[a] At 25 °C. Values of $k_N$ and $k_{cN}$ obtained from the slopes of plots of $k_{obs}$ *vs.* [nucleophile] at [CD] = zero and 10 mmol dm$^{-3}$, as described in the text. [b] Experiments in a 0.2 mol dm$^{-3}$ phosphate buffer of pH 11.60. [c] Experiments in a 0.1 mol dm$^{-3}$ phosphate buffer of pH 8.00. [d] Experiments in a 0.1 mol dm$^{-3}$ borate buffer of pH 9.00.

**Table 3** Constants for the cleavage of *p*-nitrophenyl hexanoate by nucleophiles in the absence and presence of cyclodextrins[a]

| Constant | TFE[b] | $HSCH_2CH_2OH$[b] | $NH_2OH$[c] | Imidazole[d] |
|---|---|---|---|---|
| $k_N$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 7.1 | 8.5 | 0.93 | 0.43 |
| α-CD ($K_s$ = 3.52 mmol dm$^{-3}$) | | | | |
| $k_{cN}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 1.0 | 6.8 | 0.67 | 0.43 |
| $k_{cN}/k_N$ | 0.14 | 0.80 | 0.73 | 1.0 |
| $K_{TS}$/mmol dm$^{-3}$ | 25 | 4.4 | 4.8 | 3.5 |
| β-CD ($K_s$ = 1.60 mmol dm$^{-3}$) | | | | |
| $k_{cN}$/dm$^3$ mol$^{-1}$ s$^{-1}$ | 1.7 | 2.1 | 0.42 | 0.23 |
| $k_{cN}/k_N$ | 0.24 | 0.24 | 0.45 | 0.54 |
| $K_{TS}$/mmol dm$^{-3}$ | 6.7 | 6.7 | 3.6 | 3.0 |

[a] At 25 °C. Values of $k_N$ and $k_{cN}$ obtained from the slopes of plots of $k_{obs}$ *vs.* [nucleophile] at [CD] = zero and 10 mmol dm$^{-3}$, as described in the text. Values for TFE differ from those in Table 2 which were obtained in a more complicated manner (see the text). [b] Experiments in a 0.2 mol dm$^{-3}$ phosphate buffer of pH 11.60. [c] Experiments in a 0.1 mol dm$^{-3}$ phosphate buffer of pH 8.00. [d] Experiments in a 0.1 mol dm$^{-3}$ borate buffer of pH 9.00.

presence of CDs, for which sizeable accelerations ($k_{cN}/k_N$ = 20–30 for β-CD) have been reported.[10,*]

*Transition-state Binding* ($K_{TS}$).—Here, we utilize the Kurz approach to transition-state stabilization.[19] This approach has proved particularly useful in studies of reactions catalysed by enzymes[20] and metal ions,[21] and we have employed it extensively for reactions mediated by CDs.[6–9,12] Based on transition-state theory, we define $K_{TS}$ [eqn. (9)] as the apparent dissociation constant of the transition state of the CD-mediated reaction [eqn. (6)], symbolized by TS·CD, into the transition state (TS) of the normal reaction [eqn. (5)] and the CD. The variation of $K_{TS}$, or better $pK_{TS} = -\log K_{TS}$, with substrate structure can be used as a probe of transition-state binding.[6–9,12,19–23]

$$K_{TS} = \frac{[TS][CD]}{[TS{\cdot}CD]} = \frac{k_N K_S}{k_{cN}} \quad (9)$$

As noted above, the ratios $k_{cN}/k_N$ in the present work are not very different from 1.0 and so values of $K_{TS}$ are only marginally different from $K_S$ (Tables 2 and 3). This observation implies that transition-state binding and substrate binding are virtually the same and that they involve the same structural feature of the ester in each case, although it is not necessarily the same feature for *p*NPA and *p*NPH (see above). There are minor differences in $K_{TS}$ values, notably that they are generally higher for the two anionic nucleophiles ($CF_3CH_2O^-$ and $HOCH_2CH_2S^-$) than for the two neutral ones ($NH_2OH$ and imidazole).

Studies in progress are concerned with a larger selection of esters, the intent being to find out more about transition-state binding relative to initial-state binding, and the potential of CDs for catalysing acyl transfers.

## Experimental

The cyclodextrins and nucleophiles were purchased from Aldrich and used as supplied. 'Hydroxypropyl-β-cyclodextrin' is available with different degrees of substitution: we used the material with an average molecular weight of 1500, corresponding to alkylation of six of the seven primary hydroxy groups of β-CD by 2-hydroxypropyl groups.[22] *p*NPA and *p*NPH were obtained from Sigma.

Reactions were carried out by 1:1 mixing in a stopped-flow spectrophotometer. For experiments with α-CD or Hp-β-CD, one syringe contained buffer and nucleophile and the other contained ester and a concentration of the CD twice that desired in the reaction. For β-CD, which is less soluble in water,[1] both syringes contained the desired level of CD. The buffers used were: pH 11.60, 0.4 mol dm$^{-3}$ phosphate; pH 9.00, 0.2 mol dm$^{-3}$ borate; pH 8.00, 0.2 mol dm$^{-3}$ phosphate, and the ester concentrations (μmol dm$^{-3}$) were: *p*NPA, 100; *p*NPH, 50. The final concentrations, after mixing, were half these. Substrate solutions were made by dilution of 0.1 mol dm$^{-3}$ stock solutions in spectral-grade acetonitrile.

Reactions were followed by monitoring the production of *p*-nitrophenolate ion at 405 nm, using a SX17MV stopped-flow

* We have also carried out some experiments with the amino acid alanine as the nucleophile. Our findings are quite similar to those reported in the present work but they differ significantly from those of Barra and de Rossi.[10] The discrepancy is under active investigation.

Surrey, UK). Normally, 400 absorbance values, covering 7–12 half-lives, were collected and first-order rate constants were estimated from non-linear least-squares fitting of an exponential, using the software supplied with the apparatus. The recorded rate constants ($k_{obs}$) were the averages of 5–10 determinations. The observation cell of the apparatus was kept at 25.0 ± 0.1 °C.

As explained in the main text, data such as those in Fig. 1 were analysed in terms of eqn. (8), using multiple linear regression.[23] This was executed conveniently on a Lotus 123 spreadsheet. The bulk of the experimental data, acquired at [CD] = 0 and 10 mmol $dm^{-3}$, were analysed as linear plots of $k_{obs}$ against [Nuc] (e.g., Fig. 5). From the slopes of these plots, the values of $k_N$ and $k_{cN}$ were extracted, as explained in the text.

## Acknowledgements

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