Stabilisation by Natural and Unnatural Cyclodextrins of the Transition States of Acyl Transfers of Nitrophenyl Alkanoate Esters

Timothy Adam Gadosy

A Thesis
in
The Department
of
Chemistry and Biochemistry

Presented in Partial Fulfilment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada
April, 1995

ISBN 0-612-01257-3

Abstract

Stabilisation by Natural and Unnatural Cyclodextrins of the Transition States of Acyl Transfers of Nitrophenyl Alkanoate Esters

Timothy Adam Gadosy, Ph.D.

Concordia University, 1995.

The work in this thesis falls into four major categories:

1) Kinetic studies of the basic aqueous cleavage of *m*- and *p*-nitrophenyl alkanoates (mNPAlk and pNPAlk) in the presence of "hydroxypropyl-β-cyclodextrin", "dimethyl-β-cyclodextrin", and γ-cyclodextrin (Hp-β-CD, DiMe-β-CD, and γ-CD) show that transition state binding in the cleavage by CDs depends strongly on the ester chain length: shorter esters through aryl group inclusion whereas longer esters involve acyl group inclusion.

2) An investigation of the cleavage of *p*-nitrophenyl acetate and hexanoate (pNPA and pNPH) by Hp-β-CD showed that added alcohols which bind to the CD do not inhibit the reaction by competitive inhibition and they can even accelerate it, in some cases. This observation means that the alcohol-mediated cleavage of pNPA and pNPH by Hp-β-CD can occur through a transition state where the ester resides mainly outside of the CD cavity, with an alcohol inside.

3) Dissociation constants of the complexes of cyclodextrins and series of nitrophenyl alkanoate esters, alcohols, alkanesulphonate anions, and alkylamines

were determined using kinetic or fluorescence methods. The results show that these aliphatic guests bind to CDs from the wider, secondary opening, and that binding by β-CD and Hp-β-CD occurs with virtually the same strength.

4) Investigations of the CD-mediated cleavage of pNPAlks by non-binding nucleophiles demonstrate that the CD-bound esters are generally less reactive than the free esters, and that for longer esters there is a switch from aryl group to acyl group binding in the transition state for acyl transfer. Aminolysis of the esters by *n*-alkylamines shows distinct catalysis by ß-CD (less by Hp-ß-CD), with the extent increasing with the chain length of both the amine *and* the ester. Conceivably, the CD-catalysed aminolysis occurs through a transition state involving a transient {ester.CD.amine} ternary complex, with both reactants partially included in the CD cavity.

Acknowledgements

Foremost I would like to thank my research supervisor, Prof. Oswald S. Tee, for assisting in my development, on both a professional as well as personal level. Without his presence my experience would not have been as colourful

I also wish to thank the following:

- NSERC and Concordia University for financial assistance.
- Dr. Mikkelsen and Prof. Colebrook for serving on my committee.
- The Department of Chemistry and Biochemistry.
- Dr. Rye for running mass spectra.
- My mother, who made this much easier by providing me with food and a warm loving home
- My fiancee, Johanne, for always being there for me, understanding the many long nights and weekends spent in the lab, and photocopying above and beyond the call of duty.
- The students I have worked with in this lab (A. Ako, M. Bozzi, and N. Clement) and the present members of the A Team (Javier, Mike, Alexei, Paul).
- The faculty and graduate students of this department, whose diverse personalities have made my stay at Concordia enjoyable.
- Steve, who lent a hand on many occasions with fixing equipment and keeping me company on the overnight shift.

This thesis is dedicated to the memory of my father,

the late Ferdinand Justin Gadosy.

Pro Deus Regina Atque Regio

Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

CD	cyclodextrin
CGTase	cyclodextrin glucosyltransferase
DiMe-β-CD	dimethyl-β-cyclodextrin
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DS	degree of substitution
Hp-β-CD	hydroxypropyl-β-cyclodextrin
ME	β-mercaptoethanol
MM	molecular mechanics
mNPAlk	*m*-nitrophenyl alkanoate
MS	molecular substitution
PI	potential inhibitor
pNPAlk	*p*-nitrophenyl alkanoate
TFE	trifluoroethanol

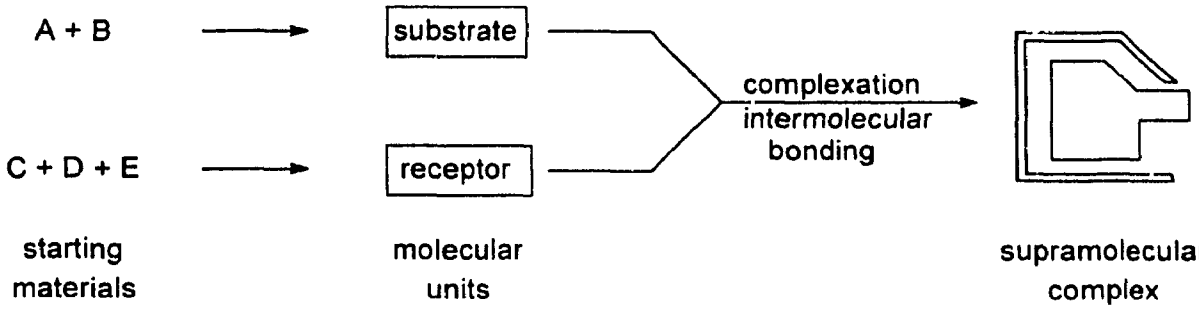
1. Introduction

1.1 Supramolecular Chemistry

Supramolecular chemistry is the study of "Supermolecules" made up of smaller, normal molecules, held together by *non*-covalent intermolecular forces,[1-3] unlike molecular chemistry, which is based predominantly on covalent interactions. Intermolecular (supramolecular) interactions are the foundation for highly specific biological processes, such as substrate binding by enzymes or receptors, the formation of protein complexes, the intercalation complexes of nucleic acids, the decoding of the genetic code, neurotransmission processes, and cellular recognition (immunology).[4]

The versatility of supramolecular systems, and the interest in them, is due to certain of their characteristics which include: self organisation (a step beyond pre-organisation), regulation, co-operativity, communication and replication. The common thread behind each of these subjects is that they utilise the supermolecule's ability to store information.[4]

The exact knowledge of the energetic and stereochemical characteristics of these non-covalent, multiple, intermolecular interactions (electrostatic forces, hydrogen bonding, van der Waals forces, etc.) within defined structural areas have been used in the design of artificial receptors, which can bind the substrate tightly *and* specifically, forming supramolecular complexes of defined structure and function.[4] Scheme 1 illustrates the formation of a supramolecular complex from simple molecules.



Scheme 1. Supermolecules are to molecules and their intermolecular bonds what molecules are to atoms and their covalent bonds.[1,2,4]

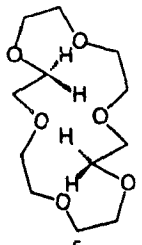
There are three basic subsections of supramolecular chemistry, which include the study of: molecular recognition; supramolecular reactivity and catalysis; and transport processes. Although the study of synthetic supramolecular systems is relatively new,[1-13] natural supramolecular systems have been receiving wide attention for many years.[5-17]

The main foundation of molecular recognition has been set up by the study of macrocyclic systems, which have helped to shape and guide research in the area. Cyclodextrins, for example, have been used to show how small molecules can be used to mimic essential steps in enzymatic reactions, using acyl transfer reactions as a probe (*vide infra*). Crown ethers have been used in the study of complexation and transport of metal ions; at the molecular level, complexation of ammonium species has been used as a vehicle for biological processes. Crown ethers have also been used to study allosteric effects due to guest binding.[9]

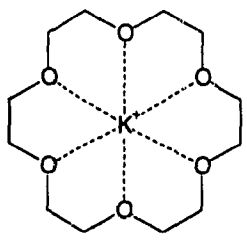
1.1.1 Molecular Recognition

The selective binding of a substrate or guest by a molecular receptor to form a supermolecule involves *molecular recognition*, which rests on the *molecular information* stored in the interacting partners.[6] It requires the design of receptors possessing steric and electronic features complementary to that of the guest, together with a balance of flexibility and rigidity to allow the supramolecular system to carry out its task.

Multiple binding sites are usually required to form a stable complex. Since the supermolecules are held together by non-covalent forces, of which each contributes very little to the overall stability of the complex, multiple interactions are required to overcome the work involved in bringing the host and guest together.[10] In some cases, there is a significant amount of work required to form the supramolecular complex. An example of this was observed in the crystal structure of [18]crown-6 in the absence (**1**) and presence (**2**) of K^+. The native host does not have a crown shape, nor does it have a cavity; only upon complexation, where the oxygens become engaged with a species such as K^+ does a *filled cavity* develop. In other words, the guest *conformationally reorganises* the host upon complexation.[10]



1[5]



2[5]

A highly structured molecular complex is composed of at least one host and one guest that possess complementary stereoelectronic binding sites. The host is defined as a molecule or ion whose binding sites converge (Figure 1A) and the guest is defined as a molecule or ion whose binding sites diverge (Figure 1B).[18,19] The functionalisation of a host's binding site allows for an improved complementarity between it and the guest. As the binding site becomes more and more complementary to a specific guest, the number of possible noncovalent interactions increases, thus allowing for stronger binding. This functionalisation also allows for a host to become more specific for a particular guest or class of guests (Figure 2).

Examples of the effect due to host size and geometry can be seen in the ability of different structurally-related dicarboxylic acid derivatives to bind a variety of different guests (**3** - **6**).[9] It is evident from **3** - **6** that even a slight change in the host's backbone causes a change in guest preference due to a change in the size of the binding site. Preference for ionic or neutral guests can also be modulated by changing the pH of the environment.

A great variety of receptor molecules have been designed for effecting the recognition of numerous and very diverse types of substrates (spherical, tetrahedral, linear or branched, charged or neutral, organic, inorganic, or biological, etc.).[1-15] Structural units have been incorporated that may respond to or be perturbed by external factors;[6] this type of allosteric behaviour is seen in many enzymes and is often the method by which their activity is regulated.

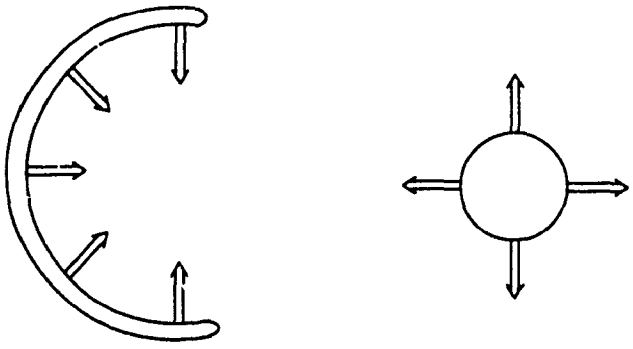


Figure 1. Binding site of host molecule is convergent (A) while that of the guest molecule is divergent (B).

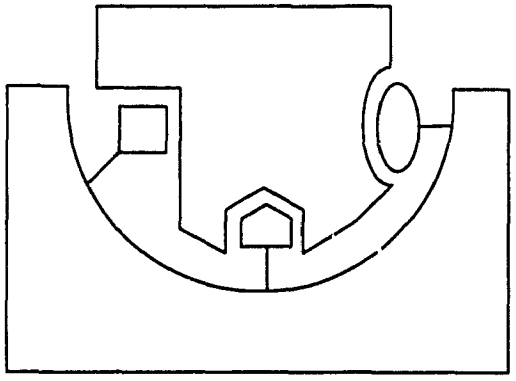
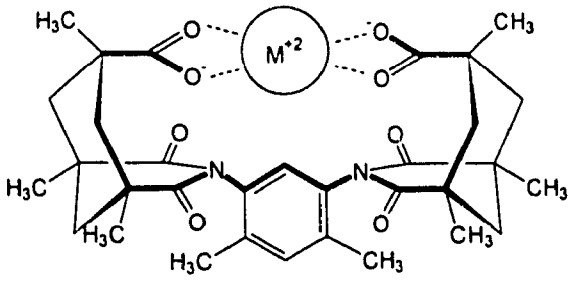
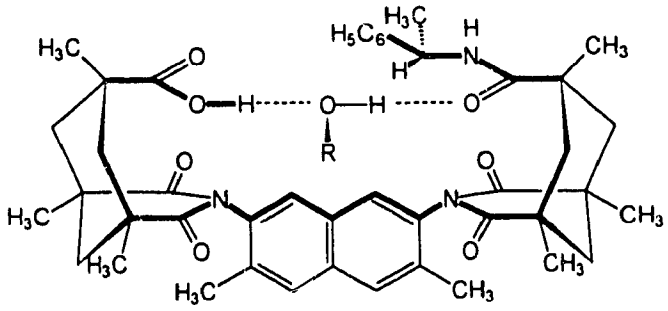


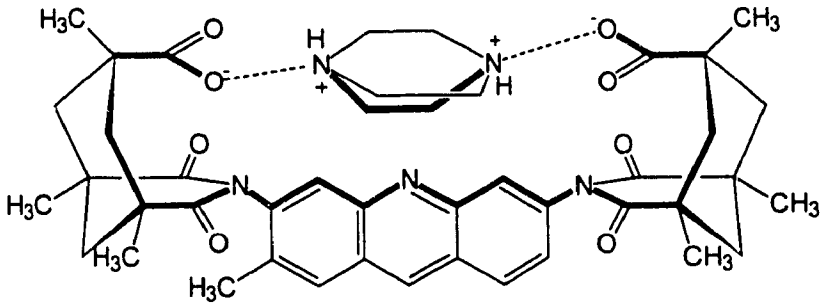
Figure 2. Surface complementarity between the host and guest increases the number of binding interactions.



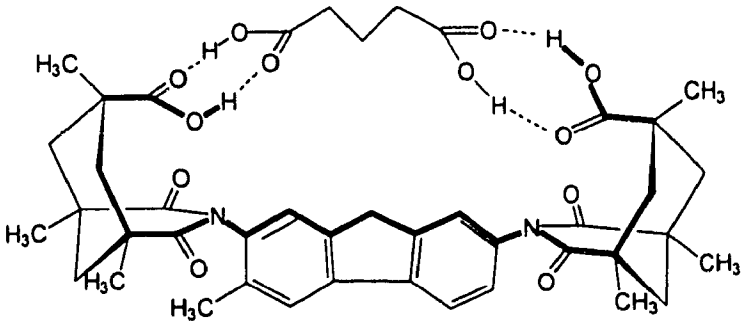
3



4



5



6

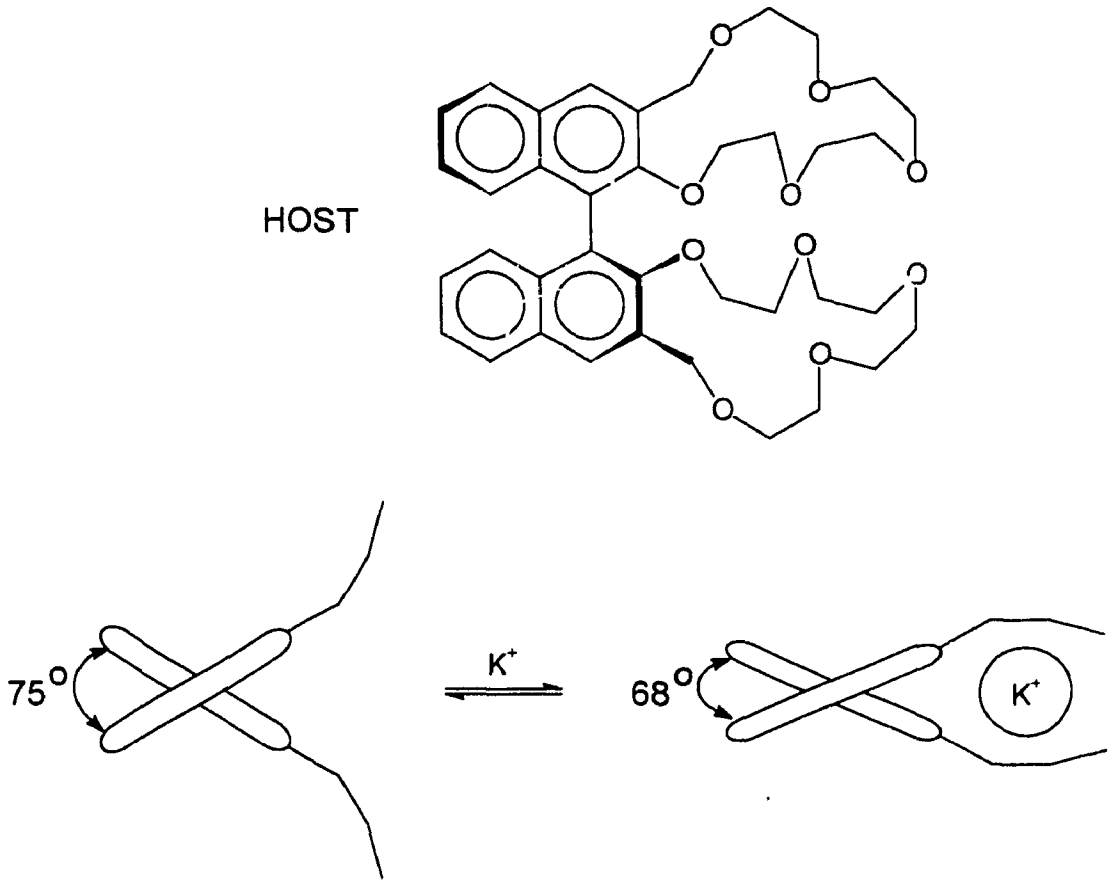
6

Molecular recognition events are the basis for information storage and retrieval in supramolecular systems. The formation of the supermolecule may be accompanied by a change in structural, optical, electrical, or ionic characteristics of the system. By making use of this "signal", the supramolecular complex may be used as a supramolecular device.

1.1.2 Supramolecular Devices

Supramolecular devices are structurally organised and functionally integrated chemical systems built into supramolecular architectures.[3] These devices are based on the spatial arrangement of the constituent parts, and how the complex or the individual parts respond to the presence of an external stimulus.[6] Molecular recognition is the backbone of these devices, and plays an intimate role in many ways: building of the device; incorporation into supramolecular arrays; guest specificity; response to stimuli; and the nature of the response to the stimuli.[6]

The stimulus may be in the form of light, ions, electrons, or a particular guest that can bind to the device, all of which have the effect of triggering some type of event on the supramolecular level. An example of this is shown in Scheme 2, where binding of a K^+ ion to the crown ether-like host causes a significant conformational change, with a decrease in the dihedral angle between the naphthyl rings and a closing up of the "molecular jaws".



Scheme 2. Binding of K^+ induces a conformational change, thus closing the molecular switch.[20]

1.1.3 Self-Organisation

Modern supramolecular chemistry relies heavily on systems which are built synthetically and are more or less arranged in the proper conformation, *i.e. pre*-organisation. The most recent trends in supramolecular chemistry are focused on advancing one step beyond pre-organisation to *self*-organisation. Self-organisation is where the starting blocks come together and spontaneously form the *functional* supermolecule. The building blocks themselves must be coded for the correct information and algorithm (the "Aufbau" plan) required to generate the supermolecule.[21-24]

Self-organisation can occur in liquid, solid or crystal matrices and can make use of hydrogen bonding, electrostatic and donor/acceptor effects, or metal-ion binding as basic interactions between the components. Multiple binding sites are required in order to allow the supramolecular assembly to grow. If the first binding facilitates the binding of subsequent building blocks, then there is the potential for the development of *molecular amplification devices*.[3]

Numerous natural examples of self-organisation exist. Systems such as: the nucleic acid double helices, viral protein coats, and multienzyme complexes are but a few examples.[6]

Many of the factors described in the previous sections are of vital importance to the chemistry of the cyclodextrins (CDs). The CDs have the ability to recognise molecules, catalyse reactions, and transport a variety of guests. For these reasons we have examined the supramolecular chemistry of the

cyclodextrins, in order to examine the difference in behaviour between natural supramolecular systems (α-, β-, and γ-cyclodextrin) and unnatural ones ("hydroxypropyl-β- and dimethyl-β-cyclodextrin").

1.2 Cyclodextrins

Cyclodextrins (CDs) were first discovered by Villers in 1891 in a bacterial culture medium,[15-17] where they are formed as the enzymatic degradation product of starch (*vide infra*). CDs have found a very large and widespread role in chemical research and industry, with much of the interest residing in the field of supramolecular chemistry, since they provide a series of water soluble host molecules, which are capable of affecting many types of reactions. Since their original discovery and isolation, over 7000 reports relating to cyclodextrins have been reported in the literature.[a]

1.2.1 Synthesis

1.2.1.1 Naturally Occurring Cyclodextrins

CDs are obtained when starch is degraded by bacteria such as *Bacillus macerans*, *Bacillus megaterium*, *Bacillus circulans*, alkalophilic *Bacillus sp.*, *Klebsiella pneumoniae*, and *Bacillus stereothermophilus*, all of which contain the

[a] Chemical Abstracts Service lists 7131 publications on cyclodextrins between 1979 and August 30th 1993.[25]

enzyme Cyclodextrin Glucosyltransferase (CGTase).[14-17,25-32] All of the CGTases act in a manner as described by Scheme 3.

$$G_n \underset{\text{Coupling}}{\overset{\text{Cyclization}}{\rightleftharpoons}} G_{(n-x)} + \textit{Cyclo}\text{-}G_x$$

Scheme 3. Action of CGTases in the formation of CDs from starch.[15-17,26,28]

Since the CGTase never completely detaches from the starch, it is possible to reversibly obtain CDs with 6 or more glucose units.[26] It has been reported[29] that CDs containing 6 to 12 glucose units have been isolated, however, it is unclear whether or not the CDs with more than 8 glucose units are actually very large CDs or simply branched forms of cyclodextrins containing 6, 7, or 8 glucose units. Rings with fewer than 6 glucose units are unlikely due to the strain involved in the formation of the 5, or fewer, membered macrocycle, as well as for steric considerations.[32]

The predominant forms of enzymatically produced CDs are those containing 6, 7, or 8 glucose units which are known as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD), respectively. The ratio in which the various enzymes produce the CDs is different for each bacterium and the conditions under which the culture is grown. Much work in recent years has focussed on developing bacteria which overproduce a specific cyclodextrin. Wacker Chemie

GmbH has reported[33] the development of a method for the overproduction of γ-CD, which until recently has been the most expensive of the three cyclodextrins.[b]

Since no CGTase forms any one CD uniquely, the starch digests must be purified in order to separate the CDs. This is usually accomplished by the addition of organic molecules to the digest that are capable of binding to the CD and forming insoluble complexes. For example, the addition of tetrachloroethylene-tetrachloroethane to a starch digest causes all three CDs to precipitate out of solution. The addition of *p*-cumene or fluorobenzene to a mixture of all three CDs causes only the β-CD to precipitate out, while the α-CD can be precipitated out using cyclohexane and γ-CD can be separated using anthracene.[17] Unfortunately, these precipitating agents are not 100% specific although they are usually chosen so as to have a maximum effect on only one CD. Table 1 shows how much CD can be recovered from a solution that initially contains 1% β-CD, 3% γ-CD and 0.5% of the complexing agent. This method has recently been taken one step further, were the complexing agent is present in the starch digest right from the beginning (Table 2). Now the amount of a γ-CD *formed* (as opposed to separated) can be enhanced relative to other CDs. This is due to the fact that the formation of the CD is reversible (Scheme 3), and as soon as γ-CD is formed, it precipitates out of solution, thus preventing the back reaction, whereas β-CD, which does not

---

[b] For the sake of discussion in this thesis, the term "all of the CDs" or "all 3 CDs" will be taken to mean α-, β-, and γ-cyclodextrin, since higher CDs are formed in insignificant amounts, and their exact nature is unclear.[16]

Table 1. Precipitation of β- and γ-Cyclodextrin by the Formation of Insoluble Inclusion Complexes with Macrocyclic Compounds.[a]

Macrocyclic Complexing Agent	Amount of CD Precipitated	
	β-CD (%)	γ-CD (%)
Cyclododecanone	91	93
Cyclotridecanone	3	72
Cyclotetradec-7-en-1-one	2	95
Cyclohexadec-8-en-1-one	4	93
Cyclohexadecan-1,9-dione	1	94
2,8-dioxa-1-oxo-cycloheptadecane	1	98
2,5-dioxa-1,6-dioxo-cyclohexadecane	3	97

[a] From reference 33.

Table 2. Relative Yield of Cyclodextrins Using Macrocyclic Complexing Reagents in an Enzymatic Conversion of Starch to Cyclodextrins.

Complexing Agent	β-CD (%)	γ-CD (%)
Cyclododecanone	98.5	1.5
Cyclotridecanone	2	98
Cyclotetradec-7-en-1-one	1	99
Cyclohexadec-8-en-1-one	1	99
Cyclohexadecan-1,9-dione	1	99
2,8-dioxa-1-oxo-cycloheptadecane	1	99
2,5-dioxa-1,6-dioxo-cyclohexadecane	1	99

[a] From reference 33.

form an insoluble complex, is in solution longer, and can be reverted back to a linear starch and then reformed as γ-CD.

1.2.1.2 Unnatural Cyclodextrins

The number of chemically modified cyclodextrins is constantly growing, with new CDs appearing in the literature every year. CDs are derivatised in order to vary their solubility behaviour, to modify their complexation properties, and to introduce groups with certain specific (e.g. catalytic) functions.[25] Since CDs are polyfunctional, they can undergo a wide variety of reactions,[25,34] a large number of which involve cleavage of one or more C-O bonds. The functionalised CDs with which we are the most interested are "hydroxypropyl-β-cyclodextrin" (Hp-β-CD) and "dimethyl-β-cyclodextrin" (DiMe-β-CD).

DiMe-β-CD is β-CD where all of the hydrogens on the primary hydroxy groups (C-6), and half of the hydrogens on the secondary hydroxy groups (C-2 and C-3) are replaced with methyl groups. Synthesis and purification of completely methylated cyclodextrin is virtually impossible on the commercial scale (while keeping the price of the CD reasonable). DiMe-β-CD is available in various degrees of substitution, and the one used in this study was DiMe-β-CD DS[c] 1.9, in which there are an average of 2 methyl groups per glucose unit. The alkylation

---

[c] DS is degree of substitution, and refers to the number of substitutions *per glucose residue*.

is performed by treating β-CD with dimethyl sulphate, $Ba(OH)_2 8H_2O$, and BaO in DMF/DMSO.[25,34,35]

Hp-β-CD is obtained by reacting β-CD with propylene oxide in a basic aqueous medium.[16,34] This yields a product in which the hydrogens on the primary hydroxy groups are replaced by 2-hydroxypropyl groups. This functionalised CD is also available in various degrees of substitution, and the one used in this study was Hp-β-CD MS[d] 0.8, in which 80% (an average of 6) of the seven primary hydroxy groups of β-CD are functionalised.

1.2.2 Structure

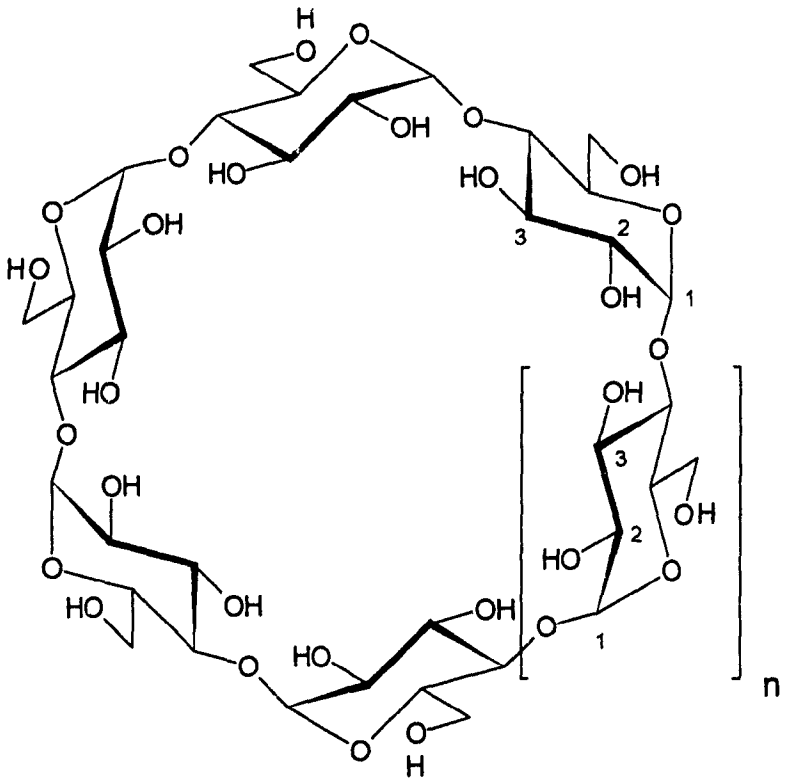
Cyclodextrins are cyclic oligomers formed from 6 (α-CD, **7a**), 7 (β-CD, **7b**; DiMe-β-CD, **8**; Hp-β-CD, **9**), or 8 (γ-CD, **7c**) α-(D)-glucopyranose units, linked α-1,4 as in amylose. Each glucopyranose unit is in its relatively undistorted C1 (chair) conformation (Figure 3).[15-17]

In most cyclodextrin structures solved so far by X-ray crystallography or predicted by force field optimisation (MM), the anomeric oxygens together with the C1 and C4 atoms form a plane, that is usually only slightly deformed, towards whose axis the glucopyranose units are tilted.[e] The CD molecule is lined on the

---

[d] MS is molar substitution, and refers to the fraction of primary hydroxy groups functionalised *per molecule*, according to the Aldrich catalog.

[e] According to MM calculations on α-CD in the absence of any water molecules, the glucose rings are tilted 14 ± 1°.[36]



	n	CD
7a	1	α-CD
7b	2	β-CD
7c	3	γ-CD

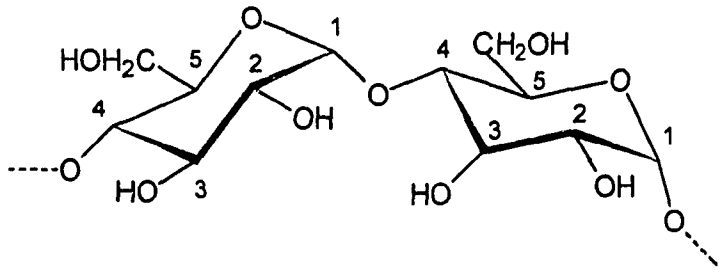
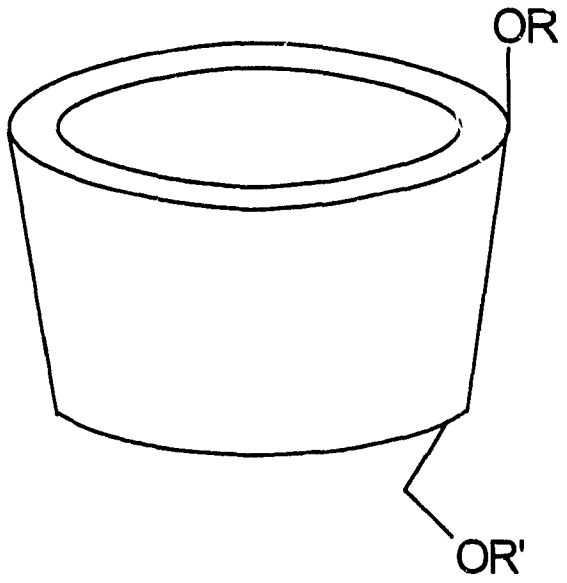


Figure 3. Schematic diagram of two glucopyranose units of a cyclodextrin molecule.[17]



	R	R'	HOST
7b	H	H	β–CD
8	Me	Me	DiMe-β-CD
9	H	CH_2CHCH_3 OH	Hp-β-CD

wider opening by secondary hydroxy groups (on C-2 and C-3), and by primary hydroxy groups on the narrow opening of the bucket (C-6) (Figure 4).[15-17] The major driving force for the slight deformation of the glucose units, and the resulting bucket shape,[f] originates in an inter-glucose hydrogen bonding network set-up between the C-2 and C-3 hydroxy groups.[36] Saenger has stressed[16] that the reduced size of α-CD provides a less than ideal geometry for this hydrogen bonding network, as compared to the larger β-CD and γ-CD. Since the hydrogen bonds are not well formed in α-CD, it tends to be the most flexible of the three CDs. The approximate dimensions of α-, β-, and γ-CD are given in Figure 5.

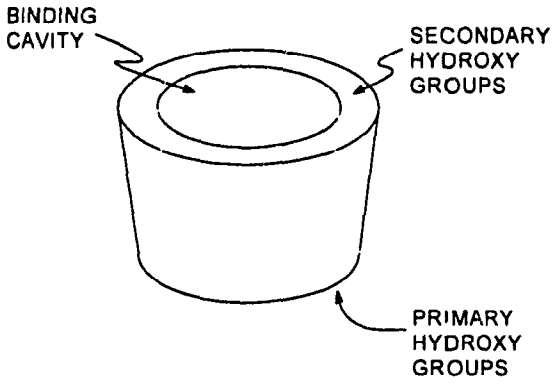


Figure 4. Schematic representation of CD, showing location of primary and secondary hydroxy groups as well as the binding cavity.

[f] The term "nanobucket" would definitely be more appropriate.

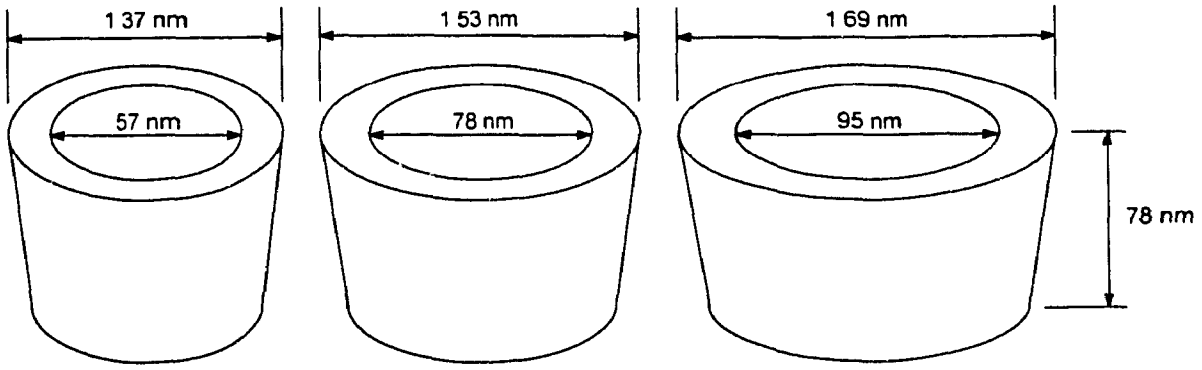


Figure 5. Molecular dimensions of α-, β-, and γ-cyclodextrin, respectively.[17]

The interior of the CD cavity is lined with methine hydrogens and glycosidic ether type linkages joining adjacent glucose units. The lone pairs of electrons on the oxygens in the glycosidic linkages are oriented into the cavity producing a high electron density, and gives the CD cavity some Lewis-base character.[16] These features lead to a very important characteristic of the CDs, which is that the polarity of the interior of the cavity is less than that of water, similar in nature to oxygenated solvents such as dioxane, ethanol, and *iso*-propyl ether.[37-40]

Evidence supporting the cyclic structure of CDs has been found by other researchers using standard chemical methods.[17] These results have also been verified by X-ray crystallography.[41-46]

The cavity shape of α-CD is perturbed in aqueous solution since 2 molecules of water[9] occupy the CD cavity, and cause two of the glucopyranose

---

[9] These water molecules are some times referred to as "high energy waters" since the complexed water molecules are at a higher energy than in the bulk solution.

units to cant inwards, so as to form hydrogen bonds between the water molecules and the primary CD hydroxy groups.[47] The expulsion of these water molecules by inclusion of another guest relaxes the conformational strain of the system. Other CDs also have high energy water molecules included in the cavity, however, the deformation observed in the case of α-CD is the greatest.

1.2.3 Physical and Chemical Properties

The cyclisation of the linear dextrin to form the cyclodextrin is an energetically unfavourable process, and is associated with a gain in free energy of 9.6, 7.2, and 8.3 kJ mol^{-1} for α-CD, β-CD, and γ-CD, respectively.[17] The cyclic structures are less energetically favourable due to a large increase in enthalpy (27.6, 18.5, and 18.4 kJ mol^{-1} for α-CD, β-CD, and γ-CD respectively), which may be viewed as the instability of cyclisation, and it is apparent that there is more conformational strain in α-CD since the enthalpy increase is 9 kJ mol^{-1} greater than for β-CD or γ-CD. The large gain in enthalpy is partially offset by a favourable gain in entropy (60, 38, and 34 J mol^{-1} K^{-1} for α-, β-, and γ-CD, respectively) which is associated with a disordering of the solvent waters around the linear dextrin upon formation of the macrocycle.[15-17] The increase in entropy is due to the formation of a highly ordered intramolecular hydrogen bonding network, which removes the ordering of the water molecules around the cyclodextrin.

The secondary hydroxy groups around the wider opening of the CD cavity are partially responsible for the interesting chemistry observed with the CDs. Not

only do they help to add stability[15,16] to the molecule, but they also influence the solubility and reactivity to varying degrees.[15-17] The stability of the CD's bucket shape is due to the formation of a hydrogen bonding network between the hydroxy group on the C-2 and the hydroxy group on the C-3 carbon of the adjacent glucose residue. The formation of the hydrogen bonding network also helps to make the CD more reactive by making the secondary hydroxy groups more acidic; the pK_a of these hydroxy groups is ~ 12.2.[48,49]

It has been shown[17] that the hydrogen bonding network is so strong that it does not break down in DMSO, a solvent which normally removes all hydrogen bonding interactions between solute molecules. The strength of these hydrogen bonds would imply that in aqueous solution hydrogen bonding occurs intramolecularly, rather than with the solvent water, which is confirmed by the increase in entropy of the cyclic dextrin compared to the linear one. According to Sandararajan and Rao,[32] the hydrogen bonding between the adjacent C-2 and C-3 hydroxy groups stabilises α-CD by 83.7 kJ mol^{-1} and β-CD by 125 kJ mol^{-1}.

Although the hydrogen bonding network helps in stabilising the CD bucket shape and increasing solubility (α-CD = 14.5; β-CD = 1.85; γ-CD = 23.2; all values in g/100 mL water),[15-17] their importance in this regard has been questioned.[17] Hp-β-CD is soluble up to 50% w/w in water while solutions containing 80% w/w DiMe-β-CD can be made.[h,50] Although the hydrogen bonding network may play a role

---

[h] The solubility of DiMe-β-CD in aqueous solution *decreases* with *increasing* temperature.[16] The exact nature of this phenomenon is unclear.

with Hp-β-CD, it *cannot* with DiMe-β-CD since half of the secondary hydroxy groups are *O*-methylated, thus destroying the hydrogen bonding network. It has also been shown that per *O*-methylated cyclodextrins retain their bucket shape, even without the hydrogen bonding network.[15-17] The increased solubility of the functionalised CDs may be due more to a destabilisation of their crystal lattices as opposed to a direct increase in hydrophilicity.

The cyclic structure of the CDs also means that there are no reducible terminal ends to the molecule, helping to improve its stability towards enzymes that are normally very efficient at degrading starch. CDs are also extremely stable towards alkaline hydrolysis, and only undergo hydrolysis in acidic media at a very slow rate (Table 3). β-CD in 1.15 N aqueous HCl at 40 °C has a half-life of over two weeks!

Table 3. Rate Constants for the Acid Hydrolysis of β-CD.[a]

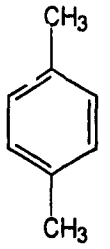
T, °C	[HCl], N	10^5 k, s^{-1}	$t_{1/2}$
100	1.15	137	500 sec
80	1.15	13.7	84.3 min
60	1.15	1.25	15.4 hrs
40	1.15	0.0533	15.1 days
26	5.00	0.617	1.3 days

[a] From reference 16.

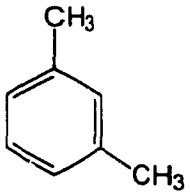
1.2.4 Industrial Applications of CDs[15-17,50]

Because of their ability to alter a wide range of chemical reactions (*vide infra*), CDs have found many uses in industry as analytic reagents, additives, solubilisers, and stabilisers.

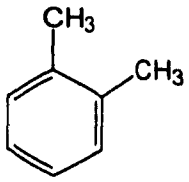
Many patents have been awarded for technologies which use CD to separate hydrocarbons. α-CD can be used to separate *p*-xylene (**10**) from a mixture of *p*-xylene (**10**), *m*-xylene (**11**), *o*-xylene (**12**), and ethylbenzene (**13**) by selectively forming an insoluble complex with only the *para* isomer. The precipitate is collected as it forms, and *p*-xylene may be removed from α-CD by heating, thus rendering the CD reusable.



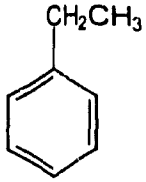
10



11



12



13

Due to the chiral nature of the CD cavity, it has received a lot of attention as a stationary phase in column chromatography. There are many examples in the literature of CDs being used to separate chiral aliphatic alcohols.[15]

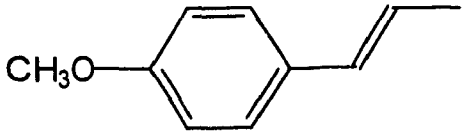
Industrial applications of CDs are by far the most predominant in the making of consumer products such as drugs, cosmetics, and food stuffs. One of the most important uses of CDs is the prevention of oxidation, thus improving stability and product shelf life. The uptake of O_2 by anethole (**14**, p. 26) is reduced by 90%

when anethole is complexed with β-CD. Uncomplexed ascaridole (**15**) undergoes total oxidation in under 200 hours, whereas the β-CD complex exhibited *no* oxidation after 8 months, four of which were at 100% relative humidity.[16]

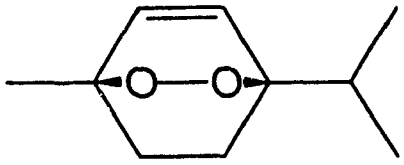
CDs can also help stabilise light sensitive chemicals. The insecticide allethrin (**16**) is light sensitive and will completely decompose in about two weeks, whereas in the allethrin β-CD complex the active allethrin concentration drops only by about 20%.[16]

Another important characteristic of CDs is that they reduce the volatility of many organic compounds. A solution of anethole (**14**) lost 30% of its initial concentration in the absence of CD, whereas the decrease in anethole concentration from a solution containing 1-2 mol β-CD/mol anethole, over the same time period, was only 5%. The ability of CDs to reduce volatility has been used extensively to help preserve the fresh taste of foods, to mask the unpleasant odour/taste of medications, and to remove the smell of cosmetics (eg. mercaptans in hair care products). Since cyclodextrins have shown no toxicological effects (within normal consumption limits) many governments have allowed the addition of CDs into food stuffs and drugs such as: prostaglandin E_1-α-CD (Germany and Japan), piroxicam-β-CD (Italy), garlic oil-β-CD (Hungary), low cholesterol butter (France), chewing gum (Denmark), spice extracts (Hungary), and the Japanese government places no restrictions on the use of natural CDs in foods.

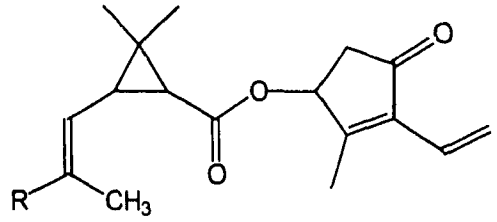
The binding of guest molecules to CDs usually increases the solubility of the guest, in water, although the solubility of the CD.guest complex is usually less than



14

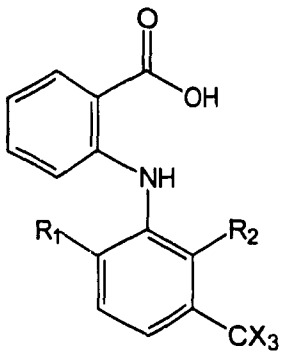


15



a R = CH_3 Allethrin I b R = $COOCH_3$ Allethrin II

16

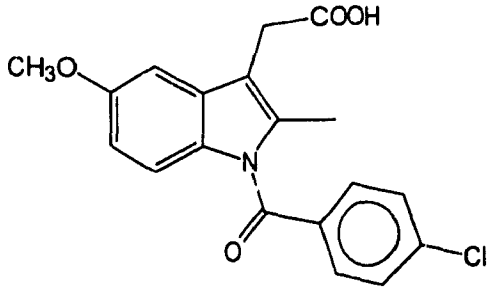


a R_1=R_2=H X=F flufenamic acid

b R_1=X=H R_2=Me mefenamic acid

c R_1=R_2=Cl X=H meclofenamic acid

17



18

the solubility of the CD alone. The improved solubility of a guest molecule is a practical way of solubilising hydrophobic drugs, especially when one takes into consideration the high solubility of modified cyclodextrins (*vide supra*). The solubilisation of drugs is one of the leading industrial applications of functionalised CDs, since they appear to have the same toxicological behaviour as the native CD, helping with the development of intravenous delivery techniques as well as allowing for a timed delivery of the drug due to the complexation equilibrium. On the other hand, liquid drugs, such as the non-steroidal anti-inflammatory agents (**17**), can be complexed with β-CD, freeze dried and then pelleted.

Indomethacin (**18**) is one of the most active and widely used non-steroidal anti-inflammatory drugs.[16] However, indomethacin has a very bad side effect, causing stomach lacerations and ulceration. Complexation of **18** with two molecules of β-CD drastically reduces the extent to which these side effects manifest themselves.

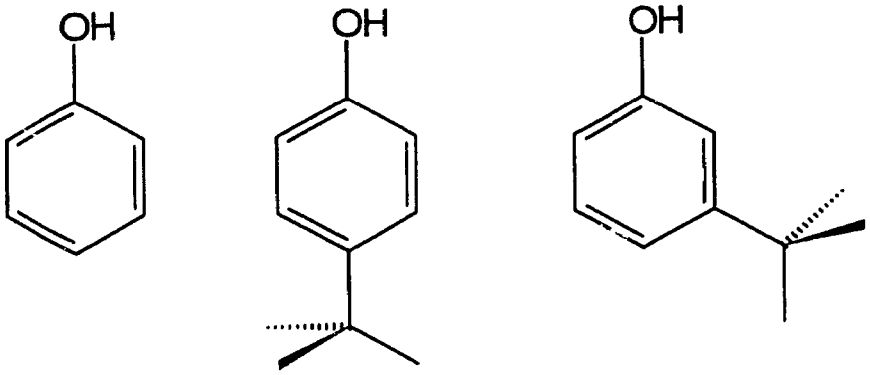
The above examples of industrial applications of CDs rely on the basic ability of the CD to form an inclusion compound with the active ingredients in the various formulations. The chemistry behind this complex formation is important, and not necessarily as simple as it may seem.

1.2.5 Host-Guest Chemistry

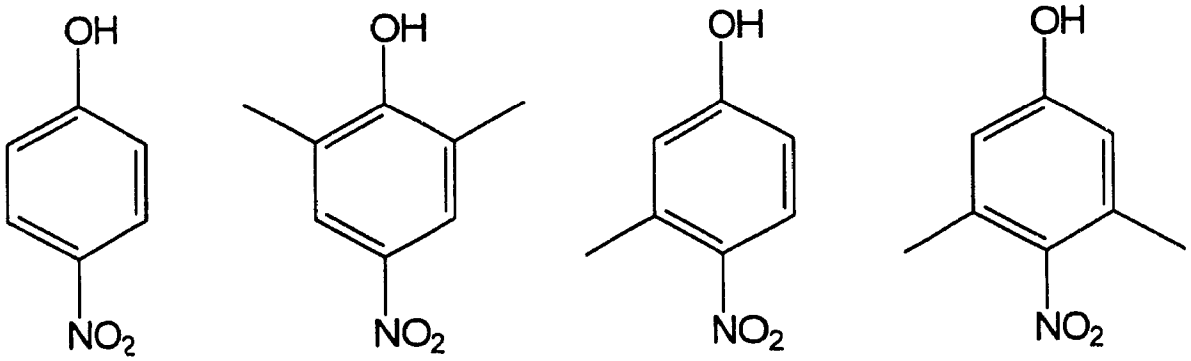
Cyclodextrins are able to interact with a large variety of ionic and molecular species, ranging in size from noble gases to fatty acid coenzyme A derivatives, forming supramolecular complexes, also known as "host-guest" complexes.[16,17]

The first evidence for the formation of CD host-guest complexes with aromatic substrates came from ^{1}H NMR data.[51] The C-3 protons and C-5 protons are all directed towards the interior of the CD cavity, while the C-1, C-2, and C-4 protons are directed away from the CD cavity. The inclusion of several guests (benzoic acid, *p*-hydroxybenzoic acid, and phenobarbital) into the β-CD cavity causes a strong up-field shift on C-3 and C-5 protons, leaving the other three relatively unaffected. The large up-field shift can be ascribed to an anisotropic shielding effect by the benzene ring of the included guest on the protons inside the CD cavity.[17] More recently, the formation of CD host-guest complexes has been verified using x-ray crystallographic techniques.[41,43,46]

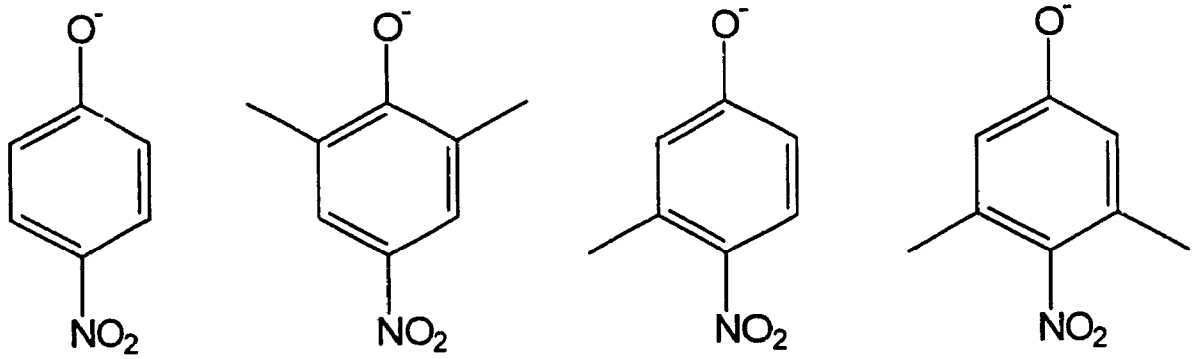
Complexation of sodium *p*-nitrophenolate with α-CD in aqueous solution caused the ^{1}H NMR resonance of the C-3 protons to shift up-field, while those on C-5 remained relatively unaffected. The meta protons on the guest were deshielded by 0.30 ppm while the ortho protons only experienced an upfield shift of 0.16 ppm. These data indicate that *p*-nitrophenolate penetrates from the wider, secondary side of the cavity, and only to a depth where the meta protons interact with the C-3 protons.[17,52] Similar evidence was observed with phenol (**19**), *p-tert*-butylphenol (**20**), and *m-tert*-butylphenol (**21**) (Table 4). In these cases the



19 **20** **21**



22 **23** **24** **25**



22$^-$ **23$^-$** **24$^-$** **25$^-$**

substrate binding still occurs via the wider opening of the CD cavity and with the phenolic hydroxy group oriented towards the outside of the cavity. The orientation of the two substituents on the substrate determines the depth to which the guest will penetrate the CD cavity, in the order **19** > **21** > **20**.

Table 4. Values of δ for the inclusion of **19**, **20**, and **21** in β-cyclodextrin as determined by ^{1}H NMR.[a]

Guest	β-CD proton	δ (ppm)
19	H-3	+0.09
	H-5	+0.26
20	H-3	+0.21
	H-5	+0.02
21	H-3	+0.20
	H-5	+0.20

[a] From reference 51.

More conclusive evidence for the formation of the inclusion compounds of nitrophenol and nitrophenolate derivatives, occurring with the hydroxy group oriented out of the wider end of the CD cavity, was presented by Bergeron *et al*[53] (Table 5). If we use **22**$^-$ as our standard, we can see that the addition of methyl groups at the 2 and 6 positions (**23**$^-$) decreases the binding strength by only a

factor of 2.4, whereas the introduction of a methyl group in the 3 position (**24**$^-$) decreases the strength of binding by a factor of 100, and the addition of a second methyl group in the 5 position (**25**$^-$) totally inhibits the formation of the host-guest complex. The situation is slightly different with the neutral analogues. The addition of methyl groups in the 2 and 6 positions (**23**) *increases* the strength of binding by a factor of 30, relative to **22**. A methyl group in position 3 and/or position 5 inhibits the formation of the complex.

Table 5. Dissociation Constants for β-Cyclodextrin Complexes of *p*-Nitrophenols and their Anions.[a,b]

Phenol	K_d (mM)	Anion	K_d (mM)
22	53	**22**$^-$	0.40
23	1.8	**23**$^-$	0.94
24	-[c]	**24**$^-$	42
25	-[c]	**25**$^-$	-[c]

[a] From reference 50. [b] Determined using UV-Vis spectroscopy. [c] These compounds did not bind to β-CD.

1.2.5.1 Requirements of Complex Formation

In order for a molecule to form a host guest complex with CD, it must have a size comparable to that of the CD cavity. Molecules that are significantly larger than the CD cavity may form host-guest complexes in which only part of the guest is included. Fujiki *et al*[54] demonstrated that naphthalene, its 1- and 2- methyl as well as 1,4-, 1,5-, and 1,8- dimethyl derivatives all bind to α-, β-, and γ-CD. They also demonstrated that the geometry of binding of any given guest to the three CDs can vary significantly. Sanemasa and coworkers[55] demonstrated that the binding of anthracene to β- and γ-CD occurs with the anthracene inside the CD cavity, but that due to the smaller cavity size of α-CD, only the ends could be partially included, and that two CD molecules, one at each end, can be bound to each anthracene.

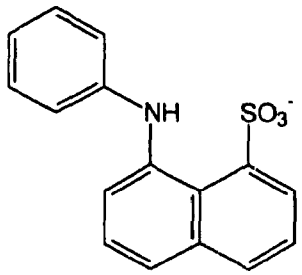
The structure of CD inclusion complexes in solution may be quite different from those in the crystalline state. In solution the guest molecule resides in the cavity, and the whole complex is surrounded by a sphere of solvating water molecules. In the crystalline state, however, the guest may occupy either the CD cavity and/or the interstitial spaces, leaving the CD cavity to be filled with water molecules. Therefore crystalline CD host-guest complexes very rarely have integral host:guest ratios, whereas aqueous complexes generally do.[16,17] The work in this thesis was all performed in aqueous media, so therefore any further discussions will be on aqueous CD complexes, unless otherwise noted.

Guest molecules tend to bind to CDs in such a way as to optimise the interaction between their hydrophobic parts and the CD cavity, at the same time trying to keep hydrophilic moieties as far away from the CD cavity as possible, ensuring maximum contact with the solvent water and/or hydrogen bonding network on the secondary side of the CD cavity.[16]

The use of molecular models and computer simulations are useful tools in the attempt to predict if and how a given guest is likely to form a complex with one of the natural or modified cyclodextrins.[16] Certain chemical groups and substituents may promote complex formation, whereas others may impede this process. There is an inverse correlation between the solubility and complex forming ability of guest compounds, yet nitro and amino groups tend to alter this relationship. The protonation state of amino groups is also very important in the ability of a guest to form a complex with CD, since the presence of an NH_3^+ group strongly disfavours complexation.[16] Since methyl and ethyl groups will decrease the solubility of a compound they would be expected to increase the stability of the complex, however, steric considerations must also be taken into account, as was discussed earlier.

It is generally assumed that α-CD provides a good binding site for benzenes, β-CD for naphthalenes, and γ-CD for larger guests. However, the data of Schneider *et al*[36] show that this is not always the case. After studying the binding of 1-anilino-8-naphthalene sulphonate (**26**) by NMR it was found that: a) although the α-CD cavity would appear to be of the correct size to include the

phenyl portion, **26** is only included very weakly;[1] b) β-CD binds preferentially to the phenyl *and not* the naphthyl moiety of **26**; c) γ-CD seems to bind both the phenyl and naphthyl moieties of **26**.



26

1.2.5.2 Energetics of Complex Formation

The energy of covalent interactions is on the order of 400 kJ mol^{-1}, while hydrogen bonds and van der Waals interactions normally account for only 40 kJ mol^{-1} and 4 kJ mol^{-1}, respectively. It is evident that a single interaction of these latter two forces is not strong enough to hold the complex together, however, if the host and guest have complementary binding sites, allowing for multiple interactions, then the formation of a stable supermolecule is likely.[16]

Although van der Waals forces are important in the stabilisation of the host-guest complex, they are not the most important ones. Hydrogen bonding cannot be the driving force either since compounds which are not capable of forming hydrogen bonds form stable complexes with CDs,[16] and the addition of a non-

[1] K_d ~ 1 M; unpublished results, Tee, O.S.; Loncke, P.G.; and Gadosy, T.A.

hydrogen bonding solvent does not increase the CD-solute interaction. This last point was clearly demonstrated by Tee and coworkers, when the addition of DMSO (60% aqueous) increased the dissociation constant of the *m-tert*-butylphenyl acetate-β-CD complex to 6.3 mM[56] from a value of 0.13 mM in pure water.[57]

Thus, inclusion complex formation proceeds by an energetically favoured interaction of a relatively non-polar guest molecule with an imperfectly solvated hydrophobic cavity. In this process entropy and enthalpy considerations may have an almost equal role.[16]

1.2.5.3 Driving Force of Complex Formation

Although the formation of CD host-guest complexes has been studied for many years, the exact driving force has not been well understood until recently. A major problem is that any explanation must take into account major differences in binding strength between substrates, as well as between hosts. As illustrated in Table 6, these dissociation constants can vary by over a factor of almost 20 000.

Early explanations of the driving force of complex formation centred on either a decrease of the ring strain resulting from complex formation, or the removal of the high energy water molecules from the CD cavity. In either case there must be a net decrease in overall free energy.[16]

Bender first suggested that water molecules bound inside the CD cavity are enthalpy rich because they cannot have a full complement of hydrogen bonds. As a substrate entered the CD cavity, one or more of these water molecules would

Table 6. Comparison of Dissociation Constants of α-CD and β-CD Complexes.

CD	Guest	K_d (mM)
α	*m*-nitrophenyl acetate[a]	25
	p-nitrophenyl acetate[a]	10
	p-nitrophenyl hexanoate[a]	2.9
	methanol[b]	1070
	n-octanol[b]	0.16
	26[c]	~1000
β	*m*-nitrophenyl acetate	12
	p-nitrophenyl acetate[a]	7.8
	p-nitrophenyl hexanoate[a]	1.8
	methanol[b]	3090
	n-octanol[b]	0.68
	26[d]	26.8

[a] From reference 58. [b] From reference 59. [c] Loncke, P.G.; Gadosy, T.A.; Tee, O.S., unpublished results. [d] From reference 60.

be returned to the bulk solvent, a process which would be associated with a favourable enthalpy term. Measurements of the thermodynamics of inclusion have shown that this is the case.[15-17]

Table 7. Approximate CD Cavity Volumes and Calculated Number of Water Molecules Contained in Aqueous Solution.[a]

CD	Volume ($Å^3$)	# of water molecules
α	176	6
β	346	11
γ	510	17

[a] From reference 61.

Using the CD cavity dimensions of Cramer *et al.*,[62] the approximate volumes and number of included water molecules has been calculated by Bergeron[61] and are summarised in Table 7. It is clear that increasing the cavity size increases the number of *potentially* high energy water molecules included in the cavity. However, as the number of water molecules increases, each water molecule becomes more capable of developing a full complement of hydrogen bonds and is therefore less enthalpy rich. This in turn means that the expulsion of a water molecule becomes less and less of a driving force for complexation as the CD gets

larger. The critical cavity volume at which expulsion of "enthalpy rich" water ceases to have any effect on the binding of a guest is unknown.[61]

The high energy water argument is consistent with the case of α-CD, which binds the smaller sodium propionate more weakly than the larger pNPA, since the latter can exclude more water molecules. Furthermore, phenyl esters can cause more water molecules to be expelled from the β-CD cavity compared to the α-CD cavity, which is consistent with the observed dissociation constants, α-CD > β-CD (Table 6), although the size of α-CD is more suited for phenyl derivatives. The fact that γ-CD binds simple aromatic guests more weakly than β-CD suggests that the critical cavity volume (*vide supra*) has been passed or that other significant contributions to the binding energy must be considered.[61]

The trends in dissociation constants based on high energy water molecules can be just as easily described by the release in conformational ring strain associated with complexation.[61] Saenger[47,63] has demonstrated by X-ray crystallography that one of the glucopyranose units of α-CD is canted inwards, with the C-6 hydroxy group oriented towards the interior of the cavity, serving as a hydrogen bond acceptor for cavity water. Saenger[47] has suggested that the strain associated with this canted glucose molecule can be relieved by complexation, which would allow the glucose unit to relax to a more "normal" state. Small substrates would be expected to relieve the ring strain to a smaller extent than would a large guest, since they require less space.[61]

If the release of ring strain is a major driving force, then methylation of the C-6 hydroxy groups should have an effect on the ability of the CD to form stable host-guest complexes. Space filling models clearly show that methylation of all of the C-6 hydroxy groups would make it more difficult for any glucose residue to achieve orthogonality.[61] It is most likely that in an effort to maximize interaction between hydrophobic groups, all of the methyl groups would be facing inwards.[64] This arrangement would sterically hinder the orthogonal placement of the glucose residues, although it would not completely prevent orthogonality. If one of the rings is orthogonal, then the 6-O-methylated CD would be at a higher energy than its native parent, due to the steric requirements of placing that ring orthogonal. The binding of a substrate should therefore release this strain energy and lead to a much stronger binding. This is not the case, however, since DiMe-α-CD binds *p*-nitrophenyl acetate only 2.3 times stronger than α-CD[61] and DiMe-β-CD[65] binds *p*-nitrophenyl hexanoate 1.7 times weaker than β-CD.[58]

Overall it is clear that relief of ring strain is not a major driving force in complex formation. The driving force for complexation is much more likely to be a combination of several factors including van der Waals forces, high energy water, London dispersion forces, hydrogen bonding, and possibly relief of ring strain.[61] Without including multiple binding forces into a discussion on complex stability, it would not be possible to explain the apparent anomalies in dissociation constants among a family of compounds or series of CDs.

1.2.6 Cyclodextrin Catalysis

Cyclodextrins are very versatile molecules. Not only do they have the ability to bind a wide variety of guest molecules, but they are also capable of inhibiting a variety of different degradative processes, both of which characteristics give them great industrial utility as solubiliser and stabiliser. CDs also have the ability to accelerate and catalyse reactions, and it is because of this latter trait that for many years CDs have been used to study reactions and model various enzymes, such as α-chymotrypsin.

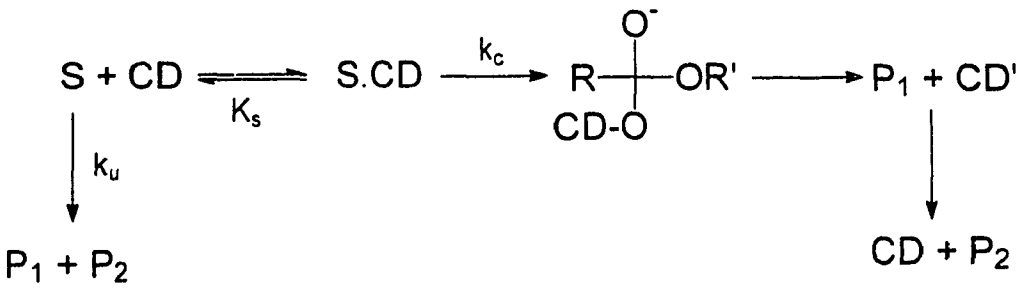
CD catalysis can arise from either the nature of the cavity or from the inherent reactivity of the secondary hydroxy groups which line the wider side of the cavity.

1.2.6.1 Covalent Catalysis

Covalent catalysis describes the situation where there is a distinct covalent interaction between the substrate and the CD during the reaction.[15-17] For convenience it will be illustrated using ester cleavage, although several sorts of reactions show this type of catalysis with CDs.[17]

The catalysis of the cleavage of an ester (S) is illustrated in Scheme 4. The substrate can either undergo a reaction in the medium, k_u, or a reaction involving CD. The first step in the CD-catalysed reaction is the formation of a non-covalent ester.CD complex (S.CD), in which the substrate is then attacked nucleophilically by an ionized secondary hydroxy group, k_c, giving a tetrahedral intermediate.[66-68]

The breakdown of this intermediate yields the first product (P_1), and a modified CD (CD'). The regeneration of the native CD, and formation of the second product (P_2), may be very slow.



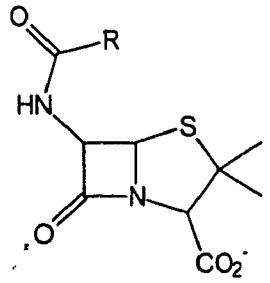
Scheme 4. Mechanism of the CD accelerated cleavage of an ester.

In the case of true catalysis, *i.e.* $k_c > k_u$, the increasing addition of CD to the system causes an increase in the observed pseudo-first order rate constant, k_{obs}, which levels off at high CD. This type of saturation behaviour supports the formation of an S.CD complex, and it is similar to the Michaelis-Menten kinetics observed in the reaction of enzymes with substrates.

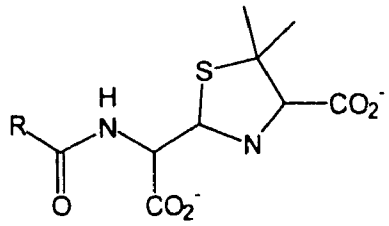
1.2.6.1.1 Amide Hydrolysis.

The use of CDs as models for enzyme behaviour arises from their ability to assist in the cleavage of a multitude of bonds. An example of this is the CD-accelerated cleavage of various amides including penicillins, N-acylimidazoles and acetanilides.[15-17] The cleavage of the penicillin amide bond has made CDs the

subject of model studies for enzymes such as penicillinase (β-lactamase), where CDs catalyse the conversion of penicillins (**27**) to penicilloic acids (**28**).



27



28

Table 8 summarises the catalytic rate constant, k_c, acceleration, k_c/k_u, and dissociation constant, K_s, for various penicillins with β-CD. Although the series depicted covers a large range of structural variations in the side chain, k_c only varies by a factor of 8 and the spread in k_c/k_u is even less, with only a factor of 4.2 between highest and lowest ratios. It is not surprising that the largest effect is seen in the dissociation constants, K_d, of the CD.**27** complexes which vary over 20-fold. It appears that in this case the orientation of the β-lactam ring of **27** is not critical in terms of catalysis, since the carbonyl carbon has access to the catalytic CD hydroxy group due to the flexible nature of **27**. As has been demonstrated elsewhere (*vide infra*), this reaction may actually be occurring with all or part of **27** outside of the CD cavity, which would explain the low sensitivity of the kinetic parameters to substrate structure. The formation of the S.CD complex in no way necessitates the inclusion of S inside of the cavity during the transition state (*vide infra*).

Table 8. Kinetic and Thermodynamic Parameters for the β-CD-Catalysed Hydrolysis of Penicillins (**27**).[a]

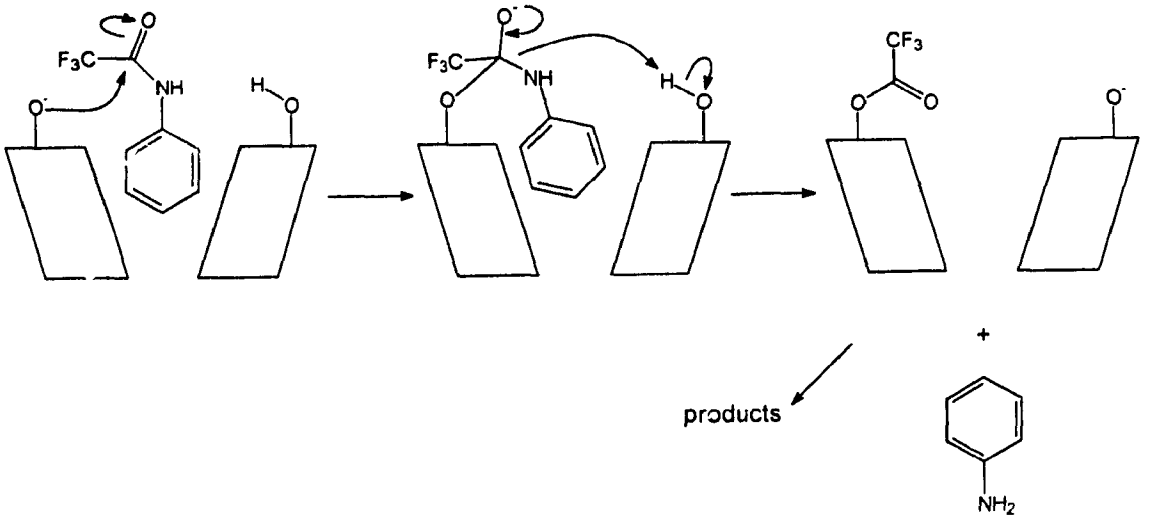
R	$10^3 k_c$ s^{-1}	k_c/k_u	K_d mM
CH_3	2.52	37	33
$CH_3(CH_2)_4$-	3.83	66	41
$CH_3(CH_2)_8$-	2.98	47	21
$PhCH_2$-	5.40	77	43
Ph_2CH-	2.68	34	4.7
Ph_3C-	10.4	40	3.9
2,6-$(MeO)_2$Ph-	1.23	21	13
1-Naphthyl-	1.82	31	16
2-Naphthyl-	7.12	89	75

[a] From reference 72.

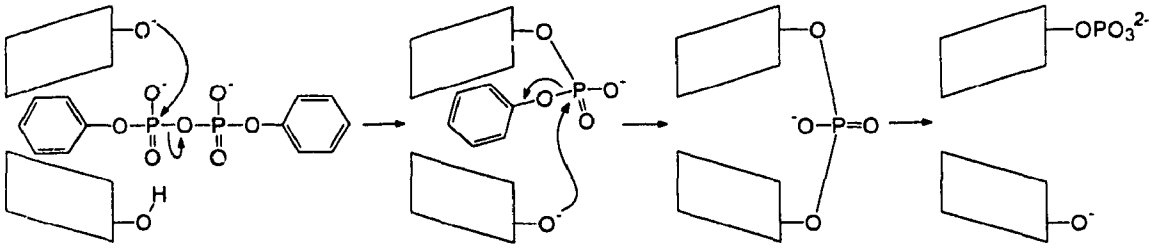
Tutt[72] demonstrated the presence of a covalent penicillin-β-CD intermediate in several ways. The initial rate of consumption of **27** was greater than the initial rate of formation of **28**, including an induction period for the formation of **28** even in the presence of excess **27**. Evidence for the covalent intermediate was also found by performing assays which are specific for penicilloyl derivatives.

N-acylimidazoles are hydrolysed through acid catalysed, neutral and base catalysed mechanisms.[73,74] The cleavage of the amide bond in N-trans-cinnamoylimidazole is accelerated by α-CD and β-CD by factors of 28 and 38, respectively. The cleavage of N-acetylimidazole is also accelerated by these two CDs, by a factor of 50 and 28 respectively.[17]

One of the first cases which demonstrated the ability of CDs to accelerate the cleavage of a normal amide was the example of *p*-nitrofluoroacetanilide reacting with α-CD,[75] which accelerated the rate by a factor of 16. The reaction proceeds through a mechanism outlined in Scheme 5, where the tetrahedral intermediate is produced by the nucleophilic attack of an ionized hydroxy group on the carbonyl carbon of the substrate, which is bound in the cavity.[75] The intermediate then breaks down via a general acid catalysed protonation, by a secondary hydroxy group on the CD of the aniline nitrogen, to yield aniline and a trifluoroacetylated CD, which hydrolyses to regenerate the CD.[17] In the case of *p*-nitroacetanilide, a less activated substrate, α-CD actually inhibits the cleavage.



Scheme 5. Mechanism of CD-accelerated cleavage of a "normal" amide.



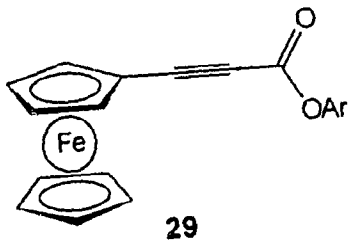
Scheme 6. Mechanism for the CD-assisted hydrolysis of diarylphosphonate esters. N.B. the hydroxy groups involved in the reaction are on adjacent residues.

1.2.6.1.2 Hydrolysis of Organophosphates

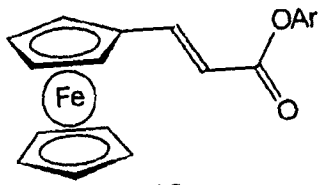
Diaryl pyrophosphates are stable in both neutral and alkaline solutions. The addition of divalent metal ions to the reaction increases the rate significantly, but the addition of CDs speeds up the reaction to an even greater extent.[76] At pH 12, the first step, as illustrated in Scheme 6, is the formation of the CD.organophosphate complex. The binding of the substrate to the CD places one of the P=O groups adjacent to an ionized hydroxy group on the CD, which is capable of attacking nucleophilically, to form a CD monophosphate intermediate. The monophosphate breaks down in a two step process, going through a cyclic CD phosphate intermediate, yielding another CD phosphate which in turn breaks down to regenerate the native CD.[17,76]

1.2.6.1.3 Approaching and Beyond Enzymatic

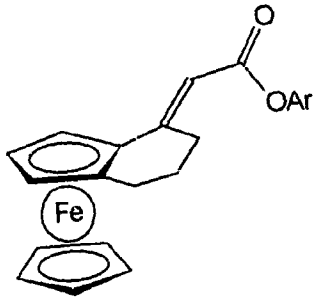
In the above examples, the host-guest complex forms in such a way that the reactive centre of the substrate is oriented away from the nucleophilic CD hydroxy groups. Breslow proposed[77] that better rate enhancements should be obtainable if the angle between the bound portion and the side-arm carrying the reactive site could be lowered to 90° or less. On the basis of CPK models, Breslow and co-workers[69,78-80] proposed that ferrocene derivatives (**29** - **32**, and the enantiomers of **31** and **32**), where the angle between the bound moiety and the side-arm is 90°, would meet this criteria. These compounds bind well to β-CD and the use of a suitable side chain places the reactive centre very close to the nucleophilic CD



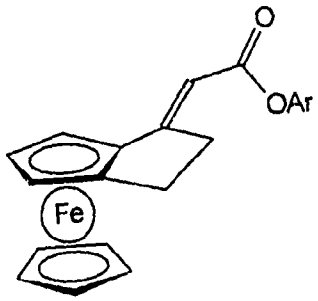
29



30



31



32

Ar = *p*-NO_2-Ph

hydroxy group, requiring that the substrate only has to move a very short distance for reaction to occur (*vide infra*).[78 80]

In 60% aqueous dimethyl sulfoxide (DMSO), β-CD accelerated the rate of cleavage of **29** by a factor of 140 000, and an even larger acceleration was observed with **30**, where the addition of CD increased the rate of cleavage by 750 000 times. The latter acceleration is even larger than that afforded to the cleavage of pNPA by the enzyme α-chymotrypsin.[77] Breslow surmised that the actual rate acceleration should be augmented by a factor of 25 to take into account the nature of the solvent relative to water, giving a total acceleration of 18 000 000 fold.

Table 9. The Best Esters for Cleavage by β-CD.[a]

Ester	10^{-5} k_c/k_u	K_s mM	10^9 K_{TS} mM	Selectivity
31	32	3.8	1.2	
Enantiomer	1.6	14.6	0.29	
				20:1
32	59	5.7	97	
Enantiomer	0.95	4.7	0.49	
				62:1

[a] From reference 81.

Breslow found it surprising[77] that **30** seemed to be cleaved more efficiently by β-CD than **29**, since **30** has more degrees of rotational freedom that must be frozen out in the transition state. In an effort to study the effect of a substrate with less rotational degrees of freedom, they examined the cleavage of **31** and **32** (and their enantiomers). As summarised in Table 9, β-CD was selective for **31** and **32** over their respective enantiomers, and accelerated the cleavage of **31** and **32** by 5 and 6 orders of magnitude, respectively. Since the binding of these two compounds is on the order of mM, the rate acceleration must be due to a very strong transition state binding.[81]

One of the most studied examples of covalent CD catalysis involves the cleavage of phenyl esters, which will be discussed thoroughly at a later point.

1.2.6.2 Non-covalent Catalysis

The ability of CDs to accelerate the rates of organic reactions is not restricted to the formation of a covalent CD substrate intermediate. CDs can alter the rate of reactions by various noncovalent means: microsolvent effects due to the apolar nature of the cavity; conformational effects due to the geometric requirements of inclusion; and by helping to preorganise reactants into a more reactive geometry.[15-17]

1.2.6.2.1 Microsolvent Effects

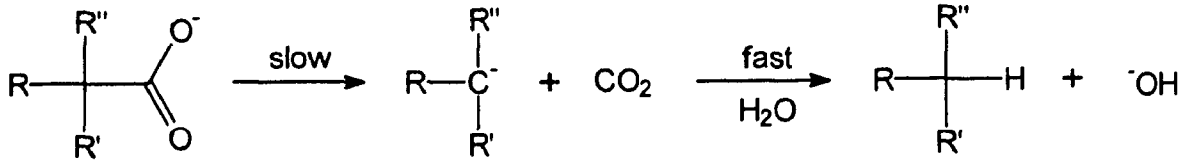
A typical example of the CD microsolvent effect is seen in the highly solvent dependent decarboxylation of activated α-cyano and β-keto acids.[82,83] The rate determining step in this reaction is the heterolytic cleavage of the carbon-carbon bond to the carboxylate group, as shown in Scheme 7.

Cyclodextrins accelerate these decarboxylations since the interior of the cavity affords a less polar, ether-like[82,83] environment for reaction. Evidence for non-covalent catalysis over covalent catalysis is shown in the facts that: the catalytic rate constant follows the Hammett relationship; the activation parameters for the CD-catalysed reactions are almost identical to those in a 2-propanol/H_2O mixture; and the position of the substituents hardly affects acceleration, whereas stereospecificity in covalent catalysis is usually quite marked.[17]

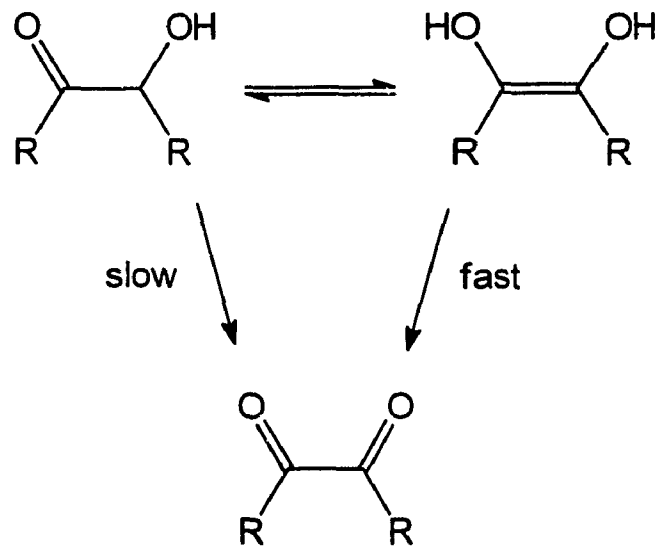
The oxidation of α-hydroxyketones to α-diketones is another example of a reaction that is accelerated due to the microsolvent effect afforded by the CD cavity. The formation of the diketone from the enol (Scheme 8) is much faster than the reaction starting with the hydroxyketone. The reaction is catalysed by virtue of a shift in the keto-enol equilibrium towards the enol caused by the CD.[17]

1.2.6.2.2 Conformational Effect

Since the cyclodextrin cavity is of more or less fixed dimensions, it may preferentially bind a guest in one conformer rather than another. If the favoured



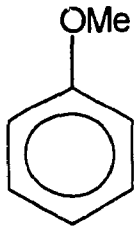
Scheme 7. Noncovalent catalysis in the decarboxylation of activated acids.



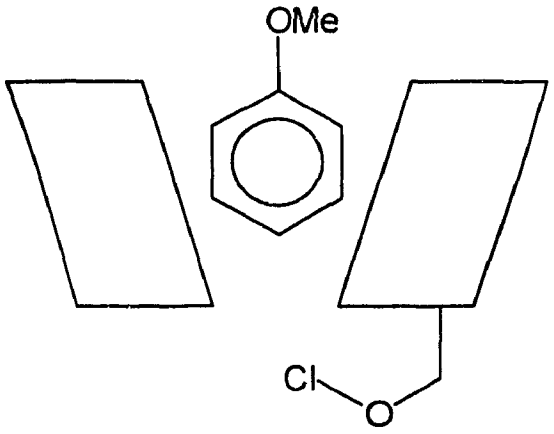
Scheme 8. CD catalysis occurs by helping to shift the equilibrium towards the more reactive enol.

conformer is more reactive than the other one, then the rate of reaction can be accelerated, other things being equal.

Under normal conditions the chlorination of anisole (**33**) produces both the *ortho*, 40%, and *para*, 60%, products. In the presence of 10 mM β-CD the formation of the *para* product is strongly favoured, with only 4% of the product being formed as the *ortho* isomer. There are two causes for this: chlorination within the anisole.CD complex (**34**) *via* a hypochlorite ester of one of the primary CD hydroxy groups (covalent catalysis) is regiospecific; the ortho positions in **34** are completely blocked by the CD,[77] so that ortho substitution is suppressed.



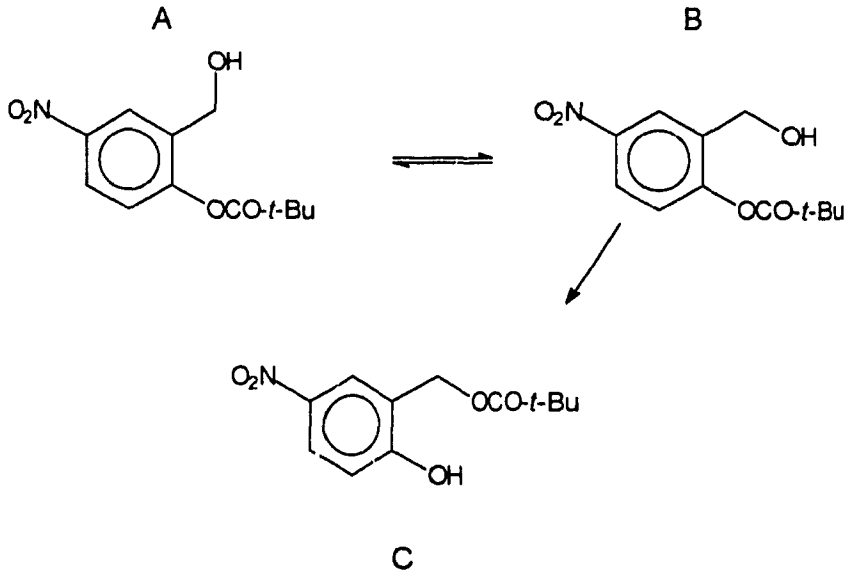
33



34

Another example of the conformational effect is shown in Scheme 9. Isomerisation of the phenyl ester A to the benzyl ester C occurs through an intramolecular nucleophilic attack of the benzylic hydroxy group on the carbonyl group. The reaction occurs through conformation B, and so, the presence of α-CD

accelerates the rate of group transfer by a factor of 6, since it promotes formation of the reactive conformer, B, from A. On the other hand, β-CD retards this reaction by about 5 fold, where the only difference between the two CDs is the size of the cavity. This result is consistent with a CD-promoted mechanism involving only conformational effects, since catalysis is only governed by the geometry of the fit of the substrate into the CD.[17,84]

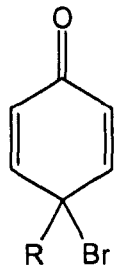


Scheme 9. Preferential binding of one conformer over another accelerates the rate of reaction.[84]

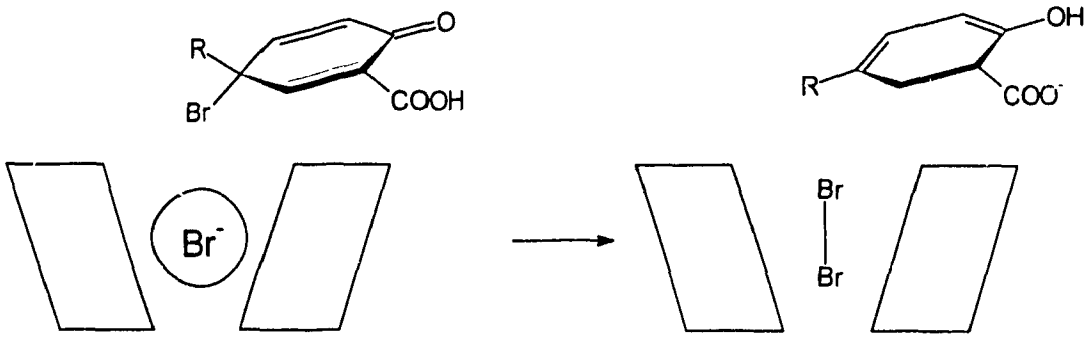
1.2.6.2.3 Catalytic Additivity

The debromination of various 4-bromo-4-alkylcyclohexadienones (**35**) is induced by Br^-, and catalysed by α-CD. The reaction seems to occur with free

ketone reacting with Br^- bound inside the CD cavity (Scheme 10). This complexation of bromide ion removes, at least partially, its normal sphere of solvating water molecules, making it more nucleophilic by factors of 2400 - 4600, depending on the substituent on the alkylcyclohexadienone.[85]



35



Scheme 10. Co-catalysis and a true approach to enzymatic enhancements.

The addition of a carboxylic acid group to **35** (R = Me) increases the rate of debromination, in the absence of α-CD, by a factor of 3500, relative to the parent compound, due to an intramolecular protonation of the carbonyl oxygen. Takasaki and Tee[86] demonstrated that this effect is additive to the catalysis

afforded by the complexation of Br^- to α-CD (3400 x), and that the two effects combined increase the rate of debromination, relative to **35** (R = Me), by a factor of 3400 x 3500 = 12 000 000! This is one of the largest rate enhancements observed in cyclodextrin chemistry.

As was stated earlier, one of the most studied reactions, which is accelerated by CDs, is the cleavage of phenyl esters. The remainder of this thesis will be devoted to discussion of the cleavage of nitrophenyl esters by various CDs and nucleophiles and the study of the binding of small aliphatic guests to both natural and modified CDs.

2. Cleavage of Nitrophenyl Esters

2.1 Introduction

As discussed in Chapter 1, CDs are capable of accelerating or retarding many reactions. One of the most widely studied reactions, which is accelerated by CDs, is the basic cleavage of phenyl esters. The extensive study of these systems has allowed researchers not only to understand the catalytic processes involved in phenyl ester cleavage, but also the manner by which CDs catalyse or accelerate other reactions Cleavage of phenyl esters by CDs has received such wide attention because of the fact that they seem to be one of the best, water soluble, substrates for CD "catalysis" (Table 10).

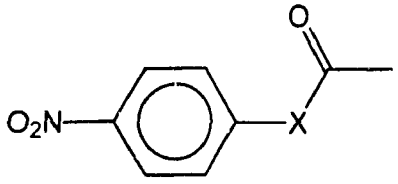
Table 10. Reactions Accelerated by Cyclodextrins.[a]

Reaction		Substrate	k_c/k_u
Cleavage of: esters		Phenyl	300
		Ferrocenyl	$>10^6$
	amides	Penicillins	89
	organophosphates	Pyrophosphates	>200
	carbonates	Aryl carbonates	7.5
	sulphates	Aryl sulphates	19
Decarboxylation		Cyanoacetate anions	44
Oxidation		α-Hydroxyketones	3.3

[a] From reference 70.

In the CD-assisted cleavage of phenyl esters, reaction occurs via nucleophilic attack of an ionized secondary hydroxy group of the CD on the carbonyl carbon of the CD-bound ester, resulting in the formation of a tetrahedral intermediate, which then breaks down to give an acylated CD (Scheme 4, p. 41).[15-17] By definition,[87] true catalysis is only obtained once the *O*-acylated CD is hydrolysed to regenerate the native CD.

Breslow *et al.*[78] found that the cleavage of phenyl esters by CDs results in a mixture of CDs which are *O*-acylated on either the C-2 or the C-3 hydroxy groups. However, more recent work by the groups of Bergeron[71] and Inozuka[88] indicates that the nucleophilic centre is actually the hydroxy group on the C-3. The probable explanation of the apparent discrepancies between the two observations is that Breslow's results were obtained in a solution of relatively high pH, where the migration of an acyl group is likely.[70]



a X = S; b X = O

36

The rate-limiting step for the cleavage of phenyl esters is the formation of the tetrahedral intermediate by attack of one of the ionized CD hydroxy groups on the carbonyl carbon of the ester. Evidence for this was obtained by examining the

rate of hydrolysis of **36** where the first order rate constants for the basic cleavage of **36a** and **36b** by α-CD are virtually identical. The same behaviour was also observed with the β-CD-catalysed reaction, indicating that the loss of the leaving group is not rate limiting.[89]

The catalysis of reactions by CDs, like enzymes, is very specific in several ways. Both CDs and enzymes are capable of selecting between enantiomeric substrates, as was illustrated in the case of the binding and cleavage of ferrocenyl substrates by CDs;[69 78-80] when more than one possible product of a reaction exists CDs can selectively form only one, as in the noncovalently catalysed chlorination of anisole in the presence of CDs;[77] and finally, a subtle change in geometry of the substrate can have a marked effect on the catalysis afforded by the CD.[57] The first two cases have been discussed previously, leaving only the last example to be introduced.

The acceleration afforded to a reaction by cyclodextrin is measured as the ratio of the rate constant at saturating levels of CD (k_c) compared to the rate constant in the absence of the CD (k_u). Table 11 summarises the kinetic rate constants for a series of phenyl esters which undergo a CD-accelerated cleavage (Scheme 4, p. 41). The values of k_c/k_u range from near unity (no acceleration) up to 300 (very good acceleration for a CD-mediated process). In each case, k_c/k_u is at least an order of magnitude greater for the *meta* esters than for the *para* esters The largest difference in acceleration is observed with the *t*-butylphenyl esters,

where the cleavage of the *meta* isomer is accelerated 200-fold more than the *para* isomer.

Table 11. Catalytic Rate Constants and Accelerations in the α-Cyclodextrin-assisted Cleavage of Phenyl Acetates.[a]

Acetate	10^2 k_c s^{-1}	k_c/k_u	*m*/*p*[b]	k_2[c] $M^{-1}s^{-1}$	*m*/*p*[d]
Phenyl	2.2	27		1.0	
m-Tolyl	6.6	95	29	3.9	20
p-Tolyl	0.22	3.3		0.20	
m-*tert*-Butylphenyl	13	260	236	65	650
p-*tert*-Butylphenyl	0.067	1.1		0.1	
m-Nitrophenyl	43	300	88	22	11
p-Nitrophenyl	2.4	3.4		2.0	
m-Carboxyphenyl	5.6	68	13	0.53	12
p-Carboxyphenyl	0.67	5.3		0.045	

[a] From reference 57. [b] Calculated as: $m/p = (k_c/k_u)_{meta} / (k_c/k_u)_{para}$. [c] Using dissociation constants, K_d, from reference 57, calculated as follows: $k_2 = k_c/K_d$. [d] Calculated as: $m/p = k_2^{meta}/k_2^{para}$.

The specificity of an enzyme for its substrate is usually discussed in terms of the second order rate constant, k_2 (= k_c/K_M). The equivalent k_2 (= k_c/K_s) for the CD-mediated processes have been calculated and included in Table 11. The second order rate constants, k_2, range over 4 orders of magnitude, but in most cases the difference in selectivity between the *meta* and *para* isomers is between 10- and 20- fold. The largest difference occurs with the *t*-butylphenyl esters, where the selectivity for the *meta* isomer is 650 times greater than that for the *para* isomer. Although the above discussion was for α-CD, the same type of behaviour can be seen with other CDs.[58 65 90]

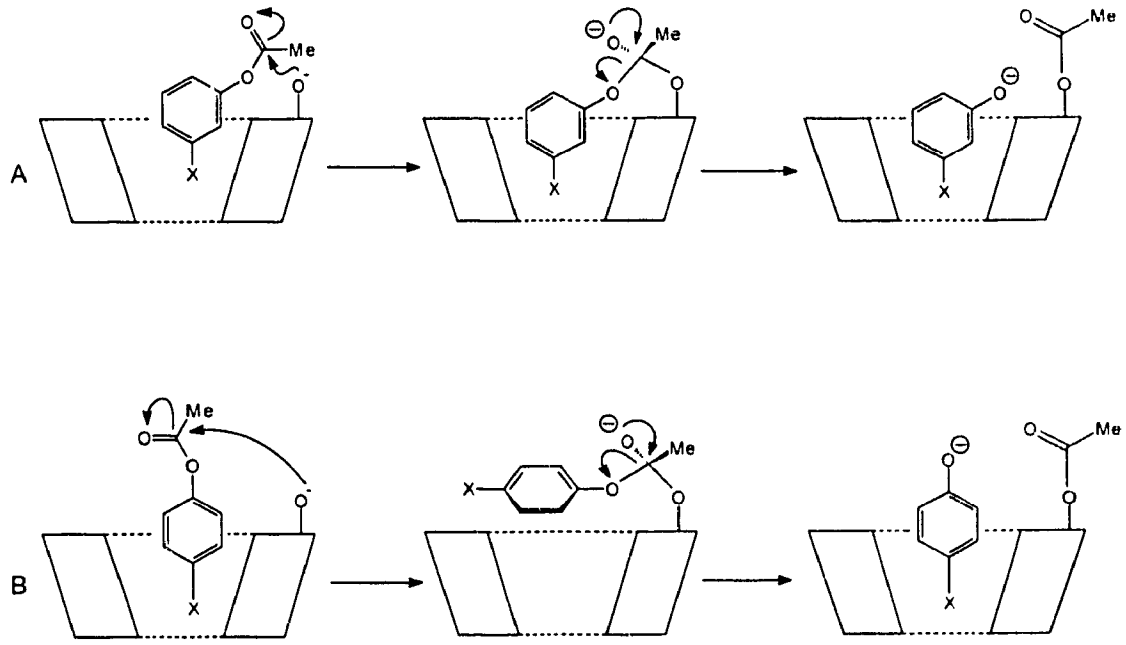
Table 12. Distance between Nucleophilic Centres of α-Cyclodextrin and Nitrophenyl Ester Carbonyl Carbon.[a]

Acetate	Distance Å	Acceleration
p-Nitrophenyl	6.0	4.4
Phenyl	5.8	14
m-Nitrophenyl	3.4	220

[a] From reference 91. Based on NMR measurements in a 1:1 mixture of 1N DCl:DMSO-d_6. We can assume that the trends, if not the distances, for the C-3 hydroxy group are the same as those for the C-2 hydroxy group.

VanEtten *et al.*[57] explained the large difference in reactivities between the isomeric *t*-butylphenyl esters in terms of different modes of binding. The *meta* ester binds with the *t*-butyl group in the CD cavity and the acyl group near the secondary hydroxy groups, whereas the *para* ester binds with the acyl group protruding through the bottom of the CD cavity, near the primary hydroxy groups, leaving the phenyl ring in the cavity and the *t*-butyl group near the secondary hydroxy groups. This orientation of *p-tert*-butylphenyl acetate, with the acyl group away from the reactive site on the CD, readily explains why the cleavage is not accelerated by α-CD ($k_c/k_u \sim 1$). The *meta/para* selectivity for the rest of the esters was explained by Komiyama and Bender[92] as being due to a smaller enthalpy of activation for the *meta* esters relative to the *para* esters. This is attributed to the fact that in order for the *para* esters to react they are required to move a great deal more than the *meta* esters, as described below. Komiyama and Hirai[91] investigated the origin of the relative reactivities using ^{1}H-NMR and found that the trends in acceleration of the rate of cleavage was inversely related to the distance between the nucleophilic centre and the carbonyl carbon of the ester (Table 12), in the order *p*-nitrophenyl acetate (pNPA) » phenyl acetate > *m*-nitrophenyl acetate (mNPA). The dependence of acceleration on distance is due to a large geometrical change that occurs in going from the initial state to the transition state (Scheme 11). As shown in Scheme 11a, mNPA is initially bound in such a way that it places the ester carbonyl group within very close proximity of the secondary hydroxy groups on C-2. This allows the nucleophilic attack, and formation of the

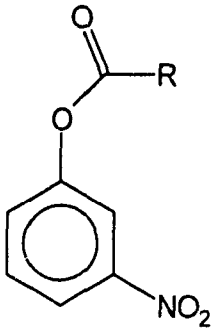
consequent transition state and tetrahedral intermediate, to occur without requiring the ester to move significantly in the cavity. pNPA, on the other hand, must lift out of the CD cavity in order to bring the carbonyl carbon close enough to the nucleophilic centre to allow for reaction (Scheme 11b). It has been recently shown that during the transition state for the cleavage of *p*-nitrophenyl esters the CD cavity is empty or only partially occupied by the guest ester.[58,81,93-98]



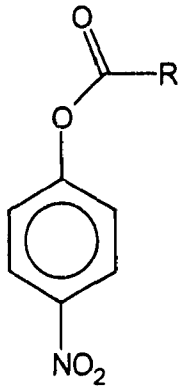
Scheme 11. Cyclodextrin assisted cleavage of A) *m*-nitrophenyl acetate; B) *p*-nitrophenyl acetate.

The focus of this chapter is the CD-mediated cleavage of *m*-nitrophenyl alkanoate esters (mNPAlk, **37**) and *p*-nitrophenyl alkanoate esters (pNPAlk, **38**). These nitrophenyl esters provide a convenient series of compounds which can be

used to examine the structural dependence of the ability of CDs to bind guests, accelerate reactions, and of phenomena occurring in the transition state.[58]



37



38

If a binding process (either in the initial or transition state) occurs via aryl group inclusion (Figure 6a) then parameters which are dependent on this binding should show little or no dependence on the length of the acyl chain of mNPAlk or pNPAlk. Conversely, parameters which are dependent on a binding process which occurs via acyl inclusion (Figure 6b), should be independent of the position of the substituent nitro group (*meta* or *para*). Using this hypothesis it has been shown previously that mNPAlk with shorter acyl chains, 2 to 6 carbons long, undergo cleavage by α-CD with a transition state where the aryl portion of the ester is bound inside the CD cavity. This is in contrast to the behaviour observed with pNPAlk, where the transition state for ester cleavage involves the inclusion of the acyl group, past the acetate (C_3 to C_{12}).[58,99] The trends observed for cleavage by β-CD are similar, if not as clear cut. Both series of esters, past the acetates, bind

to α-CD and β-CD in the initial state via acyl group inclusion, as is evidenced by the linear increase of pK_s (= - log K_s) with the length of the acyl chain.

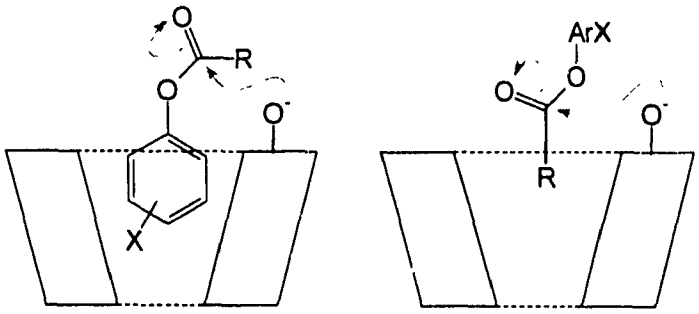


Figure 6. *Meta* versus *para* binding of nitrophenyl alkanoates to cyclodextrins.

Previous research in this group has centred on the mediation of the basic cleavage of mNPAlk and pNPAlk by α-CD and β-CD, mainly because other CDs were either very rare or too costly. Due to recent advances in the technology associated with the formation and synthesis of CDs, it is now possible to study the effect of other CDs, such as γ-CD, Hp-β-CD, and DiMe-β-CD on the cleavage of these esters. The chemistry of these CDs should prove to be interesting and informative, as they have many present and possible applications in both industry and research.[15-17]

2.2 Results

Kinetic investigations into the basic cleavage of mNPAlk and pNPAlk in the presence of Hp-β-CD,[90] DiMe-β-CD,[65] and γ-CD,[65] have been carried out, and the raw data are collected in Appendix 1. The pseudo first-order release of nitrophenolate anion was used to monitor the progress of the reaction, and reactions were performed over a range of CD concentrations in order to determine the dependence of the first-order rate constant, k_{obs}, on [CD].

For Hp-β-CD (**9**, p. 18) reacting with the short esters (C_2 to C_6) in both series, the dependence of k_{obs} on [Hp-β-CD] follows simple saturation kinetics,[15-17] as shown in the examples in Figures 7 and 8. This behaviour is ascribed to two kinetic processes, the first being the uncatalysed reaction of the substrate, S, in the basic medium as shown in equation [1]; and the second is the reaction of the reversibly formed ester.CD complex, S.CD, as shown in equation [2]. The combination of these two processes affords a relationship between k_{obs} and [CD] as given in equation [3].

$$S \xrightarrow{k_u} P \qquad [1]$$

$$S + CD \underset{K_s}{\rightleftharpoons} S.CD \xrightarrow{k_c} P \qquad [2]$$

$$k_{obs} = \frac{k_u K_s + k_c[CD]}{K_s + [CD]} \qquad [3]$$

The use of eqn. [3] requires that we maintain the inequality, $[S.CD] \leq [S]_o \ll [CD]$, which was possible under our experimental conditions. Eqn. [3] was fitted

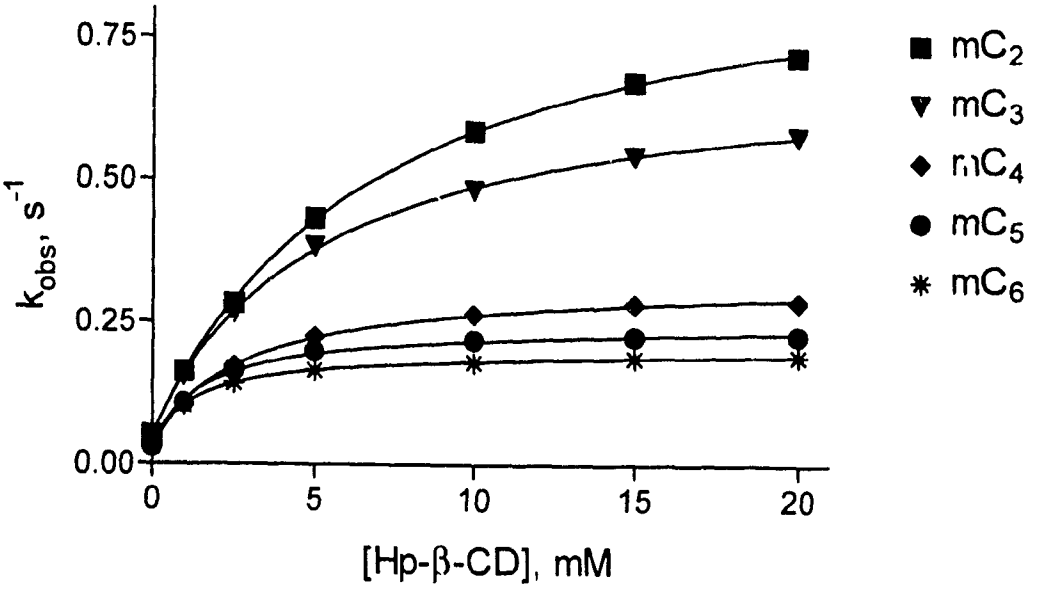


Figure 7. Simple saturation type dependence of k_{obs} on [Hp-β-CD] for the cleavage of mNPAlk (C_2 to C_6).

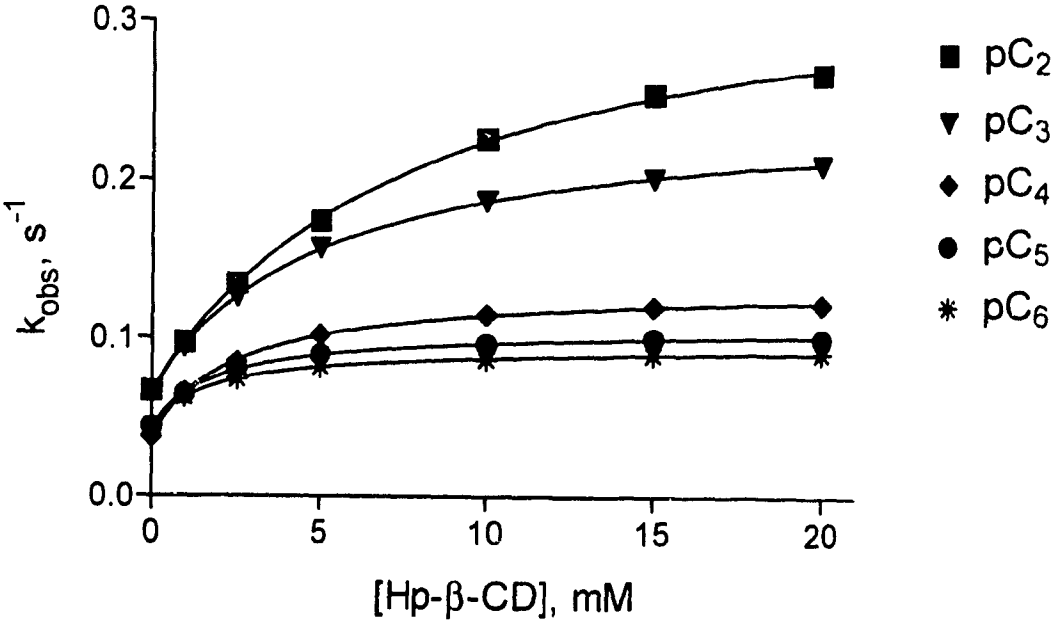


Figure 8. Simple saturation type dependence of k_{obs} on [Hp-β-CD] for the cleavage of pNPAlk (C_2 to C_6).

Table 13. Constants for the Cleavage of Short Chain (C_2 to C_6) *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by "Hydroxypropyl-β-cyclodextrin".[a,b]

Ester	k_u s^{-1}	k_c s^{-1}	K_s mM
m-Nitrophenyl alkanoates			
C_2	0.0502	0.956 ± 0.010	6.98 ± 0.19
C_3	0.0515	0.707 ± 0.007	5.08 ± 0.16
C_4	0.0293	0.325 ± 0.004	2.68 ± 0.12
C_5	0.0306	0.247 ± 0.004	1.63 ± 0.13
C_6	0.0306	0.202 ± 0.001	1.33 ± 0.03
p-Nitrophenyl alkanoates			
C_2	0.0653	0.343 ± 0.007	8.18 ± 0.48
C_3	0.0660	0.247 ± 0.002	5.05 ± 0.15
C_4	0.0402	0.135 ± 0.001	2.67 ± 0.15
C_5	0.0431	0.105 ± 0.001	1.94 ± 0.09
C_6	0.0444	0.0932 ± 0.0005	1.59 ± 0.08

[a] In aqueous phosphate buffer (0.2 M) pH 11.40, 25 °C. Values of k_u were determined experimentally, k_c and K_s were obtained by fitting of equation [3] to the obs ed data (see text). The errors are the standard error obtained from the fitting. [b] Reference 90.

to the data, by non-linear least squares analysis, using the measured k_u values, in order to obtain K_s and k_c. These constants are collected in Table 13 and are used with equation [3] to calculate the curves in Figures 7 and 8.

It was not possible to fit equation [3] reasonably to the data for the longer esters (C_7 to C_{10}) reacting with Hp-β-CD. This was taken to mean that there are other processes acting in conjunction with those in equations [1] and [2], as has been demonstrated with longer carboxyphenyl esters reacting with α-CD and β-CD.[100,101]

The observed rate constants for *m*-C_7 and *p*-C_7 to *p*-C_{10} do not level off at high [Hp-β-CD], as would be predicted by simple saturation kinetics, but continue to rise linearly, as the [Hp-β-CD] is increased, after the initial curvature. Therefore a process involving a second molecule of Hp-β-CD, attacking the S.CD complex is proposed, as shown in equation [4]. The combination of this process with those in equations [1] and [2] now yields an expression for the dependence of k_{obs} on [Hp-β-CD] as given by equation [5] (Figures 9 and 10). The fitted parameters K_s, k_c, and k_{c2} are given in Table 14.

$$S.CD + CD \xrightarrow{k_{c2}} P \qquad [4]$$

$$k_{obs} = \frac{k_u K_s + k_c[CD] + k_{c2}[CD]^2}{K_s + [CD]} \qquad [5]$$

Equation [5] is still not capable of adequately describing the observed data for the remaining esters, *m*-C_8 to *m*-C_{10} and *p*-C_{10}. At high [Hp-β-CD] the data do

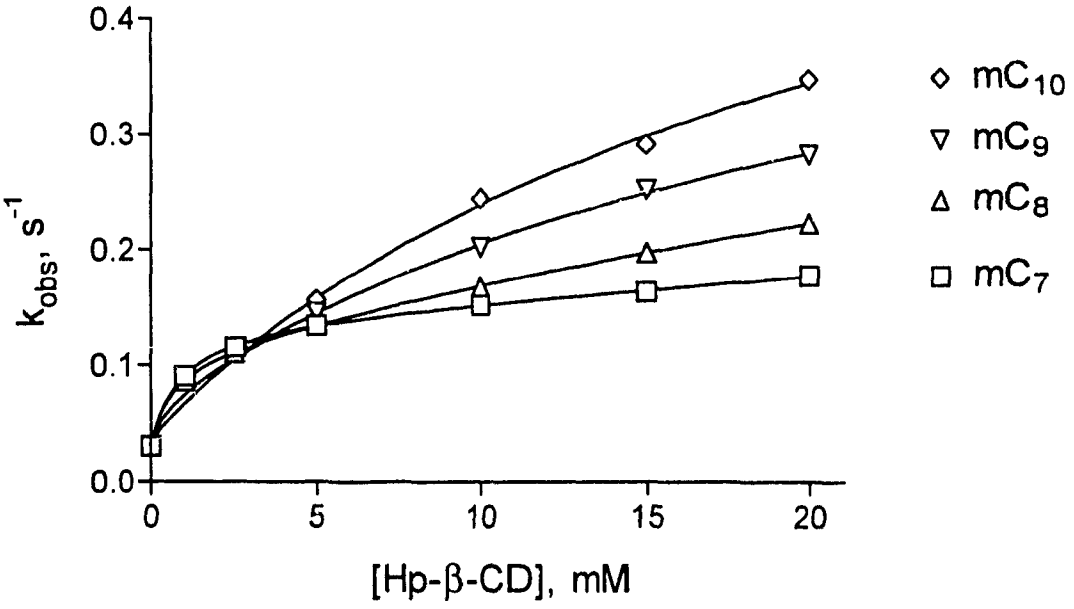


Figure 9. Dependence of k_{obs} on [Hp-β-CD] for the cleavage of mNPAlk (C_7 to C_{10}), with processes involving two CDs.

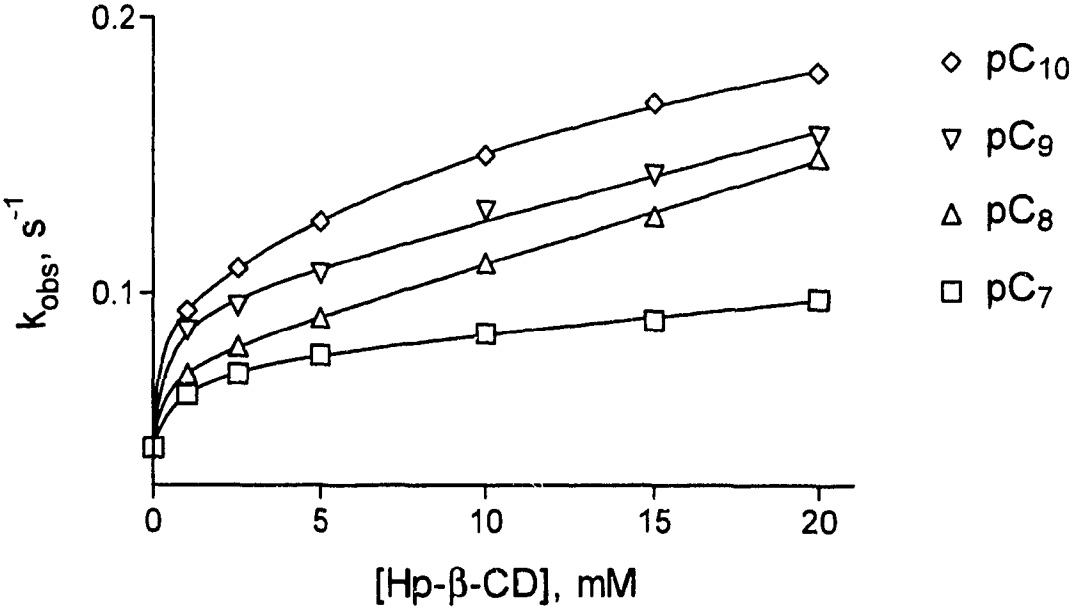


Figure 10. Dependence of k_{obs} on [Hp-β-CD] for the cleavage of pNPAlk (C_7 to C_{10}), with processes involving two CDs.

Table 14. Constants for the Cleavage of Longer Chain (C_7 to C_{10}) *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by "Hydroxypropyl-β-cyclodextrin".[a b]

Ester	k_c s^{-1}	K_s mM	k_{c2} $M^{-1}s^{-1}$	K_{s2} mM	k_{cc} s^{-1}
m-Nitrophenyl alkanoates					
C_7	0.143±0.003	0.899±0.060	2.06±0.13		
C_8	0.112±0.010	0.587±0.211	8.7[c]	75.9±8.0	0.666±0.043
C_9	0.0721±0.0156	0.372[d]	22.2[c]	25.1±9.1	0.557±0.084
C_{10}	0.0495±0.0369	0.245[d]	29.8[c]	23.2±14.0	0.692±0.178
p-Nitrophenyl alkanoates					
C_7	0.0769±0.0018	0.792±0.146	1.13±0.09		
C_8	0.0858±0.0014	0.497±0.068	2.17±0.07		
C_9	0.0980±0.0048	0.391±0.170	3.14±0.03		
C_{10}	0.0925±0.0055	0.159±0.100	13.7[c]	19.5 ± 3.9	0.268 ± 0.014

[a] In aqueous phosphate buffer (0.2 M) at pH 11.40 and 25.0 ± 0.1 °C. Values of k_u are taken to be the same as that of the C_6 ester (see text), the other constants were obtained by non-linear least squares fitting of equation [5] or [7] to the observed data. The quoted errors are the standard error obtained from the fitting. [b] Reference 65. [c] Calculated as k_{cc}/K_{s2}. [d] Estimated by extrapolation (see text).

not rise linearly with [Hp-β-CD], but rise in a curved manner, suggesting the onset of a second binding process, and so the formation and reaction of a discrete, reactive, 1:2 (ester.CD) complex is proposed as in equation [6]. The combination of this process with those shown in equations [1] and [2] requires that equation [7] now be used to describe the observed dependence of k_{obs} on [Hp-β-CD].

$$S.CD + CD \underset{K_{s2}}{\rightleftharpoons} S.CD_2 \xrightarrow{k_{cc}} P \qquad [6]$$

$$k_{obs} = \frac{k_u K_s K_{s2} + k_c K_{s2}[CD] + k_{cc}[CD]^2}{K_s K_{s2} + K_{s2}[CD] + [CD]^2} \qquad [7]$$

Equation [7] provides an adequate fit to the remaining data, with the fitted parameters k_c, K_s, K_{s2} and k_{cc}, given in Table 14. The values of k_{c2} (= k_{cc}/K_{s2}) are calculated and are also included in Table 14, to allow for a comparison between both of the processes involving two molecules of Hp-β-CD.

Due to the limited solubility of the esters with longer acyl chains (>C_6) it was difficult to obtain reliable values for k_u in the absence of any CD. It has been shown previously[58] that the k_u for the reaction of these longer esters is relatively constant past the C_4, providing that the formation of ester aggregates can be avoided. Therefore, for these longer esters, the k_u value was assumed to be the same as that of the C_6 ester. The addition of Hp-β-CD to the reaction solutions causes an increase in solubility of the esters, which alleviates this problem. A related problem with solubility occurred with the *m*-C_9 and *m*-C_{10} esters, where we were forced to work at [Hp-β-CD] too high to allow us to determine K_s accurately.

Since we found a linear relationship between the pK_s value and length of the acyl chain we were able to estimate the dissociation constants by extrapolation (*vide infra*).

Similar kinetics studies with the two series of esters were carried out with γ-CD. The data were fit well by equation [3], as is shown in Figures 11 and 12. However, we were forced to limit the number of esters studied due to solubility problems. γ-CD seemed to form stable complexes with the longer *m*-nitrophenyl alkanoates, and these complexes tend to precipitate out of solution. Due to the low molar absorptivities of the anion liberated from the *meta* esters, and the necessarily low concentration, we were not able to proceed past the *m*-C_6. The measured values of k_u and fit values of K_s and k_c are summarised in Table 15.

In initial exploratory studies we did observe the formation of 1:2 ester:CD complexes at high [γ-CD] (up to 50 mM). These complexes seemed to be non-reactive, so we worked at lower [γ-CD] and limited our investigation to the first binding process and the associated cleavage.

The final CD examined in this study was DiMe-β-CD (**8**, p. 18). Figures 13 through 16 show the dependence of k_{obs} on [DiMe-β-CD], all of which show the same downward curvature. Although the rate constant decreases with increased [CD], the reaction is not totally inhibited (*ie*. $k_c \neq 0$), as had been presumed in a previous, limited study on DiMe-β-CD.[57,66] The data are described well by equation [3], with k_c now being lower than k_u. The experimental values of k_u and fitted values of K_s and k_c are summarised in Table 16.

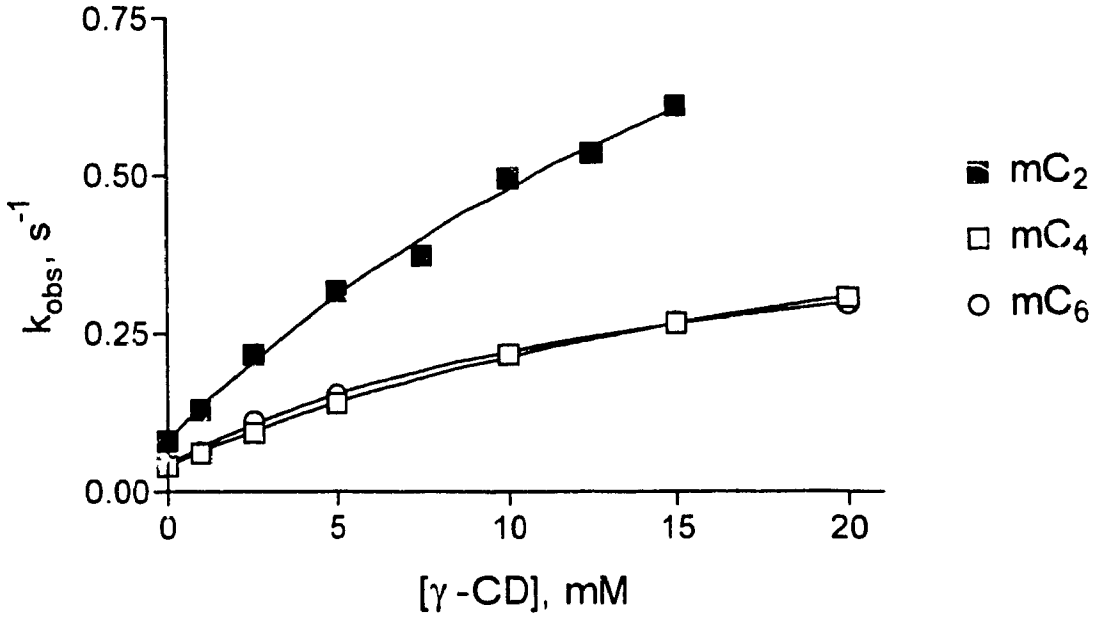


Figure 11. Simple saturation type dependence of k_{obs} on [γ-CD] for the cleavage of mNPAlk.

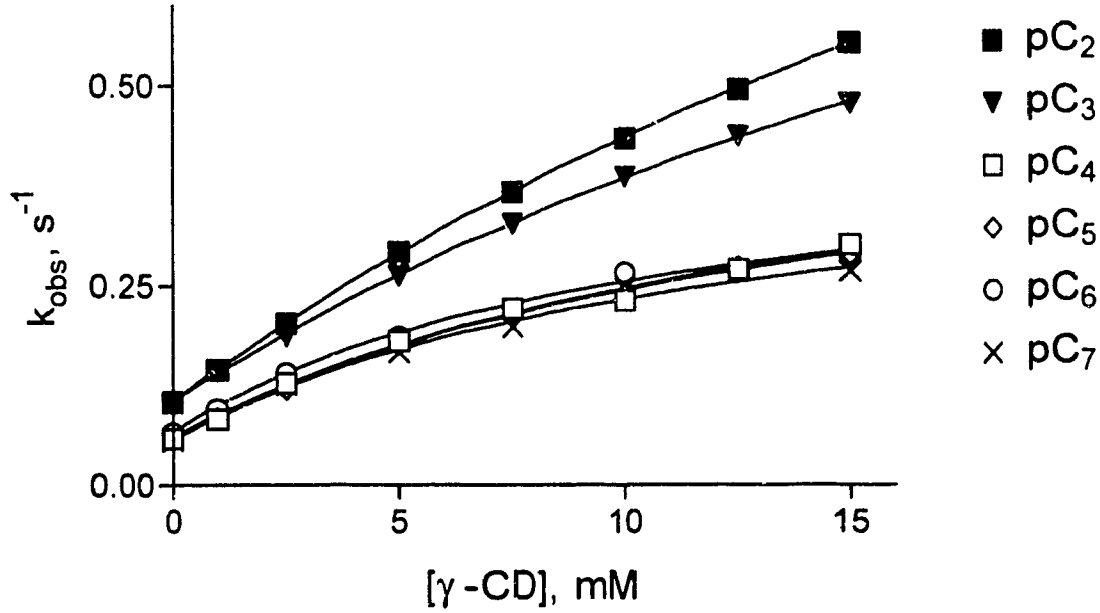


Figure 12. Simple saturation type dependence of k_{obs} on [γ-CD] for the cleavage of pNPAlk.

Table 15. Constants for the Cleavage of *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by γ-Cyclodextrin.[a,b]

Ester	k_u s^{-1}	k_c s^{-1}	K_s mM
m-Nitrophenyl alkanoates			
C_2	0.0805	1.39 ± 0.10	22.6 ± 2.6
C_4	0.0401	0.624 ± 0.027	23.7 ± 1.7
C_6	0.0414	0.482 ± 0.077	14.4 ± 1.7
p-Nitrophenyl alkanoates			
C_2	0.104	1.61 ± 0.04	35.6 ± 1.3
C_3	0.105	1.28 ± 0.05	31.9 ± 1.8
C_4	0.0589	0.530 ± 0.032	14.7 ± 1.7
C_5	0.0594	0.515 ± 0.034	14.6 ± 1.8
C_6	0.0660	0.443 ± 0.010	10.7 ± 0.5
C_7	0.0558	0.434 ± 0.017	12.0 ± 1.0

[a] In aqueous phosphate buffer (0.2 M) at pH 11.60 and 25.0 ± 0.1 °C. Values of k_u are those obtained experimentally while those of k_c and K_s were obtained by non-linear least squares fitting of equation [3] to the observed data. The quoted errors are the standard error obtained from the fitting. [b] Reference 65.

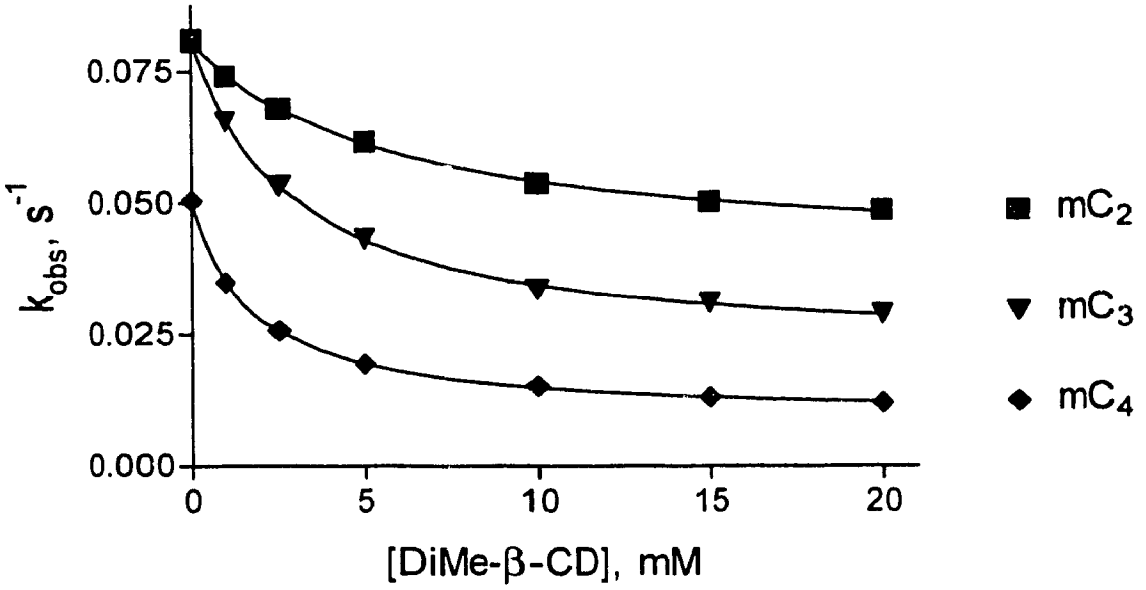


Figure 13. Simple saturation type dependence of k_{obs} on [DiMe-β-CD] for short mNPAlk.

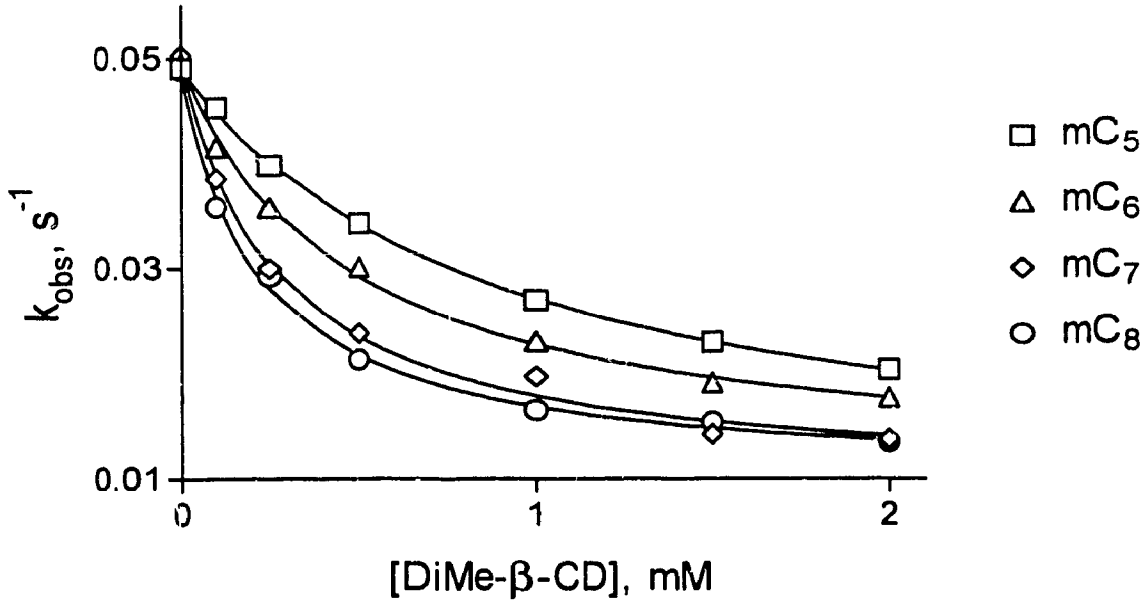


Figure 14. Simple saturation type dependence of k_{obs} on [DiMe-β-CD] for longer mNPAlk.

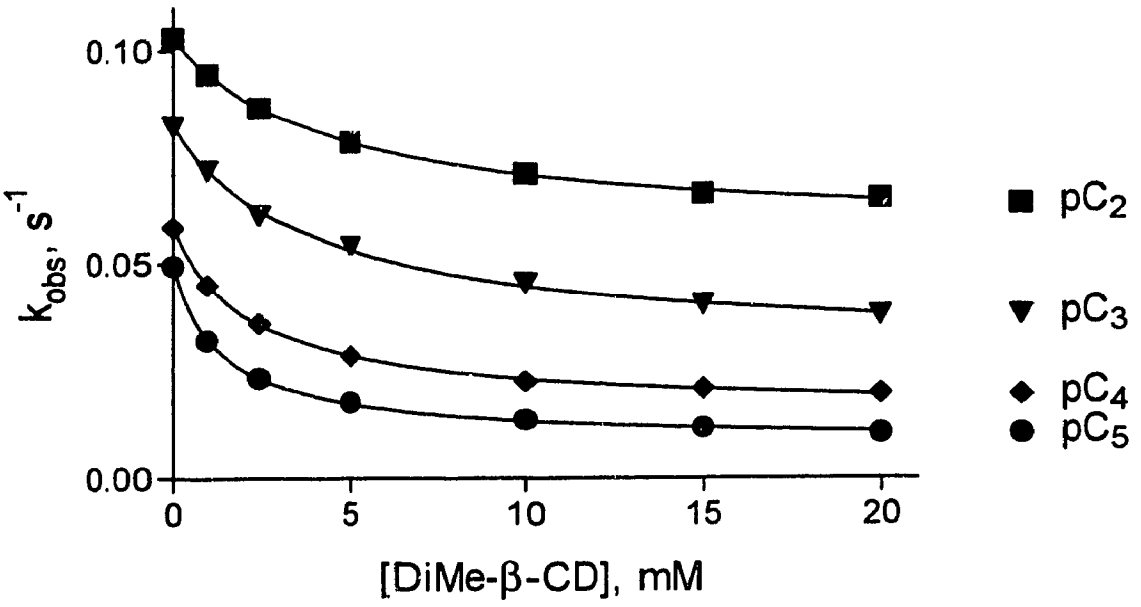


Figure 15. Simple saturation type dependence of k_{obs} on [DiMe-β-CD] for short pNPAlk.

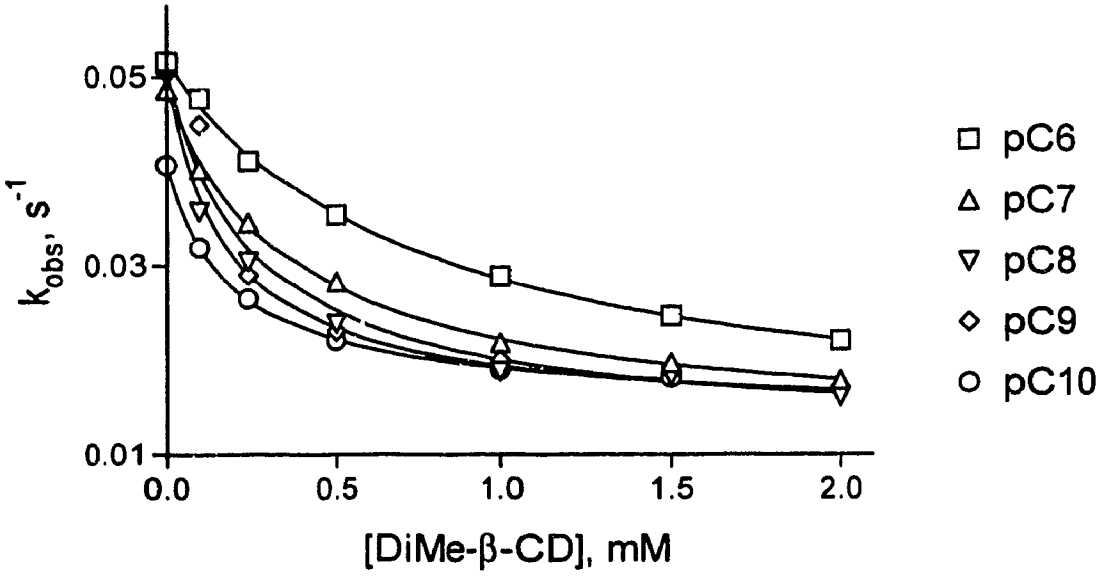


Figure 16. Simple saturation type dependence of k_{obs} on [DiMe-β-CD] for longer pNPAlk.

Table 16. Constants for the Cleavage of *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by "Dimethyl-β-cyclodextrin".[a,b]

Ester	k_u s^{-1}	k_c s^{-1}	K_s mM
m-Nitrophenyl alkanoates			
C_2	0.0807	0.0392 ± 0.0006	5.66 ± 0.24
C_3	0.0812	0.0210 ± 0.0005	2.88 ± 0.08
C_4	0.0504	0.00887 ± 0.00017	1.69 ± 0.03
C_5	0.0491	0.00650 ± 0.00004	0.942 ± 0.024
C_6	0.0497	0.0102 ± 0.0012	0.467 ± 0.041
C_7	0.0503	0.00933 ± 0.00119	0.264 ± 0.028
C_8	0.0489	0.00983 ± 0.00075	0.219 ± 0.017
p-Nitrophenyl alkanoates			
C_2	0.103	0.0558 ± 0.0006	4.64 ± 0.18
C_3	0.0823	0.0292 ± 0.0011	4.15 ± 0.28
C_4	0.0586	0.0150 ± 0.0003	2.27 ± 0.06
C_5	0.0496	0.00824 ± 0.00031	1.42 ± 0.05
C_6	0.0516	0.0108 ± 0.0012	0.767 ± 0.060
C_7	0.0487	0.0122 ± 0.0007	0.378 ± 0.022

(*continued ...*)

C_8	0.0505	0.0138 ± 0.0010	0.176 ± 0.020
C_9	0.0505	0.0149 ± 0.0004	0.160 ± 0.009
C_{10}	0.0406	0.0149 ± 0.0020	0.197 ± 0.006

[a] In aqueous phosphate buffer (0.2 M) at pH 11.60 and 25.0 ± 0.1°C. Values of k_u are those obtained experimentally while those of k_c and K_s were obtained by non-linear least squares fitting of eqn. [3] to the observed data. The quoted errors are the standard error obtained from the fitting. [b] Reference 65.

DiMe-β-CD showed no evidence of forming 1:2 ester:CD complexes, reactive or unreactive, even at high [DiMe-β-CD].[J]

2.3 Discussion

The strategy behind this project is the same as was used in the study of the cleavage of mNPAlk and pNPAlk by α-CD and β-CD.[58] If the reaction occurs through a transition state where the aryl group is bound in the CD cavity (Figure 6A), then any kinetic parameters which are dependent on this binding process should not be sensitive to the length of the acyl chain, and should be dependent on whether the NO_2 group is *para* or *meta*. Conversely, if the transition state for the rate limiting process occurs via inclusion of the acyl group (Figure 6B), then the appropriate parameters should be dependent on the length of the acyl chain, and not the location of the nitro substituent.

2.3.1 Substrate Binding (K_s & K_{s2})

The ability of *m*-nitrophenyl alkanoates and *p*-nitrophenyl alkanoates to form stable host-guest complexes is very similar with each CD (Table 13 to 16), and virtually identical in the case of Hp-β-CD, although there is a marked difference between the CDs (Figures 17 & 18).

[J] Recent work by other researchers in our lab has shown that DiMe-β-CD does actually form 2:1 (ester.CD) complexes, but only at high [DiMe-β-CD].

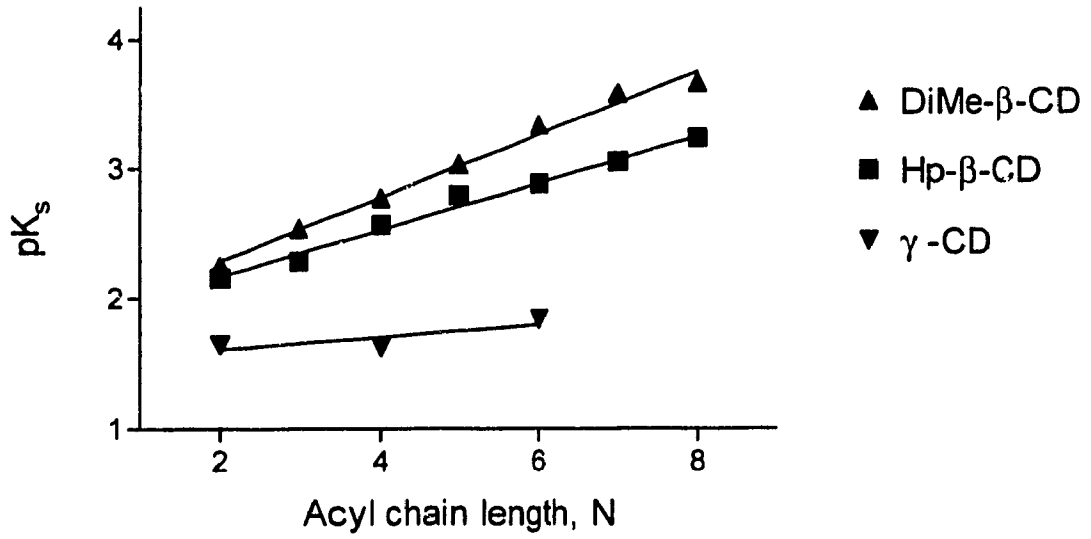


Figure 17. Dependence of *m*-nitrophenyl alkanoate binding strength to CDs (pK_s) on acyl chain length.

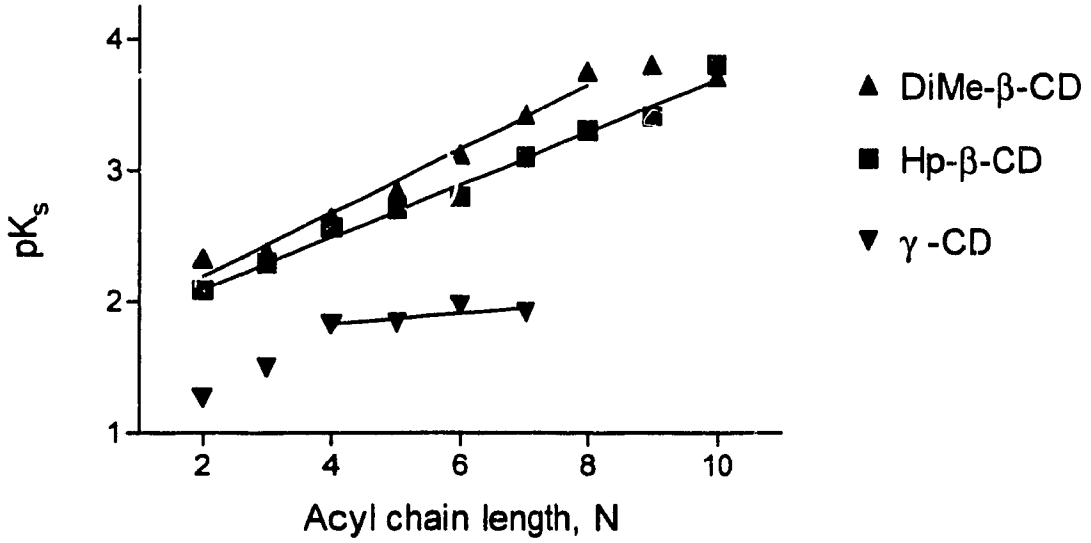


Figure 18. Dependence of *p*-nitrophenyl alkanoate binding strength to CDs (pK_s) on acyl chain length.

The stability of the complexes formed by the binding of mNPAlk and pNPAlk to Hp-β-CD shows a linear dependence on the length of the acyl chain over the whole range of esters examined. This trend in behaviour is similar to what has been observed with α-CD and β-CD[58,99] and is attributed to the binding of the esters to the CD occurring via their acyl group, for esters longer than the acetate. The strength of binding of the esters to Hp-β-CD is virtually identical to that with β-CD,[102] and will be discussed in detail in Chapter 4. As the length of the acyl chain, N, is increased, both the size of the ester and its hydrophobicity increase monotonically, making the relationship between N and pK_s (= -log K_s) a linear free energy relationship (LFER), as previously discussed in terms of the binding of these same esters to α-CD and β-CD.[81,93] The LFERs between pK_s and N are given below (equations [8] and [9]) and shown as solid lines on Figures 17 and 18, respectively.

mNPAlk + Hp-β-CD: $pK_s = (0.180 \pm 0.009)\ N + (1.81 \pm 0.05)$

$(r = 0.993;\ 7\ points)$ [8]

pNPAlk + Hp-β-CD: $pK_s = (0.201 \pm 0.009)\ N + (1.70 \pm 0.06)$

$(r = 0.993;\ 9\ points)$ [9]

One feature of the binding of mNPAlk and pNPAlk to Hp-β-CD that is different from all the other CDs, so far investigated, is that with certain nitrophenyl esters (*m*-C_8 to *m*-C_{10} and *p*-C_{10}) there appears to be the formation of a 1:2

ester:CD complex (see footnote, p. 79). This type of behaviour has previously been seen with carboxynitrophenyl alkanoates and α-CD (K_{s2} ~ 20 mM) and β-CD (K_{s2} ~ 50 mM).[58] The K_{s2} values for the mNPAlk and pNPAlk with Hp-β-CD are ~ 20 - 70 mM,[k] and most probably correspond to the binding of the aryl group to the second molecule of CD. One possible explanation why this behaviour has not been observed with α-CD and β-CD is that they are much less soluble in water than HP-β-CD which prevents preparing solutions with a high enough [α-CD] or [β-CD] to detect the 1:2 complexes. Another possible explanation is that when Bonora *et al.*[99] analyzed their data to obtain K_s values they used a Lineweaver-Burke type approach which assumes that complex formation is only 1:1, and they used low [CD] (≤ 5 mM).

The trends in binding behaviour of the *m*-nitrophenyl alkanoates with DiMe-β-CD are similar to that observed with Hp-β-CD (Figure 17), with DiMe-β-CD binding the esters more strongly than either β-CD or Hp-β-CD. The pK_s values increase monotonically with N, and the LFER is given by equation [10] (shown as the solid line in Figure 17). By comparing equations [8] and [9] with [10] and [11] we can see that there is a stronger dependence of pK_s on N with DiMe-β-CD than with Hp-β-CD.

---

[k] The K_{s2} value of 76 mM for *m*-C_8 may not be very accurate since it lies well outside of the range of [Hp-β-CD] used in this study.

mNPAlk + DiMe-β-CD: $pK_s = (0.245 \pm 0.012)N + (1\ 79 \pm 0.06)$

$(r = 0.994;\ 7\ points)$ [10]

pNPAlk + DiMe-β-CD: $pK_s = (0.244 \pm 0.017)N + (1.71 \pm 0.09)$

$(r = 0.988;\ 7\ points)$ [11]

The situation with the binding of *p*-nitrophenyl alkanoates by DiMe-β-CD is not a clear as with the *meta* isomers. Although there is virtually identical behaviour up to *p*-C_7, with a linear relationship between pK_s and N as given by Equation 11 (solid line in Figure 18), after the heptanoate, the pK_s values level off. This levelling off is also seen in the transition state binding (*vide infra*), and has been seen with longer (>C_8) alkanes and aliphatic surfactants binding with α-CD and β-CD,[103-105] and can be ascribed to the finite depth of the CD cavity. A possible reason for the lack of a plateau in pK_s with the other CDs could be that in DiMe-β-CD, the *O*-methylated primary hydroxy groups arrange themselves in a way which would maximize hydrophobic-hydrophobic interactions, creating an intrusive floor on the CD cavity, thus preventing penetration past 6 or 7 methylene units. Since inclusion of the *meta* and *para* esters occurs via the acyl group, the levelling-off of pK_s should also be observed in the binding of longer *m*-nitrophenyl alkanoates to DiMe-β-CD, however, it is unclear from Figure 17 whether the point for the C_8 has begun to form a plateau or if it is part of the straight line, since the series could only be examined up to the octanoate.

In contrast to the behaviour reported for α-CD, β-CD,[58] Hp-β-CD,[90] and DiMe-β-CD,[65] the binding of mNPAlk and pNPAlk to γ-CD seems to exhibit almost no dependence[1] on chain length (Figures 17 and 18), and the binding of the esters is much weaker than any of the other four CDs. The K_s values are also hardly different for the two series of esters (Table 15) and so it is concluded that formation of the ester.CD complex occurs via inclusion of the aryl moiety of the ester, up to at least the C_7. However, it is conceivable that inclusion does occur via the acyl group, but since the γ-CD cavity is large, the fit of the acyl chain is very loose, resulting in a LFER with a very shallow slope.

2.3.2 Introduction to the Kurz Method

Before discussing the rate accelerations (and retardations) found in the present studies with cyclodextrins, it would be worthwhile to introduce the concept of transition state stabilisation. Following the initial ideas of Haldane[106] and Pauling,[107] it is largely accepted that the catalysis afforded by an enzyme to a reaction arises through the stabilisation of the transition state of the enzymatic

---

[1] As determined by the statistical package Prism (GraphPad Software, San Diego, CA), using the following algorithm: Is the slope significantly different than zero? Prism reports the P value testing the null hypothesis that the overall slope is zero. The P value answers this question: If there were no linear relationship between X and Y overall, what is the probability that randomly selected points would result in a regression line as far from horizontal as we observed? The P value is calculated from an F test.

reaction.[108-110] This is most probably the mechanism by which otner catalysts work,[81] therefore any method that allows us to probe the effect of various reaction parameters on transition state stabilisation is a useful tool to the physical organic chemist.

Kurz developed a method[111] based on transition state theory which allows one to estimate the stabilisation afforded to a transition state by a catalyst (enzymatic or non-enzymatic). This method was first used to probe the acidity of the transition state of reactions catalysed by H^+ or ^-OH,[112] and it then found wide-spread although not universal[113] use in enzymology.[109,114-116] The application of this method to cyclodextrin-mediated processes has only begun within the last ten years, and has recently been reviewed.[81,93]

The use of this method, with regards to CD-mediated reactions, begins by considering two processes, one of which is uncatalysed (equation [12]) and one of which is "catalysed" by CD (equation [13]). According to simple transition state theory[87] the rate constant for the uncatalysed reaction, is given by equation [14], while that for the CD-mediated process is given by [15].

$$S \xrightarrow{k_u} P \qquad [12]$$

$$S + CD \xrightarrow{k_2} P \qquad [13]$$

$$k_u = \nu[TS]/[S] \qquad [14]$$

$$k_2 = \nu[TS.CD]/[S][CD] \qquad [15]$$

For mathematical and thermodynamic reasons, TS.CD is considered to be the transition state of the uncatalysed reaction bound to the CD. The frequency of transitions over the energy barrier, ν (= $k_B T/h$), is assumed to be the same for both the uncatalysed and catalysed process, as are the transmission coefficients.[81] With these assumptions, division of equation [14] by [15] affords equation [16], which is the definition of the *apparent* dissociation constant for the process shown in equation [17].

$$K_{TS} = \frac{[TS][CD]}{[TS.CD]} = \frac{k_u}{k_2} = \frac{k_u K_s}{k_c} \qquad [16]$$

$$TS.CD \longrightarrow TS + CD \qquad [17]$$

One important factor regarding the derivation presented above is that *no assumptions regarding mechanism have been made*. This means that we can probe variations in transition state structure by analysing how pK_{TS} (= -log K_{TS}) varies with changes in substrate structure, or some other variable of the reaction.[81,93 112] This treatment loses direct utility if the mechanism of the catalysed and uncatalysed reactions are vastly different. However, it may be able to point out this fact and help in assessing how they differ.[81]

The parameter K_{TS} is a *quasi*-dissociation constant, since the actual dissociation of the TS.CD complex (equation [17]) is unlikely, if not impossible. This parameter does however provide a way to examine the stabilisation afforded to a transition state of a catalysed process, relative to the uncatalysed reaction under standard conditions, as shown in Figure 19. Although K_{TS} may be a

constant of dubious physical nature, as a measure of the stabilisation that the catalyst affords to the transition state, in terms of the Gibbs free energy (equation [18]), it is a "real" parameter.

$$\Delta G^{\circ}_{TS} = -RT \ln K_{TS} \qquad [18]$$

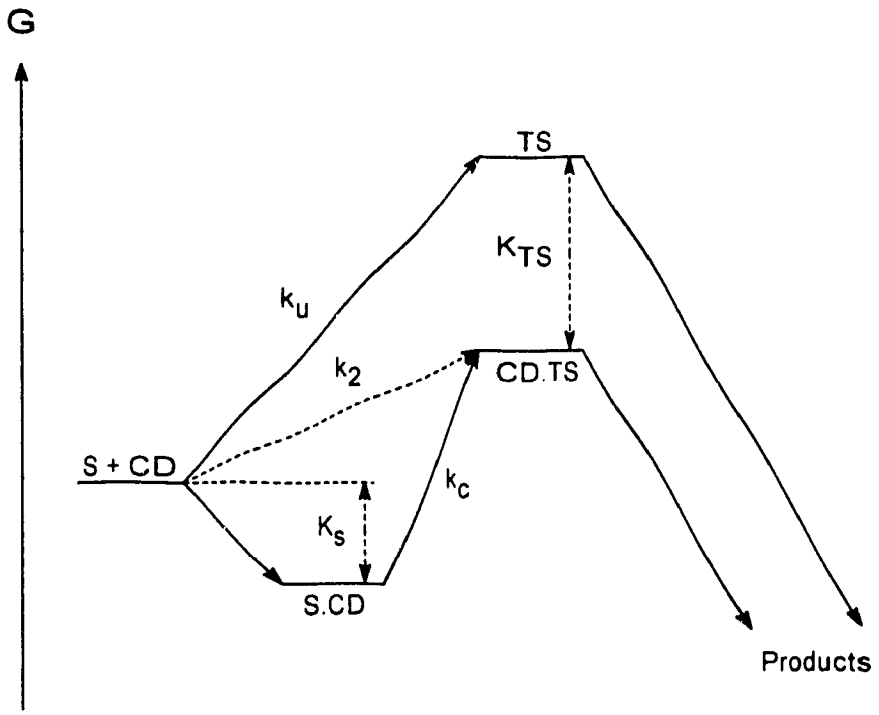


Figure 19. Gibbs free energy diagram for the stabilisation afforded to the transition state by a CD catalyst.

Figure 19 represents the different processes schematically, and is not necessarily to scale. The process labelled k_2 (equation [13]) takes into account the formation of the S.CD and the reaction of the complex (k_c process on Figure 19).

Figure 19 also demonstrates that if we measure the values of k_u, k_c, and K_s (or k_u and k_2), providing that they are measured under the same conditions, we can obtain the relative free energies of the various species, and thus assess the stabilisation afforded to the transition state by the CD, as measured by K_{TS}.

We can see that the main function of the catalyst is to stabilise the transition state, and thus lower the energy required to surmount the overall barrier to reaction. However, if the catalyst binds the substrate very strongly, then there is a decrease in catalysis, since more and more energy is required to surmount the barrier of the k_c process. According to equation [16] the acceleration afforded to a reaction, k_c/k_u, is related to the relative binding energies of the substrate and transition state as given by equation [19].[109]

$$\frac{k_c}{k_u} = \frac{K_s}{K_{TS}} \qquad [19]$$

Therefore a catalyst which binds the substrate more strongly than the transition state, will in fact retard the reaction rather than accelerate it.

2.3.3 Rate Acceleration (k_c/k_u)

The maximum acceleration, k_c/k_u, is a measure of the ratio of the rate constant at saturating [CD] compared to the uncatalysed rate constant. Cleavage of mNPAlk and pNPAlk is accelerated ($k_c > k_u$) by both Hp-β-CD (Table 17 and Figure 20) and γ-CD (Table 18 and Figure 21), as was previously observed for the

Table 17. Derived Constants for the Cleavage of *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by "Hydroxypropyl-β-cyclodextrin".[a]

Ester	k_c/k_u	k_2 $M^{-1}s^{-1}$	K_{TS} mM	$K_{TS'}$ mM
m-Nitrophenyl alkanoates				
C_2	19.0	137	0.367	
C_3	13.7	139	0.370	
C_4	11.1	121	0.242	
C_5	8.07	152	0.202	
C_6	6.60	152	0.201	
C_7	4.67	159	0.192	69.4
C_8	3.66	191	0.160	12.8
C_9	2.36	193	0.158	3.25
C_{10}	1.62	202	0.151	1.66
p-Nitrophenyl alkanoates				
C_2	5.25	41.9	1.56	
C_3	3.74	48.9	1.35	

(continued...)

C_4	3.36	50.6	0.795	
C_5	2.44	54.1	0.796	
C_6	2.10	58.6	0.757	
C_7	1.75	97.1	0.453	68.1
C_8	1.95	173	0.255	39.5
C_9	2.23	251	0.176	31.2
C_{10}	2.10	582	0.0756	6.73

[a] Using values of k_u, k_c, K_s, k_{c2}, and K_{s2} from Table 13 and 14, calculated as follows: $k_2 = k_c/K_s$; $K_{TS} = k_uK_s/k_c$; $K_{TS'} = k_c/k_{c2} = k_cK_{s2}/k_{cc}$.

Table 18. Derived Constants for the Cleavage of *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by γ-Cyclodextrin.[a]

Ester	k_c/k_u	k_2 $M^{-1}s^{-1}$	K_{TS} mM
m-Nitrophenyl alkanoates			
C_2	17.3	61.5	1.31
C_4	15.6	26.3	1.52
C_6	11.6	33.5	1.24
p-Nitrophenyl alkanoates			
C_2	15.5	45.2	2.30
C_3	12.2	40.1	2.62
C_4	9.00	36.1	1.63
C_5	8.67	35.3	1.68
C_6	6.71	41.4	1.59
C_7	7.78	36.2	1.54

[a] Using values of k_u, k_c, and K_s from Table 15, calculated as follows: $k_2 = k_c/K_s$; $K_{TS} = k_uK_s/k_c$.

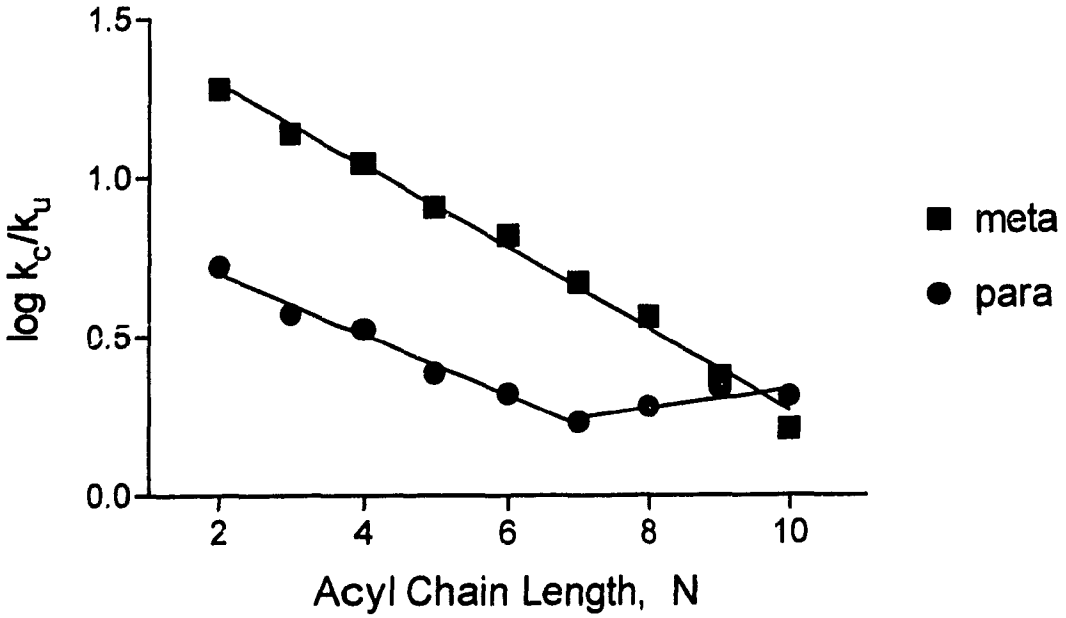


Figure 20. Chain length dependence of log k_c/k_u for the basic cleavage of nitrophenyl alkanoates by Hp-β-CD.

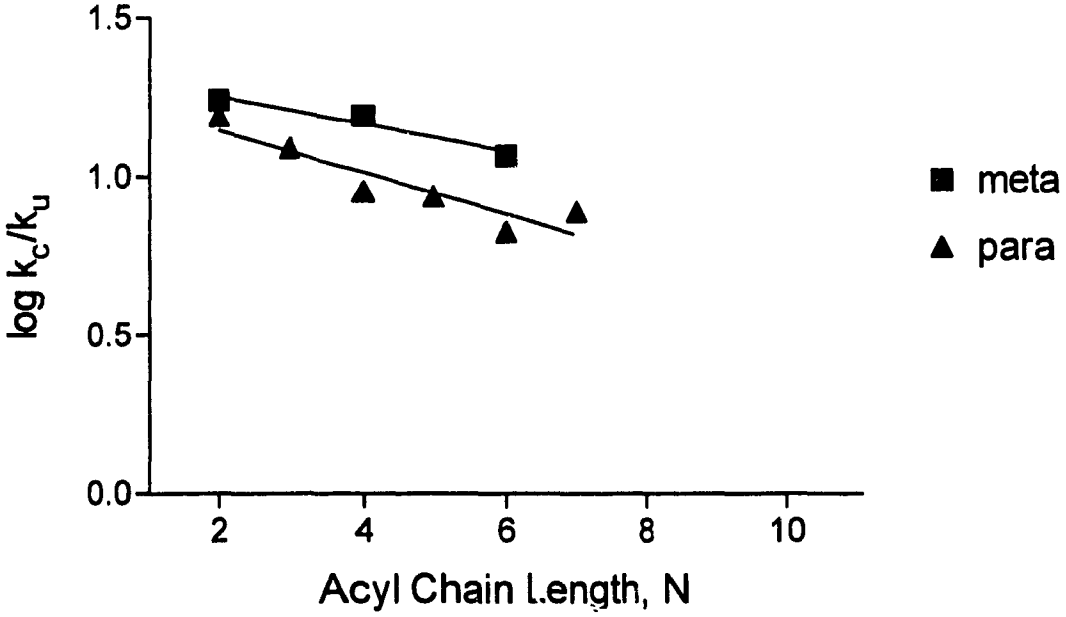


Figure 21. Chain length dependence of log k_c/k_u for the basic cleavage of nitrophenyl alkanoates by γ-CD.

cleavage of these esters in the presence of α-CD and β-CD, however, there were two major differences in acceleratory behaviour. The first lies in the fact that the acceleration afforded to the cleavage of mNPAlk by Hp-β-CD and γ-CD is much less than observed with α-CD and β-CD.[56] The former two CDs only accelerate the rate of cleavage of mNPA by factors of 19[90] and 17,[65] respectively, whereas the later two accelerate the cleavage of mNPA by factors of 290 and 62,[58] respectively. Secondly, both α-CD and β-CD generally show a much larger acceleration in the cleavage of mNPAlk relative to pNPAlk (below the C_{10}), whereas with Hp-β-CD the difference in acceleration is somewhat smaller, and γ-CD appears to make virtually no distinction between the two isomeric esters.

The cleavage of mNPAlk and pNPAlk is retarded by the addition of DiMe-β-CD (Table 19 and Figure 22), a mode of behaviour which is opposite to that of all other CDs studied to date with the same esters.[58,65,90] The retardation most likely arises from the fact that some of the nucleophilic secondary hydroxy groups of DiMe-β-CD have been *O*-methylated, thus hindering the attack of ionized CD hydroxy groups on the substrate. Furthermore, the cleavage of mNPAlk, which is normally more reactive towards β-CD,[58] is retarded to a further extent than the cleavage of pNPAlk. For example, the cleavage of the *m*-C_6 ester by β-CD is accelerated by a factor of 27, whereas DiMe-β-CD retards the same reaction by a factor of 4.9, so that the overall reduction in k_c is approximately 130 fold. For the isomeric *para* ester, the overall reduction in k_c is only 18 fold. DiMe-β-CD appears to wipe out the preference of β-CD for the *meta* esters, as discussed above.

Table 19. Derived Constants for the Cleavage of *m*-Nitrophenyl and *p*-Nitrophenyl Alkanoates by "Dimethyl-β-cyclodextrin".[a]

Ester	k_c/k_u $M^{-1}s^{-1}$	k_2 mM	K_{TS}
m-Nitrophenyl alkanoates			
C_2	0.486	6.93	11.7
C_3	0.259	7.29	11.1
C_4	0.176	5.25	9.60
C_5	0.132	6.90	7.12
C_6	0.205	21.8	2.28
C_7	0.185	35.3	1.42
C_8	0.201	44.9	1.09
p-Nitrophenyl alkanoates			
C_2	0.542	12.0	8.56
C_3	0.355	7.04	11.7
C_4	0.256	6.61	8.86
C_5	0.166	5.80	8.54

(continued...)

C_6	0.209	14.1	3.66
C_7	0.251	32.3	1.51
C_8	0.273	78.4	0.645
C_9	0.295	93.1	0.542
C_{10}	0.367	75.6	0.537

[a] Using values of k_u, k_c, and K_s from Table 16, calculated as follows: $k_2 = k_c/K_s$; $K_{TS} = k_u K_s/k_c$.

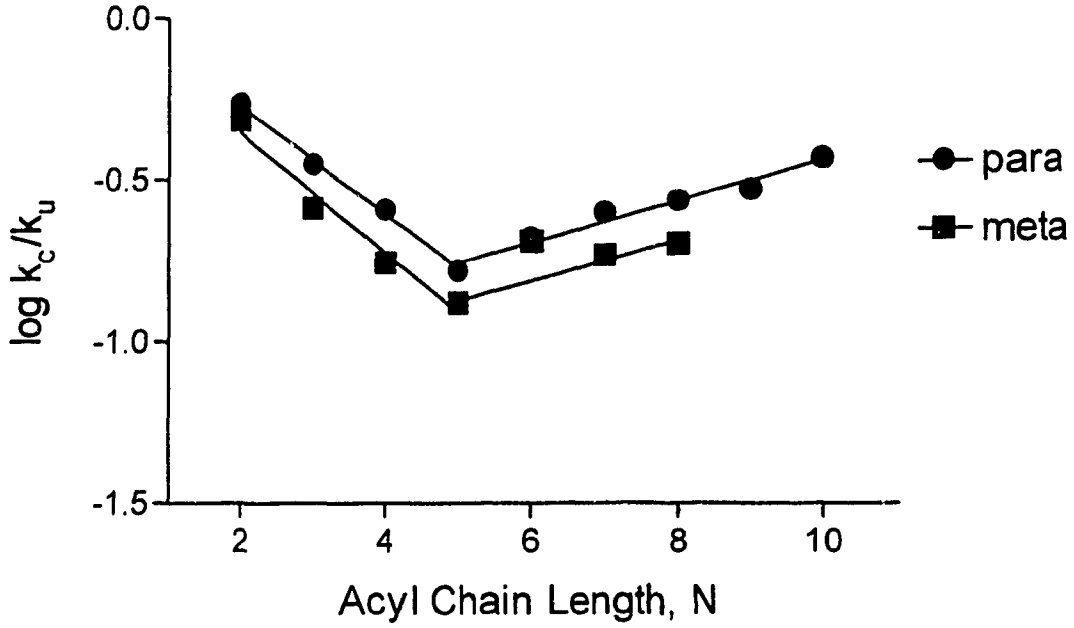


Figure 22. Chain length dependence of log k_c/k_u for the basic cleavage of nitrophenyl alkanoates by DiMe-β-CD.

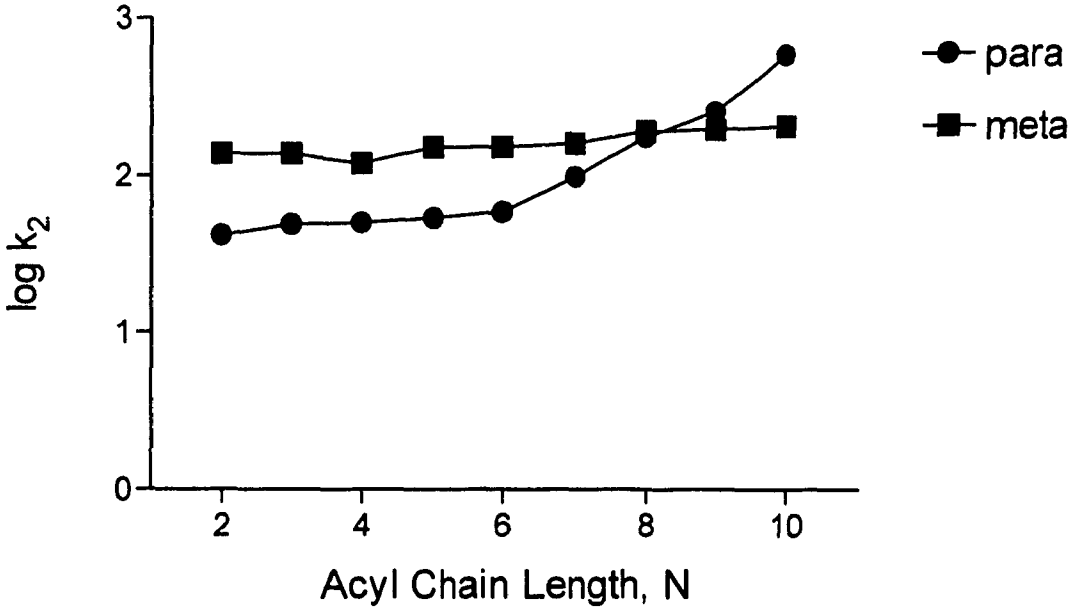


Figure 23. Chain length dependence of the substrate selectivity for the basic cleavage of nitrophenyl alkanoates by Hp-β-CD.

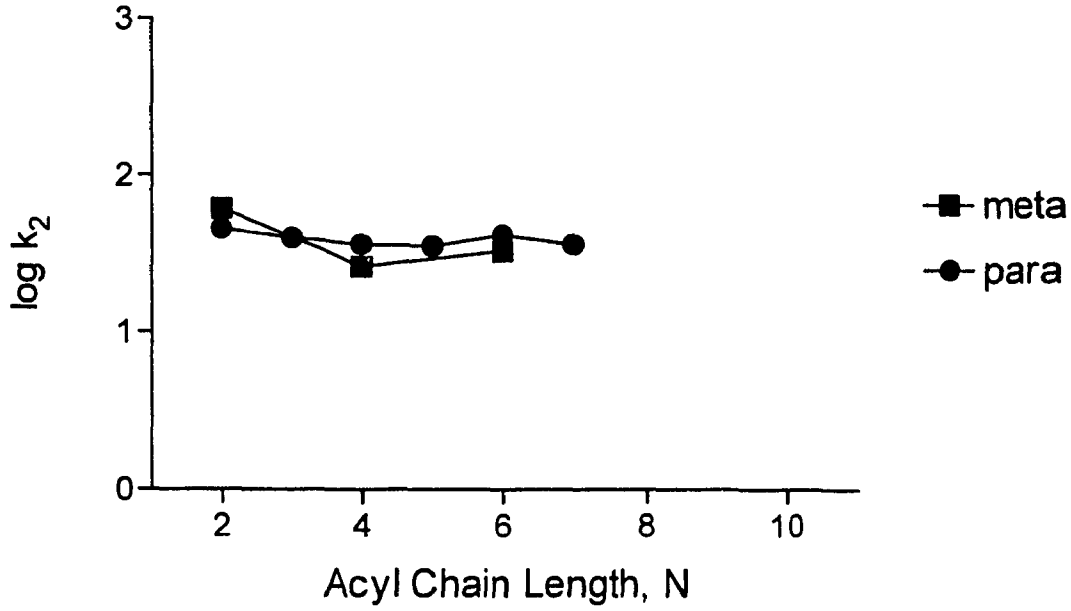


Figure 24. Chain length dependence of the substrate selectivity for the basic cleavage of nitrophenyl alkanoates by γ-CD.

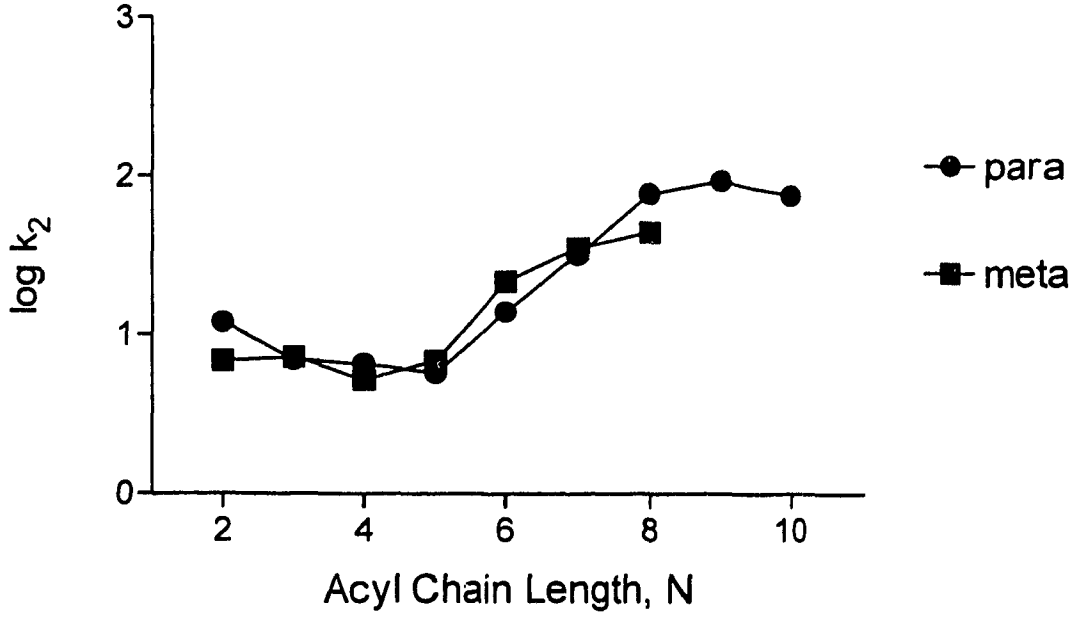


Figure 25. Chain length dependence of the substrate selectivity for the basic cleavage of nitrophenyl alkanoates by DiMe-β-CD.

For mNPAlk reacting with Hp-β-CD there is a monotonic decrease in log k_c/k_u over the whole range of esters examined (Figure 20). The behaviour of pNPAlk is markedly different, in that log k_c/k_u decreases up to the *p*-C_7, at which point it begins to rise (Figure 20). Both series of esters reacting with γ-CD exhibit a decrease in log k_c/k_u over the range examined (Figure 21), however, the dependence on N is very shallow (Figures 20 through 22 have been drawn on the same scale for easier comparison). Both series of ester reacting with DiMe-β-CD also exhibited a similar, biphasic behaviour. Log k_c/k_u decreases monotonically up to the C_5, after which it rises steadily for the remainder of the range. The biphasic behaviour observed in three of the above cases is suggestive of a change in mechanism occurring at the inflection point, which will be discussed later in this chapter.

According to equation 19, the acceleration by the CD is due to the ratio of the binding of the transition state relative to the binding of the initial state. In terms of the Kurz approach (and equation [19]) and the monotonic dependence of pK_s on N (Figures 17 and 18), deviations in behaviour away from linearity must be due to processes affecting the binding in the transition state, a feature which will be discussed shortly.

2.3.4 Substrate Selectivity

The selectivity of a catalyst for various substrates can be measured in terms of the second order rate constant, k_2 (= k_c/K_s), for the process outlined in equation

[13]. We can see from the data for the cleavage of the alkanoate esters by Hp-β-CD (Table 17 and Figure 23, p. 96) that this CD is more reactive with the *meta* esters up to *m*-C_8, at which point the *para* esters become more reactive. Another feature of Figure 23 is that while log k_2 for the cleavage of mNPAlk is relatively constant, it shows two distinct modes of behaviour for pNPAlk, with log k_2 being constant up to *p*-C_6, after which it rises sharply. γ-CD appears to have no selectivity towards the *meta* and *para* isomers, with log k_2 remaining essentially constant, and virtually the same for both series of ester (Table 18 and Figure 24, p. 97). DiMe-β-CD appears to be equally reactive towards mNPAlk and pNPAlk up to about C_7, after which a slight preference seems to exist for the *para* isomers, and the trends in log k_2 for the reaction of DiMe-β-CD with the esters shows a more complicated behaviour than any of the other CDs (Table 19 and Figure 25, p. 97). Log k_2 remains relatively constant and similar for both series of esters up to the C_5, at which point it starts to rise sharply. Both series of esters seem to level off at around the C_8, although the break is much more evident for the *para* isomers.[m]

In the present case of mNPAlk and pNPAlk, the esters have similar reactivities, and thus pose no problem in comparing the k_2 values. However, if the intrinsic reactivities of the esters differed markedly, then the comparison of k_2, which does not correct for this, could be problematic.[81] A more prudent approach

---

[m] This is probably due to the fact that the series of *meta* esters was limited compared to the *para* esters.

would be to look at the binding in the transition state, K_{TS} (equation [16]), where the intrinsic reactivities have been accounted and corrected for.

2.3.5 Transition State Stabilisation (K_{TS})

In order to explain the mechanism of the cleavage reaction in the presence of Hp-β-CD, γ-CD, and DiMe-β-CD, we will now look at the stabilisation afforded to the transition state by these CDs, and compare them to the results previously obtained with α-CD and β-CD.[58]

With α-CD as the "catalyst" there is a clear distinction between the modes of transition state binding for the cleavage of mNPAlk (Figure 26) and pNPAlk (Figure 27), as evidenced by the dependence of pK_{TS} (= -log K_{TS}) on chain length. For the cleavage of the *meta* compounds by α-CD there is virtually no dependence of pK_{TS} on the acyl chain length of the esters (slope = 0.04 ± 0.03), which is markedly different from the behaviour of the *para* esters, where the transition state stabilisation increases linearly (slope = 0.25 ± 0.01) with the length of the acyl chain, past *p*-C_2. The slope of the dependence of the transition state stabilisation on acyl chain length N is very similar to the slope of pK_s vs N (~ 0.20).[58] This difference in behaviour has been ascribed[58] to different modes of transition state binding; the *m*-nitrophenyl alkanoates reacting via aryl inclusion (Figure 6A) while the *p*-nitrophenyl alkanoates react via acyl inclusion (Figure 6B). β-CD exhibits the same trend with the mNPAlk, whereas the trend with the pNPAlk is not as clear cut, as shown in Figure 27. There is a general upwards drifting of the pK_{TS} values

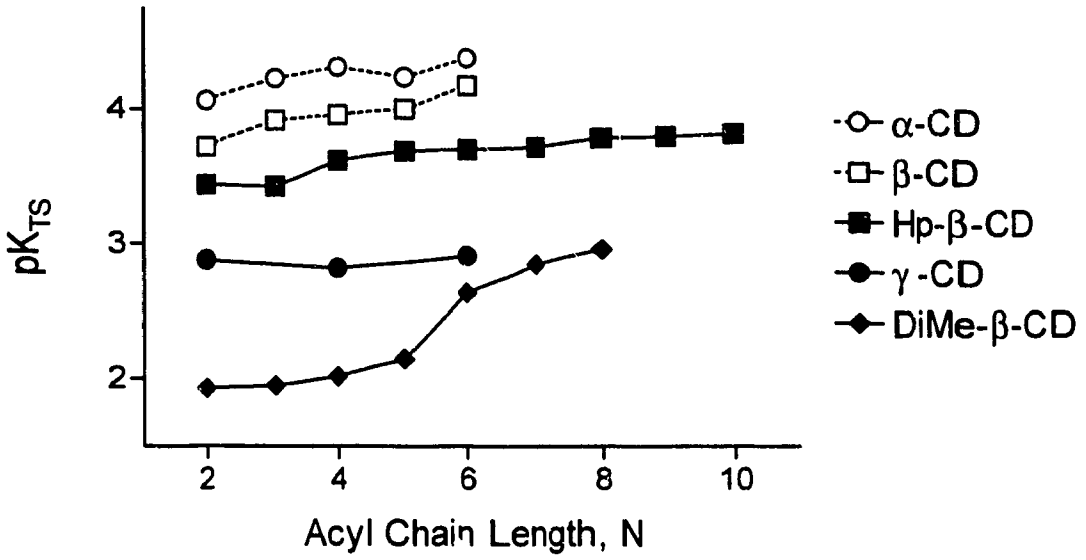


Figure 26. Chain length dependence of transition state stabilisation (pK_{TS}) for the basic cleavage of mNPAlk by five CDs.

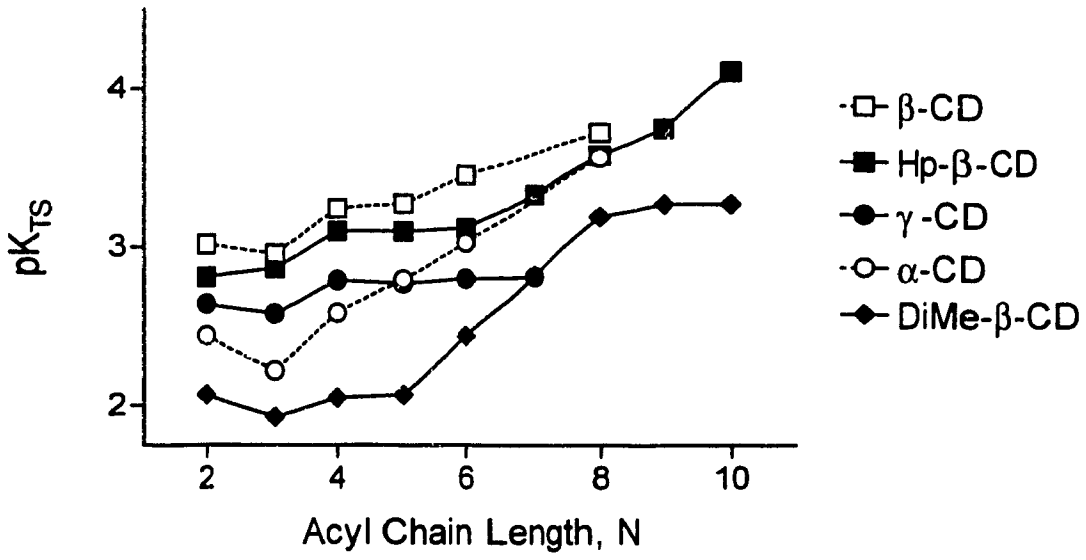


Figure 27. Chain length dependence of transition state stabilisation (pK_{TS}) for the basic cleavage of pNPAlk by five CDs.

with acyl chain length; however, there is a break in the graph with the longer *para* esters[99] (C_8 and C_{12}; the latter is not included on Figure 27).

For the Hp-β-CD mediated cleavage of mNPAlk, the trend in behaviour is virtually the same as observed with α-CD and β-CD, except that the transition state binding is about two thirds of a pK unit weaker. There is a very small upward drift in the pK_{TS} values with increasing N, but the values are virtually constant past the *m*-C_3. With the pNPAlk, there is a slow upwards drift of pK_{TS} up to the *p*-C_6 at which point there is a change in behaviour and the pK_{TS} values begin to rise sharply (slope = 0.24 ± 0.02), with the same slope as the plot of pK_s vs N (equation [9]). These observations are taken to mean that the *meta* esters (*m*-C_2 to *m*-C_{10}) and some *para* esters (< *p*-C_6) react through a transition state involving aryl group inclusion, while the cleavage of the remaining esters (> *p*-C_6) passes through a transition state where the longer, hydrophobic, acyl chain in included in the CD cavity.

With regards to the cleavage of the *meta* esters, γ-CD behaves in a manner similar to what has already been discussed, with the stabilisation afforded to the transition state by inclusion being the least of all the cases where the addition of CD accelerated the reaction (Figure 26). This weak binding can be attributed to the large cavity size of γ-CD, which precludes the "tight" formation of a host-guest complex. γ-CD differs from the previous three CDs in regards to the stabilisation of the transition state for the cleavage of the *para* esters, where pK_{TS} shows no dependence on acyl chain length, and is virtually constant up to the *p*-C_7 (Figure

27). This is either due to the reaction of the esters with γ-CD occurring through aryl group inclusion, or if the reaction occurs through acyl group inclusion then the fit is so poor that the stabilisation is the same regardless of the chain length.

Since DiMe-β-CD retards the rate of esterolysis, one would not be surprised if the trends in transition state stabilisation for this CD were vastly different to the other four CDs. Surprisingly, the trends in stabilisation afforded to the transition state for the cleavage of pNPAlk by DiMe-β-CD, are remarkably similar, although significantly weaker, than α-CD, β-CD, and Hp-β-CD (Figure 27). There is an initial flat region where pK_{1S} remains relatively constant (up to the *p*-C_5), after which point there is a break in the graph, and the pK_{TS} values then begin to rise (slope = 0.375 ± 0.002) at a rate significantly faster than the binding of the esters to the CD in the initial state. The increasing trend in pK_{TS} ceases at *p*-C_8, after which the value remains constant. This triphasic behaviour is mirrored in the dependence of log k_2 on acyl chain length (Figure 25), and accounts for the sharp bend in the plot of log k_c/k_u vs N. This changeover arises from the cleavage of the shorter esters (< *p*-C_5) occurring via aryl inclusion and the cleavage of the longer esters (> *p*-C_5) taking place by acyl inclusion, as discussed previously for the other CDs. The plateau region for the long esters is most likely the result of an intrusive floor formed by the *O*-methylated primary hydroxy groups (*vide supra*).

The most striking difference between DiMe-β-CD and the other CDs lies in the stabilisation of the transition state for the cleavage of the *m*-nitrophenyl

alkanoates. Not only is the binding significantly weaker with the *m*-C_2,[n] there is also a *linear dependence* of pK_{TS} vs N for the cleavage of mNPAlk (> C_5) by DiMe-β-CD. There is a shallow dependence up to *m*-C_5, after which it rises with a slope of 0.26 ± 0.06 virtually the same as the slope for the binding of the esters to DiMe-β-CD (equation [10]) in the initial state. It seems that the steric constraints due to the *O*-methylation of the secondary hydroxy groups favours transition state formation, for the cleavage of the longer esters (> *m*-C_5), via acyl group inclusion *rather than* aryl group inclusion, a phenomenon that has been unobserved with mNPAlk up until now.

Both initial state and transition state binding processes of the esters to DiMe-β-CD show a plateau region with the longer esters. This is not surprising since one would expect the *O*-methyl groups to impede, if not hinder, the penetration of the acyl portion of the ester into the CD cavity. The proposal for formation of an intrusive floor on the CD cavity has already been made for esters reacting with other β-CD derivatives, where modification occurs on the primary hydroxy groups.[78,117,118] What is surprising is that there is *no plateau* region in the corresponding plots for Hp-β-CD. We have taken this to mean that the 2-hydroxypropyl groups do not intrude into the cavity and only serve to extend it.[65] In support of this view is the fact that the strength of binding of the esters in the

---

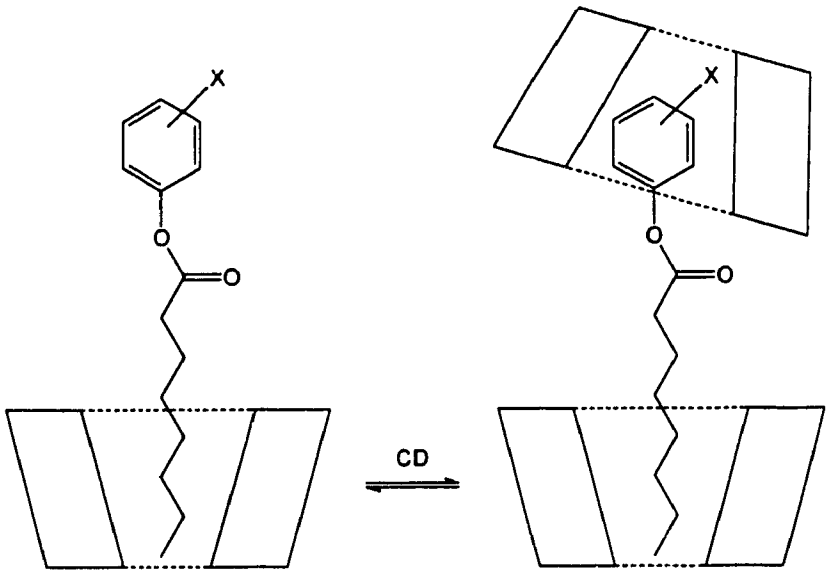
[n] The transition state for the cleavage of the *m*-C_2 ester is stabilised ~5.7 kJ mol^{-1} less by DiMe-β-CD compared to γ-CD and ~11.5 kJ mol^{-1} less compared to α-CD.

initial state, to Hp-β-CD is virtually identical to that with β-CD (*vide infra*), whereas with DiMe-β-CD the substrate binding is stronger than β-CD, while the transition state binding is weaker.

The above considerations are crucial in the explanation of the retardation of cleavage experienced in the presence of DiMe-β-CD. Recalling that $k_c/k_u = K_s/K_{TS}$, an increase in initial state binding strength (a decrease in K_s) accompanied by a decrease in transition state binding (increase in K_{TS}) predicts that there will be an overall decrease in k_c/k_u and in the case of DiMe-β-CD that results in an overall retardation of the rate of cleavage. The destabilisation of the transition state is most likely the result of steric considerations arising from the *O*-methyl groups adjacent to the nucleophilic centres of the CD.

2.3.6 Reactions Involving two Molecules of Hp-β-CD

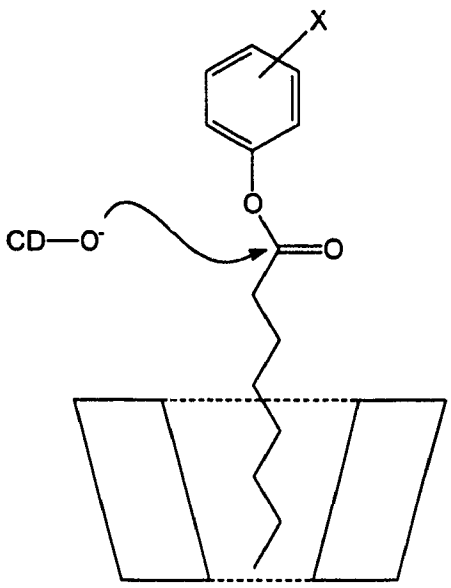
Cleavage reactions involving two molecules of Hp-β-CD are evident within both series of esters (Figures 9 and 10) past the C_6. Presumably these processes are only possible when the acyl chain of the ester is sufficiently long, that after binding to the first CD the ester protrudes from the CD cavity enough to allow for the approach of a second molecule of Hp-β-CD (**39** → **40**). Evidence for the intimate association of a second molecule of Hp-β-CD in the transition state for the rate limiting step arises from the fact that the value of k_{obs} does not level off as would be predicted by simple saturation kinetics.



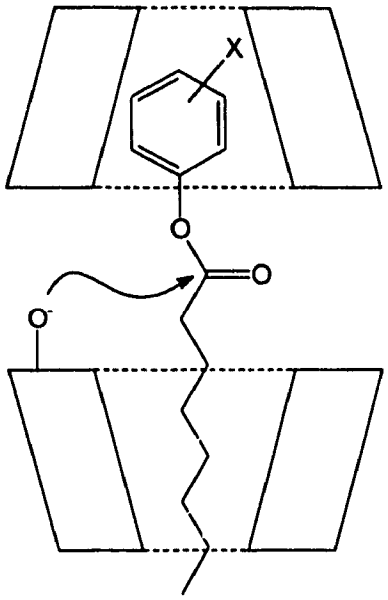
39 **40**

Rate constants for the cleavage of the esters by two CD molecules ($k_{c2} = k_{cc}/K_{s2}$, Table 14) are dependent on the length of the acyl chain of the ester, presumably since the longer the ester, the more accessible the carbonyl group is to an external nucleophile, possibly, but not necessarily, on the second CD molecule (**41**). However, in view of the clear evidence of 1:2 (ester:CD) binding with some of the esters, is seems likely that these esters react via an $S.CD_2$ complex as in **42** or **43**. Deciding which is the actual mechanism is difficult in that all three mechanisms (**41**, **42**, **43**) are kinetically indistinguishable.

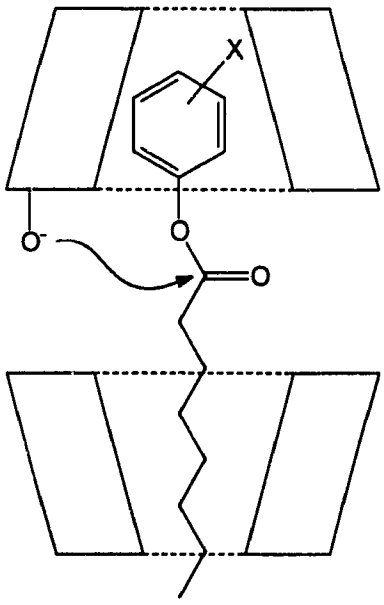
It is worth noting that the second order rate constants, k_{c2}, (Table 14) are larger for the *meta* esters compared to those for the *para* esters, which is contrary to the trend in intrinsic reactivities. This could be caused by the *meta* isomers reacting as in **42** and making use of the superior geometry for reaction due to the



41



42



43

meta nitro group.[90] Consistent with this hypothesis is the fact that the values of k_{cc} for *m*-C_8, *m*-C_9, and *m*-C_{10} are essentially constant.

We can apply the Kurz method to these termolecular processes and obtain an expression for the stabilisation afforded to the transition state by the second CD, $K_{TS'}$, as given by equation [20]. If the first process in the cleavage of these long chain *meta* esters, k_c, corresponds to a reaction occurring via aryl inclusion, as discussed above, then the second process, k_{c2}, must occur with binding of the acyl chain to the second molecule of Hp-β-CD. The decrease in $K_{TS'}$ given in Table 17 converts into a linear dependence of $pK_{TS'}$ on acyl chain length with a very steep slope (= 0.55 ± 0.07; r = 0.984), supporting the argument that the reaction involving the second molecule of HP-β-CD occurs with the binding of the second CD to the acyl chain of the ester (**42** or **43**). There is much less dependence of $pK_{TS'}$ on acyl chain length for the reaction of the *p*-nitrophenyl alkanoates, presumably since the first process already involves the binding of the acyl chain, and therefore the second molecule of CD is limited to binding the aryl moiety.

$$K_{TS'} = \frac{[TS.CD][CD]}{[TS.CD_2]} = \frac{k_c}{k_{c2}} \qquad [20]$$

Another interesting aspect of these ternary $S.CD_2$ complexes is that they are more reactive, as measured by the ratio of k_{cc}/k_c, than their binary counterparts, by

factors of 6, 8, 14, and 3 for *m*-C_8, *m*-C_9, *m*-C_{10}, and *p*-C_{10}, respectively. The apparent enhancements afforded to the *meta* series can be ascribed to a combination of two factors: the improved geometric positioning of a meta substituent *and* the driving force for removal of the hydrophobic acyl chain from the bulk water by complexation,[90] as outlined by the mechanism given in **42**.

2.4 Conclusions

We are able to draw several conclusions from the results presented in this chapter: (a) Both the *m*- and *p*-nitrophenyl alkanoates bind to Hp-β-CD and DiMe-β-CD, by their acyl chains, to form 1:1 host-guest complexes. (b) Binding of both series of esters to the wider γ-CD is less clear, although it appears that inclusion occurs via the aryl group rather than the acyl group. (c) mNPAlk undergo cleavage by Hp-β-CD and γ-CD through a transition state where the ester is bound via its aryl group. (d) Cleavage of the short mNPAlk (< C_5) by DiMe-β-CD occurs via aryl group inclusion whereas the cleavage of the longer mNPAlk (> C_5) occurs via acyl group inclusion. (e) Short pNPAlk (< C_6 - Hp-β-CD; < C_7 - γ-CD; < C_5 - DiMe-β-CD) react less efficiently via aryl group inclusion but longer esters (> C_6 - Hp-β-CD; > C_5 DiMe-β-CD) react more efficiently via acyl group inclusion. (f) The stability afforded to the transition state for the cleavage reaction of pNPAlk by DiMe-β-CD levels off after C_7, probably due to the formation of an intrusive floor by the *O*-methylated primary hydroxy groups. (g) Longer esters (> C_6) undergo cleavage by two molecules of Hp-β-CD, whereas DiMe-β-CD and γ-CD show no

evidence for the involvement of a second CD. (h) For reactions involving two molecules of CD the ternary complexes ($S.CD_2$) are more reactive than the corresponding binary complexes (S.CD), with *meta* isomers most probably undergoing cleavage as shown in **42**. (i) The rate of esterolysis of mNPAlk and pNPAlk is retarded by DiMe-β-CD, but, the reaction is not totally inhibited ($k_c > 0$).

2.5 Experimental

Most of the *p*-nitrophenyl alkanoates were purchased from the Sigma Chemical Company and used without further purification. "Hydroxypropyl-β-cyclodextrin" and sodium phosphate (dibasic, heptahydrate) was obtained from the Aldrich Chemical Company, while γ-CD was purchased, and "dimethyl-β-cyclodextrin" was a gift, from Wacker-Chemie, GmbH, Munich, Germany. All cyclodextrins were used without further purification.

Hydroxypropyl-β-cyclodextrin (molecular substitution = 0.8) supplied by Aldrich Chemical Company has an average molecular weight of 1500 g mol^{-1}, which corresponds to the functionalisation of approximately 6 of the 7 primary hydroxy groups of β-CD. This material is not homogeneous and so the absolute values of measured parameters may vary from sample to sample, but we observed no such variation. However, we feel that trends in parameters are meaningful, especially in the present case where the values of K_s for Hp-β-CD and β-CD are virtually identical (*vide infra*), but the kinetic parameters differ noticeably.

The experiments with Hp-β-CD were carried out in a phosphate buffer nominally pH 11.6. It was later discovered that the pH of this buffer was actually 11.4 (measured using an Accumet combination pH electrode with a silver/silver chloride reference), due to a problem with pH measurements, which accounts for the low values of k_u for the uncatalysed cleavage of the esters, compared to experiments with other CDs. This error in pH does not affect the overall results or conclusions since our interests lie in the comparison of the rates of reaction in the presence and absence of CD, at the same pH. The dissociation constants, K_s, are not dependent on pH within this narrow range.[15-17]

The *m*-nitrophenyl esters along with the *p*-C_7 and *p*-C_9 were all synthesised by mixing 1.1 equivalent of the carboxylic acid with 1.0 equivalent of the nitrophenol and 1.0 equivalent of 1,3-dicyclohexylcarbodiimide in freshly distilled dichloromethane. The solution was refluxed overnight, and then allowed to cool and dicyclohexylurea was filtered off. The volume of the remaining solution was reduced by 50% and any precipitated dicyclohexylurea was again filtered off. The remaining dichloromethane and any remaining dicyclohexylurea were then removed. The resulting solution was a dark brown oil, which was further purified by column chromatography on silica gel, using dichloromethane as the mobile phase and the desired product was in the first pale yellow band. The fractions containing the product were collected and combined, and the dichloromethane was removed by rotary evaporation, yielding the final product (yellow oil or pale yellow solid). The solid *m*-C_{10} was recrystallised from methanol to give pale yellow

needles. The identity and purity of the products were determined by TLC, their NMR spectra, and by the spectral change that occurred on hydrolysis in aqueous base.

The experiments with Hp-β-CD were carried out by 1:1 stopped-flow mixing of a 0.40 M phosphate buffer (pH 11.40) at 25.0 ± 0.1 °C, with the ester (10 - 100 μM for pNPAlk; 4 - 400 μM mNPAlk) dissolved in water or a solution containing Hp-β-CD, so that the final concentrations were half of these. Substrate solutions were made by diluting a 0.1 M stock made in HPLC grade methanol or acetonitrile. The conditions for the experiments with DiMe-β-CD and γ-CD are similar to those listed above except that the buffer was at pH 11.60 and the ester concentrations ranged from 20 - 100 μM for the mNPAlk and 4-50 μM for the pNPAlk. The ester stock solutions were stored in a freezer at -4 °C to guard against degradation.

The concentration of the longer chain esters (> C_5) was of vital importance since aggregate formation can complicate the reaction kinetics. According to Guthrie,[119] a solution that appears clear to the eye may still contain aggregates, especially if the substrate is highly hydrophobic. A first warning of possible aggregate formation was the lack of well defined absorbance value at infinite time (i.e. after 10 half-lives). When this occurred, substrate concentrations were dropped as low as instrument sensitivity allowed. If aggregate formation persisted, the data were analysed using the Kezdy-Swinbourne method, as described later.

The reactions in the presence of Hp-β-CD were followed by monitoring the formation of the product nitrophenolate anion, 390 nm for mNPAlk and 405 nm for

pNPAlk, using a stopped-flow apparatus from Tri-Tech Dynamic Instruments, Winnipeg, Manitoba, Canada. The output of the photomultiplier was fed to a Metrabyte 16F A/D converter, installed in a an Olivetti M24 (IBM 8088 compatible) personal computer. The input voltage was converted to transmittance and the finally into absorbance (A). Usually 100 absorbance values covering 10 half-lives were acquired, or 99.9% reaction completion.

Since the absorbance of nitrophenolate anion is directly related to its concentration (at low concentrations; Beer-Lambert law), the change in absorbance can be used to determine the rate of formation of nitrophenolate anion, and thus the rate of consumption of the substrate. Providing that [ester] « [CD], then the reaction followed pseudo-first order kinetics (equation 21):

$$S \longrightarrow P \qquad [21]$$

$$\text{rate} = -\frac{d[S]}{dt} = k_1[S] \qquad [22]$$

Integration of equation [22]:

$$-\int\frac{d[S]}{[S]} = k_1 \int dt \qquad [23]$$

$$-\ln [S] = k_1 t + c \qquad [24]$$

To find "c", focus on initial conditions, *i.e.* t = 0. Thus:

$$-\ln [S]_o = c \qquad [25]$$

therefore, $$-\ln [S] = k_1 t - \ln [S]_o \qquad [26]$$

or $$\ln [S] = \ln [S]_o - k_1 t \qquad [27]$$

Since we are monitoring the formation of the product, we can use the mass balance relationships given in equations [28] and [29] to convert from substrate to product:

$$[S]_o = [P]_\infty \quad [28]$$

$$[S] = [S]_o - [P] = [P]_\infty - [P] \quad [29]$$

substituting equations [28] and [29] into equation [27]:

$$\ln ([P]_\infty - [P]) = \ln [P]_\infty - k_1 t \quad [30]$$

The concentration of the products is directly proportional to the change in absorbance, as given by equations [31] and [32] (where ε is the extinction coefficient for the nitrophenolate anion):

$$[P]_\infty = (A_\infty - A_o)/\varepsilon \quad [31]$$

$$[P] = (A - A_o)/\varepsilon \quad [32]$$

substituting equations [31] and [32] into equation [30] yields equation [33]:

$$\ln (A_\infty - A) = \ln (A_\infty - A_o) - k_1 t \quad [33]$$

Therefore, by linearly regressing $\ln (A_\infty - A)$ versus time, the pseudo first-order rate constant is obtained as the negative of the slope. The use of this method does not require the knowledge of A_o, however, knowledge of A_∞ is essential.

In the case where reliable estimates of A_∞ were not possible, we were forced to use the Kezdy-Swinbourne method.[120,121] This method uses two series of data points separated by a constant time interval, δ, A at time t and A' at time $t+\delta t$. Rearrangement of the exponential form of equation [33] with the use of these two sets of data yields:

$$(A_\infty - A) = (A_\infty - A_0)\, e^{-k_1 t} \qquad [34]$$

and

$$(A_\infty - A') = (A_\infty - A_0)\, e^{-k_1 (t+\delta)} \qquad [35]$$

Dividing equation [34] by equation [35] gives:

$$\frac{(A_\infty - A)}{(A_\infty - A')} = e^{k_1 \delta} \qquad [36]$$

Rearrangement of equation [36] gives:

$$A = A_\infty (1 - e^{k_1 \delta}) + A' e^{k_1 \delta} \qquad [37]$$

so that a plot of A versus A' linearly regresses with a slope equal to $\exp(k_1\delta)$.

Measurements in the presence of DiMe-β-CD and γ-CD were made under identical conditions except that we used a SX17MV stopped-flow spectrophotometer obtained from Applied Photophysics (Leatherhead, Surrey, U.K.). Absorbance traces consisting of 400 points covering 5 to 15 half-lives were collected and first-order rate constants were estimated from non-linear least squares fitting of a first-order exponential to the traces, using software provided by the instrument manufacturer. The recorded rate constants are the average of 5 to 15 determinations.

3. Potential Inhibition

3.1 Introduction

The cleavage of mNPAlk and pNPAlk by various CDs was discussed extensively in the last chapter, so only the points that are of particular importance to this section will be reintroduced.

It has been shown previously that *m*-nitrophenyl alkanoate esters are generally more reactive than their *para* isomers° towards α-CD, β-CD,[58] and "hydroxypropyl-β-cyclodextrin" (Hp-β-CD).[90] These results support a mechanism whereby the carbonyl group of the mNPAlk is closer to an ionized secondary CD hydroxy group, in the ester.CD complex, than is the carbonyl group of the analogous pNPAlk.CD complex, as discussed in Chapter 2.

In the case of the acetates, both mNPA and pNPA undergo initial state binding to the CD via aryl group inclusion (Figure 6A, p. 64). The structure of mNPA is such that it is bound to the CD with its carbonyl group in close proximity to secondary hydroxy groups, thus facilitating the rate limiting nucleophilic attack, since little reorganisation to form the transition state is required (Scheme 11A, p. 62). However, in order for the *para* ester to undergo nucleophilic attack, the ester must first lift out of the CD cavity in order to bring its carbonyl group closer to the ionized hydroxy group, and then undergo nucleophilic attack by the CD (Scheme

---

° We note that for the nitrophenyl esters reacting with Hp-β-CD the difference in reactivity between the *meta* and *para* isomers decreases with increasing acyl chain length, and that the *p*-nitrophenyl decanoate is more reactive than the *meta* (see Chapter 2).

11B).[96] This means that the CD cavity is virtually empty[p] during the transition state for the cleavage of pNPA.[94 96 97] It is conceivable therefore, that a species with the correct geometry and size might be able to occupy the cavity during the transition state of the cleavage reaction.

It has been shown that the cleavage of mNPA by α- and β-CD is inhibited by small alkyl-bearing molecules such as simple aliphatic alcohols, alkanoate and alkanesulphonate anions,[96 97] whereas the analogous cleavage of pNPA in the presence of the "potential inhibitors" (PIs) is retarded to a much lesser degree, or even *accelerated* in some instances. The inhibition of the CD-assisted cleavage of mNPA by PIs is consistent with a mechanism whereby the binding of a PI disrupts the normal transition state geometry and thus prevents the reaction (Scheme 11A). Since the transition state for the cleavage of pNPA involves little or no binding of the ester to the CD, the inclusion of a PI in the CD cavity does not greatly affect the normal transition state geometry, thus having little effect on the rate (Scheme 11B).[96]

Both *m*- and *p*-nitrophenyl alkanoates, longer than the C_2 bind to α-CD, β-CD, and Hp-β-CD in the initial state via their acyl chains, and the stability of the ester.CD complexes shows a strong dependence on the ester chain length, with pK_s (= - log K_s) increasing linearly with the length of the acyl chain.[58 90] The behaviour of these esters with regard to binding in the transition state is somewhat

---

[p] In this case we use the term "empty" to mean lack of species other than water.

more complicated. The *meta* alkanoates bind to CDs via aryl group inclusion (Figure 6A), as demonstrated in Chapter 2, for the whole series of esters. Binding of the *para* isomers is biphasic in nature, with the shorter esters (< C_5) binding via their aryl groups (Figure 6A) and the longer esters (> C_6) binding via their acyl groups (Figure 6B).[58,90]

It has been demonstrated that the cleavage of pNPH by β-CD is not inhibited by the presence of various PIs,[95,96] although the ester is bound by its acyl chain, in both the initial state and transition state for the acyl transfer reaction. Not only is the reaction not inhibited, it is *catalysed* by some alcohols acting as the PI. The above observations indicate that during the transition state for the cleavage of these longer esters by CDs, the acyl chain is oriented in such a way that it is either mainly outside of the cavity, or, if it is included, then inclusion occurs in such a manner as to allow for the complexation of another guest (albeit a small one), without overly disrupting the normal transition state. In some cases this disruption is actually advantageous, and the presence of the PI in the CD cavity helps the system to attain an even more reactive geometry. The involvement of a molecule of PI in the transition state is further supported by the fact that saturation kinetics indicate the onset of a second binding process which has been assigned to the formation of a ternary, pNPH.β-CD.PI complex.[95] Such behaviour was not observed with α-CD.

The strength of binding of small molecules containing alkyl groups (alcohols, alkanoate esters, and alkanesulphonate ions) to Hp-β-CD is virtually identical to

that for binding of the same molecules to β-CD[102] (*vide infra*). However, some larger compounds bind more strongly to Hp-β-CD than to β-CD. For example, the 1-anilino-8-naphthalenesulphonate anion (1,8-ANS) is bound more tightly to Hp-β-CD than to β-CD so that K_d is smaller by more than an order of magnitude.[39,60] This preferential binding of 1,8-ANS by Hp-β-CD cannot be accounted for solely on the basis of size, since β-CD and Hp-β-CD bind 4-(2-pyridylazo)-N,N-dimethylaniline equally well, with dissociation constants of 2.9 and 3.3 mM, respectively.[122]

The binding of aliphatic molecules such as alcohols, alkanesulphonate ions, and alkanoate esters to β-CD[58,95,96] and Hp-β-CD[60,90,102] are virtually identical, yet the kinetic parameters for the cleavage of *p*- and *m*-nitrophenyl alkanoates by these CDs are quite different, as was discussed in the last chapter. This led us to ask whether the cleavage reactions of pNPA and pNPH by Hp-β-CD in the presence of PIs behave in a fashion similar to those by the parent, β-CD, or if they exhibit radically different modes of behaviour, similar to the differences in binding of the 1,8-ANS anion to these CDs.

The aim of this chapter is to demonstrate how alcohols mediate the cleavage of pNPA and pNPH by Hp-β-CD, and to compare these results with those obtained previously for α-CD and β-CD.

3.2 Results

We have examined the effect of various alcohols, containing 3 to 6 carbons, as potential inhibitors (PIs) on the rates of cleavage of pNPA and pNPH in basic aqueous medium containing Hp-β-CD. The reactions were carried out in a 0.2 M phosphate buffer at pH 11.60, and the raw data are collected in Appendix II. Before discussing the results, we review the pertinent kinetic models used to understand them.

In the absence of any PI, the kinetics for the cleavage of pNPA and pNPH are the same as described in the previous chapter. The addition of an inert PI, capable of binding to Hp-β-CD (equation [35]), causes a reduction in the concentration of free CD. As the concentration of free CD is lowered, we would expect there to be a corresponding decrease in k_{obs}, as predicted by equation [3] (Chapter 2, p. 65).

$$CD + PI \underset{K_I}{\rightleftharpoons} CD.PI \qquad [35]$$

In the CD-mediated cleavage of *m*-nitrophenyl acetate, the addition of a PI to the reaction medium caused a reduction in k_{obs}, which obeys a model for simple competitive inhibition. Analysis of the dependence of k_{obs} on the concentration of potential inhibitor by a method[96] described in detail in Chapter 4 allows us to determine the dissociation constant (K_I) for the CD.PI complex, and values of K_I determined by this method for the binding of PIs to α-CD and β-CD[96] generally agree well with results in the literature that have been obtained by other

methods.[60,123] The K_I values for the binding of aliphatic alcohols to Hp-β-CD (Table 20) were determined by this method, and they have been reported elsewhere.[102]

For the present study, only aliphatic alcohols of 3 to 6 carbons were examined as PIs, spanning a wide structural variety, including straight and branched chain (primary, secondary, and tertiary), as well as cyclic. Overall, the 14 alcohols studied span two orders of magnitude in K_I, providing a significant range in ability to bind to Hp-β-CD. The addition of these alcohols to the reaction mixture of the cleavage of pNPA and pNPH by Hp-β-CD did not inhibit the reaction in the manner predicted for competitive inhibition (equation [35]), with the sole exception being the inhibition of the cleavage of pNPA by Hp-β-CD upon the addition of 2-hexanol (2-HexOH) (*vide infra*). In some cases the addition of a PI caused a slight depression in k_{obs}, while in others there was a net *acceleration* (Figures 28 & 29). The solid lines in Figures 28 and 29 were calculated using equation [37] (p. 125) and the constants in Tables 20 and 21. The dashed lines were calculated using equation [3], correcting for the decrease in [Hp-β-CD] as predicted by a competitive inhibition model (equation [35]). The data were scaled so that the points at [PI] = 0 all coincide.

As stated above, the cleavage of pNPA in the presence of Hp-β-CD was inhibited by the addition of 2-HexOH. Analyzing the dependence of k_{obs} for the cleavage of pNPA by Hp-β-CD on [2-HexOH] affords a K_I for the 2-HexOH.Hp-β-CD complex of 14.0 ± 0.9 mM, which is virtually identical to the one obtained from inhibition of the cleavage of mNPA by Hp-β-CD (13.2 ± 1.4 mM). It is interesting

Table 20. Constants for the Cleavage of *p*-Nitrophenyl Acetate by "Hydroxypropyl-β-cyclodextrin" in the Presence of Various Alcohols.[a,b]

PI	K_I mM	k_a $M^{-1}s^{-1}$	k_b $M^{-1}s^{-1}$	K_{TS} mM
1-PrOH	319	0.349	13.6	983
iso-PrOH	279	0.432	14.7	795
2-BuOH	83.6	0.851	8.70	403
1-BuOH	64.0	1.18	9.23	291
2-PenOH	41.4	1.21	6.14	282
tert-BuOH	40.1	1.31	6.42	262
iso-BuOH	37.6	1.58	7.24	218
1-PenOH	16.7	3.03	6.18	113
2-HexOH	13.2	-[c]	-[c]	-[c]
c-PenOH	10.7	5.00	6.53	68.6
iso-PenOH	9.28	6.76	7.67	50.7
1-HexOH	4.56	7.39	4.12	46.4
neo-PenOH	2.86	11.6	4.07	29.5
c-HexOH	2.19	17.4	4.67	19.7

[a] In 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] K_I values from reference 123. [c] Not determined because 2-HexOH inhibited the reaction, see text.

Table 21. Constants for the Cleavage of *p*-Nitrophenyl Hexanoate by "Hydroxypropyl-β-cyclodextrin" in the Presence of Various Alcohols.[a,b]

PI	k_a $M^{-1}s^{-1}$	k_b $M^{-1}s^{-1}$	K_{TS} mM
1-PrOH	0.104	21.0	891
iso-PrOH	0.0593	11.9	1570
2-BuOH	0.209	11.0	445
1-BuOH	0.237	9.52	393
2-PenOH	0.253	6.57	368
tert-BuOH	0.205	5.18	454
iso-BuOH	0.336	7.94	277
1-PenOH	0.653	6.85	142
2-HexOH	1.06	9.29	87.8
c-PenOH	1.02	6.86	91.0
iso-PenOH	1.06	6.21	87.4
1-HexOH	1.38	3.96	67.3
neo-PenOH	2.06	3.71	45.1
c-HexOH	3.01	4.15	30.9

[a] The reactions were carried out in 0.20 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] The dissociation constants are given in Table 19.

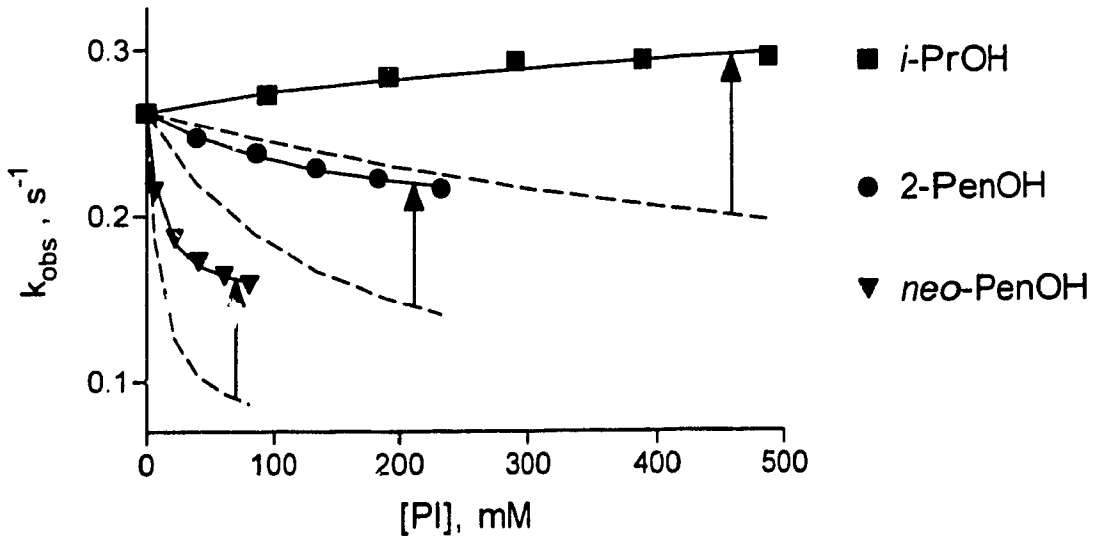


Figure 28. The effect of various potential inhibitors on the Hp-β-CD-mediated cleavage of pNPA.

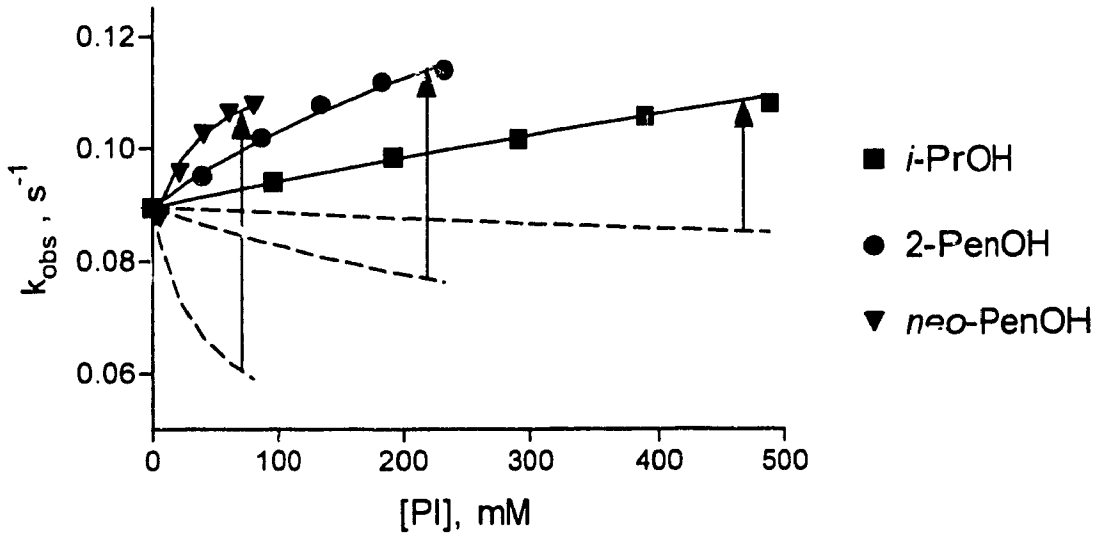


Figure 29. The effect of various potential inhibitors on the Hp-β-CD-mediated cleavage of pNPH.

to note that 2-HexOH *did not* inhibit the cleavage of pNPA in the presence of β-CD, although it binds to β-CD with similar strength (K_I = 11 mM).[96 123]

In analyzing our data for the Hp-β-CD-mediated cleavage of pNPA and pNPH in the presence of various PIs we have taken as our working hypothesis that a reaction occurs between the PI and the ester.CD complex (equation [36]), following earlier work.[94-97] Combining the process in equation [36] with those in equations [1] and [2] (Chapter 2, p. 65), for the reaction in the absence of PI, leads to a dependence of k_{obs} on both the free concentrations of Hp-β-CD and PI, as described by equation [37].

$$S.CD + PI \xrightarrow{k_a} Products + PI \qquad [36]$$

$$k_{obs} = \frac{k_u K_s + (k_c + k_a[PI])[CD]}{K_s + [CD]} \qquad [37]$$

Equation [37] is not particularly convenient since it is both non-linear and multi-variate. The equation is made more tractable by linearising it, which is accomplished by dividing equation [37] by the fraction of free substrate, f_s (equation [38]), which also allows for the separation of variables, as seen in equation [39]. The parameter k_{corr} in equation [39] is a rate constant in which the binding of the substrate to the CD, and the background reaction have been corrected for,

enabling us to separate out the terms involving CD (both in the presence and absence of PI).

$$f_s = \frac{K_s}{K_s + [CD]} \qquad [38]$$

$$k_{corr} = \frac{k_{obs}(K_s + [CD]) - k_u K_s}{[CD]} = k_c + k_a[PI] \qquad [39]$$

Use of either equation [37] or [39] requires knowledge of the actual concentrations of free CD and PI, and not simply their initial concentrations, which may be very different. The free concentrations of both species can be calculated from their initial concentrations, providing that we know the dissociation constant (K_I) of the PI guest.[94,96,97]

According to equation [39], variations in k_{corr} as a function of [PI] should afford a linear plot whose slope is equal to the rate constant k_a. Several examples of this analysis are shown in Figure 30. Tables 20 and 21 summarise the k_a values for pNPA and pNPH reacting with Hp-β-CD in the presence of 13 and 14 alcohols, respectively. The linearity of these plots indicates that the assumption of only *one* molecule of the PI being involved in the transition state for the cleavage of the esters by Hp-β-CD is a valid one.

In general, the values of k_a increase with the ability of the alcohol to bind to Hp-β-CD (Tables 20 and 21, Figure 31). For the cleavage of pNPA by Hp-β-CD

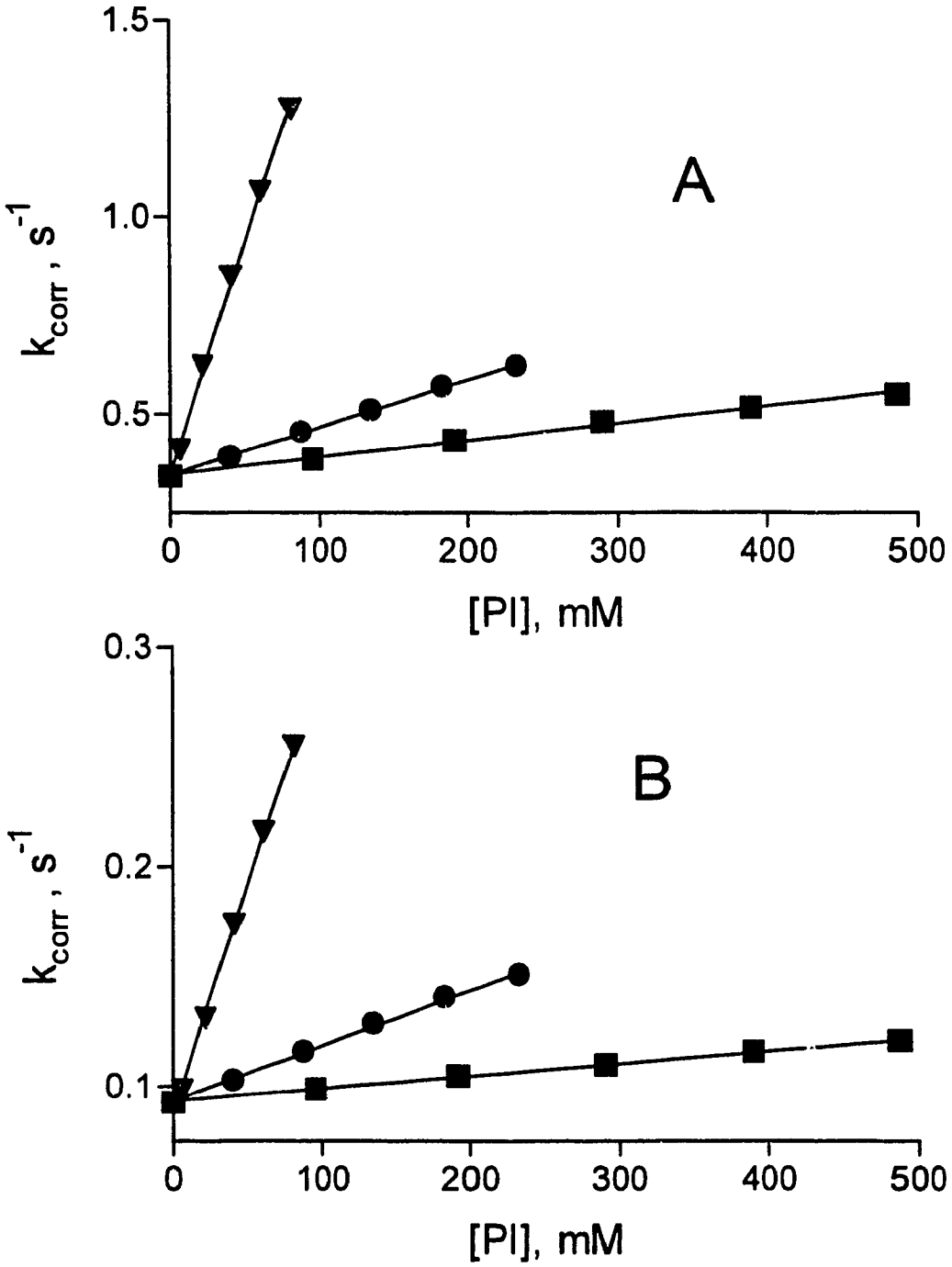


Figure 30. Dependence of k_{corr} on [PI] (eqn. [39]) for pNPA (A) and pNPH (B) in the presence of: *i*-PrOH, ■; 2-PenOH, ●; *neo*-PenOH ▾.

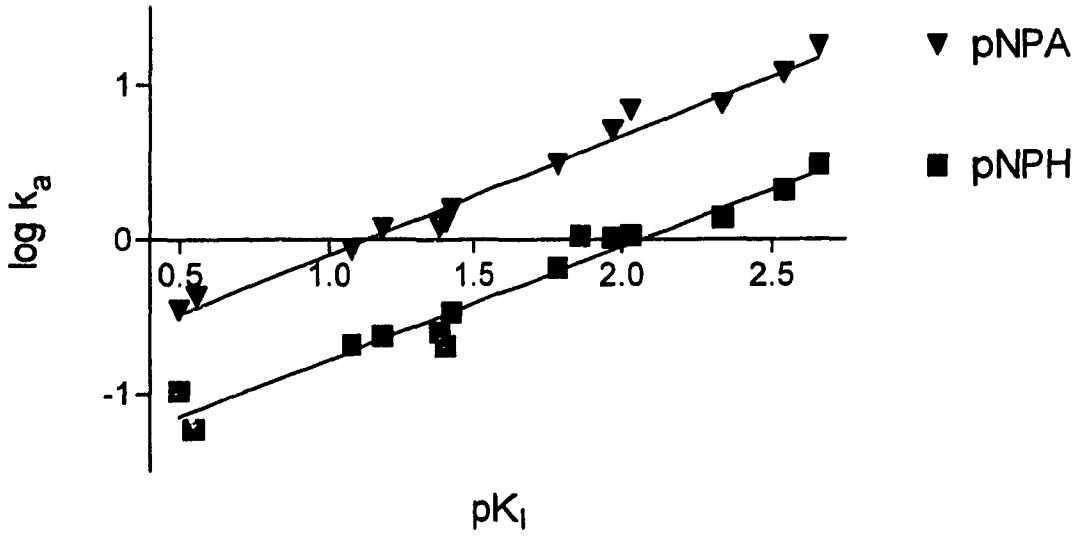


Figure 31. Dependence of log k_a on the ability of the alcohol to bind to Hp-β-CD in the initial state.

the k_a values range from 0.35 to 17 $M^{-1}s^{-1}$, an increase of almost 50-fold, and they are decidedly lower than those observed with β-CD (2.8 to 83 $M^{-1}s^{-1}$)[96] although the span is larger with Hp-β-CD. The k_a values for the cleavage of pNPH by Hp-β-CD ranged from 0.10 to 1.1 $M^{-1}s^{-1}$, an increase of only a factor of 11, which is decidedly lower than the results obtained with β-CD, where the k_a values ranged from 1.4 to 61 $M^{-1}s^{-1}$,[97] a fact which is most probably linked to the ability of β-CD to form 1:1:1 {S.CD.PI} complexes in the initial state, whereas there was no evidence for such ternary complex formation with Hp-β-CD (*vide infra*).

3.3 Discussion

The results reported above clearly support a transition state for a cleavage process involving the ester (pNPA or pNPH), Hp-β-CD, and *one* molecule of PI. Since the alcohols span a wide array of structural varieties, and are thus greatly different in terms of reactivities, it is unlikely that the interaction between the transition state for the cleavage reaction and the alcohol is covalent in nature. It is most likely that the role played by these potential inhibitors is one in which they act as inert space fillers, or "molecular spectators", simply occupying the CD cavity during the "normal" transition state for the cleavage reaction, affecting the geometry to a small extent, in a deleterious or advantageous manner. This is the same conclusion as was drawn for the analogous cleavage processes involving both α-CD and β-CD.[96,97]

The conclusion that the PI occupies the CD cavity during the transition state for the cleavage of pNPA by Hp-β-CD is consistent with the mechanism that has been proposed for the cleavage of pNPA in the absence of PI (Scheme 11B).[90] For the cleavage of pNPH by Hp-β-CD in the presence of PIs it may be that during the transition state the acyl portion of the ester is either completely outside of the cavity, or intrudes only slightly, since there is clear evidence for the PI being inside the cavity. Since we observed no evidence of S.CD.PI complex formation we are led to believe that the acyl chain of pNPH cannot fully penetrate into the Hp-β-CD cavity which contains a PI, either in the initial state or the transition state. As detailed below, our analysis of the data with the various PIs supports these interpretations.

3.3.1 Reactivity of the PI with the S.CD complex (k_a)

The rate constants for the reaction of the PI with the S.CD complex (k_a) depend strongly on the ability of the alcohol to bind to the CD in the initial state. For each ester there is a good linear free energy relationship[q] (LFER) between the second order rate constants and the dissociation constants for the CD.PI complexes, as given by equations [40] and [41], where pK_I = -log K_I (Figure 31):

---

[q] Although we are aware that the reliability of LFERs has been recently brought into question,[124,125] we feel that they are still valid tools for the analysis of physical organic data and continue to use them.

pNPA: $\log k_a = 0.77\ pK_I - 0.87$ [40]

$(N = 13, r = 0.992)$

pNPH: $\log k_a = 0.74\ pK_I - 1.52$ [41]

$(N = 14, r = 0.979)$

The strong dependence of these rate constants (k_a) on pK_I presumably reflects the fact that the PI is bound inside the cavity during the transition state. Since the dependence of log k_a on pK_I is virtually identical, for both pNPA and pNPH, the interaction between the ester and the PI must be very similar, supporting the conclusion that the ester is largely *outside* the cavity during the transition state for the cleavage of pNPH by Hp-β-CD, when the cavity is occupied by a PI. However, we are not able to ascertain whether the ester is oriented with its acyl or aryl group pointing towards the cavity. This ester-excluded transition state differs markedly from what has been observed in the absence of PI, where stabilisation of the transition state for the cleavage of pNPAlk (> C_5) by Hp-β-CD is directly dependent on the length of the acyl chain, which has been assumed to mean that the acyl chain occupies the cavity (*vide supra*).[90]

The slope of equation [41] is the same as that (0.75) of the analogous equation for β-CD and lower than that observed with α-CD, (1.02)[97] (Figure 32, p. 133). This indicates that the functionalisation of β-CD on the primary side has little, if any, effect on the dependence of k_a (for the CD-mediated cleavage of pNPH), on K_I, but it does have a significant effect on the absolute values since the

k_a values for Hp-β-CD are an order of magnitude lower than those for β-CD (*vide infra*). The width of the CD cavity does appear to play a more significant role, with the smaller α-CD showing a stronger dependence of k_a on pK_I than β-CD. Although the dependence of log k_a on pK_I, for the cleavage of pNPH, is stronger for α-CD than for β-CD and Hp-β-CD, this does not mean that the situation is better, since log k_a values for α-CD are lower than those for β-CD. The shallower dependence of log k_a on pK_I for β-CD and Hp-β-CD can be ascribed to the wider cavities of these CDs which allows more latitude in positioning PI and pNPH in the transition state. Thus, there is a decreased sensitivity of the transition state binding to the nature of the PI, since the PI can more easily reposition itself to accommodate pNPH, if necessary.

The results in the case of pNPA cleavage by Hp-β-CD are somewhat different, in that the slope of equation [40] falls in between those found for β-CD (0.67) and α-CD (1.02)[96] (Figure 33), meaning that the cleavage of pNPA is *more* sensitive to the ability of the PI to bind to HP-β-CD than to β-CD. This increase in sensitivity, relative to β-CD, could be explained with a mechanism whereby the pNPA sits outside of the cavity during the nucleophilic attack, orienting the phenyl ring directly above the CD cavity (Scheme 11B), forcing the PI further down into the CD cavity. By penetrating further down into the CD cavity, the PI would come into greater contact with the hydroxypropyl groups on the primary side of the CD, thus causing more interaction between the PI and the CD. On the other hand, since k_a for the cleavage of pNPH by β-CD and Hp-β-CD both show the same

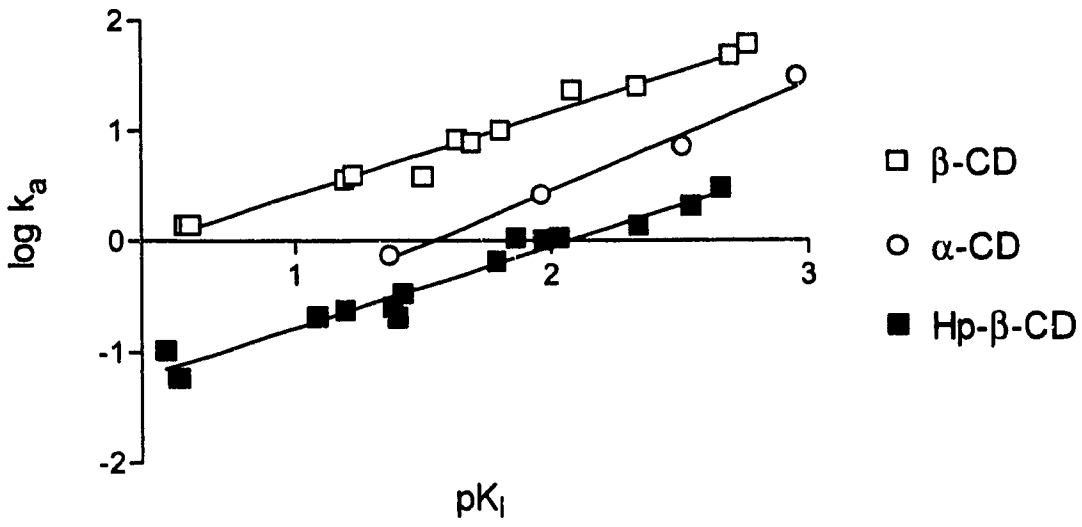


Figure 32. Dependence of log k_a on pK$_I$ for the cleavage of pNPH in the presence of PIs. Data for α-CD and β-CD from reference 97.

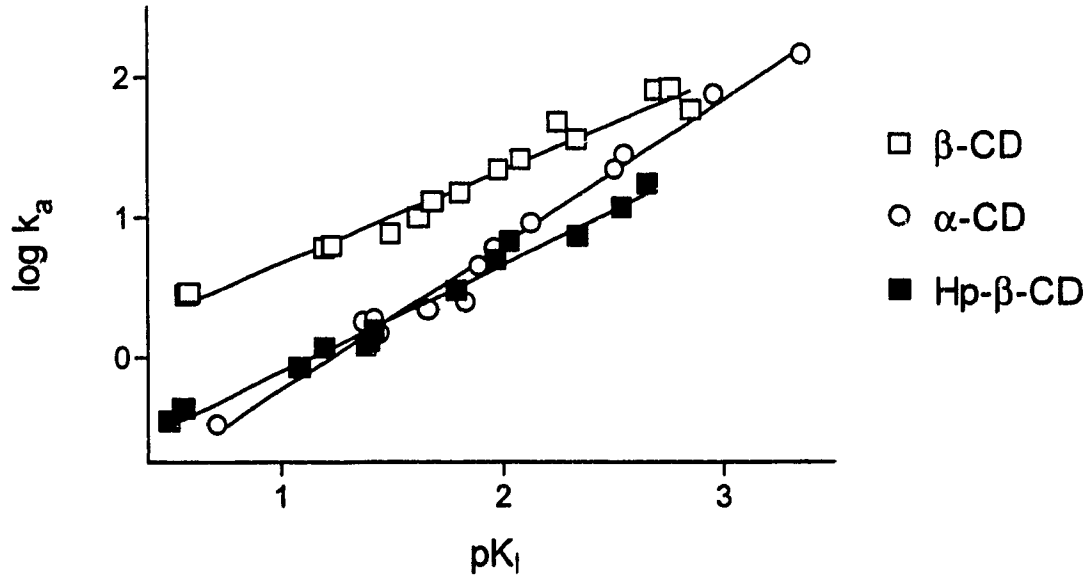


Figure 33. Dependence of log k_a on pK$_I$ for the cleavage of pNPA in the presence of PIs. Data for α-CD and β-CD from reference 96.

dependence on pK_I, it is likely that the transition state is similar to that depicted in Figure 6B, where the acyl group is oriented towards the cavity and positioned either completely outside or only intruding slightly into the CD cavity. By assuming this orientation, pNPH is not effectively "capping" the CD, and the PI is not forced down further into the cavity, where it would interact with the primary 2-hydroxypropyl groups.

Since the binding of the PI in both the initial and transition states of the Hp-β-CD-mediated cleavage of pNPA and pNPH seems to be similar (from slopes of log k_a vs pK_I and pK_{TS} vs pK_I (*vide infra*)), it may be more appropriate to consider the reaction occurring via a kinetically equivalent pathway, as given by equation [42]:

$$S + CD.PI \xrightarrow{k_b} Products + PI \qquad [42]$$

The rate constant for this process can be determined from that of the equivalent third order reaction shown in equation [43]:

$$S + CD + PI \xrightarrow{k_3} P \qquad [43]$$

where $k_3 = k_a/K_s$ or k_b/K_I depending on whether one chooses equation [36] or [42]. Therefore, k_b can be evaluated as k_aK_I/K_s and values of k_b calculated in this manner are collected in Tables 20 and 21.

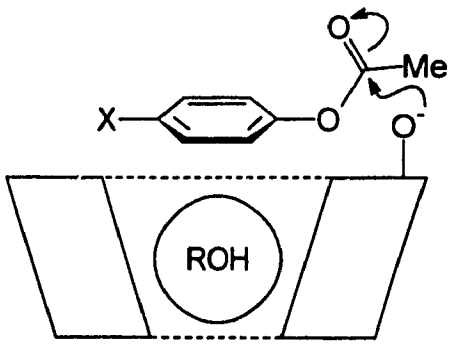
The values of k_b do not show much systematic variation with the ability of the PI to bind to Hp-β-CD, and fall within a range much narrower than the k_a

values, 4.07 to 14.7 $M^{-1}s^{-1}$ for pNPA and 3.96 to 21.0 $M^{-1}s^{-1}$ for pNPH, with most of the values falling within the ranges of 5 to 9 $M^{-1}s^{-1}$ and 4 to 12 $M^{-1}s^{-1}$, respectively. These values are lower than the analogous reactions with β-CD,[96,97] as are the second order rate constants, k_2 (= k_c/K_s), for the reaction of the CD with the esters (Table 22). These results are consistent with reaction occurring as described in equation [42], as long as the ester is mainly outside of the cavity during the transition state, so that the PI may be accommodated inside (transition states **44** for pNPA; **45** for pNPH).

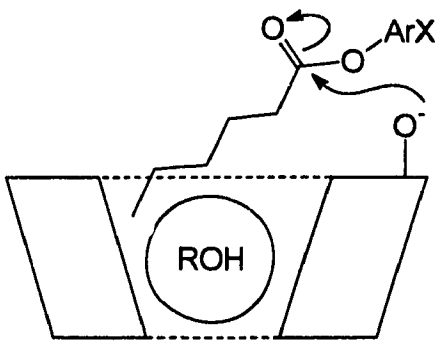
Table 22. Parameters for the Cleavage of pNPA and pNPH by CDs.[a]

ester	CD	k_u s^{-1}	k_c s^{-1}	K_s mM	k_2 $M^{-1}s^{-1}$
pNPA[b]	Hp-β	0.0653	0.343	8.18	41.9
pNPH[b]	Hp-β	0.0444	0.0932	1.59	58.6
pNPA[c]	β	0.0772	0.660	7.92	83.3
pNPH[d]	β	0.0451	0.137	1.60	85.6

[a] For reaction in 0.2 M phosphate buffer at 25.0 ± 0.1 °C at pH 11.40 or 11.60. [b] Previous chapter and reference 90. [c] Reference 96. [d] Reference 97.



44



45

3.3.2 Transition State Binding (K_{TS})

In order to further examine the transition state phenomena we have used the Kurz approach, as detailed in the Chapter 2, but with minor adjustments. In order to apply transition state formalism to this case we must compare the processes occurring in the absence (equation [2]) and presence of PI (equation [36]), for which the rates of reaction are as given by equations [44] and [45]:

$$\text{rate} = k_c[S.CD] = v[TS] \qquad [44]$$

$$\text{rate} = k_a[S.CD][PI] = v[TS.PI] \qquad [45]$$

Applying the same considerations and restrictions as before, we can obtain an expression for the *pseudo*-dissociation constant, K_{TS}, as given by equation [46], for the *apparent* dissociation of the PI from the PI-containing transition state (equation [47]).

$$K_{TS} = \frac{[TS][PI]}{[TS.PI]} = \frac{k_c}{k_a} = \frac{k_2}{k_3} \qquad [46]$$

$$TS + PI \underset{K_{TS}}{\rightleftharpoons} TS.PI \qquad [47]$$

The values of K_{TS} for the cleavage of pNPA and pNPH by Hp-β-CD in the presence of PI are summarised in Tables 20 and 21.

As discussed in detail in the preceding chapter, variations in pK_{TS} (= -log K_{TS}) can be used as a probe of transition state binding. In this study we examined the LFERs between pK_{TS}, for the binding of the PI to the transition state, and pK_I for the initial state binding in the CD.PI complex. For the cleavage of pNPA and pNPH by Hp-β-CD in the presence of a large number of alcohols we found the two relationships given by equations [48] and [49] (Figure 34):

pNPA: $pK_{TS} = 0.77\ pK_I - 0.41$ (N = 13, r = 0.992) [48]

pNPH: $pK_{TS} = 0.74\ pK_I - 0.49$ (N = 14, r = 0.979) [49]

Except for the intercept terms, these equations are identical to those in equations [40] and [41] since $K_{TS} = k_c/k_a$ and k_c is a constant for each ester (Table 22).

We interpret the slopes of equations [48] and [49] to mean that the binding of the PI in the initial and transition states is similar for the cleavage of both pNPA and pNPH by Hp-β-CD in the presence of PIs. Also, since equations [48] and [49]

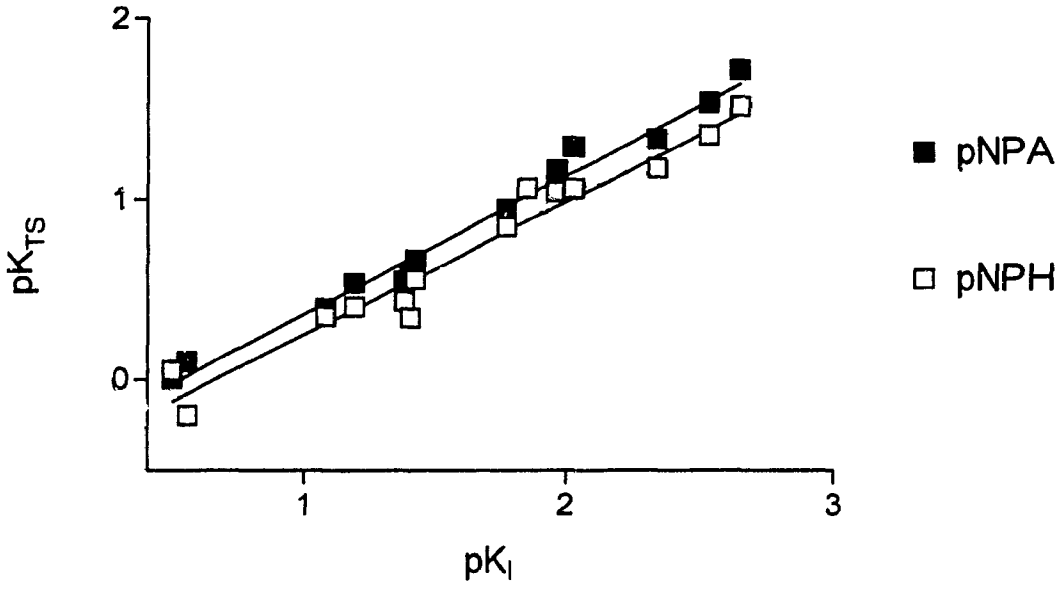


Figure 34. Dependence of transition state binding on the ability of the PI to bind to Hp-β-CD in the initial state.

are almost identical, the PI must be bound in a similar manner for the reaction of both esters, which could well imply that both pNPA and pNPH are mostly, if not totally, excluded from the CD cavity during the transition state of the PI-mediated reaction. If the acyl portion of pNPH was included in the CD cavity during the transition state, to a significant degree, we would expect this to be manifested in equation [49], at least as an alteration of the intercept term. The fact that the points for pNPH (Figure 34) lie slightly below those for pNPA may indicate that there is a *slight* inclusion of the acyl chain of pNPH in the cavity during the transition state (**45**), or simply a modest steric effect. The results observed with β-CD were not this similar, as can be seen from the LFERs in equations [50][96] and [51]:[97]

β-CD + PI + pNPA: $pK_{TS} = 0.67\ pK_I + 0.19$ [50]

β-CD + PI + pNPH: $pK_{TS} = 0.75\ pK_I + 0.53$ [51]

These results for β-CD indicate that there is a stronger dependence of transition state stabilisation on initial state binding of the PI for the cleavage of pNPH as compared to pNPA (slopes), and that the PI is bound more tightly to the transition state for the cleavage of pNPH than pNPA (intercept terms), which is the opposite of what we have observed with Hp-β-CD.

One of the major differences in behaviour between the PI-mediated cleavage of pNPH by β-CD and Hp-β-CD is the formation of 1:1:1 (ester:CD:PI) complexes. Although these complexes were evident with β-CD,[97] we found no

evidence for their existence with Hp-β-CD. Tee *et al*[97] found that in the transition state for the cleavage of pNPH by β-CD the alcohol is bound in a manner very similar to the way in which it is bound in the ternary complex, although the binding in the transition state is weaker. The dependence of pK_{TS} on pK_I for the PI-mediated cleavage of pNPH by both β-CD and Hp-β-CD is virtually identical, however, the strength of transition state binding is stronger in the case of β-CD. This observation substantiates the claim that the ester is included in the transition state for the cleavage of pNPH by β-CD, but not to a significant degree in the cleavage by Hp-β-CD. The exact origin of these differences is unclear and is most probably due to the 2-hydroxypropyl groups on the primary side of the Hp-β-CD cavity.

The differences in the kinetic results for the cleavage of pNPA by β-CD and Hp-β-CD in the presence of PIs are not as easy to explain, in view of the fact that we know that both pNPA and the alcohols studied bind virtually identically to β-CD and Hp-β-CD, at least as regards to strength.[102] Since the dissociation constants with both CDs are so similar, we have concluded that the binding of the guest to the CD occurs at the wider, secondary, side of the CD cavity. Presumably, the alcohols approach the CD cavity from the wider secondary face of the cavity, since even alcohols which would be too large to fit through the narrower end of the cavity are bound. In order to accommodate a molecule of PI in the CD cavity, one or more molecules of water must be excluded (see Chapter 1). If, during the rate-limiting step the PI is required to reposition itself in some manner, then water

molecules may be required to leave or enter the CD cavity, probably through the narrower, primary side of the CD, a process which may lead to a destabilisation of the transition state due to the 2-hydroxypropyl groups. Comparing the pK_{TS} values for the cleavage of pNPA in the presence of PIs, we can estimate this destabilisation to account for approximately 0.6 pK units. Should the 2-hydroxypropyl groups destabilise the transition state, either through interaction with the PI or by impeding water transport, we would expect to see an effect in the PI-mediated cleavage of pNPH by β-CD and Hp-β-CD similar to that observed with pNPA. Due to the similarity in the slopes of pK_{TS} vs pK_I for β-CD- and Hp-β-CD-mediated cleavage of pNPH (equations [51] and [49], respectively) in the presence of alcohols we assume that the PI is not required to reorient itself during the cleavage of pNPH, which would be the case if the ester is not included in the cavity during the transition state, to any significant degree.

Since the transition state for the CD-mediated cleavage of pNPA (**44**), and pNPH (**45**), in the presence of PIs occurs with the esters mainly outside of the CD cavity, is it likely, or even possible, that the CD is simply acting as a nucleophile attacking the ester? In order to clarify this point we examined the cleavage of pNPA and pNPH by trifluoroethanol under the same conditions as the cleavage by CDs (Figures 35 and 36).[r] Both α-CD and TFE have similar reactivities towards pNPA, whereas Hp-β-CD and β-CD are slightly more reactive; in the case of

---

[r] The data in Figures 35 and 36 have been scaled so that the points at [CD] = [TFE] = 0 mM coincide.

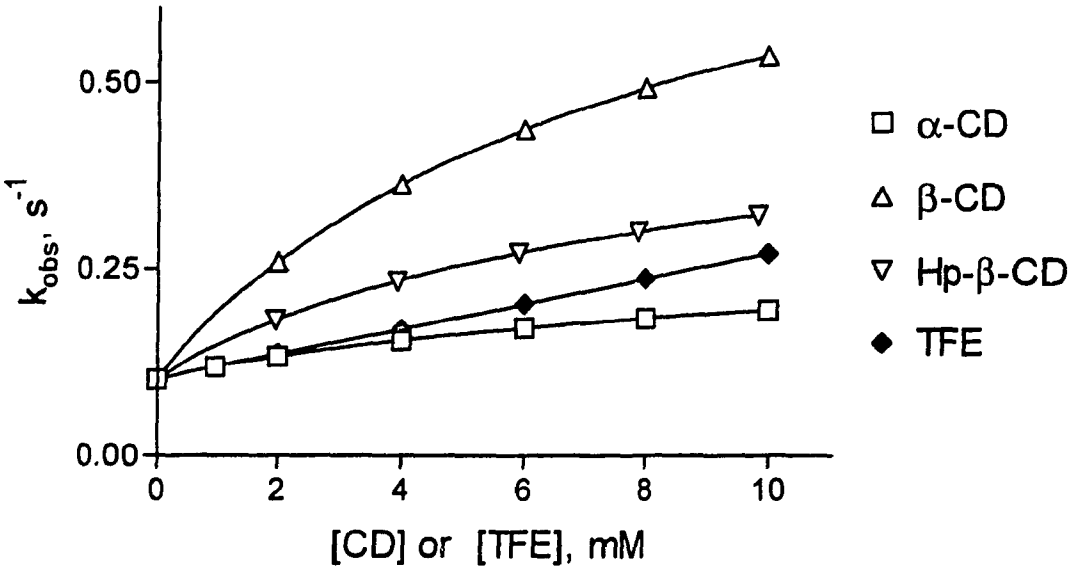


Figure 35. Comparison of the relative ability of 3 CDs and TFE to cleave pNPA in basic aqueous solution.

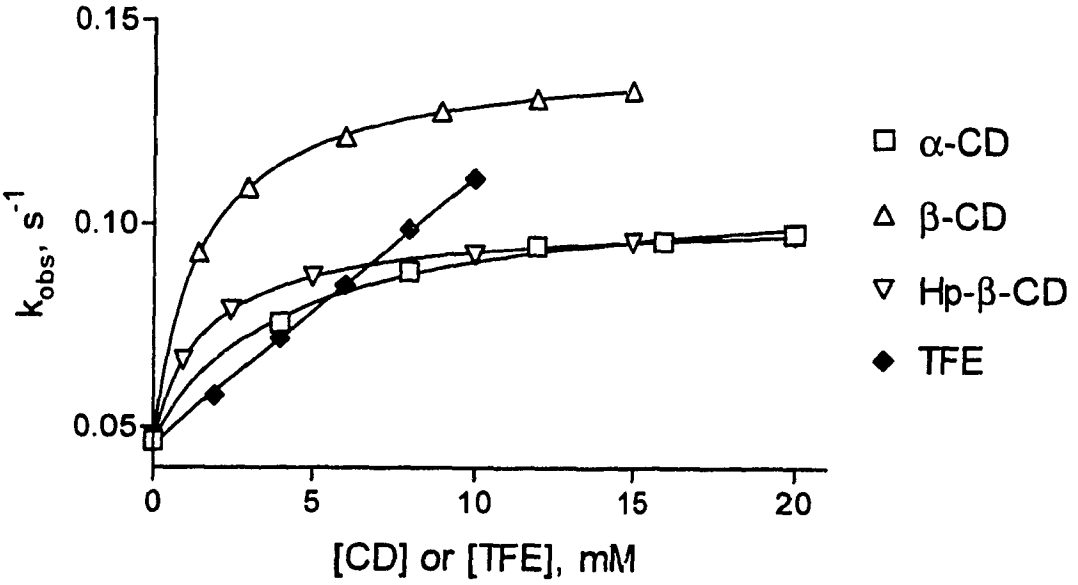


Figure 36. Comparison of the relative ability of 3 CDs and TFE to cleave pNPH in basic aqueous solution.

pNPH, the 3 CDs are more reactive than the TFE. We can see from these figures that the CDs do not seem to be unusually reactive towards either of the esters discussed, given that the pK_as (12.2 and 12.3)[15-17,48,49] of the secondary hydroxy groups are very similar to that of TFE (12.37).[126,127]

3.4 Conclusions

This chapter contains a large amount of data that supports the hypothesis that the cleavage of pNPA and pNPH by Hp-β-CD can occur with a molecule of PI in the CD cavity, as do the analogous reactions with α-CD and β-CD.[96,97] Although both pNPA and pNPH bind to Hp-β-CD, as evidenced by saturation kinetics,[90] and that pNPH is included in the CD cavity, via its acyl chain, during the transition state for the cleavage reaction in the absence of PI,[90] this in no way requires that the esters are in the CD cavity during the transition state for the PI-mediated cleavage. The PI may allow for the formation of a transition state of differing geometry, which may be more or less stable. The presence of a PI as a benign "spectator" in the transition state does not necessarily mean the situation will be an "all-or-nothing" affair, the effect of the PI may vary from total inhibition to slight retardation to acceleration.[94-97]

We conclude that during the transition state for the PI-mediated cleavage of pNPA and pNPH by Hp-β-CD that the esters reside largely outside of the CD cavity. We note that there are difference between the cleavage of these esters by β-CD and Hp-β-CD, while the only differences between the two systems are the

2-hydroxypropyl groups on the Hp-β-CD, which are by all accounts located away from the centre of activity. Although the exact nature of the effect due to the 2-hydroxypropyl groups is unclear, we feel that they act to restrict the movement of species in and out of the CD via the narrow side of the cavity, which may be necessary to allow for reorientation of the PI during the transition state.

3.5 Experimental

pNPA and pNPH were purchased from the Sigma Chemical Company and used without further purification. The alcohols used as PIs were all purchased from the Aldrich Chemical Company, and were all of the highest grade possible. In all cases the secondary alcohols were tested for peroxide formation by mixing a few millilitres with an equal volume of saturated KI solution. If peroxides were present the solution would turn yellow, due to the formation of I_2 and I_3^-, alcohols found to contain peroxides were discarded and fresh supplies ordered.

Hydroxypropyl-β-cyclodextrin was purchased from the Aldrich Chemical Company, and from Wacker-Chemie, Munich, Germany, and used without any further purification. Due to the amorphous nature of "hydroxypropyl-β-cyclodextrin" we were initially concerned that a change in suppliers may affect the nature and/or quality of the Hp-β-CD. In order to confirm that this was not the case, we reexamined the cleavage of pNPA by Hp-β-CD in a basic aqueous medium as described in the previous chapter. The results are listed in Table 23, noting that

the difference in k_u and k_c is due to a difference in pH and not in the Hp-β-CD (see Chapter 2.5).

Table 23. Comparison of "Hydroxypropyl-β-cyclodextrin" as Supplied by the Aldrich Chemical Company (ACC) and Wacker-Chemie (WC).[a,b]

supplier	k_u s^{-1}	k_c s^{-1}	K_s mM	k_c/k_u
WC[c]	0.1024	0.501	8.01	4.89
ACC[d]	0.0653	0.343	8.18	5.25

[a] For reaction in 0.2 M phosphate buffer at 25.0 ± 0.1 °C. [b] Differences in k_c and k_u are due to differences in pH, not Hp-β-CD. See text. [c] pH 11.60. [d] pH 11.40.

The reactions were carried out in the same manner as detailed in Chapter 2.5, with the sole exceptions being that now the initial concentration of Hp-β-CD is fixed, and there were varying concentrations of alcohol (as a PI) added to the solutions.

To examine the kinetic results in terms of equation [39] we were limited in the number of alcohols we could use. Longer alcohols have a very low solubility, which prevents getting enough into solution to have a sufficiently large effect on

k_{corr}, to give reliable values of k_a. On the other hand, small alcohols like methanol and ethanol bind to β-CD (and presumably to Hp-β-CD as well[102]) very weakly, thus the [ROH] required to have a significant effect on k_{corr} might be so high as to cause concern about solvent effects.

Using k_{obs} to calculate k_{corr} and its subsequent use in equation [39] requires the use of known values of k_u, k_c, and K_s. Since the former two constants vary significantly with pH, we attempted to minimise this problem by scaling all of the data to a master run, based on the value of k_{obs} in the absence of PI. Sample calculations are given in Table 24. In order to calculate k_{corr} and perform the analysis we needed to know the free concentrations of CD and PI. The quadratic equation [52] can be solved (equation [53]) to calculate [CD], and the concentration of free PI was calculated using the mass balance equation, $[PI] = [PI]_o - [CD]_o + [CD]$.

$$K_i = \frac{[CD]([PI]_o - ([CD]_o - [CD]))}{([CD]_o - [CD])} \qquad [52]$$

$$[CD] = \frac{[CD]_o - [PI]_o - K_i + ((([PI]_o - [CD]_o + K_i)^2 + 4K_i[CD]_o)^{1/2}}{2} \qquad [53]$$

Table 24. Example of Potential Inhibition Calculations for the 1-Propanol-mediated Cleavage of *p*-Nitrophenyl Acetate by "Hydroxypropyl-β-cyclodextrin".[a,b]

[1-Propanol]	k_{obs} s^{-1}	k_{scaled}[c] s^{-1}	[CD][d] mM	[PI][e] mM	k_{corr} s^{-1}
0	0.288	0.262	20.0	0.00	0.343
100	0.302	0.275	15.4	95.4	0.387
200	0.304	0.277	12.5	193	0.416
300	0.311	0.284	10.5	290	0.454
400	0.313	0.285	9.01	389	0.485
500	0.316	0.288	7.91	488	0.519

Regression of k_{corr} versus [PI] gives slope = k_a = 0.349 ± 0.013 s^{-1} (r=0.997)

Therefore, $k_b = k_a K_I / K_s$ = 13.6 s^{-1} and $K_{TS} = k_c / k_a$ = 983 mM

[a] For reaction at pH 11.6 (0.2 M phosphate buffer) and 25.0 ± 0.1 °C, $[Hp\text{-}\beta\text{-}CD]_o$ = 20.0 mM. [b] K_I = 319 mM. [c] Scaled to a master run with k_u = 0.0653 s^{-1}; k_c = 0.343 s^{-1}; K_s = 8.18 mM. [d] Calculated using equation [53]. [e] Calculated as: $[PI] = [PI]_o - [CD]_o + [CD]$.

4. Binding of Guests to β-CD and Hp-β-CD

4.1 Introduction

As discussed previously, the acceleration of many types of reactions by CDs requires, at some point, the binding of at least one molecule of CD to the substrate, although this in no way requires the presence of the substrate in the CD cavity during the transition state (see Chapter 3). The studies discussed in this thesis required the knowledge of the complex forming ability of various CDs with a wide variety of guests (nitrophenyl alkanoates, alcohols, alkanesulphonate anions, and alkylamines).

In order to discuss the stabilisation afforded to the transition state of the cleavage reaction of mNPAlk and pNPAlk by CDs, we were required to know the dissociation constant of the ester.CD complex. These results came naturally out of the analysis of the variation k_{obs} with [CD], as presented in Chapter 2.

As discussed in detail in the previous chapter, the cleavage of pNPA and pNPH is not inhibited by various alcohols having the potential to act as inhibitors, and in some cases the cleavage is actually accelerated. This situation is vastly different to that for the cleavage of mNPA by Hp-β-CD, which *is* inhibited by the same alcohols. In order to probe the effect of various alcohols on the Hp-β-CD-mediated cleavage of pNPA and pNPH, it is necessary to have estimates of the dissociation constants of the alcohol.CD complexes. These constants can be determined by examining the dependence of k_{obs} for the cleavage of mNPA on the concentration of added alcohol; we refer to this as the kinetic method.

The use of the kinetic method for the determination of the dissociation constants has one main limitation: the guest molecules must be inert towards the probe reaction. This condition is met with alcohols, but in the next chapter, where we investigate the effect of CDs on aminolysis, we required a different method for determining the dissociation constants of various CD.amine complexes.

One feature of CD chemistry which is observed in almost every case examined is the enhancement of fluorescence caused by the binding of a fluorophore to the CD.[37-39,123,128,129] This enhancement is due to a combination of effects resulting from complex formation, including: the protecting of the bound fluorophore from external quenchers such as oxygen; the inhibition of the "free rotor" effect for the bound fluorophore; and the placement of the fluorophore in a less polar environment, inside the CD cavity.[37] The CD complexes of naphthalene and its derivatives have been widely studied[40,130,131] because of the large fluorescence enhancement obtained upon complexation.

The method we developed uses the competition between an amine guest and a fluorescent probe for the CD binding cavity. The decrease in fluorescence upon addition of the amine allows us to estimate the dissociation constant for the CD.amine complex. This approach we call the fluorescence method.

4.2 Results

Before discussing the results, it is important to discuss the pertinent models used in the determination of the dissociation constants. Since the different

methods (and their mathematical models) are of integral importance they will be discussed in detail and not relegated to the experimental section.

4.2.1 Esters

As shown in Chapter 2, the dissociation constant of the ester.CD complex is obtained by a non-linear least squares fitting of equation [3] (Chapter 2, p. 65) to the observed data. The dissociation constants of mNPAlk and pNPAlk from Hp-β-CD are summarised earlier in Tables 13 and 14 (Chapter 2) while those for the analogous β-CD complexes are given in Table 25. We can see that the binding of both series of esters to a particular CD is very similar, and that the strength of binding to β-CD is very similar to the binding to Hp-β-CD.

4.2.2 Alcohols and Alkanesulphonate Anions

The dependence of k_{obs} on the [CD] for the cleavage of mNPA by CDs is given by equation [3] (Chapter 2, p. 65). We can rearrange this equation to allow us to estimate the concentration of free CD, providing that the determination of k_{obs}, k_u, K_s, and k_c were all performed at the same pH (equation [54]).

$$[CD] = \frac{(k_{obs} - k_u)K_s}{(k_c - k_{obs})} \qquad [54]$$

$$CD + PI \underset{K_I}{\rightleftharpoons} CD.I \qquad [55]$$

In the presence of an inhibitor capable of binding to the CD (equation [55]) there is an overall decrease in the concentration of free CD, which manifests itself

Table 25. Dissociation Constants of mNPALk and pNPAlk from β-CD.[a,b]

Ester	K_s (mM)	
	meta	*para*
C_2	12	7.8
C_3	5.2	5.2
C_4	3.7	2.7
C_5	2.4	2.0
C_6	1.8	1.3
C_8		1.9[c]
C_{12}		0.75[c]

[a] In a 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Reference 58. [c] In a sodium carbonate buffer, pH 10.4 at 25 °C; reference 99.

as a decrease in k_{obs} as described by equation [3]. Traditionally, these experiments are carried out under conditions with $[I]_o$ » $[CD]_o$ so that the approximation given by equation [56] is reasonably valid. Combining equations [54] and [56] one can obtain an equation of use for determining K_I graphically.[57 96]

$$[CD] = \frac{[CD]_o K_I}{(K_I + [I]_o)} \qquad [56]$$

The major limitation with the above method is the requirement for large $[I]_o$. With the smaller alcohols and alkanesulphonate anions this was not a problem, but with the larger inhibitors it was not possible to maintain this conditions due to their low solubilities. Our approach does not have the above limitation, and it has been applied to a wide range of alcohols and alkanesulphonate anions.[60,102] We estimate the concentration of free CD by using equation [54] at various $[I]_o$ and then calculate the concentration of free inhibitor (equation [57]) and that of the CD.I complex (equation [58]). Knowing these three concentrations, we can estimate the dissociation constant using equation [59] (p. 156), and several values obtained at different $[I]_o$ are averaged to obtain our best estimate of K_I. These calculations were easily accomplished in a computer spreadsheet, and a sample calculation is provided in Table 26. The K_I values determined by this method for various alcohols and alkanesulphonate anions with Hp-β-CD and β-CD are summarised in Table 27.

$$[I] = [I]_o - [CD]_o + [CD] \qquad [57]$$

Table 26. Sample Calculation for the Determination of the Dissociation Constant of Guests from CDs using the Kinetic Method.[a b]

INHIBITION CALCULATION -- Expt # = D-53

Guest (G) = $HexSO_3^-$ Ester = mNPA CD = HP-β-CD

k_u = 0.0502 s^{-1}; k_c = 0.956 s^{-1}; K_s = 6.98 mM

$[CD]_o$ = 2.00 mM; At this $[CD]_0$ k_{calc}[c] = 0.252 s^{-1}

$[G]_0$ mM	k_{obs} s^{-1}	k_{scal}[d] s^{-1}	[CD] mM	[CD.G] mM	[G] mM	K_I mM
0.00	0.229	0.252	2.00	0.00	0.00	---
15.0	0.131	0.145	0.812	1.19	13.8	9.43
30.0	0.101	0.112	0.507	1.49	28.5	9.68
45.0	0.0872	0.0959	0.371	1.63	43.4	9.87
60.0	0.0771	0.0848	0.277	1.72	58.3	9.37
75.0	0.0715	0.0786	0.226	1.77	73.2	9.32

Average K_I, mM = 9.54

± 0.23

[a] In a 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Values of k_u, k_c, and K_s are from a previous experiment. [c] Observed rate constants were scaled to this. [d] Calculated as k_{calc} / $k_{obs}([G]_c=0\ mM)$ * k_{obs} in order to correct for variations in pH.

Table 27. Dissociation Constants for Aliphatic Guests from β-Cyclodextrin and "Hydroxypropyl-β-cyclodextrin".[a]

Guest	K_I (mM)	
	β-CD	Hp-β-CD
Alcohols[b,c]		
Ethanol	1100	2100
Propanol	270	320
Butanol	60	64
Pentanol	16	17
Hexanol	4.6	4.6
Heptanol	1.4	1.51[d]
2-Butanol	65	84
2-Pentanol	32	41
2-Hexanol	11	13
3-Pentanol	45	32.3[d]
iso-propanol	260	280
iso-butanol	24	38
iso-pentanol	5.6	9.3

(continued...)

tert-Butanol	21	40
neo-pentanol	1.7	2.9
Cyclopentanol	8.3	10.7
Cyclohexanol	2.0	2.2
2-Methoxyethanol	602	1200[d]
Alkanesulphonate anions[e,f]		
Butanesulphonate	89	105
Pentanesulphonate	17	26.6
Hexanesulphonate	5.6	9.5
Heptanesulphonate	2.3	3.38
Octanesulphonate	0.97	1.07

[a] At 25.0 °C. [b] β-CD in a 0.05 M citrate-phosphate buffer, pH 6.4; from reference 123 and 59. [c] Hp-β-CD in a 0.2 M phosphate buffer, pH 11.60; from reference 102. [d] This work. [e] β-CD by conductometric method; from reference 103. [f] Hp-β-CD In a 0.2 M phosphate buffer, pH 11.60; this work.

$$[CD.I] = [CD]_o - [CD] \quad [58]$$

$$K_I = \frac{[CD][I]}{[CD.I]} \quad [59]$$

4.2.3 Amines

The next chapter discusses the effect of CDs on aminolysis, and it was for this study that we needed to obtain the dissociation constants of CD.amine complexes. For this purpose we developed a method based on the displacement of a fluorescent probe from the CD cavity which is mathematically similar to the kinetic method, but it has some special considerations.

The binding of the probe, in this case the anion of 1-anilino-8-naphthalenesulphonate anion (1,8-ANS), to the CD greatly increases its fluorescence in aqueous solution. The increase in fluorescence follows a saturation type of behaviour, as described by equation [60], which is the fluorescence analog of the kinetic equation [3].

$$F = \frac{F_{ANS}K_{AI} + F_{CD\,ANS}[CD]}{K_{AI} + [CD]} \quad [60]$$

In this equation F_{ANS} is the fluorescence in the absence of any CD, $F_{CD\,ANS}$ is the fluorescence of a 0.1 mM 1,8-ANS solution at saturating concentrations of CD and K_{AI} is the dissociation constant of the CD.ANS complex. Since $[CD]_o$ is generally much larger than $[ANS]_o$ we can make the assumption that $[CD] = [CD]_o$. The fluorescence measurements, F, are always relative to the fluorescence in the

absence of CD, to eliminate the deviations in the lamp from day to day. The value of K_{AI} was estimated by a non-linear fitting of equation [60] to the data, and the fitted parameters F_{ANS}, $F_{CD\,ANS}$ and K_I are summarised in Table 28. The dependence of the observed fluorescence on CD concentration is shown in Figures 37 and 38, for Hp-β-CD and β-CD, respectively.

Table 28. Equilibrium and Fluorescence Parameters for the Fluorescence Enhancement of 1,8-ANS due to Complexation with β-Cyclodextrin and "Hydroxypropyl-β-cyclodextrin".[a]

CD	F_{ANS}	$F_{CD\,ANS}$	K_{AI} mM
β-CD	1.00	46.7 ± 1.7	26.8 ± 1.3
Hp-β-CD	1.00	430 ± 2	1.71 ± 0.04

[a] Constants were obtained by the non-linear least squares fitting of equation [60] to the experimental data. Aqueous phosphate buffer (0.2 M), pH 11.60 at 25.0 ± 0.1 °C.

Several points are immediately evident from the data in Table 28. First of all, the binding of 1,8-ANS is much stronger to Hp-β-CD than to β-CD, by more than an order of magnitude. This is the first time that we have observed a large

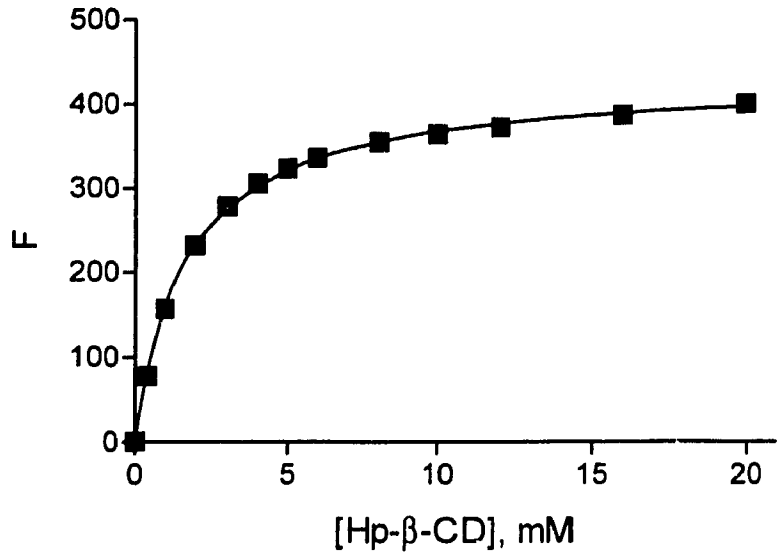


Figure 37. Dependence of fluorescence enhancement due to binding of 1,8-ANS to Hp-β-CD.

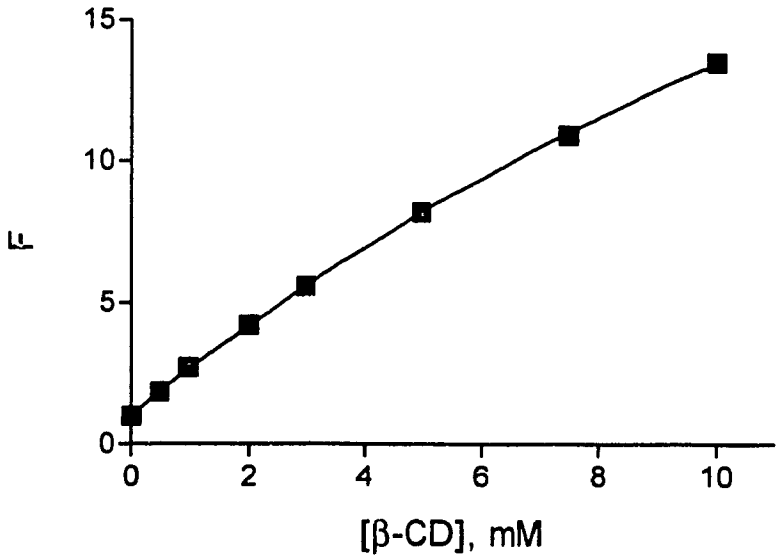


Figure 38. Dependence of fluorescence enhancement due to binding of 1,8-ANS to β-CD.

difference in binding behaviour of β-CD and Hp-β-CD. This type of result has led some to conclude wrongly that Hp-β-CD has significantly different binding abilities than β-CD, based on limited results with a few fluorophores.[39] Second, at saturating levels of CD, the fluorescence of the β-CD.1,8-ANS complex is 47 times greater than that of free 1,8-ANS (Figure 38), whereas Hp-β-CD affords a fluorescence enhancement of 430-fold (Figure 37). This difference is much more marked in practical terms, since the determination of the K_I values for the amines is done at CD concentrations below saturation. Typically the [β-CD] for the binding studies was 10 mM, where the fluorescence enhancement is only 13 fold. With Hp-β-CD we worked in a range of concentrations, from 2.5 to 10 mM, and still had a fluorescence enhancement of 250 - 360 fold. This means that within typical working concentration ranges of 2 to 10 mM, we have anywhere from 55 to 28 fold enhancement of fluorescence by Hp-β-CD over β-CD, for equal [CD].

If we now add another species (RNH_2), capable of binding to the CD, into the system, we have competitive binding as illustrated in equation [61]. Since we have already determined the dissociation constant of the CD.ANS complex, we can calculate K_I by considering the following. The concentration of free 1,8-ANS can be calculated using the quadratic equation [62], whose solution is given in equation [63].

$$CD.1,8\text{-}ANS \underset{K_{AI}}{\overset{1,8\text{-}ANS}{\longleftarrow}} CD \underset{K_I}{\overset{RNH_2}{\longleftarrow}} CD.RNH_2 \qquad [61]$$

$$K_{AI} = \frac{[ANS]([CD]_o - ([ANS]_o - [ANS]))}{([CD]_o - [CD])} \quad [62]$$

$$[ANS] = \frac{-B + \sqrt{B^2 + 4K_{AI}[ANS]_o}}{2} \quad [63]$$

where $B = [CD]_o + K_{AI} - [ANS]_o$

The concentrations of CD.ANS and free CD are then determined using the mass balance equations [64a] and [64b].

$$[CD.ANS] = [ANS]_o - [ANS] \quad [64a]$$

$$[CD] = [CD]_o - [CD.ANS] \quad [64b]$$

By knowing the concentrations of free 1,8-ANS and CD, we can calculate a reference fluorescence due to both free and bound 1,8-ANS, F_{ref}, using equation [65]. We then convert our observed fluorescence into relative fluorescence, F_{rel}, using the values of F_{ref} and the fluorescence in the absence of any added amine, F_{NG}, as shown in equation [66].

$$F_{ref} = \frac{(F_{ANS}K_{ANS} + F_{CD\,ANS}[CD])}{(K_{ANS} + [CD])} \quad [65]$$

$$F_{rel} = \frac{F_{ref}\,F_{obs}}{F_{NG}} \quad [66]$$

Finally use F_{rel} to calculate the concentration of [CD.ANS] in the presence of added amine, according to equation [67]. Knowing the [CD.ANS] allows us to use equation [68] to calculate the concentration of free CD, which in turn allows us to calculate the concentration of CD.amine complex and free amine using the mass balance equations [69] and [70].

$$[CD.ANS] = \frac{F_{rel} - F_{ANS}}{F_{CD\ ANS} - F_{ANS}} \times [ANS]_o \qquad [67]$$

$$[CD] = \frac{K_{AI}[CD.ANS]}{[ANS]_o - [CD.ANS]} \qquad [68]$$

$$[CD.RNH_2] = [CD]_o - [CD] - [CD.ANS] \qquad [69]$$

$$[RNH_2] = [CD]_o - [CD.RNH_2] \qquad [70]$$

After having determined [CD], $[CD.RNH_2]$, and $[RNH_2]$ we can use equation [59], with I = RNH_2, to calculate the K_I values, as shown by the example in Table 29. K_I values determined in this way for both β-CD and Hp-β-CD are collected in Table 30. These results show much the same behaviour as other alkyl-bearing guests in that there is a linear dependence of the binding strength on the number of carbons (N), and in the case of the linear alkyl amines, this corresponds to an increasing chain length (Figure 39). The LFERs for the binding of the alkylamines to the CDs are given by equations [71] and [72], inclusion of the two cyclic amines with each series barely changes the equations, since these points lie virtually on

Table 29. Sample Calculation for the Determination of the Dissociation Constants for Various Amines from β-Cyclodextrin and "Hydroxypropyl-β-cyclodextrin", Based on the Displacement of a Fluorescence Probe.[a,b]

INHIBITION CALCULATION -- Expt # D - 264

Guest (G)= 1-PrNH2 | CD = β-CD | Probe = 1,8-ANS

$[CD]_o$ = 10.00 mM | $[ANS]_o$ = 0.075 mM | K_{AI} = 26.79 mM

F_{ANS} = 1.000 | $F_{CD\,ANS}$ = 46.7 | F_{NG} = 0.0310[c]

Solve quadratic equation [62]; B = 36.715 (see text, equation [63])

[ANS] = 0.0546 mM | f_{ANS} = 0.7286

[CD.ANS] = 0.0204 mM | F_{ref} = 13.4

[CD] = 9.9796 mM | $f_{CD\,ANS}$ = 0.2714

$[G]_o$ (mM)	F_{obs} ($x10^2$)	F_{rel}[d]	[CD.ANS][e] (mM)	[CD][f] (mM)	[CD.G][g] (mM)	[Guest][g] (mM)	K_I[h] (mM)
0.00	3.10	13.4	0.0204	9.98	-	-	-
44.3	2.50	10.8	0.0161	7.32	2.66	41.6	115
88.6	2.08	8.99	0.0131	5.68	4.31	84.3	111
177	1.56	6.74	0.00943	3.85	6.14	171	108

(continued...)

199	1.47	6.36	0.00879	3.56	6.44	193	107
221	1.36	5.88	0.00801	3.20	6.79	215	101
						Average K_I, mM =	108
							± 5

[a] Aqueous solution, pH 11.60, 25.0 ± 0.1 °C. [b] Values of K_{AI}, F_{ANS}, and $F_{CD\,ANS}$ were determined in a previous experiment. [c] Fluorescence in the absence of any guest. [d] Using given values of F_{ref} and F_{NG}, calculated as follows: $F_{rel} = F_{ref}F_{obs}/F_{NG}$. [e] Calculated using equation [67]. [f] Calculated using equation [68]. [g] Calculated using mass balance equations [69] and [70]. [h] Calculated using equation [59].

Table 30. Dissociation Constants for Various Amines from β-Cyclodextrin and "Hydroxypropyl-β-cyclodextrin".[a]

RNH_2	K_I mM	
	β-CD	Hp-β-CD
C_3	108 ± 5	141 ± 4
C_4	35.6 ± 1.2	42.2 ± 2.2
C_5	11.4 ± 0.2	13.7 ± 0.8
C_6[c]	2.62 ± 0.27	4.81 ± 0.36
C_7[c]	0.955 ± 0.041	1.32 ± 0.05
C_8[c]	-[b]	0.520 ± 0.02
cyclo-C_5	13.5 ± 2.1	16.1 ± 0.9
cyclo-C_6	1.83 ± 0.24	5.21 ± 0.21

[a] Constants were obtained by the method outlined in section 4.2.3. Aqueous solution, pH 11.60, 25.0 ± 0.1 °C. [b] Not determined due to the limited solubility of the β-CD.OctNH_2 complex. [c] 0.2 M aqueous phosphate buffer, pH 11.60.

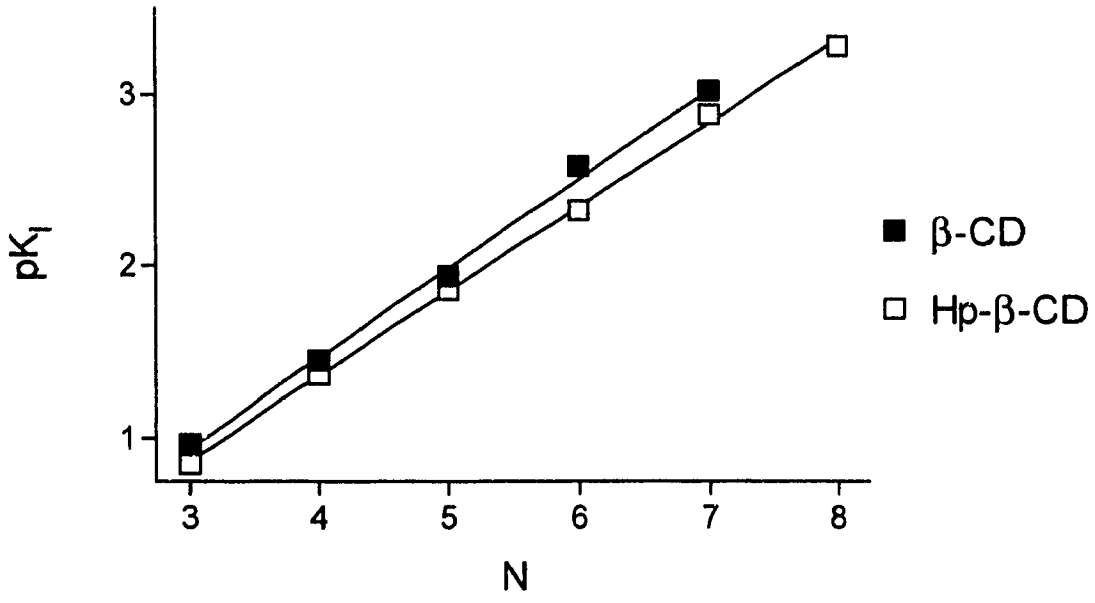


Figure 39. Dependence of pK_I on chain length of *n*-alkylamines.

top of their straight chain analogues. Since equations [71] and [72] are very similar, a plot of pK_I(Hp-β-CD) versus pK_I(β-CD) has a slope very close to unity

RNH_2 + β-CD: $pK_I = (0.524 \pm 0.016)\ N - (0.627 \mp 0.085)$ [71]

(N = 5; r =0.9985)

RNH_2 + Hp-β-CD: $pK_I = (0.490 \pm 0.008)\ N - (0.599 \mp 0.049)$ [72]

(N = 6; r = 0.9994)

4.3 Discussion

We can see from equations [71] and [72] that the alkylamines bind to both CDs with about the same strength (Table 30 and Figure 39) This is similar to what was observed with the nitrophenyl alkanoates (Chapter 2), and other alkyl-bearing guests, such as alkanesulphonate anions, alcohols, and ketones.[60] This behaviour is not limited to straight chain alkyl compounds, since in some instances the correlation between pK_I and the number of carbons in the alkyl portion is equally good when including the cyclic isomers.

Table 31 summarises the dependence of complex forming ability on chain length for various series of straight chain alkyl-bearing compounds. We can see that the dependence of pK_I on N, with Hp-β-CD, for the amines and alkanesulphonate anions is virtually identical, 0.488 and 0.490, respectively. This would support a binding which occurs with the alkyl portion of the guest in the CD cavity, while the "head" group is oriented such that it is largely exposed to the

Table 31. Chain Length Dependence of the Binding of *n*-alkyl Compounds to β-Cyclodextrin and "Hydroxypropyl-β-cyclodextrin".[a]

Substrate	CD	N	slope	r
Aliphatic				
R-OH	β[b]	1 - 7	0.571	0.999
	Hp-β[c]	3 - 7	0.580	0.998
RCH(OH)Me	β[b]	1 - 4	0.450	0.993
	Hp-β[d]	1 - 4	0.428	0.995
$R\text{-}SO_3^-$	β[d]	4 - 8	0.479	0.998
	Hp-β[c]	4 - 8	0.488	0.999
$R\text{-}NH_2$[c]	β	3 - 7	0.524	0.998
	Hp-β	3 - 8	0.490	0.999
$R\text{-}COCH_3$[d]	β	1 - 6	0.459	0.999
	Hp-β	1 - 6	0.456	0.999
Aromatic				
$R\text{-}CO_2\text{-}m\text{-}NO_2Ph$	β[e]	2 - 5	0.157	0.997

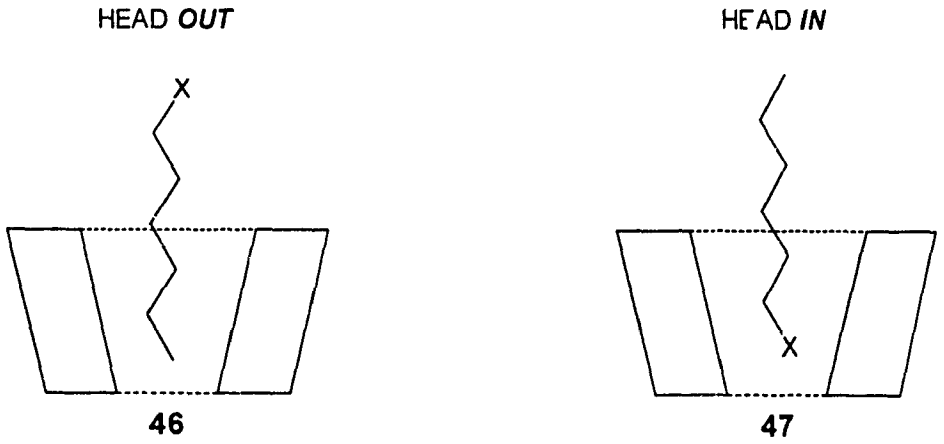
(continued...)

	Hp-β^c	1 - 7	0.180	0.993
R-CO_2-*p*-NO_2Ph	β^e	2 - 5	0.194	0.988
	Hp-β^c	1 - 9	0.201	0.993

---

[a] Aqueous solution at 25.0 ± 0.1 °C. The slope and the correlation coefficient (r) are from the least squares analysis of pK_i versus N. [b] Reference 123. [c] This work and reference 60. [d] Reference 60. [e] Reference 58.

surrounding medium (**46** versus **47**). It is very unlikely that the sulphonate anion group would bind inside the cavity due to its hydrophillic nature, therefore it is most likely that this compound binds as in **46**. Since the dependence of the binding strength of alkylamines to Hp-β-CD on chain length is virtually identical to that of the alkanesulphonate anions, it is most likely that the alkylamines also bind with their alkyl chains in the CD cavity.

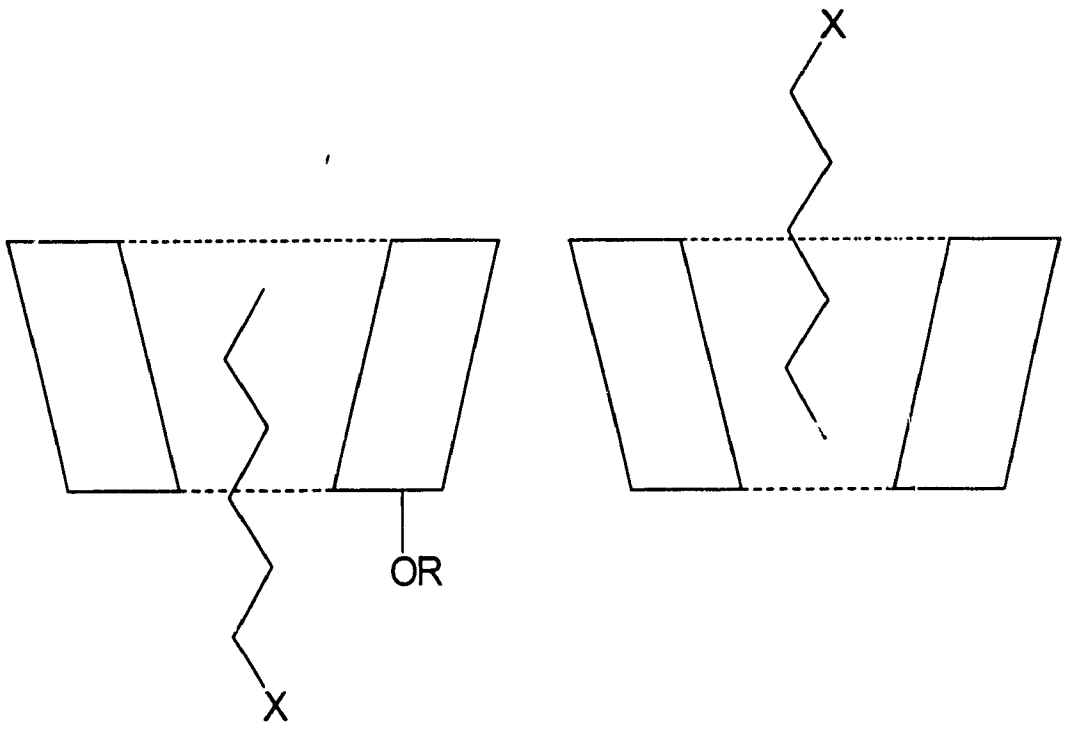


46 **47**

By stating that the alkyl-bearing compounds bind to CDs with their chains in the cavity, we are not making any assumptions as to whether the head group is outside on the primary (**48**, "down") or secondary side (**49**, "up") of the cavity. Until recently, this question could not be answered, since even NMR experiments were not able to provide conclusive evidence for either mode of binding.

By considering the data in Tables 13, 14, 25, 27, 30 and Figure 40, we conclude that the binding of these guests occurs via the wider secondary side of the CD cavity (**49**).[102] The solid line in Figure 40 is given by equation [73] which

"DOWN" "UP"



48 **49**

β-CD; R = H

Hp-β-CD; R = $CH_2CH(OH)CH_3$

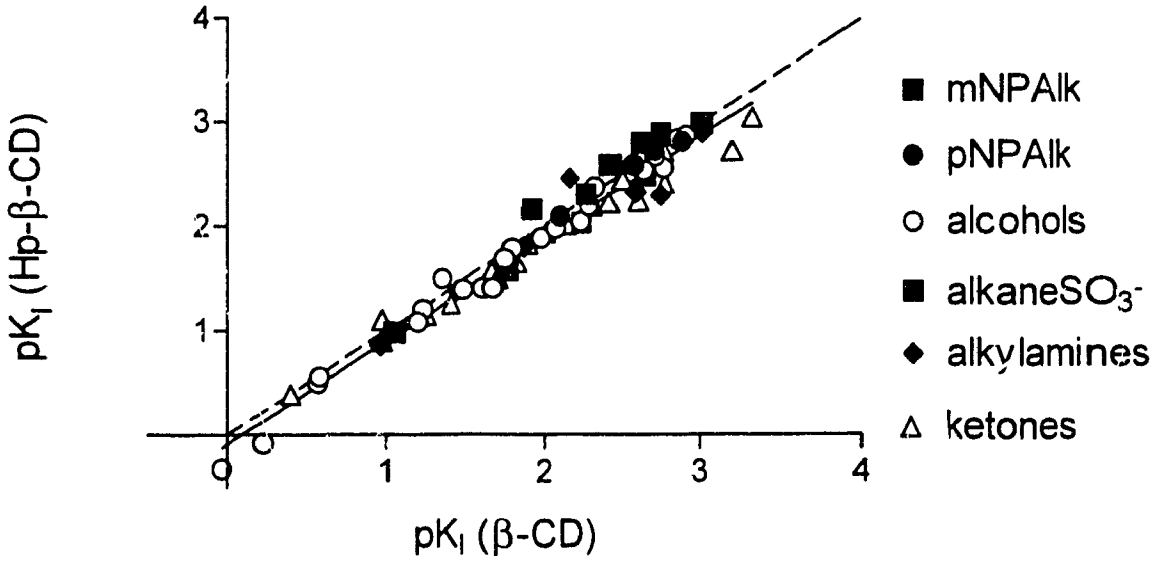


Figure 40. Correlation of the binding strengths of 65 compounds to β-CD and Hp-β-CD. Dashed line has slope 1.00 and passes through the origin.

was determined by a linear regression of pK_I (Hp-β-CD) versus pK_I (β-CD), while the dashed line has unit slope and passes through the origin. Since the slope and intercept terms are virtually unity and zero, respectively, it means that the strength of binding of these 65 compounds (Figure 40) (10 nitrophenyl alkanoate esters, 22 alcohols, 5 alkanesulphonates, 8 amines, an 20 ketones) to β-CD and Hp-β-CD, are virtually identical. The only way that this could occur is if the binding occurs by access through the unmodified, wider, secondary opening of the CD cavity. Although the intercept is slightly negative, meaning a slightly tighter binding to β-CD than to Hp-β-CD, the difference is too small to denote significant interference by the 2-hydroxypropyl groups. These distant groups may account for the difference in binding to the secondary side of the CD, but the mechanism of this action is unclear. If the 2-hydroxypropyl groups act to extend the depth of the CD cavity or partially block off the bottom opening, then they may make it more difficult for guest compounds to expel higher energy water molecules from the CD cavity, which is one of the driving forces of complexation (see Chapter 1).

$$pK_I\{\text{Hp-β-CD}\} = (0.999 \pm 0.028)\, pK_I\{\text{β-CD}\} - (0.088 \mp 0.061) \qquad [73]$$

$$(N = 65;\ r = 0.987)$$

The above discussion has dealt with all of the compounds examined so far, except for one, 1,8-ANS. This is the only guest molecule we have studied that exhibited a major difference in behaviour between β-CD and Hp-β-CD. This is not

the only reported compound to show this anomaly,[39] although they are the exception rather than the rule. Most of the anornalous compounds are aromatic in nature, such as pyrene, and not alkyl-bearing molecules. NMR experiments have shown[129] that 1,8-ANS binds to β-CD in such a way that only the phenyl ring is included in the cavity and that the naphthyl group is in the bulk solvent, although one might expect the reverse based on size considerations.

One possible explanation for this anomalous behaviour is that the addition of 2-hydroxypropyl groups to the CD alters the physical properties of the cavity in such a way that a different mode of binding of 1,8-ANS is now favoured, compared to β-CD. If the 2-hydroxypropyl groups on the primary side of the CD promote a deeper inclusion of 1,8-ANS into the CD cavity, it is then easy to understand the great increases in fluorescence enhancement observed with Hp-β-CD, since the molecule would now be more removed from the bulk aqueous solution, compared to β-CD where the majority of the molecule *is in* the bulk aqueous solution. The basis of the difference in binding behaviour needs to be examined in more detail, since it may also provide information about the differences observed in the transition states for Hp-β-CD accelerated cleavage reactions.

4.4 Conclusions

From the data presented in this chapter we can draw three major conclusions: a) Binding of the alkyl-bearing guests examined so far occurs via inclusion of the alkyl group into the CD cavity. b) Binding of *most* compounds to

β-CD and Hp-β-CD occurs via the wider secondary opening of the CD cavity, since both CDs show almost identical binding behaviour. c) The binding of 1,8-ANS to Hp-β-CD is radically different than the binding to β-CD, since the Hp-β-CD complex is more than an order of magnitude more stable, in terms of K_i, and the fluorescence enhancement afforded by Hp-β-CD is also at least 10 fold greater than by β-CD at sub-saturating [CD].

4.5 Experimental

The experiments with the nitrophenyl alkanoates were outlined in Chapter 2, and for the determination of the dissociation constants of alcohols and alkanesulphonate anions, by the kinetic method, the experiments were done as in Chapter 3, with the only difference being the ester used, *m*-nitrophenyl acetate.

The alkanesulphonate ions were purchased as their sodium salts from the Aldrich Chemical Company and used without further purification. Hp-β-CD was purchased from Wacker-Chemie (Munich, Germany). The 1-anilino-8-naphthalenesulphonic acid, β-CD, alkanesulphonates, and the amines were purchased from the Aldrich Chemical Company and used without further purification.

The fluorescence experiments were carried out using the stopped-flow spectrophotometer mentioned earlier, but set up in the fluorescence mode. The excitation wavelength was selected with a monochromator, and then the light was passed into the sample cell. The fluorescence signal was measured at 90° to the

incident light by an end-window photomultiplier tube, after having passed through a second monochromator. This set up allowed us to scan both the excitation and emission wavelengths.

The sample cell was irradiated at 383 nm, which corresponds to an excitation maximum in the emission spectrum of 1,8-ANS and CD.ANS. The β-CD.ANS emission was monitored at 474 nm, while that of Hp-β-CD was monitored at 468 nm. These wavelengths were not necessarily the maxima, but merely the most convenient to work with, using our experimental set up.

It was important to rinse out the sample cell with fresh solution before each measurement, because we found that 1,8-ANS deteriorates with time when it is left in the light path.

The determination of the K_I for 1,8-ANS was done by mixing equal volumes of 0.4 M phosphate buffer, pH 11.60, containing varying concentrations of β-CD or Hp-β-CD, with an aqueous solution containing 0.2 mM 1,8-ANS. The fluorescence of the solution was measured three times, and then averaged, at varying concentrations of added CD, allowing us to determine the K_I for 1,8-ANS with β-CD or Hp-β-CD as detailed in the text.

The K_I values for the amines determined by mixing a solution containing varying concentrations of amine, pH 11.60 (0.4 M phosphate buffer for the straight chain C_6, C_7, and C_8 amines, a buffer of the amine itself for all of the others), and CD (β-CD = 10.0 mM; Hp-β-CD = 5.0 mM) with an aqueous solution containing 1,8-ANS (β-CD - 0.2 mM; Hp-β-CD - 0.05 mM) and CD (β-CD = 10.0 mM; Hp-β-

CD = 0 mM). Much higher [β-CD] were required since the fluorescence enhancement is much lower for β-CD than Hp-β-CD. The decrease in fluorescence as the concentration of amine was increased was analyzed as outlined in the text to afford an estimate of the K_I values.

5. Nucleophilic Attack in the Presence of CDs

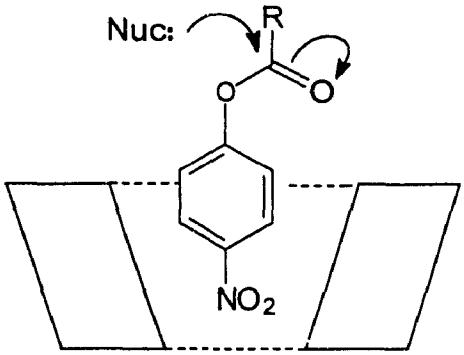
5.1 Introduction

As discussed in Chapter 2, we found that long chain alkanoate esters reacting with Hp-β-CD do so via processes involving two molecules of CD. These processes involve either the attack of the second CD on a molecule of ester bound to a CD (equation [4], Chapter 2) or reaction within a discrete 1:2 {ester.CD_2} complex (equation [6], Chapter 2).

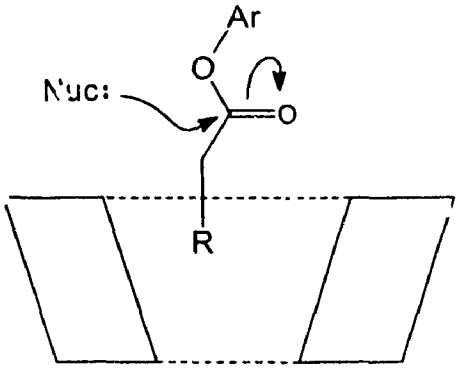
These results have prompted us to ask the question, "How do CDs mediate the reaction of *p*-nitrophenyl alkanoates with other, 'external' nucleophiles?" It has recently been reported, that the cleavage of pNPA by α-amino acids is catalysed by CDs, in a reaction that proceeds through a ternary {ester.CD.AA} complex.[132] However, we have not been able to reproduce these results (*vide infra*).

One of the first points we needed to investigate was whether or not the ester is more or less reactive when bound to a CD, and how the reactivity varies with nucleophile, ester and CD. Should binding of the ester to the CD occur in such a way that the carbonyl carbon is situated mainly in the surrounding medium, and thus exposed to external nucleophiles (**50** or **51**), its reactivity towards such nucleophiles may not be greatly affected. If, on the other hand, the ester is bound with the carbonyl group buried in the CD cavity, then it should be much less reactive towards an external nucleophile. There also exists the possibility that the CD plays a role other than simply binding the ester, and that the secondary hydroxy groups may become involved in the reaction, either through general base

catalysis or hydrogen bonding, as was proposed in the case of the aminolysis of pNPA by α-amino acids.[132]



50



51

The mode of binding of the ester to the CD, aryl vs acyl group inclusion (**50** vs. **51**), was also a feature we were seeking to characterise. We have discussed, in previous chapters, how the mode of ester binding may be probed by varying the ester chain length, and we will use this method to determine the mode of ester binding during cleavage by external nucleophiles. If appropriate kinetic parameters exhibit only minimal dependence on the length of the acyl chain, then reaction most likely occurs via aryl group inclusion (**50**), whereas a steep dependence of these parameters on acyl chain length would provide evidence for the reaction occurring through acyl group inclusion (**51**).

Another factor which must be taken into consideration when studying the cleavage of pNPAlk by larger nucleophiles is the binding of the nucleophiles to the

CD. It was previously determined that the cleavage of pNPH by β-CD in the presence of alcohols occurs via a ternary ester.CD.ROH complex,[95 96] where the alcohols appear to be acting as an inert space filler. We were interested in seeing if *n*-alkylamines, which are similar in size and structure to *n*-alkanols, also react via a ternary complex, and if the formation and reaction through this {ester.CD.amine} complex affords significant catalysis.

5.2 Results

In order to investigate the various points discussed in the introduction, we performed three sets of experiments. First, to probe the relative reactivity of the CD-bound esters towards small, non-binding, nucleophiles we studied the reaction of pNPA and pNPH with trifluoroethanol (TFE) and β-mercaptoethanol (ME) in the presence of α-CD, β-CD and Hp-β-CD, as well as with hydroxylamine and imidazole in the presence of α-CD and β-CD. We also did some exploratory work on the cleavage of pNPA and pNPH by α-amino acids in the presence of β-CD. Second, the mode of ester binding was investigated by studying the cleavage of *p*-nitrophenyl alkanoates (C_2 to C_{10}) by TFE and ME in the presence of α-CD, β-CD, and Hp-β-CD. The final series of experiments were conducted to investigate the cleavage of *p*-nitrophenyl alkanoates (C_2 to C_6) by alkylamines (C_3 to C_8) in the presence of β-CD and Hp-β-CD. All experiments were carried out in basic aqueous buffers chosen to have pHs appropriate to the pK_a of the nucleophiles,

knowing their reactivities towards *p*-nitrophenyl esters.[126 127 133-137] The raw data are collected in Appendix IV.

5.2.1 *p*-Nitrophenyl Hexanoate and Trifluoroethanol

Our first studies in this area centred on the cleavage of pNPH by the anion of TFE, which was chosen as being an appropriate nucleophile for several reasons. Since ethanol binds very weakly to CDs[59 123] we assumed, that for the low TFE concentrations used the binding of TFE to the CDs would be insignificant Earlier work had already established the reactivity of the TFE anion towards pNPA[96 97 126 127 137] and pNPH.[96 97] The pK_a of TFE[138] (12 4) is very similar to that of the secondary CD hydroxy groups,[48 49] meaning that the TFE anion can compete efficiently with CD as a nucleophile. Also, the similarity in pK_as of TFE and the CDs provides a possibility that ester cleavage will be general base catalysed, either by CD-assisted attack of TFE or the TFE-assisted attack of CD.

As discussed previously, the CD-assisted cleavage of pNPH in basic aqueous medium, in the absence of TFE, occurs via the reactions given in equations [1] and [2] (Chapter 2). It has been shown[126 127 137] that pNPA is cleaved by various nucleophiles, through a reaction as given by equation [74] We take as a working hypothesis that the nucleophile also reacts with the CD-bound ester (equation [75]). Combining equations [74] and [75] with equations [1] and [2] yields an expression for k_{obs} which is dependent on both the concentrations of CD and TFE, as given by equation [76].

$$S + TFE \xrightarrow{k_N} P \qquad [74]$$

$$S.CD + TFE \xrightarrow{k_{cN}} P \qquad [75]$$

$$k_{obs} = \frac{(k_u + k_N[TFE])K_s + (k_c + k_{cN}[TFE])[CD]}{K_s + [CD]} \qquad [76]$$

As shown by the calculated curves in Figure 41, equation [76] fits the data very well, which supports our hypothesis that the TFE is capable of reacting with the bound ester. At every level of α-CD the addition of TFE raises k_{obs} although at higher [α-CD] this effect is diminished, indicating the bound ester is *less* reactive than the free ester.

$$\frac{k_{obs}}{f_s} = k_u + k_N[TFE] + \frac{k_c[CD]}{K_s} + \frac{k_{cN}[TFE][CD]}{K_s} \qquad [77]$$

Equation [76] is not amenable to easy analysis, since it is both non-linear and bivariate. Therefore, in order to obtain the rate constants k_N and k_{cN} we linearised the equation by dividing through by the fraction of free substrate, $f_s = K_s/(K_s+[CD])$, yielding equation [77]. We used this equation, and a K_s value of 3.52 mM for the binding of pNPH to α-CD, to *fit to all of the data* shown in Figure 41 by a multiple linear regression, with [CD], [TFE], and [CD][TFE] as our independent variables, (see Figure 42). We obtained a very strong correlation (r = 0.9999) and were able to obtain the values of k_u, k_N, k_c/K_s, and k_{cN}/K_s, from which k_u = 0.0476

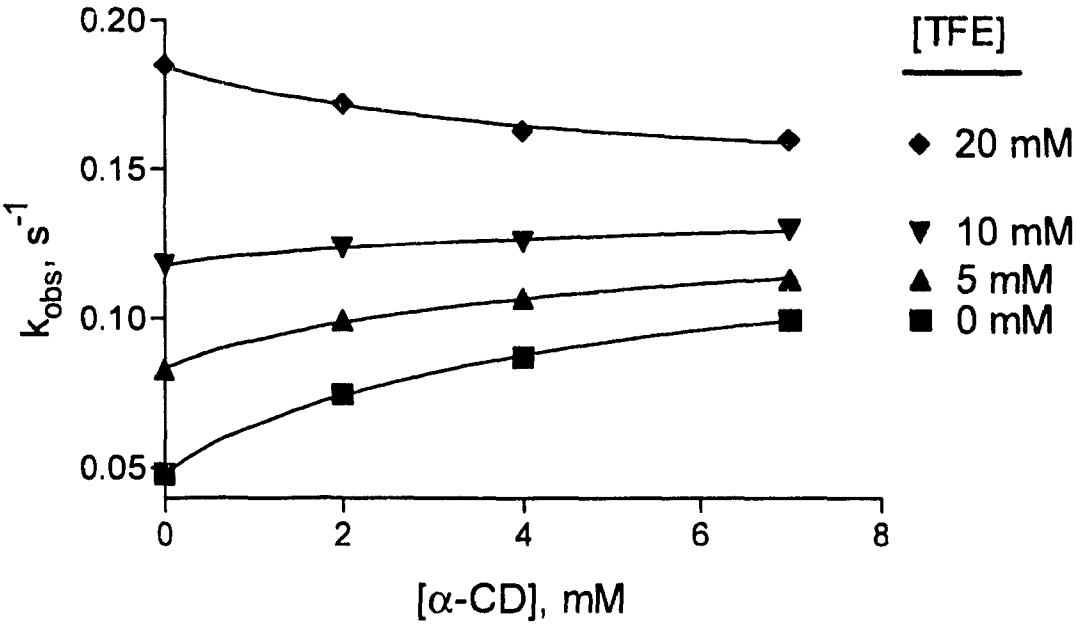


Figure 41. Dependence of k_{obs} on [α-CD] at four concentrations of TFE. Solid lines calculated using equation [76].

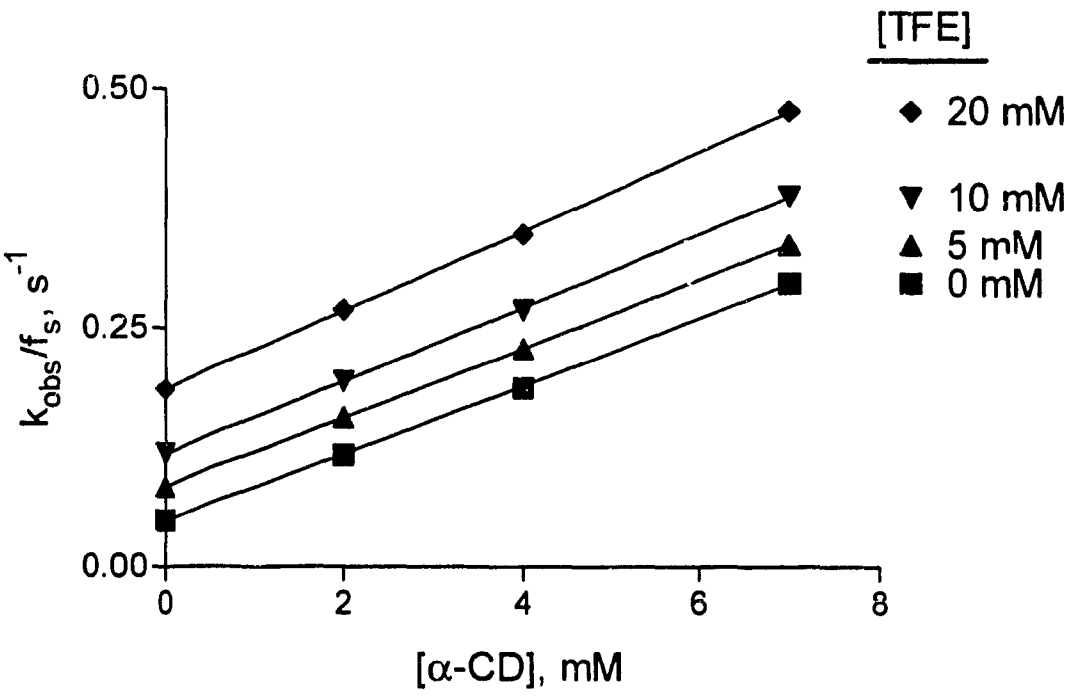


Figure 42. Dependence of k_{obs}/f_s on [α-CD] at four concentrations of TFE. Solid lines calculated using equation [77].

$\pm$ 0.0019 s^{-1}, k_N = 6.89 $\pm$ 0.10 $M^{-1}s^{-1}$, k_c = 0.124 $\pm$ 0.001 s^{-1}, and k_{cN} = 1.09 $\pm$ 0.09 $M^{-1}s^{-1}$. The values of k_u and k_c are very similar to earlier reported values,[97] and help to confirm the validity of this analysis. Using these fitted parameters and equation [76], we are able to reproduce the data very well (Figure 41). Comparable data for β-CD and Hp-β-CD were analysed in the same way and gave equally good results (Figures 43 and 44); values of k_N and k_{cN} are summarised in Table 32.

5.2.2 *p*-Nitrophenyl Acetate and Trifluoroethanol

Although the above described method yields very good results, it is limited in one aspect: the number of measurements required make it very time consuming. We therefore developed a method, based on the above, which allowed us to obtain the same information while performing fewer experiments. The first step was to carry out experiments in the absence of CD, which causes equations [76] and [77] to reduce down to equation [77b], from which we can easily obtain k_N as the slope of k_{obs} versus [TFE]. In order to determine k_{cN}, we perform separate experiments at high [CD] (10 mM), and analyse the data using the full form of equation [76] with known values of k_N and K_s. The slope of k_{obs} versus [TFE] (= $(k_N K_s + k_{cN}[CD])/(K_s + [CD])$) allows us to estimate k_{cN}, for the reaction of the CD-bound ester. Figure 45 shows the effect of TFE on the observed rate constant for the cleavage of pNPA in both the absence and presence of CDs.

$$k_{obs} = k_u + k_N[TFE] \qquad [77b]$$

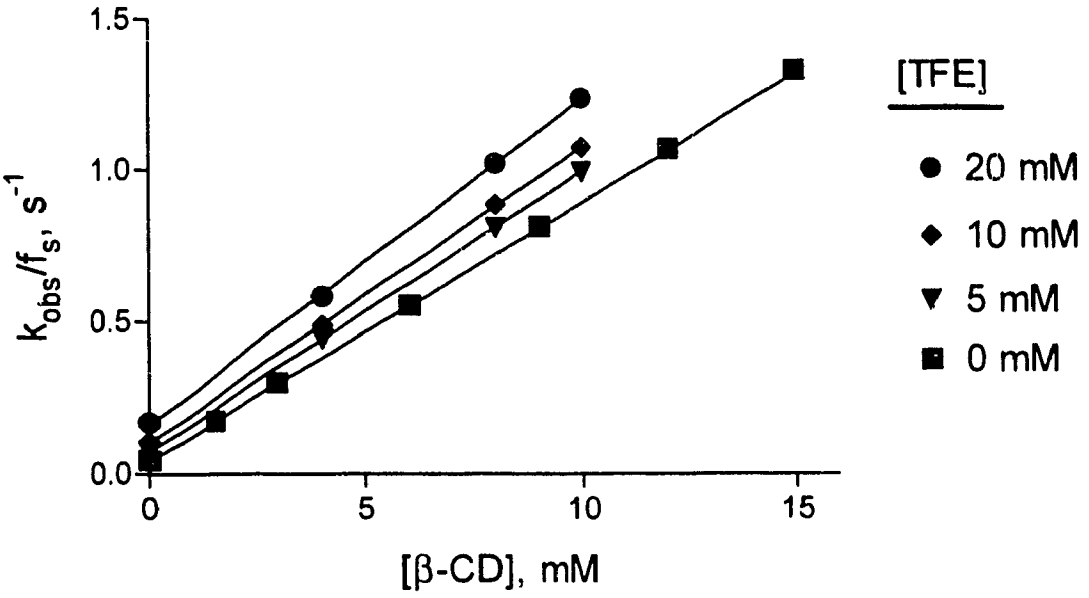


Figure 43. Dependence of k_{obs}/f_s on [β-CD] at four concentrations of TFE. Solid lines calculated using equation [77].

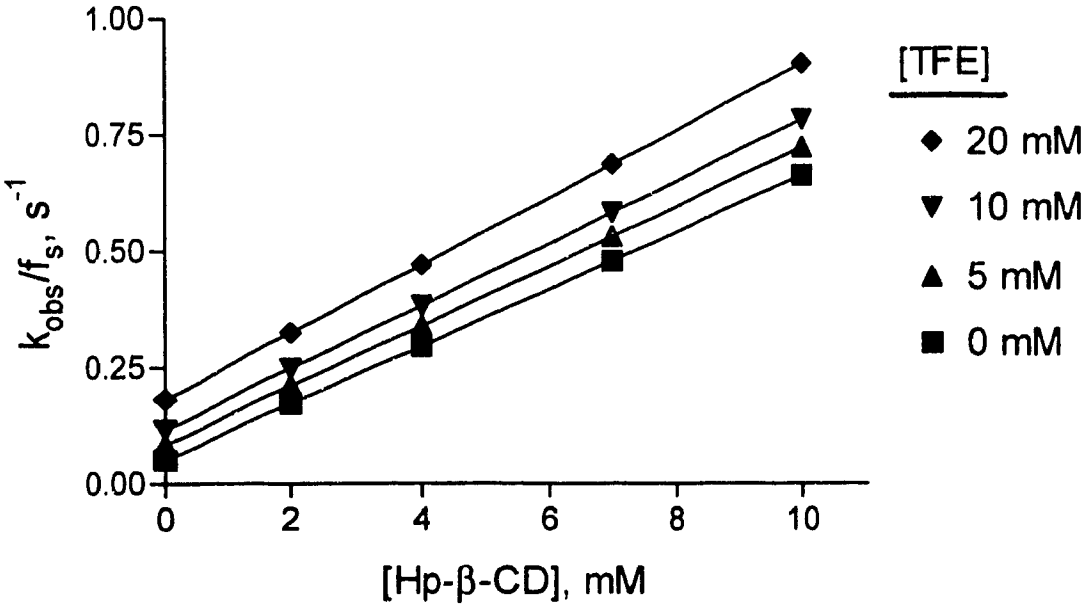


Figure 44. Dependence of k_{obs}/f_s on [Hp-β-CD] at four concentrations of TFE. Solid lines calculated using equation [77].

Table 32. Constants for the Cleavage of *p*-Nitrophenyl Hexanoate and Acetate by TFE and CDs.[a]

CD	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
	p-nitrophenyl hexanoate[b]		
α-CD	6.89 ± 0.10	1.09 ± 0.09	0.16
β-CD	5.87 ± 0.26	1.75 ± 0.06	0.30
Hp-β-CD	6.50 ± 0.29	0.878 ± 0.078	0.14
	p-nitrophenyl acetate[c]		
α-CD	12.8 ± 0.1	8.06 ± 0.09	0.63
β-CD	12.7 ± 0.1	10.4 ± 0.1	0.82
Hp-β-CD	12.8[d]	3.88 ± 0.10	0.30

[a] At 25.0 ± 0.1 °C and pH = 11.60 (0.2 M phosphate buffer). [b] Obtained by fitting equation [77] to the data. [c] k_N obtained in the absence of CD, k_{cN} obtained at high [CD], see text. [d] Same experiment as α-CD

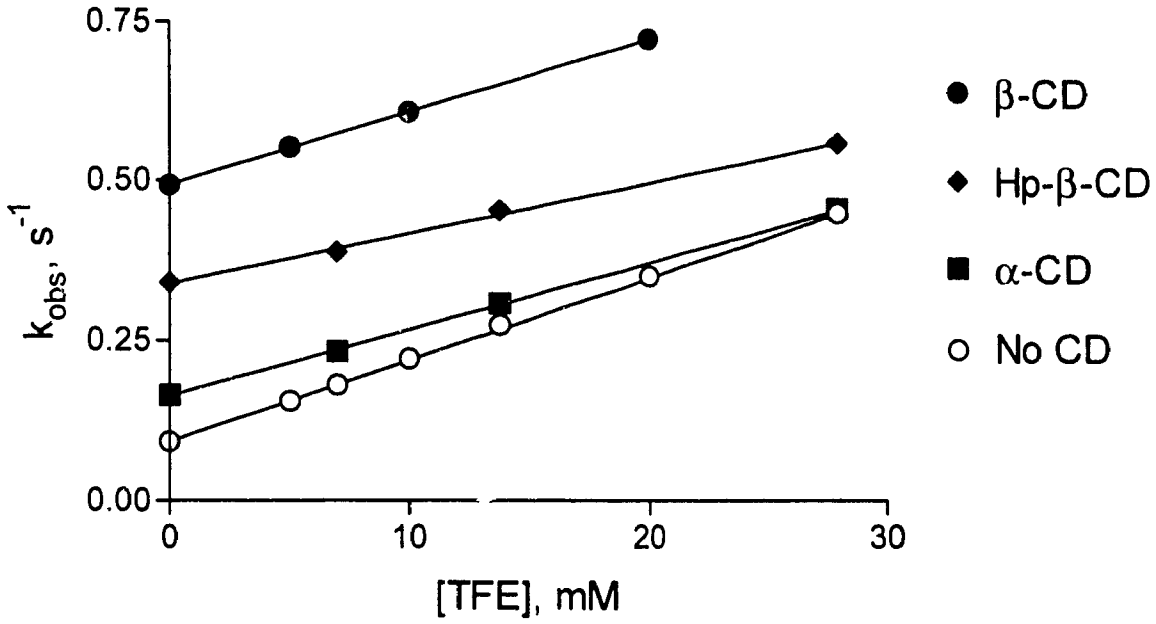


Figure 45. Effect of TFE on k_{obs} for the cleavage of pNPA in the absence and presence of CDs. The data for no CD are taken from two replicate sets.

5.2.3 pNPA and pNPH with other Nucleophiles

We also examined the cleavage of pNPA and pNPH in the presence of several other nucleophiles with α-CD and β-CD. These nucleophiles (Tables 33 and 34) were chosen since they appeared to be too small to bind significantly to α-CD or β-CD, and because we wanted to attempt to reproduce the catalysis reported to occur in the CD-mediated cleavage of pNPA by α-amino acids.[132] For β-mercaptoethanol, hydroxylamine, and imidazole the reactions were carried out at a pH at least 2 units above the pK_a of the nucleophiles, in order to assure that each nucleophile was at least 99% in the reactive form. The second order rate constants, k_{cN} for the cleavage of pNPA and pNPH by these nucleophiles are summarised in Tables 33 and 34. We have included the results for TFE, determined by the simplified method, for comparison.

The values of k_{cN}/k_N vary only slightly in the range of 0.6 to 1.6 and 0.2 to 1.0 for pNPA and pNPH, respectively. It is worth noting that none of the nucleophiles give $k_{cN}/k_N > 1$ for reaction with pNPH, whereas some do with pNPA. The k_{cN}/k_N values for pNPH are significantly lower than those for pNPA, which may reflect stronger binding or a different mode of binding (*vide infra*).

5.2.4 *p*-Nitrophenyl Alkanoates with TFE and ME

The rate constants for the cleavage of a series of pNPAlk by TFE and ME, in the presence and absence of CDs, were obtained by the simplified method described above; they are summarised in Tables 35 and 36, respectively. The

Table 33. Constants for the Cleavage of *p*-Nitrophenyl Acetate by Various Nucleophiles in the Presence of α-CD and β-CD.[a]

Nucleophile	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
	α-CD		
Trifluoroethanol[b]	12.7 ± 0.5	8.03 ± 0.09	0.63
β-Mercaptoethanol[b]	12.5 ± 0.1	14.0 ± 0.3	1.1
Hydroxylamine[c]	2.11 ± 0.03	3.30 ± 0.02	1.6
Imidazole[d]	0.551 ± 0.005	0.776 ± 0.011	1.4
	β-CD		
Trifluoroethanol[b]	-[g]	10.4 ± 0.1	0.82
β-Mercaptoethanol[b]	-[g]	9.78 ± 0.52	0.78
Hydroxylamine[c]	-[g]	1.55 ± 0.01	0.73
Imidazole[d]	-[g]	0.784 ± 0.017	1.4
L-Histidine[e]	0.0740 ± 0.0005	0.0591 ± 0.0001	0.80
L-Alanine[f]	0.291 ± 0.001	0.271 ± 0.001	0.93

[a] In aqueous solution at 25.0 ± 0.1 °C. [b] pH 11.60, 0.2 M phosphate buffer. [c] pH 8.00, 0.1 M phosphate buffer. [d] pH 9.00, 0.1 M borate buffer. [e] pH 8.00, 0.1 M borate buffer. [f] pH 9.878, 0.1 M borate buffer. [g] Unlisted k_N values are taken to be the same as those given for α-CD.

Table 34. Constants for the Cleavage of *p*-Nitrophenyl Hexanoate by Various Nucleophiles in the Presence of α-CD and β-CD.[a]

Nucleophile	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
	α-CD		
Trifluoroethanol[b]	7.06 ± 011	1.88 ± 0.65	0.27
β-Mercaptoethanol[b]	8.53 ± 0.09	6.80 ± 0.25	0.80
Hydroxylamine[c]	0.926 ± 0.016	0.672 ± 0.030	0.73
Imidazole[d]	0.426 ± 0.063	0.426 ± 0.006	1.0
	β-CD		
Trifluoroethanol[b]	-[f]	1.71 ± 0.02	0.24
β-Mercaptoethanol[b]	-[f]	2.06 ± 0.01	0.24
Hydroxylamine[c]	-[f]	0.417 ± 0.002	0.45
Imidazole[d]	-[f]	0.231 ± .002	0.54
L-Alanine[e]	0.125 ± 0.002	0.0297 ± 0.0001	0.24

[a] In aqueous solution at 25.0 ± 0.1 °C. [b] pH 11.60, 0.2 M phosphate buffer. [c] pH 8.00, 0.1 M phosphate buffer. [d] pH 9.00, 0.1 M borate buffer. [e] pH 9.878, 0.1 M borate buffer. [f] Unlisted k_N values are taken to be the same as those given for α-CD.

Table 35. Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by TFE in the Presence of α-CD, β-CD, and Hp-β-CD.[a]

Ester	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
		α-CD	
C_2	12.7 ± 0.1	8.03 ± 0.09	0.63
C_3	11.4 ± 0.3	5.67 ± 0.14	0.50
C_4	6.38 ± 0.02	1.73 ± 0.06	0.27
C_5	7.02 ± 0.09	1.56 ± 0.02	0.22
C_6	7.06 ± 0.11	1.88 ± 0.06	0.27
C_7	7.39 ± 0.12	1.46 ± 0.07	0.20
C_8	7.22 ± 0.42	1.67 ± 0.05	0.23
C_9	5.14 ± 0.03	2.57 ± 0.11	0.50
C_{10}	8.94 ± 0.75	1.79 ± 0.01	0.20
		β-CD	
C_2	-[b]	10.4 ± 0.07	0.82
C_3	-[b]	6.28 ± 0.24	0.55
C_4	-[b]	2.09 ± 0.08	0.33

(continued...)

C_5	-[b]	2.05 ± 0.04	0.29
C_6	-[b]	1.71 ± 0.02	0.24
C_7	-[b]	2.26 ± 0.33	0.31
C_8	-[b]	4.18 ± 1.25	0.58
C_9	-[b]	4.11 ± 0.13	0.80
C_{10}	-[b]	7.73 ± 2.13	0.86
		Hp-β-CD	
C_2	-[b]	7.28 ± 0.18	0.57
C_3	-[b]	4.52 ± 0.12	0.40
C_4	-[b]	2.25 ± 0.03	0.35
C_5	-[b]	1.18 ± 0.05	0.17
C_6	-[b]	1.28 ± 0.01	0.18
C_7	-[b]	1.58 ± 0.02	0.21
C_8	-[b]	1.83 ± 0.04	0.25
C_9	-[b]	1.86 ± 0.10	0.36
C_{10}	-[b]	3.02 ± 0.05	0.34

[a] In basic aqueous solution (0.2 M phosphate buffer), pH 11.60 and 25.0 ± 0.1 °C.

[b] Unlisted k_N values are taken to be the same as given for α-CD.

Table 36. Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by ME in the Presence of β-CD.[a]

Ester	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
C_2	12.5 ± 0.1	9.78 ± 0.52	0.78
C_4	8.56 ± 0.21	3.86 ± 0.05	0.45
C_6	8.53 ± 0.09	2.06 ± 0.01	0.24
C_8	6.02 ± 0.07	2.67 ± 0.01	0.44
C_{10}	9.08 ± 0.47	15.6 ± 0.86	1.7

[a] In 0.2 M aqueous phosphate buffer pH 11.60 at 25.0 ± 0.1 °C.

determination of k_{cN} requires the use of the K_s values for the binding of the ester to the CD, which are presented in Table 37. Most of the K_s values have been determined in earlier work, with the remainder estimated as follows: K_s values for α-CD (C_7, C_9, and C_{10}) were interpolated between the known values of the other esters bearing in mind the linear dependence of pK_s on chain length.[58 90] Values of K_s for the long chain esters with β-CD were taken to be the same as for Hp-β-CD, since the binding of esters is virtually the same to both CDs (see Chapters 2 and 4).

Figure 46 shows the effect of TFE on the cleavage of several pNPAlk, in both the presence and absence of β-CD. For the esters past the propanoate, the value of k_N remains virtually unchanged, with the data for the C_5 and C_6 being superimposable. The k_{cN} values on the other hand tend to decrease up to about the C_6, and then begin to rise again, which accounts for the biphasic behaviour in k_{cN}/k_N (Table 35). It is also evident from the data in Table 35 that the values of k_{cN}/k_N are universally less than unity, indicating that the free esters are more reactive towards TFE than the bound esters.

5.2.5 pNPA and pNPH with Alkylamines

The method of analysis discussed up until now makes the assumption that the nucleophile does not bind to the CD, which is not valid for alkylamines (see Chapter 4). With non-binding nucleophiles we were able to make the assumption that $[CD] = [CD]_o$ and $[Nuc] = [Nuc]_o$, provided that $[CD]_o \gg [S]_o$. Since alkylamines

Table 37. Dissociation Constants for the pNPAlk.CD Complexes.[a]

Ester	K_s, mM		
	α-CD	β-CD	Hp-β-CD
C_2	10	7.9[b]	8.2
C_3	7.4	5.2	5.1
C_4	5.0	2.7	2.7
C_5	3.4	2.0	1.9
C_6	2.9	1.3	1.6
C_7	1.8[c]	0.79[d]	0.79
C_8	0.98[e]	0.50[d]	0.50
C_9	0.91[c]	0.39[d]	0.39
C_{10}	0.65[c]	0 16[d]	0.16
C_{12}	0.37[e]		

[a] In basic aqueous solution at 25.0 ± 0.1 °C. Data are taken from: α-CD and β-CD - reference 58; Hp-β-CD - This work. [b] Reference 96. [c] Interpolated using the correlation equation: $pK_s = 0.15\ N + 1.7$ ($r = 0.993$), based on the pK_s values for N = 2, 3, 4, 5, 6, 8, and 12. [d] Assumed to be the same as Hp-β-CD. [e] Reference 99.

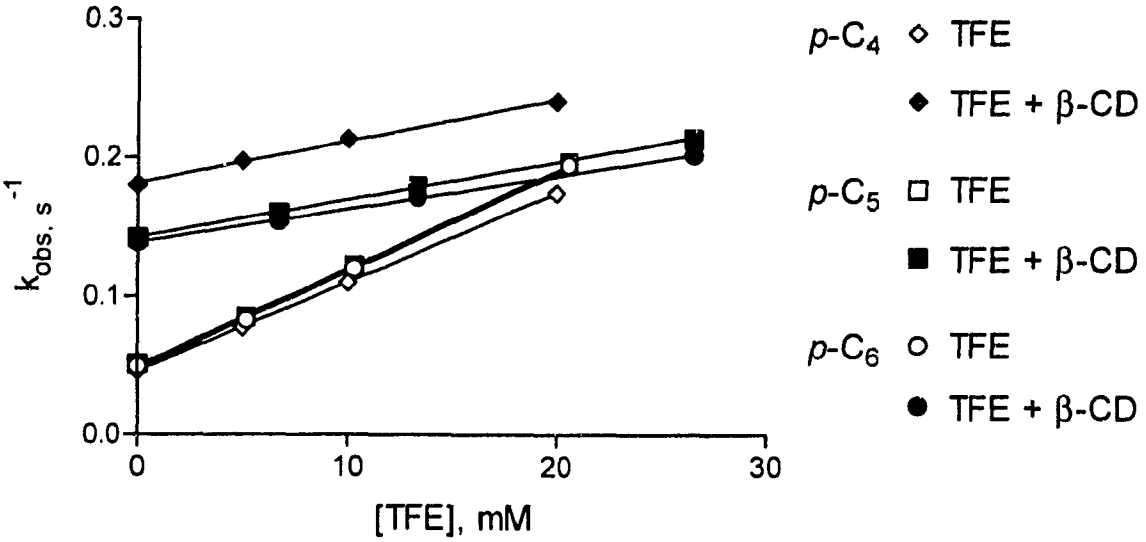


Figure 46. Effect of added TFE on k_{obs} for the cleavage of several pNPAlk, in the absence (open symbols) and presence (closed symbols) of 10 mM β-CD.

do bind to CDs (*vide supra*) we must use their dissociation constants, K_I, to calculate the [CD] and [Nuc], by solving the appropriate quadratic equation. Once we have the free concentrations of both species, the analysis is performed in the same way as detailed in section 5.2.2. The use of this approach gave the values of k_N and k_{cN}, for reaction in the presence of β-CD or Hp-β-CD, collected in Table 38.

Figures 47 and 48 show that the dependence of k_{obs} and k_{obs}/f_s on the concentration of nucleophile, in this case heptylamine, are now decidedly curved. The solid lines in Figure 48 were drawn using equation [77] and the corrected concentrations of CD and nucleophile, and the rate constants which were obtained by performing a multiple linear regression on all of the data shown.

Compared to the results obtained with non-binding nucleophiles, the k_{cN}/k_N ratios for the smaller *n*-alkylamines (< C_6) are about the same, near unity, whereas those for the longer *n*-alkylamines (> C_5) are much larger. This trend in k_{cN}/k_N is due to very large second order rate constants, k_{cN}, for the long amines reacting with pNPA and pNPH in the presence of CDs.

The reactions were carried out at pH 11.60, in either a 0.2 M phosphate buffer (*n*-hexylamine, *n*-heptylamine, and *n*-octylamine) or a buffer made up of the amine itself. Since the C_6, C_7, and C_8 amines have a relatively low solubility in water at this pH, we could not make the solutions concentrated enough to supply adequate buffering.

Table 38. Constants for the Cleavage of *p*-Nitrophenyl Acetate and Hexanoate by Alkylamines in the Presence of β-CD and Hp-β-CD.[a]

Amine		β-CD		Hp-β-CD	
	k_N	k_{cN}	k_{cN}/k_N	k_{cN}	k_{cN}/k_N
	$M^{-1}s^{-1}$	$M^{-1}s^{-1}$		$M^{-1}s^{-1}$	
		p-nitrophenyl acetate			
Propyl	10.6 ± 0.1	14.3 ± 0.8	1.4	5.13 ± 0.78	0.48
Butyl	13.0 ± 0.1	17.2 ± 2.0	1.3	8.55 ± 1.08	0.66
Pentyl	13.4 ± 0.1	24.7 ± 0.5	1.8	6.67 ±1.14	0.50
Hexyl	15.8 ± 0.3	181 ± 2	12	57.2 ± 5.1	3.6
Heptyl	16.7 ± 0.2	244 ± 5	15	59.1 ± 6.4	3.5
Octyl	16.7[b]	1265 ± 4	76	185 ± 4	11
c-Pentyl	2.73 ± 0.01	9.78 ± 0.67	3.6	4.82 ± 0.30	1.8
c-Hexyl	1.71 ± 0.01	19.2 ± 2.5	11	-[c]	
		p-nitrophenyl hexanoate			
Propyl	4.97 ± 0.08	1.58 ± 0.19	0.32	1.01 ± 0.27	0.21
Butyl	6.67 ± 0.10	3.84 ± 0.22	0.58	0.512 ± 0.194	0.077
Pentyl	6.43 ± 0.03	6.89 ± 0.12	1.1	2.24 ± 0.11	0.35

(continued...)

Hexyl	8.46 ± 0.05	43.5 ± 2.1	5.1	9.32 ± 0.45	1.1
Heptyl	7.05 ± 0.10	63.9 ± 2.0	9.1	12.1 ± 1.0	1.7
Octyl	7.05[b]	271 ± 17	38	23.1 ± 2.6	3.28
c-Pentyl	1.30 ± 0.02	3.01 ± 0.06	2.3	0.709 ± 0.039	0.55
c-Hexyl	0.837 ± 0.016	7.78 ± 0.17	9.3	0.328 ± 0.158	0.39

[a] In basic aqueous solution, pH 11.60 and 25.0 ± 0.1 °C. [b] Assumed to be the same as that of *n*-heptylamine. [c] Unable to analyse using model presented in the text.

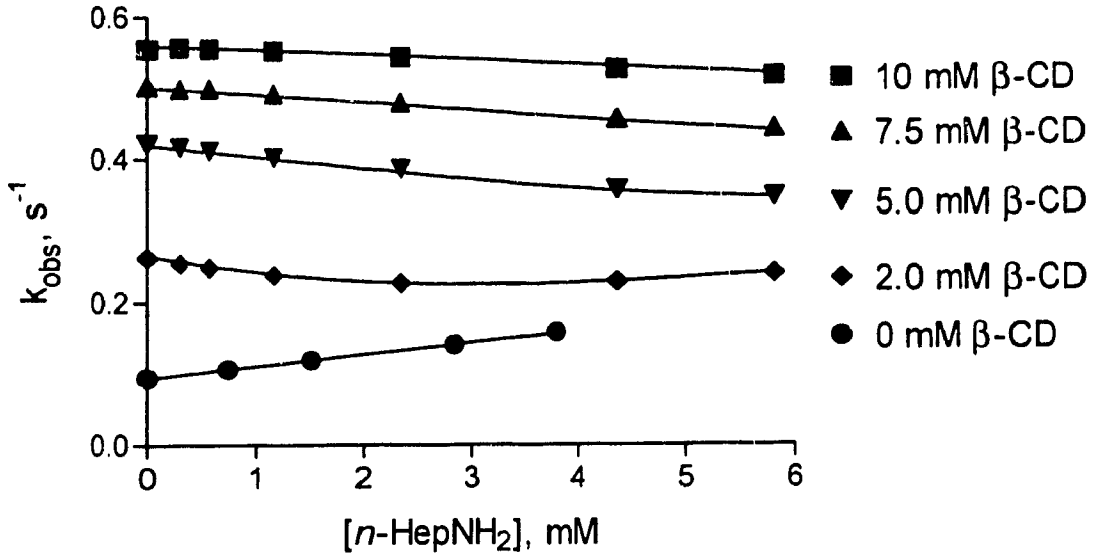


Figure 47. Effect of added *n*-heptylamine on k_{obs} for the cleavage of pNPA in the presence and absence of β-CD.

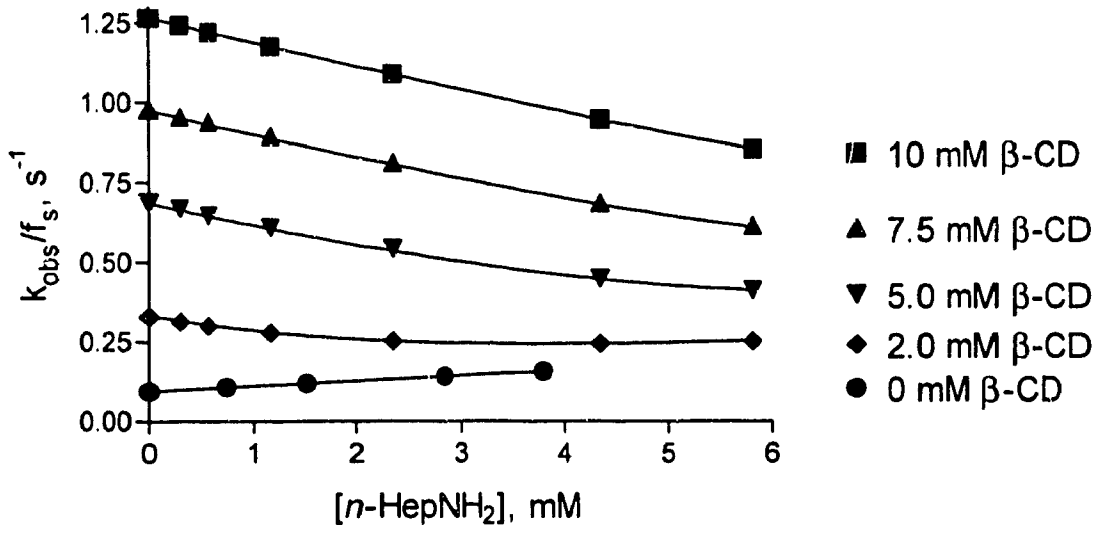


Figure 48. Effect of added *n*-heptylamine on k_{obs}/f_s for the cleavage of pNPA in the presence and absence of β-CD.

5.2.6 *p*-Nitrophenyl Alkanoates with *n*-Heptylamine

The analysis of these data was performed exactly as described in the previous section, except that we were varying the length of the ester acyl chain, as opposed to the length of the amine. Table 39 contains a summary of the values of k_N, k_{cN}, and k_{cN}/k_N for pNPAlk (C_2 to C_6) reacting with *n*-heptylamine in the presence of β-CD and Hp-β-CD.

5.3 Discussion

The data presented in this chapter support our hypothesis that nucleophiles are capable of reacting with both the free and CD-bound esters, and that the kinetic model presented in section 5.2.1 is reasonable, since, with one exception, all of the data are reproduced well by equation [77], regardless whether or not the nucleophiles bind. The assumption that the smaller nucleophiles (TFE, ME, hydroxylamine, imidazole, and amino acids) do not bind significantly to CDs at the concentrations used is a valid one; if these nucleophiles did bind to the CDs then we would expect to see curvature in the plot of k_{obs}/f_s, as we did with the amines (Figures 42-44 versus 48), which do bind.

It is important to note that the rate constants presented in Table 32 were calculated using the longer method, and that the determination of k_N was repeated for each CD. The second order rate constants in the remainder of the tables were all obtained using the simplified method, using the same value for k_N for each nucleophile, determined in a separate experiment. This difference in analyses

Table 39. Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by *n*-Heptylamine in the Presence of β-CD and Hp-β-CD.[a]

Ester	k_N $M^{-1}s^{-1}$	k_{cN} $M^{-1}s^{-1}$	k_{cN}/k_N
		β-CD	
C_2	16.7 ± 0.2	244 ± 5	15
C_3	12.0 ± 0.1	105 ± 3	8.8
C_4	7.41 ± 0.04	60.7 ± 8.3	8.2
C_5	7.78 ± 0.06	54.0 ± 2.5	6.9
C_6	7.05 ± 0.10	63.9 ± 2	9.1
		Hp-β-CD	
C_2	-[b]	59.1 ± 6.4	3.5
C_3	-[b]	16.0 ± 2.8	1.3
C_4	-[b]	8.36 ± 0.57	1.1
C_5	-[b]	14.0 ± 0.9	1.8
C_6	-[b]	12.1 ± 1.0	1.7

[a] In basic aqueous solution (0.2 M phosphate buffer), pH 11.60 and 25.0 ± 0.1 °C. [b] Missing k_N values are taken to be the same as the ones given for β-CD.

accounts for the slight differences between k_{cN}/k_N values reported in Table 32 and elsewhere.

Throughout all of the experiments with the different nucleophiles, esters, and CDs, one factor remains constant, namely, in no case did $k_{cN}/k_N = 0$. This means that in all cases the carbonyl carbon of the ester is at least partially exposed to the surrounding medium, accessible to external attack. It is still to be seen if the binding occurs via aryl inclusion (**50**, p. 178) or if it occurs via acyl inclusion (**51**, p. 178). These different modes of binding may account for the apparent differences in reactivities of CD-bound pNPA and pNPH towards external nucleophiles.

5.3.1 Reactivity of pNPAlk Towards Small Nucleophiles (k_N)

The values of the second order rate constants for reaction of pNPA with the six small nucleophiles agree well with reported literature values, taking into account differences in pH.[126,127,132,137] The reactivity of these nucleophiles towards pNPH is about half of that with pNPA.

The reactivity of the C_2 and C_3 esters towards TFE are virtually the same, as is expected, since the acetate and propanoate generally have almost equal reactivities. The value of k_N then drops by a factor of about 2, past the propanoate, due to steric considerations, and remains constant, providing that the formation of aggregates can be avoided.[119] The constancy of the k_N values indicates that the

acyl chain of the ester is not interfering with the nucleophilic attack of small nucleophiles on the ester.

The reactivities of the pNPAlk towards ME are virtually the same as TFE, which is what would be expected considering the experimental pH and the difference in pK_a between TFE and ME.[127]

5.3.2 Reactivity of Bound pNPA and pNPH towards Small Nucleophiles (k_{cN})

The values of k_{cN} for the CD-mediated reaction of pNPA and pNPH with small nucleophiles are not very different from the values of k_N, which indicates a similar reactivity of the free and bound esters. The ratios of k_{cN}/k_N for the cleavage of pNPA range from 0.63 to 1.6 for α-CD and 0.73 to 1.4 for β-CD (Table 33). These values indicate that whatever the mode by which pNPA binds to these CDs, it leaves the carbonyl carbon at least partly exposed to the medium. In some cases the binding of the ester to the CD actually promotes the cleavage reaction ($k_{cN}/k_N > 1$), although the CD is not involved in any covalent manner with the reaction. This is a case where the CD is acting as a true, non-covalent, catalyst.

The k_{cN}/k_N value for the cleavage of pNPA by TFE in the presence of Hp-β-CD (0.57; Table 35) is somewhat smaller than the value for β-CD (0.82; Table 35), indicating that the ester is bound to Hp-β-CD in such a way that the carbonyl carbon is more removed from solution than with β-CD. This is quite interesting, and part of the "hydroxypropyl anomaly", since pNPA binds to both CDs with virtually the same strength.

The situation with pNPH is slightly different, where the best case scenario is that the binding of the ester to the CD has no effect (k_{cN}/k_N = 1.0; pNPH + Imidazole + α-CD; Table 34). The CD-bound pNPH is generally less reactive, as is evidenced by k_{cN}/k_N values which range from 0.27 to 1 and 0.24 to 0.54, for α-CD and β-CD, respectively (Table 34). The value of k_{cN}/k_N for the Hp-β-CD-mediated cleavage of pNPH by TFE (0.18; Table 35) is again significantly lower than the value for β-CD, indicating that the binding of the ester is occurring in such a way that the ester carbonyl carbon is deeper in the Hp-β-CD cavity.

The values of k_{cN}/k_N show little dependence on the nature of the nucleophile, which is not unexpected since changes in reactivity due to differences in nucleophilicity should manifest themselves to more or less the same extent in both the k_N and k_{cN} processes. There does appear to be a small trend, whereby the k_{cN}/k_N values (Table 33 and 34) for the anionic nucleophiles (TFE, ME, and the amino acids) are slightly lower than those of the neutral species (hydroxylamine and imidazole). This may be due to the fact that the ester carbonyl group is in a hydrophobic environment when it is bound to the CD, and having the negatively charged nucleophile enter this area to attack the ester is slightly energetically unfavourable. The exact degree to which this effect will retard the reaction will depend on how deep into the CD cavity the ester is buried.

In some cases, with the non-binding nucleophiles, the k_{cN}/k_N value is marginally greater than unity, but they are not large enough to warrant the discussion of any "true" catalysis by the CD in the cleavage of pNPA and pNPH.

Overall, our results with the small nucleophiles differ quite strongly from the reported catalytic effect of β-CD on the cleavage of pNPA by α-amino acids, where the k_{cN}/k_N values reported ranged from 20 to 30.[132] However, a recent study of this reaction in our laboratory has shown that the earlier one was flawed, and k_{cN}/k_N are generally more modest and close to 1.[139]

5.3.3 Reactivity of Bound pNPAlk towards TFE and ME (k_{cN})

The second order rate constants, k_{cN}, listed in Tables 35 and 36 all show a very similar dependence on the length of the acyl chain, decreasing up to the hexanoate after which point they begin to rise. We take this to mean that in all cases the carbonyl carbon is exposed for attack by TFE and ME, but addition of each methylene unit, past the hexanoate, serves to make the carbonyl group more accessible. Although the increased length of the acyl chain may make the bound ester more reactive, the carbonyl group is not "free" in the bulk solution, since in all cases, except for the reaction of *p*-nitrophenyl decanoate with ME and β-CD, the value of k_{cN}/k_N is decidedly less than unity.

5.3.4 Reactivity of pNPA and pNPH towards Alkylamines (k_N)

For both pNPA and pNPH reacting with the *n*-alkylamines the values of k_N remained almost constant, with a slight upward trend with increasing chain length of the amine (Table 38). This difference in reactivity cannot be accounted for

solely on the basis of intrinsic difference in nucleophilicity, since all of the amines from the C_3 to the C_8 have virtually identical pK_as (see Table 40).

The reactivity of pNPH is about half that of the reactivity of pNPA, which is as expected (*vide supra*). The cyclic amines are less reactive towards the esters, due to branching at the α-carbon, and relative to one another, the *c*-C_5 is *more* reactive than the *c*-C_6, opposite to the trend with the straight chain amines. In view of the limited number of cyclic amines examined, and some problems encountered in their analysis and the experiments (see Experimental), we will limit further discussion to the results obtained with the straight chain amines.

5.3.5 Reactivity of pNPAlk with Heptylamine (k_N)

The trend in reactivities with heptylamine is the same as has been observed with the other nucleophiles reacting with the series of esters (*cf.* TFE and ME). The acetate and propanoate have approximately equal k_N values, while for longer esters the reactivity drops off by about a factor of two and remains relatively constant throughout the rest of the series of esters. This would rule out the increase in reactivity, in the presence of Cr^{3} (k_{cN}), of the longer chain amines being due to hydrophobic interactions with the acyl chain of the esters, since we would expect there to be an increase in k_N from the acetate to the hexanoate.

Table 40. pK_a values for the *n*-alkylamines.[a]

Amine	pK_a
Propyl[b]	10.57
Butyl[b]	10.64
Pentyl[c]	10.63
Hexyl[b]	10.64
Heptyl[d]	10.64
Octyl[c]	10.65
Cyclopentyl[c]	10.65
Cyclohexyl[b]	10.64

[a] In aqueous solution at 25.0 °C. [b] Reference 140. [c] Reference 141. [d] Assumed since Hexylamine and Octylamine have pK_as of 10.64 and 10.65 respectively.

5.3.6 Reactivity of Bound pNPA and pNPH with *n*-Alkylamines (k_{cN})

The values of k_{cN} for both esters reacting with the series of amines in the presence of β-CD and Hp-β-CD all show more or less the same behaviour, k_{cN} is virtually constant up to the C_5 (up to C_4 in the case of pNPH + Hp-β-CD) and then rises as a function of the number of carbons in the alkyl chain. The values of log k_{cN} with β-CD are all higher than the corresponding values for reaction in the presence of Hp-β-CD (Figure 49), indicating that although both CDs bind the esters in a manner which allows for nucleophilic attack by the amine, the binding to Hp-β-CD renders the carbonyl group slightly less accessible.

The trend in the observed values of k_{cN} is mirrored in the values of k_{cN}/k_N, which are generally near unity for the shorter amines (< C_6). The reactivity ratio for the C_6, C_7, and C_8 amines is significantly larger than that of the smaller ones, with pNPA and β-CD the values are 12, 15, and 76 (Table 38), respectively. The k_{cN}/k_N ratio increases in every case, past propylamine, with increasing length of the alkylamine, although the values for Hp-β-CD and pNPH seem to be quite modest However, even with Hp-β-CD and pNPH, the overall increase in k_{cN}/k_N is quite significant, since it rises from 0.077 with C_4 up to 3.3 with C_8 (Table 38).

The break in Figure 49 and the trends in k_{cN}/k_N most probably indicate a situation where we have gone from the amine reacting from the bulk solution to one where the amine is bound in the CD cavity during the transition state We cannot say at this point if and how the ester is bound, but this will be elaborated on within the next several sections.

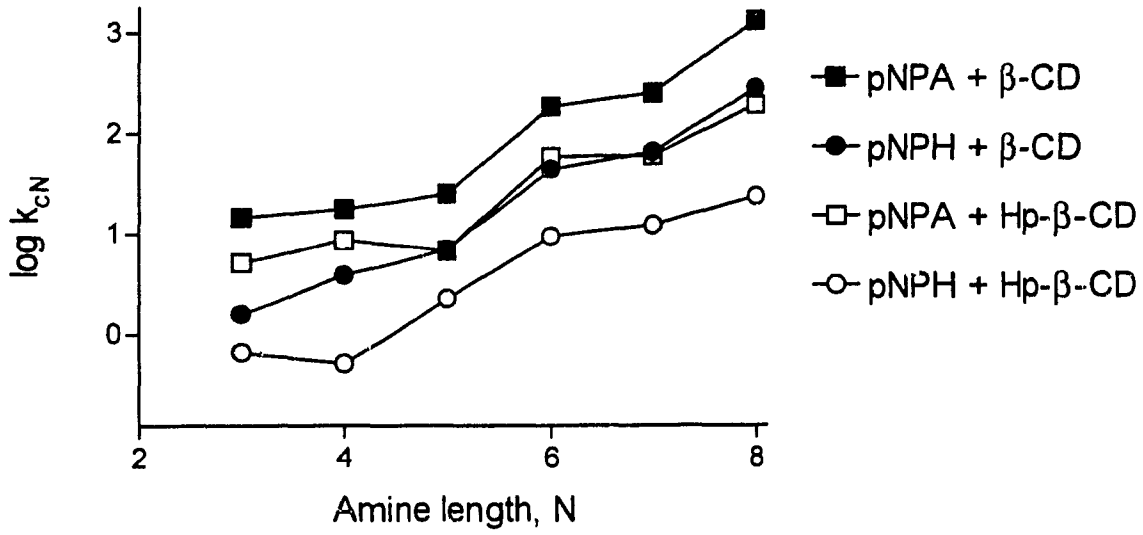


Figure 49. Dependence of log k_{cN} on the length of the amine nucleophile, for pNPA and pNPH reacting in the presence of β-CD and Hp-β-CD.

5.3.7 Reactivity of Bound pNPAlk with *n*-Heptylamine (k_{cN})

Varying the length of the ester while maintaining the same amine appears to have the reverse effect to varying the size of the amine with a constant ester, is that the k_{cN} values start off high, and then fall off after the propanoate, and then remain relatively constant up to the hexanoate. The largest values are seen with β-CD, which is about 4 times more reactive than the analogous Hp-β-CD complexes.

Since both the k_{cN} and k_N values decrease in going from the acetate to the hexanoate, the effect of the ratio k_{cN}/k_N is minimal, with k_{cN}/k_N for pNPH being only about a factor of two less than that for the acetate. The implications of these trends on binding in the transition state will soon be discussed.

5.3.8 Binding in the Transition State (K_{TS})

Our method of probing the binding in the transition state for the cleavage of the esters by various nucleophiles is based on examining the effect of varying the structure of the amine, ester, and CD on: values of k_{cN}/k_N; the third order rate constant, k_3, for the reaction of nucleophiles, ester, and CD, as given in equation [78]; and the concept of transition state binding, K_{TS} (equation [79]). We can probe transition state binding with a method which is similar to the one employed earlier, except that now we compare the termolecular reaction (equation [78]) to the reaction in the absence of CD (equation [74], with TFE replaced by any nucleophile, Nuc).

$$S + CD + Nuc \xrightarrow{k_3} P \qquad [78]$$

$$K_{TS} = \frac{[TS][CD]}{[TS.CD]} = \frac{k_N K_s}{k_{cN}} = \frac{k_N}{k_3} \qquad [79]$$

The values of k_3 can be calculated as k_{cN}/K_s due to the fact the equations [75] and [78] are kinetically indistinguishable. Values of k_3 and K_{TS} calculated in this manner, for all of the various combinations of nucleophiles/esters/CDs discussed previously, have been collected in Tables 41 to 44, 46 and 47. As previously, we can use variations in k_3 and pK_{TS} (= -log K_{TS}) as a probe of the mode of transition state binding.[81 93]

For the reaction of small, non-binding nucleophiles with pNPA in the presence of α-CD and β-CD the values of k_3 vary by several orders of magnitude for both CDs, with neither CD showing exceptional reactivity relative to the other. However, since the values of k_{cN}/k_N are relatively close to unity (Table 33), the values of K_{TS} (Table 41) are similar to those of K_s (Table 37). This implies that during the transition state the ester is bound in pretty much the same way as in the initial state. The same trends are observed with pNPH (Table 42), in that for the reaction in the presence of α-CD the K_{TS} values are virtually the same as the K_s values, while with β-CD the K_{TS} values are slightly larger than the corresponding K_s values. The similarity in K_s and K_{TS} values means that for the cleavage of pNPH in the presence of α-CD and β-CD the ester is again bound virtually the same in the initial and transition states. Although the binding does not change

Table 41. Calculated Constants for the Cleavage of *p*-Nitrophenyl Acetate by Various Nucleophiles in the Presence of α-CD and β-CD.[a]

Nucleophile	k_3 $M^{-2}s^{-1}$	K_{TS} mM
	α-CD	
Trifluoroethanol	795	16.0
β-Mercaptoethanol	1390	8.99
Hydroxylamine	327	6.45
Imidazole	76.8	7.17
	β-CD	
Trifluoroethanol	1310	9.69
β-Mercaptoethanol	1230	10.2
Hydroxylamine	196	10.8
Imidazole	99.0	5.57
L-Histidine	7.46	9.92
L-Alanine	36.7	7.93

[a] Using values of k_N and k_{cN} from Table 33 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

Table 42. Calculated Constants for the Cleavage of *p*-Nitrophenyl Hexanoate by Various Nucleophiles in the Presence of α-CD and β-CD.[a]

Nucleophile	k_3 $M^{-2}s^{-1}$	K_{TS} mM
	α-CD	
Trifluoroethanol	534	13.2
β-Mercaptoethanol	1931	4.42
Hydroxylamine	191	4.85
Imidazole	121	3.52
	β-CD	
Trifluoroethanol	1070	6.60
β-Mercaptoethanol	1290	6.61
Hydroxylamine	261	3.55
Imidazole	144	2.96
L-Alanine	18.6	6.72

[a] Using values of k_N and k_{cN} from Table 34 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

during the course of the reaction, this in no way implies that the acetate and hexanoate are bound in the same way.

For the reaction of TFE with pNPAlk in the presence of α-CD, β-CD, and Hp-β-CD, we see that the values of k_{cN}/k_N all exhibit similar trends, there is a decrease in going from the C_2 to the C_6 ester, at which point the values begin to rise (Table 35). This stems from the biphasic behaviour of k_{cN} while k_N remains virtually unchanged after the C_3. The behaviour of the third order rate constant k_3 (Table 43) is very different in that it is almost constant for the shorter esters and then it rises, for the longer esters, by maximum factors of 8, 63, or 31 for α-CD, β-CD, and Hp-β-CD, respectively. The values of K_{TS} reflect this trend, remaining constant for the shorter esters (< C_6) and then decreasing rapidly with increasing chain length (> C_6) (Table 43). Figures 50 and 51 show clearly the biphasic behaviour of the chain length dependence of log k_3 and pK_{TS} for the cleavage of pNPAlk by TFE in the presence of the three CDs.

Figure 51 shows the LFER between the transition state binding (pK_{TS}) and the ester chain (N). In all cases there is distinct biphasic behaviour. for the shorter esters (< C_6) the LFERs given by equations [80], [81], and [82] show that there is a very shallow dependence of pK_{TS} on N, that is not significantly different from zero; for the longer esters (> C_6) there is a much steeper slope as given by equations [83], [84], and [85]. The interesting point about these long chain esters is that the slopes of equations [83] - [85] are steeper than the corresponding slopes of the LFERs between pK_s and N, for substrate binding (see Chapter 2),

Table 43. Calculated Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by TFE in the Presence of α-CD, β-CD, and Hp-β-CD.[a]

Ester	α-CD		β-CD		Hp-β-CD	
	k_3	K_{TS}	k_3	K_{TS}	k_3	K_{TS}
	$M^{-2}s^{-1}$	mM	$M^{-2}s^{-1}$	mM	$M^{-2}s^{-1}$	mM
C_2	795	15.8	1310	9.77	888	14.5
C_3	764	14.8	1210	9.33	837	13.5
C_4	346	18.6	774	8.32	776	8.32
C_5	459	15.1	1030	6.92	621	11.2
C_6	648	11.0	1320	5.37	800	8.91
C_7	811	9.12	2850	2.57	2000	3.72
C_8	1704	4.27	8410	0.851	3680	1.95
C_9	2824	1.82	10500	0.490	4760	1.07
C_{10}	2754	3.24	48600	0.182	19000	0.468

[a] Using values of k_N and k_{cN} from Table 35 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

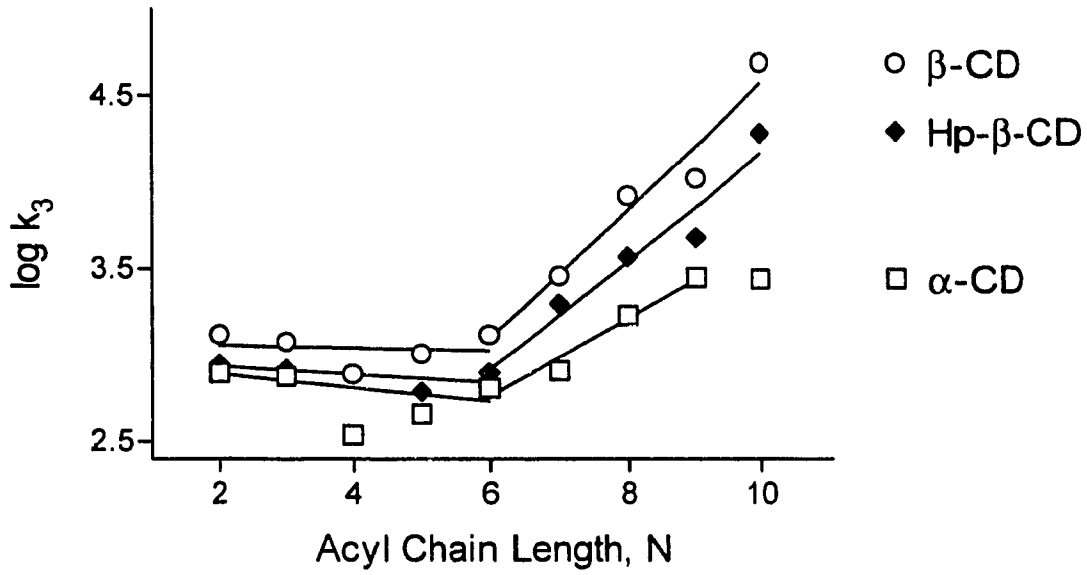


Figure 50. Dependence of log k_3 on acyl chain length for the cleavage of pNPAlk by TFE in the presence of CDs.

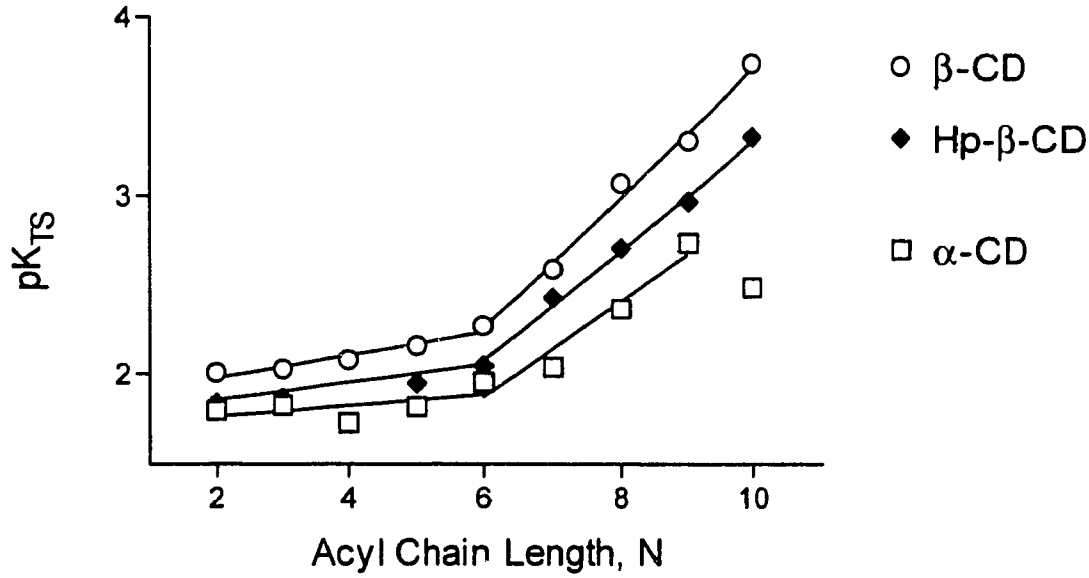


Figure 51. Dependence of transition state stabilisation on the length of the ester acyl chain for the cleavage of pNPAlk by TFE in the presence of CDs.

indicating that the sensitivity of the binding of the ester in the transition state to structure is stronger than in the initial state.

Shorter esters:

α-CD: $pK_{TS} = (0.0310 \pm 0.0247)\ N + (1.70 \pm 0.10)$ [80]

β-CD: $pK_{TS} = (0.0650 \pm 0.0102)\ N + (1.85 \pm 0.04)$ [81]

Hp-β-CD: $pK_{TS} = (0.0500 \pm 0.0260)\ N + (1.76 \pm 0.11)$ [82]

Longer esters:

α-CD: $pK_{TS} = (0.267 \pm 0.048)\ N + (0.275 \pm 0.365)$ [83]

β-CD: $pK_{TS} = (0.366 \pm 0.018)\ N + (0.068 \pm 0.148)$ [84]

Hp-β-CD: $pK_{TS} = (0.310 \pm 0.012)\ N + (0.218 \pm 0.097)$ [85]

It has been shown that the *p*-nitrophenyl alkanoates bind to the CDs in the initial state via acyl group inclusion (see Chapters 2 and 4), at least past the acetate. Since the dependence of pK_{TS} on N is greater than the dependence of pK_s, on N, the break in Figures 50 and 51 is attributed to a change in the mode of transition state binding. The shorter esters bind to the CD via aryl group inclusion, as in **50**, while the longer esters bind via acyl group inclusion, as in **51**. We have observed this type of biphasic behaviour for the cleavage of the same esters by Hp-β-CD, alone. In this latter case we also observed the break point to be at the C_6 ester (see Figure 27, Chapter 2).

We can see from Table 44 and Figure 52 that the pK_{TS} values for the cleavage of pNPAlk by TFE and ME in the presence of β-CD are virtually identical. Likewise, the pK_{TS} values for the β-CD mediated cleavage of pNPA and pNPH by hydroxylamine, imidazole, L-alanine, and L-histidine are also virtually the same (Table 41). This indicates that the nature of the nucleophile, neutral vs. anionic and sulphur vs. oxygen, has no great effect on the stabilisation afforded to the transition state by the CD. This supports our hypothesis that the CD-mediated reaction occurs with the ester in the CD cavity, while the nucleophile is situated outside, in the aqueous medium.

From the results presented so far, it is apparent that the ester is included in the CD cavity during the transition state, and that the mode of binding switches from aryl inclusion to acyl inclusion (**50** → **51**) as the acyl chain of the ester lengthens beyond C_6. We have attributed this change in behaviour to the shorter esters being bound by their acyl chains and having their carbonyl groups deep inside the CD cavity (Scheme 12). In order for this "unreactive" S.CD complex to undergo reaction, it must rearrange to a less stable arrangement, involving aryl group inclusion, which leaves the carbonyl group much more exposed to the surrounding medium. It is only when the acyl chain is 6 carbons or longer that the carbonyl group protrudes sufficiently from the CD cavity (**51**) to allow for reaction to occur without a need for this rearrangement.

Table 44. Calculated Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by ME in the Presence of β-CD.[a]

Ester	k_3 $M^{-2}s^{-1}$	K_{TS} mM
C_2	1240	10.2
C_4	1430	6.03
C_6	1590	5.37
C_8	5370	1.12
C_{10}	98100	0.0933

[a] Using values of k_N and k_{cN} from Table 36 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

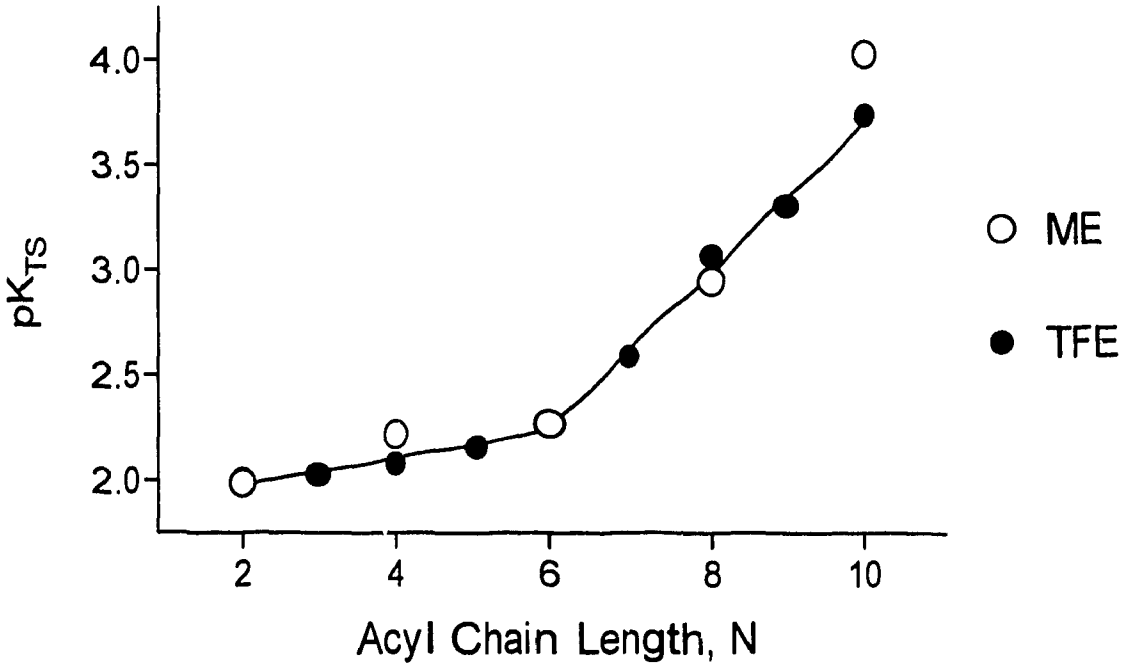
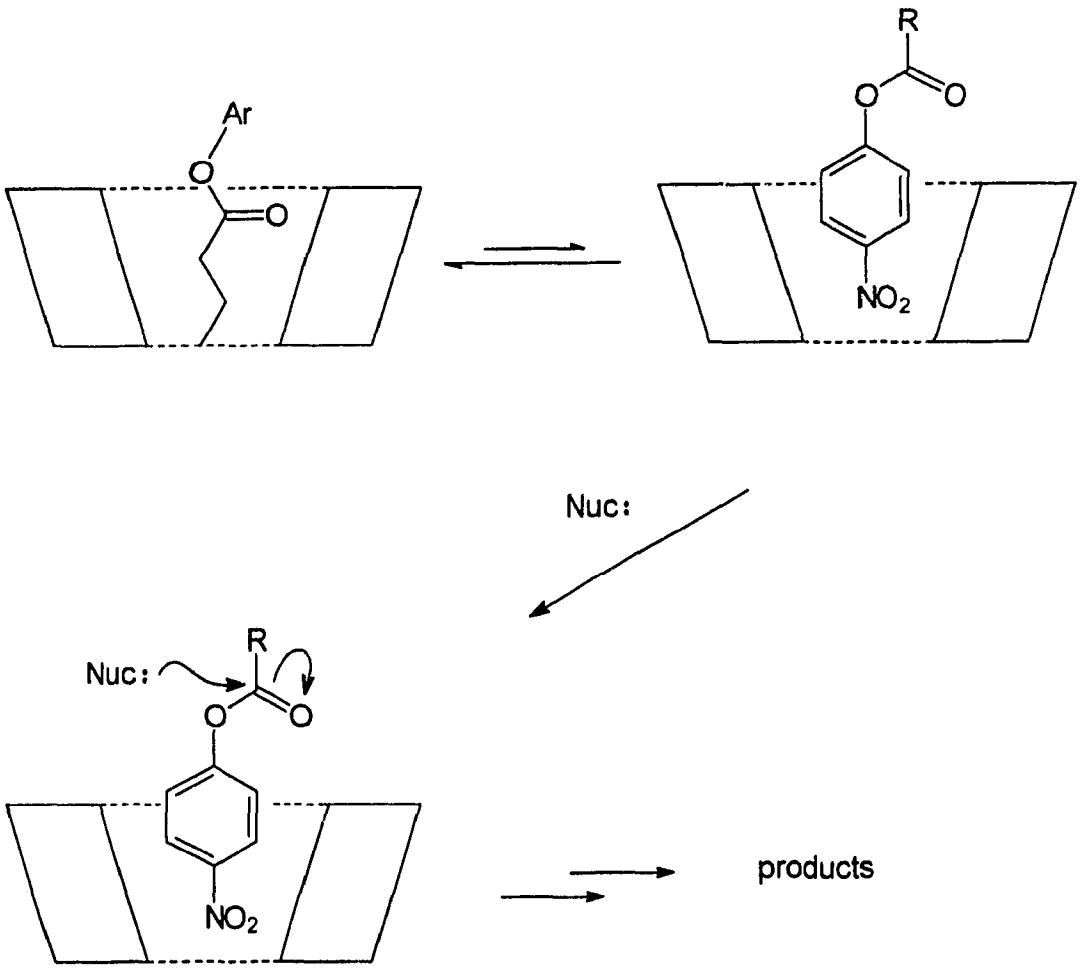


Figure 52. Comparison of the transition state stabilisation for the cleavage of pNPAlk by TFE or ME in the presence of β-CD.



Scheme 12. Switching between acyl and aryl group inclusion.

$$\underset{\text{(acyl-bound)}}{\text{S.CD}} \rightleftharpoons \underset{\text{(aryl-bound)}}{\text{CD.S}} + \text{Nuc:} \xrightarrow{k_{cN}'} \text{P} \qquad [86]$$

$$k_3 = \frac{k_{cN}'}{K_s'} = \frac{k_{cN}}{K_s} \qquad [87]$$

We can estimate values of k_{cN}' (equation [87]), for the reaction of aryl-bound CD.S (equation [86]), for the shorter ester by considering the kinetic equivalence of equation [86] and [78]. We must now use K_s', for the binding of the aryl group, instead of K_s, and we can estimate this dissociation constant as 10 mM (for α-CD), which is the same as the K_s value for pNPA which is known to bind via aryl inclusion.[58,65,90] Values of k_{cN}' have been estimated for all three CDs, using equation [87], and they are collected in Table 45. These values span a much narrower range than do than values of k_{cN} , 3.5 - 8.0, 6.1 - 10.4 and 5.1 - 7.3 $M^{-1}s^{-1}$ for α-CD, β-CD, and Hp-β-CD, respectively, giving values of k_{cN}'/k_N (Table 45) which fall in the very narrow range of 0.55 - 1.5, with no correlation on length of the acyl chain, meaning that the reaction is neither accelerated nor inhibited by more than a factor of two, which is totally consistent with the mechanism proposed in Scheme 12.

Although there is little difference in the values of the transition state stabilisation for the cleavage of pNPAlk by TFE in the presence of α-CD, β-CD, and Hp-β-CD (K_{TS}, Table 43), there is a trend in stability of β-CD > Hp-β-CD > α-CD. The interesting aspect of this, is that the same trend is observed in the transition state for cleavage of the nitrophenyl esters by the same CDs (Figure 27,

Table 45. Calculated Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by TFE in the Presence of α-CD, β-CD, and Hp-β-CD.[a]

Ester	α-CD		β-CD		Hp-β-CD	
	k_{cN}' $M^{-1}s^{-1}$	k_{cN}'/k_N	k_{cN}' $M^{-1}s^{-1}$	k_{cN}'/k_N	k_{cN}' $M^{-1}s^{-1}$	k_{cN}'/k_N
C_2	8.0	0.63	10.3	0.81	7.3	0.58
C_3	7.6	0.67	9.6	0.84	6.9	0.61
C_4	3.5	0.55	6.1	0.96	6.4	1.0
C_5	4.6	0.66	8.1	1.2	5.1	0.73
C_6	6.5	0.92	10.4	1.5	6.6	0.93

[a] Using values of k_3 from Table 43, k_N from Table 35 and K_s' values of 10 mM (α-CD), 7.9 mM (β-CD), 8.2 mM (Hp-β-CD), calculated as follows: $k_{cN}' = k_3K_S'$.

Chapter 2). This indicates that there are similar binding phenomena occurring during the transition state for the two reactions, regardless of whether binding occurs via acyl or aryl group inclusion. The fact that the transition state of the reaction in the presence of Hp-β-CD is stabilised less than that in the presence of β-CD is still puzzling, especially since the strength of binding of the esters to these two CDs are virtually identical (see Chapters 2 and 4). It is possible that the binding of the esters to Hp-β-CD occurs in such a way that the ester sits deeper in the cavity, making the carbonyl group less accessible to the external nucleophile.

We based our kinetic model on a reaction occurring with ester in the CD cavity during the reaction, and the nucleophile outside of the cavity, since the first nucleophiles examined were too small to bind significantly to CD under our reaction conditions. This leads logically to the next question, "What will happen with nucleophiles, such as alkylamines, that are capable of binding to CDs?" We can see from Table 46, for the *n*-alkylamines reacting with pNPA and pNPH in the presence of β-CD or Hp-β-CD, that the k_3 values remain relatively constant for the C_3 through C_5 amines, then rise sharply for the C_6, C_7, and C_8 amines. This biphasic behaviour signals that there may be some significant differences between the transition states for the reaction of pNPA and pNPH with the short amines as compared to the longer ones.

Figure 53 illustrates the dependence of the pK_{TS} on the ability of the amine to bind to the CD. For the aminolysis of pNPA, with both CDs, we see that for the

Table 46. Calculated Constants for the Cleavage of *p*-Nitrophenyl Acetate and Hexanoate by Alkylamines in the Presence of β-CD and Hp-β-CD.[a]

Amine	β-CD		Hp-β-CD	
	k_3	K_{TS}	k_3	K_{TS}
	$M^{-2}s^{-1}$	mM	$M^{-2}s^{-1}$	mM
	p-nitrophenyl acetate			
Propyl	1810	5.87	626	16.9
Butyl	2170	5.99	1040	12.5
Pentyl	3120	4.30	813	16.5
Hexyl	22900	0.692	6980	2.27
Heptyl	30800	0.541	7210	2.32
Octyl	160000	0.102	22600	0.741
	p-nitrophenyl hexanoate			
Propyl	988	5.03	416	11.9
Butyl	2400	2.78	322	20.7
Pentyl	4310	1.49	1410	4.56
Hexyl	27200	0.311	5870	1.44

(continued...)

Heptyl	39900	0.177	7610	0.930
Octyl	169000	0.0417	14500	0.490

[a] Using values of k_N and k_{cN} from Table 38 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

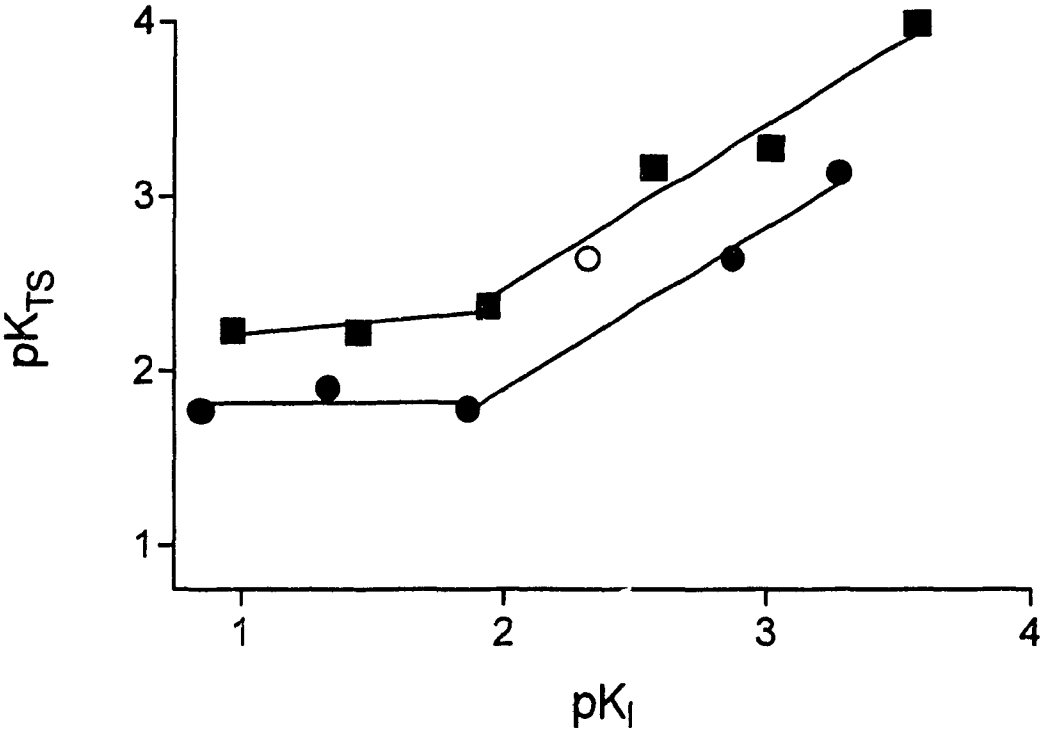
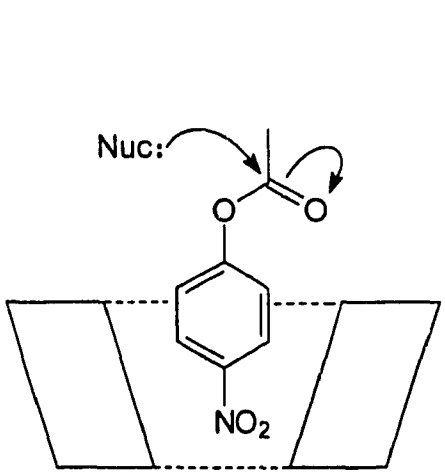


Figure 53. Dependence of transition state stabilisation on the binding strength of the amine to β-CD (■) and Hp-β-CD (●) for the cleavage of pNPA. Open point was not included in the regression analysis.

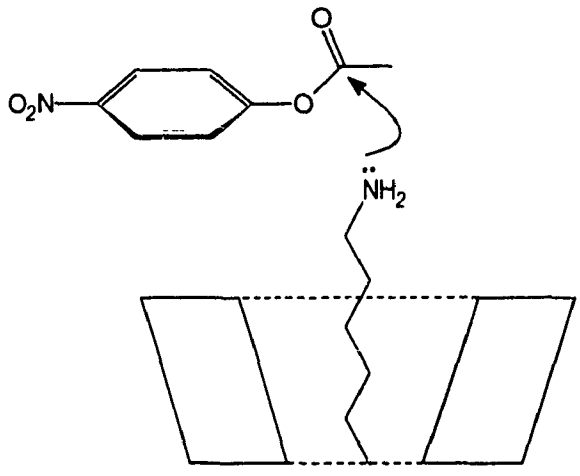
smaller amines (< C_6), the values of pK_{TS} are more or less constant, with those for β-CD being higher than Hp-β-CD. After pentylamine, there is a sharp break in the plots, and a steep increase in pK_{TS}, as described by equations [88]. The fact that the trends are identical with both CDs indicates that after pentylamine, the reaction occurs with the amine in the CD cavity and pNPA mainly outside of the CD cavity (**52** → **53**). This is not totally unexpected since the cleavage of pNPA by the CDs, in the absence of amine, occurs with the ester outside of the cavity during the transition state,[94] as discussed earlier in Chapter 3.

β-CD/pNPA: $pK_{TS} = (0.941 \pm 0.132)\ pK_I + (0.584 \pm 0.375)$ [88a]

Hp-β-CD/pNPA: $pK_{TS} = (0.930 \pm 0.084)\ pK_I + (0.032 \pm 0.230)$ [88b]



52



53

With pNPH as the ester, the situation is clearly different, as shown in Figure 54. In this case, the pK_{TS} rises linearly with the ability of the amine to bind to the

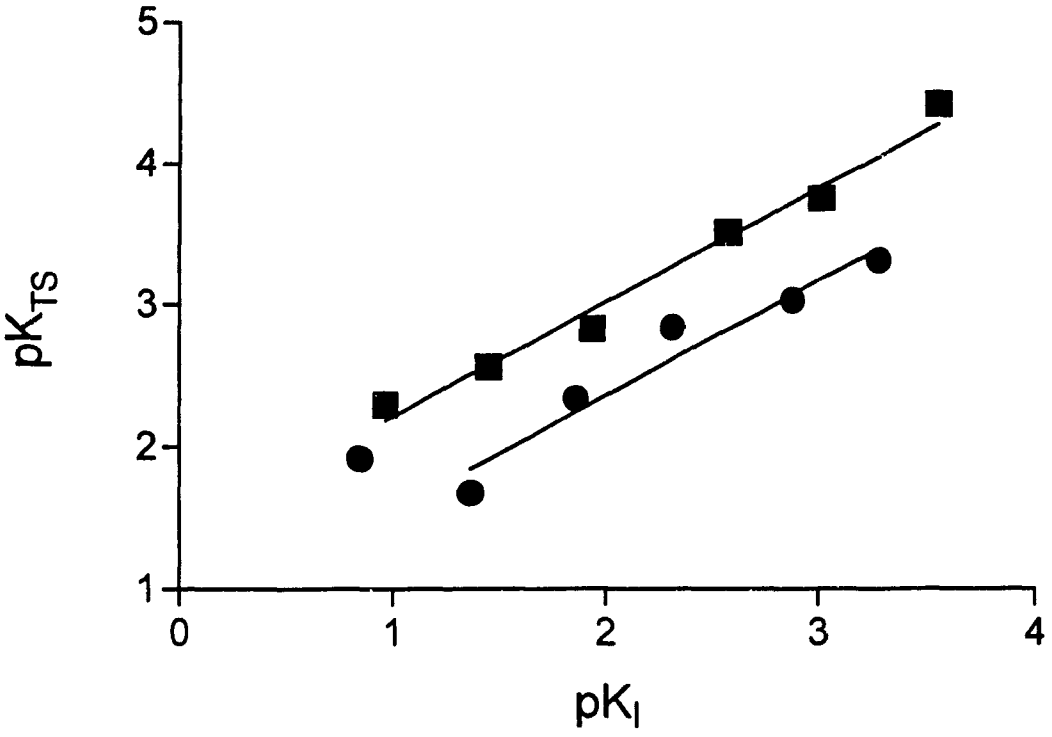


Figure 54. Dependence of transition state stabilisation on binding strength of the amine to β-CD (■) and Hp-β-CD (●) for the cleavage of pNPH.

CD, past the propylamine. The LFERs for both of the CDs are given in equations [89a] and [89b], and the slopes of these lines, 0.81 for β-CD and 0.82 for Hp-β-CD, indicate that the amine is bound in the transition state in a manner which is very similar to the binding in the initial state for the amines longer than propylamine. The observation that the dependence of pK_{TS} on pK_I is virtually the same for both CDs, while the stabilisation afforded by β-CD is larger, is in agreement with other results obtained with these two CDs, in that the binding abilities are virtually identical, although the transition states are better stabilised by β-CD than by Hp-β-CD.

β-CD/pNPH: $pK_{TS} = (0.809 \pm 0.055)\ pK_I + (1.40 \pm 0.13)$ [89a]

Hp-β-CD/pNPH: $pK_{TS} = (0.815 \pm 0.116)\ pK_I + (0.732 \pm 0.284)$ [89b]

The LFERs given by equations [88a] through [89b] clearly support a mechanism where the amine is included in the CD cavity during the aminolysis of pNPA (past C_5) and pNPH (past C_3), prompting us to replace equation [75], the attack of the nucleophile on the CD bound ester, by equation [90], for the attack of a CD bound amine on free ester. The rate constants k_{Nc} are simply evaluated as $k_{cN}K_I/K_s$, since the third order rate constant for the reaction shown in equation [78], k_3 (Table 46), equals k_{cN}/K_s or k_{Nc}/K_I, depending on whether the reaction occurs as in [75] or [90]. The values of k_{Nc} for the CD-mediated reaction of pNPA and pNPH with the amines are given in Table 47.

Table 47. Calculated Second Order Rate Constants for the Reaction of CD Bound *n*-Alkylamine with *p*-Nitrophenyl Acetate and Hexanoate in the Presence of β-CD and Hp-β-CD.[a]

Amine	β-CD		Hp-β-CD	
	k_{Nc} $M^{-1}s^{-1}$	k_{Nc}/k_N	k_{Nc} $M^{-1}s^{-1}$	k_{Nc}/k_N
	p-nitrophenyl acetate			
Pentyl	36	2.7	11	0.83
Hexyl	60	3.8	34	2.12
Heptyl	29	1.8	9.5	0.57
Octyl	53	3.2	12	0.70
	p-nitrophenyl hexanoate			
Butyl	85	13	14	2.0
Pentyl	49	7.6	19	3.0
Hexyl	71	8.4	28	3.3
Heptyl	38	5.4	10	1.4
Octyl	56	7.9	7.5	1.0

[a] Using values of k_N and k_{cN} from Table 38, K_s from Table 37, and K_I from Table 30 (Chapter 4), calculated as follows: $k_{Nc} \simeq k_{cN}K_I/K_S$

$$S + CD + Amine \underset{K_I}{\rightleftharpoons} S + CD.Amine \xrightarrow{k_{Nc}} P \qquad [90]$$

The data in Table 47 show that the values of k_{Nc} now fall into a much smaller range than do the corresponding values of k_{cN}. These values are consistent with the mechanism proposed in equation [90], for the reaction occurring after the amine is bound, although there are several points worth noting. First, the values of k_{Nc} for the CD-mediated reaction of pNPH are generally larger than the second order rate constants, k_N, for aminolysis by the same amines (k_{Nc}/k_N, Table 47). The values of k_{Nc}/k_N for Hp-β-CD (1 - 3) are considerably lower than those for β-CD (5 - 13). Second, the values of k_{Nc} for the reaction of pNPA in the presence of β-CD are only slightly larger than the corresponding k_N values, whereas in the case of Hp-β-CD, the values of k_{Nc}/k_N are generally < 1.

The fact that the k_{Nc}/k_N values for the β-CD-mediated reaction of pNPH are significantly greater than unity indicates that our system is now more reactive than nucleophile attacking free ester, whereas in the case of Hp-β-CD, the bound amine is only slightly more reactive than the unbound. For aminolysis of pNPA, on the other hand, β-CD-bound amine is only slightly more reactive, whereas binding of the amine to Hp-β-CD tends to make it marginally less reactive. The observation that CD-bound amine is generally more reactive than free amine is slightly puzzling, since it appears that the role played by the CD is simply to provide a binding site for the nucleophile. We therefore decided to investigate whether or not the esters are bound during the transition state, and if so, in which orientation.

In order to probe ester binding during the transition state we investigated the aminolysis of a series of pNPAlk by *n*-heptylamine in the presence of β-CD or Hp-β-CD. Table 48 and Figure 55 show that the stability afforded to the transition state by the CD rises linearly past the acetate, with the LFERs given in equations [91] and [92]. The slopes of these equations are close to unity, suggesting that the binding of the ester in the transition state is virtually unchanged from that in the initial state. As discussed in Chapter 2, initial state binding occurs via acyl group inclusion (past the acetate) indicating that during the transition state for aminolysis, the esters are also included in the CD cavity via their acyl chains.

β-CD: $pK_{TS} = (0.94 \pm 0.16)\ pK_s + (1.1 \pm 0.4)$ [91]

Hp-β-CD: $pK_{TS} = (1.27 \pm 0.23)pK_s - (0.54 \mp 0.61)$ [92]

It was shown above that during CD-mediated aminolysis of pNPA and pNPH longer amines (pNPA > C_5; pNPH > C_3; *cf.* equations [89a] and [89b]) are included in the cavity during the transition state. Equations [91] and [92] and Figure 55 indicate that during CD-assisted aminolysis by *n*-heptylamine, esters longer than the acetate are also included in the cavity. We therefore propose a mechanism, for several amine/ester combinations, which is consistent with these observations, whereby the transition state involves a transient ternary {S.CD.amine} complex, as shown in **54**.

Table 48. Calculated Constants for the Cleavage of *p*-Nitrophenyl Alkanoates by *n*-Heptylamine in the Presence of β-CD and Hp-β-CD.[a]

Ester	β-CD		Hp-β-CD	
	k_3	K_{TS}	k_3	K_{TS}
	$M^{-2}s^{-1}$	mM	$M^{-2}s^{-1}$	mM
C_2	30800	0.537	7210	2.29
C_3	20200	0.589	3170	3.80
C_4	22500	0.331	3130	2.34
C_5	27000	0.288	7220	1.07
C_6	39900	0.178	7610	0.933

[a] Using values of k_N and k_{cN} from Table 39 with K_s from Table 37, calculated as follows: $k_3 = k_{cN}/K_S$; $K_{TS} = k_N/k_3$.

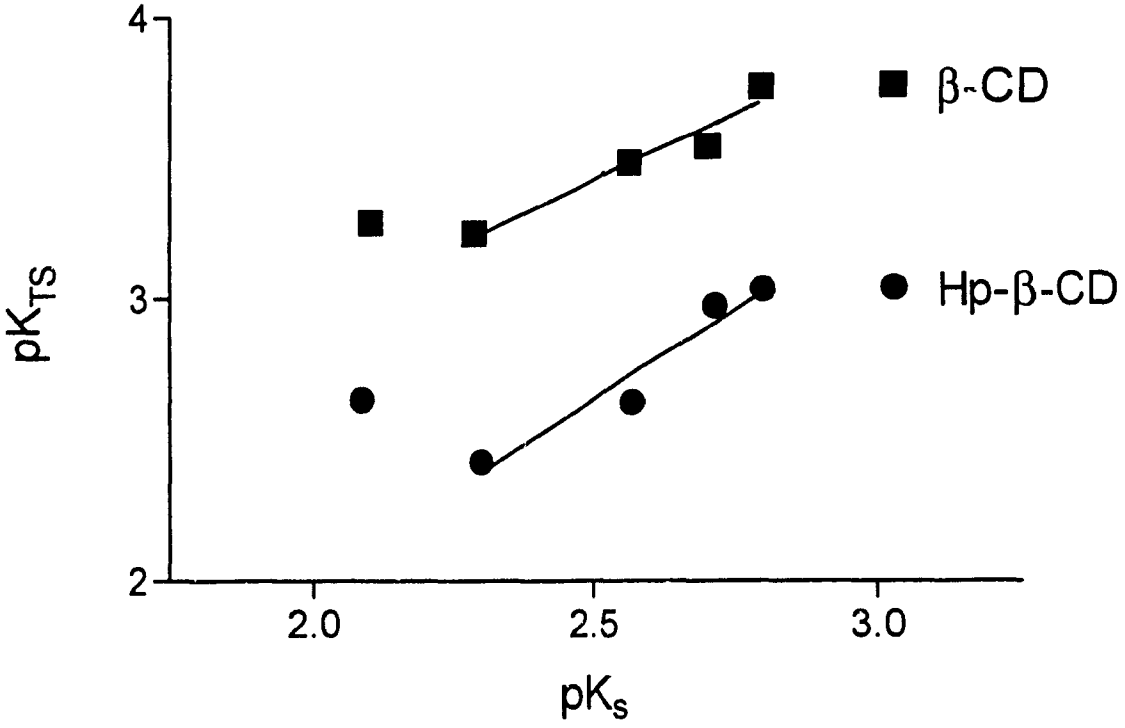
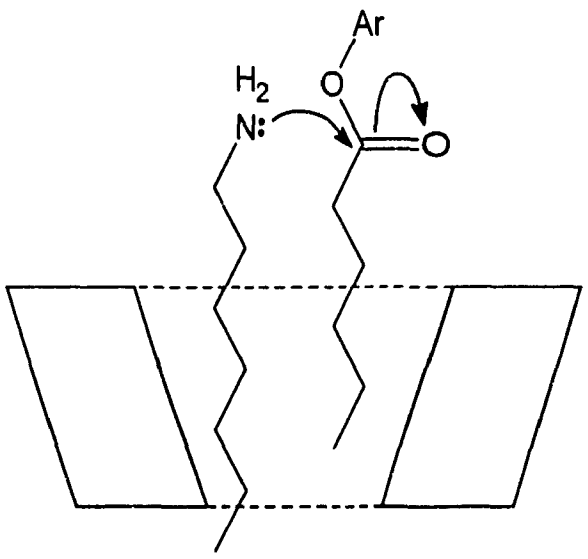


Figure 55. Dependence of pK_{TS} on the ability of pNPAlk to bind to CDs, for aminolysis by *n*-heptylamine.



54

5.4 Conclusions

This chapter contains a large body of data which helps to elucidate the mechanism and transition state phenomena of CD-mediated cleavage of *p*-nitrophenyl alkanoates by a variety of nucleophiles.

From the results presented in this chapter we can draw several conclusions a) For the cleavage of pNPA and pNPH by small nucleophiles such as TFE, ME, hydroxylamine, imidazole, L-alanine, and L-histidine, the reaction occurs with the nucleophile outside of the CD and the ester inside of the CD cavity, as in **50** and **51**. b) The chain length dependence of transition state stabilisation for the cleavage of pNPAlk by TFE and ME show that there is biphasic behaviour, with the shorter ester (< C_6) reacting via aryl group inclusion (**50**) and the longer esters (> C_6) via acyl group inclusion (**51**). c) The smaller *n*-alkylamines (C_3 and C_4) react with pNPA in a manner similar to the small, non-binding nucleophiles (**52**),

although these amines do bind significantly. d) Reaction of the esters longer than the acetates with amines longer than propylamine occurs with both the ester and the amine included in the CD cavity during the transition state (**54**).

These results help to confirm the mechanism proposed in Chapter 2, where the cleavage of certain long chain alkanoate esters occurs via a process involving a second CD nucleophilically attacking a CD-bound substrate.

With all the results presented in this chapter which compare transition state stabilisation between the β-CD-mediated and Hp-β-CD-mediated processes, there is one constant factor. Hp-β-CD universally provides less stabilisation to the transition state, though the trends displayed by the two CDs are essentially identical. This "hydroxypropyl anomaly" is not completely understood, since the molecules investigated in this chapter all bind to both CDs initially with the same strength. Since the 2-hydroxypropyl groups are located far away from the centre of activity, they most probably affect the reaction either by altering the way in which guest molecules bind to CD, although they do not affect the strength of binding, or by influencing the ease by which molecules can rearrange themselves in the transition state, to allow for reaction involving a nucleophile, a CD and an ester.

5.5 Experimental

The α- and β-cyclodextrins, trifluoroethanol, β-mercaptoethanol, and amines were all purchased from the Aldrich Chemical Company, the "hydroxypropyl-β-cyclodextrin was purchased from Wacker-Chemie, the α-amino acids were

purchased from ICN Biomedicals, and the *p*-nitrophenyl alkanoates (C_2 - C_6, C_8, and C_{10}) were purchased from the Sigma Chemical Company. All chemicals were used without further purification. The *p*-nitrophenyl heptanoate and nonanoate were synthesised as described earlier.

Reactions were carried out by 1:1 mixing in a stopped-flow UV-VIS spectrophotometer. For experiments with α-CD and Hp-β-CD, one syringe contained buffer and the nucleophile, and the other contained ester and CD. For the reactions with β-CD, which is less soluble, both syringes contained the desired amount of CD. The reactions were monitored, by following *p*-nitrophenolate release, as described earlier.

The buffer used as the medium varied depending on the nucleophile under examination and are as follows: TFE, ME, *n*-hexylamine, and *n*-heptylamine were studied in a 0.2 M phosphate buffer at pH 11.60. Hydroxylamine was examined in a 0.1 M phosphate buffer at pH 8.00. A 0.1 M borate buffer was used for imidazole, L-alanine, and L-histidine at pHs of 9.00, 9.878, and 8.00, respectively. The remaining amines were studied in buffers made up from the amines themselves.

Most of the data were analysed in terms of the simplified version of equation [77], at [CD] = 0 and 10 mM, where corrections were made to [CD] and [Nuc] because of the binding of the amines before performing the multiple linear regression.

The cyclic amines gave us some problems in both the determination of the dissociation constant and in the analysis of the data by equation [77]. The dissociation constant for cyclohexylamine from Hp-β-CD is relatively high compared to the value for β-CD, although the measurements were repeated and the same values obtained. The analysis of the data for cyclohexylamine with pNPA and Hp-β-CD gave a negative k_{cN} value, which implies that we have not properly modeled the system. Due to the limited number of cyclic amines available, we have excluded them from the discussion since it is at present not exactly clear as to what is happening in these cases.

References

1. Lehn, J.-M., *Pure Appl. Chem.*, **1978**, *50*, 871.
2. Lehn, J.-M., *Science*, **1985**, 227, 849.
3. Lehn, J.-M., *Angew. Chem. Int. Ed. Engl.*, **1988**, 27, 89.
4. Vögtle, F., *Supramolecular Chemistry*, John Willey and Sons, Toronto, 1991.
5. Cram, D.J., *Angew. Chem. Int. Ed. Engl.*, **1986**, 25, 1039.
6. *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, Schneider, H.J., Dürr, H. (Eds.), VCH, New York, 1991.
7. *Inclusion Compounds*, Vol. 2, Atwood, J.L., Davies, J.E.D., MacNicol, D.D. (Eds.), Academic Press, Montreal, 1984.
8. *Inclusion Compounds*, Vol. 3, Atwood, J.L., Davies, J.E.D., MacNicol, D.D. (Eds.), Academic Press, Montreal, 1984.
9. *Inclusion Phenomena and Molecular Recognition*, Atwood, J.L. (Ed.), Plenum Press, New York, 1990.
10. Various authors in *Topics in Current Chemistry*, **1981**, *98*.
11. Various authors in *Topics in Current Chemistry*, **1982**, *101*.
12. *Advances in Supramolecular Chemistry*, Vol. 1, Gokel, G.W. (Ed.), JAI Press, London, 1990.
13. *Advances in Supramolecular Chemistry*, Vol. 2, Gokel, G.W. (Ed.), JAI Press, London, 1992.

14. *Proceeding of the First International Symposium on Cyclodextrins*, Szejtli, J. (Ed.), D. Reidel Publishing Company, Boston, 1982.

15. Szejtli, J., *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.

16. Szejtli, J., *Cyclodextrins and their Inclusion Compounds*, Akademiai Kiado, Budapest, 1982.

17. Bender, M. and Komiyama, M., *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978.

18. Cram, D.J., *Angew. Chem. Int. Ed. Engl.*, **1988**, *27*, 1009.

19. Cram, D.J., *Nature*, **1992**, *356*, 29.

20. Cram, D.J. and Trueblood, K.N., Chapter 2, in Ref. 10.

21. Lehn, J.-M., Chapter 1, in Ref. 6.

22. Hill, C.H. and X. Zhang, *Chem. Eng. News*, **Jan. 30 1995**, 7.

23. Hill, C.H. and X. Zhang, *Nature*, **1995**, *373*, 324.

24. Ranganathan, D.; Vaish, N.K.; Chandramouli, G.V.R.; Varghese, B.; Muthukumuran, R.B.; and Manoharan, P.T, *J. Am. Chem. Soc.*, **1995**, *117*, 1643.

25. Wenz, G., *Angew. Chem. Int. Ed. Engl.*, **1994**, *33*, 803.

26. Saenger, W., *Angew. Chem. Int. Ed. Engl.*, **1980**, *19*, 344.

27. Nakamura, A.; Haga, K.; and Yamane, K., *Biochem.*, **1993**, *32*, 6624.

28. Klein, C. and Schulz, G.E., *J. Mol. Biol.*, **1991**, *217*, 737.

29. Pulley, A.O. and French, D., *Biochem. Biophys. Res. Commun.*, **1961**, *5*, 11.

30. French, D., *Adv. Carbohydr. Chem.*, **1957**, *12*, 635.

31. Kitahata, S. and Okada, S., *J. Biochem.*, **1976**, *79*, 641.

32. Sundararajan, P.R. and Rao, V.S.R., *Carbohydr. Res.*, **1970**, *13*, 351.

33. Schmid, G., In *New Trends in Cyclodextrins and Derivatives*, Duchêne, D. (Ed.), Editions de Santé, Paris, 1991.

34. Croft, A.P. and Bartsch, R.A., *Tetrahedron*, **1983**, *39*, 1417.

35. Boger, J.; Corcoran, R.J.; and Lehn, J.-M., *Helv. Chim. Acta*, **1978**, *61*, 2190.

36. Schneider, H.-J.; Blatter, T.; Cuber, U.; Juneja, R.; Schiestel; T., Schneider, U.; Tehis, I.; and Zimmerman, P . in Ref. 9., pp. 29-56.

37. Fraiji, E.K. Jr.; Cregan, T.R.; Werner, T.C., *Appl. Spec.*, **1994**, *48*, 79.

38. Frankewich, R.P.; Thimmaiah, K.N.; and Hinze, W.L., *Anal. Chem.*, **1991**, *63*, 2924.

39. Zung, J.B.; Ndou, T.T.; and Warner, I.M, *Appl. Spec.*, **1990**, *44*, 1491.

40. Cox, S.G.; Turro, N.J.; Yang, N.C.; and Chen, M., *J. Am. Chem. Soc.*, **1984**, *106*, 422.

41. Shibakami, M. and Sekiya, A., *Carbohydr. Res.*, **1994**, *260*, 169.

42. Sharff, A.J.; Rodseth, L.E.; and Quiocho, F.A., *Biochem.*, **1993**, *32*, 10553.

43. Ding, J.; Steiner, T.; and Saenger, W., *Acta Crystallogr.*, **1991**, *B 47*, 731.

44. Fujiwara, T.; Tanaka, N.; and Kobayashi, S., *Chem. Lett.*, **1990**, 739.

45. Hausler, O. and Muller-Goymann, C.C., *Starch*, **1993**, *45*, 183.

46. Harata, K., *J. Chem. Soc. Chem. Commun.*, **1993**, 546.

47. Manor, P.C. and Saenger, W., *J. Am. Chem. Soc.*, **1974**, *96*, 3630.

48. Gelb, R.I.; Schwartz, L.M.; Bradshaw, J.J.; and Laufer, D.A., *Bioorg. Chem.*, **1980**, *9*, 299.

49. Gelb, R.I.; Schwartz; L.M.; and Laufer, D.A., *Bioorg. Chem.*, **1982**, *11*, 274.

50. Wacker Chemie, GmbH. Marketing brochure.

51. Demarco, P.V. and Thakkar, A.L., *J. Chem. Soc., Chem. Commun.*, **1970**, 2.

52. Yamamoto, Y.; Kanda, Y.; Inoue, Y.; Chujo, R.; and Kobayashi, S., *Chem. Lett.*, **1988**, 495.

53. Bergeron, R.J.; Channing, M.A.; Gibeily, G.J.; and Pillor, D.M., *J. Am. Chem. Soc.*, **1977**, *99*, 5146.

54. Fujiki, M.; Deguchi, T.; and Sanemasa, I., *Bull. Chem. Soc. Jpn.*, **1988**, *61*, 1163.

55. Sanemasa, I.; Takuma, T.; and Deguchi, T., *Bull. Chem. Soc. Jpn.*, **1989**, *62*, 3098.

56. Tee, O.S.; Mazza, C.; Lozano-Hemmer, R.; Giorgi, J.B., *J. Org. Chem.*, **1994**, *59*, 7602.

57. VanEtten, R.L.; Sebastian, J.F.; Clowes, G.A.; Bender, M.L., *J. Am. Chem. Soc.*, **1967**, *89*, 3242.

58. Tee, O.S.; Mazza, C.; and Du, X.-X., *J. Org. Chem.*, **1990**, *55*, 3603.

59. Matsui, Y.; Nishioko, T.; and Fujita, T., *Top. Curr. Chem.*, **1985**, *128*, 61.

60. Tee, O.S.; Gadosy, T.A.; and Giorgi, J.B., Submitted to *Can. J. Chem.*

61. Bergeron, R.J., Chapter 12, in Ref. 8.

62. Cramer, F.; Saenger, W.; and Spatz, H.-Ch., *J. Am. Chem. Soc.*, **1967**, *89*, 14.

63. Fayos, J.; Saenger, W.; McMullan, R.K.; and Mootz, D., *Carbohydr. Res.*, **1973**, *31*, 211.

64. Siegel, B. and Breslow, R., *J. Am. Chem. Soc.*, **1975**, *97*, 6869.

65. Tee, O.S. and Gadosy, T.A., *J. Chem. Soc. Perkin Trans. 2.*, **1994**, 2191.

66. VanEtten, R.L.; Clowes, G.A.; Sebastian, J.F.; and Bender, M.L., *J. Am. Chem. Soc.*, **1967**, *89*, 3253.

67. Paton, R.M. and Kaiser, E.T., *J. Am. Chem. Soc.*, **1970**, *92*, 4723.

68. Flohr, K.; Paton, R.M.; and Kaiser, E.T., *J. Chem. Soc., Chem. Commun.*, **1971**, 1621.

69. Breslow, R.; Trainor, G.L.; and Ueno, A., *J. Am. Chem. Soc.*, **1983**, *105*, 2739.

70. Komiyama, M. and Bender, M.L., in *The Chemistry of Enzyme Action*, M.I. Page, Ed., Elseiver, New York, **1984**.

71. Bergeron, R.J. and Burton, P.S., *J. Am. Chem. Soc.*, **1982**, *104*, 3664.

72. Tutt, D.E. and Schwartz, M.A., *J. Am. Chem. Soc.*, **1971**, *93*, 767.

73. Fife, T.H.; Natarajan, R.; and Werner, M.H., *J. Org. Chem.*, **1987**, *52*, 740.

74. Brown, R.S. and Clewley, R.G., *J. Org. Chem.*, **1987**, *52*, 1216.

75. Komiyama, M. and Bender, M.L., *J. Am. Chem. Soc.*, **1977**, *99*, 8021.

76. Hennrich, N. and Cramer, F., *J. Am. Chem. Soc.*, **1965**, *87*, 1121.

77. Breslow, R., Chapter 14, in Ref. 8.

78. Breslow, R.; Czarniecki, M.F.; Emert, J.; and Hamaguchi, H., *J. Am. Chem. Soc.*, **1980**, *102*, 762.

79. Trainor, G.L. and Breslow, R., *J. Am. Chem. Soc.*, **1981**, *103*, 154.

80. Czarniecki, M.F. and Breslow, R., *J. Am. Chem. Soc.*, **1978**, *100*, 7771.

81. Tee, O.S., *Adv. Phys. Org. Chem.*, **1994**, *29*, 1.

82. Straub, T.S. and Bender, M.L., *J. Am. Chem. Soc.*, **1972**, *94*, 8881.

83. Straub, T.S. and Bender, M.L., *J. Am. Chem. Soc.*, **1972**, *94*, 8875.

84. Griffiths, D.W. and Bender, M.L., *J. Am. Chem. Soc.*, **1973**, *95*, 1679.

85. Tee, O.S. and Bennett, J.M., *J. Am. Chem. Soc.*, **1988**, *110*, 3226.

86. Takasaki, B.K. and Tee, O.S., *Can. J. Chem.*, **1989**, *67*, 193.

87. Laidler, K.J., *Chemical Kinetics*, 3rd. edn., Harper & Row, New York, **1987**.

88. Onozuka, S.; Kojima, M.; Hattori, K.; and Toda, F., *Bull. Chem. Soc. Jpn.*, **1980**, 53, 3221.

89. Komiyama, M. and Bender, M.L., *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 1073.

90. Gadosy, T.A. and Tee, O.S.,*J. Chem. Soc. Perkin Trans. 2.*, **1994**, 715.

91. Komiyama, M. and Hirai, H., *Chem. Lett.*, **1980**, 1471.

92. Komiyama, M. and Bender, M.L., *J. Am. Chem. Soc.*, **1978**, *100*, 4576.

93. Tee, O.S., *Carbohydr. Res.*, **1989**, *192*, 181.

94. Tee, O.S. and Hoeven, J.J., *J. Am. Chem. Soc.*, **1989**, *111*, 8318.

95. Tee, O.S. and Bozzi, M., *J. Am. Chem. Soc.*, **1990**, *112*, 7815.

96. Tee, O.S.; Bozzi, M.; Hoeven, J.J.; and Gadosy, T.A., *J. Am. Chem. Soc.*, **1993**, 115, 8990.

97. Tee, O.S.; Bozzi, M.; Clement, N.; and Gadosy, T.A., submitted to *J. Org. Chem.*

98. Gadosy, T.A. and Tee, O.S., manuscript in preparation.

99. Bonora, G.M.; Fornasier, R.; Scrimin, P.; and Tonellato, U., *J. Chem. Soc. Perkin Trans. 2.*, **1985**, 367.

100. Tee, O.S. and Du, X.-X., *J. Am. Chem. Soc.*, **1992**, *114*, 620.

101. Tee, O.S. and Du, X.-X., *J. Org. Chem.*, **1988**, *53*, 1837.

102. Tee, O.S.; Gadosy, T.A.; and Giorgi, J.B., *J. Chem. Soc. Perkin Trans. 2.*, **1993**, 1705.

103. Satake, I.; Yoshida, S.; Hayakawa, K.; Meada, T.; and Kusumoto, Y., *Buli. Chem. Soc. Jpn.*, **1986**, *59*, 3991.

104. Palepu, R.; Richardson, J.E.; Reinsborough, V.C., *Langmuir*, **1989**, *5*, 218.

105. Sanemasa, I.; Osajima, T.; and Deguchi, T., *Bull. Chem. Soc. Jpn.*, **1990**, *63*, 2814.

106. Haldane, J.B.S., *Enzymes*, Longmans, Green & Co., London, **1930**.

107. Pauling, L., *Chem. Eng. News*, **1946**, *24*,1375.

108. Jencks, W.P., *Catalysis in Chemsistry and Enzymology*, McGraw-Hill, New York, **1969**.

109. Leinhard, G.E., *Science (Washington, D.C.)*, **1973**, *180*, 149.

110. Fersht, A., *Enzyme Structure and Mechanism*, 2nd. edn., W.H. Freeman, Reading, **1985**.

111. Kurz, J.L., *J. Am. Chem. Soc.*, **1963**, *85*, 987.

112. Kurz, J.L., *Acc. Chem. Res.*, **1972**, *5*, 1.

113. Personal communications with Professor R.V. Wolfenden.

114. Wolfenden, R., *Acc. Chem. Res.*, **1972**, *5*, 1.

115. Schowen, R.L., In *Transition State in Biochemical Processes* (R.D. Gandour and R.L. Schowen Eds.), Chapt. 2, Plenum, New York, **1978**.

116. Kraut, J., *Science (Washington, D.C.)*, **1988**, *242*, 533.

117. Emert, J. and Breslow, R., *J. Am. Chem. Soc.*, **1975**, *97*, 670.

118. Fujita, K.; Shinoda, A.; and Imoto, T., *J. Am. Chem. Soc.*, **1980**, *102*, 1161.

119. Guthrie, J.P., *Can. J. Chem.*, **1973**, *51*, 3494.

120. Swinbourne, E.S., *J. Chem. Soc.*, **1960**, 2371.

121. Swinbourne, E.S., *Analysis of Kinetic Data*, Thomas Nelson and Sons Ltd, London, **1971**.

122. Demont, P.M. and Reinsborough, V.C., *Aust. J. Chem.*,**1991**, *44*, 759.

123. Matsui, Y. and Mochida, K., *Bull. Chem. Soc. Jpn.*, **1979**, *52*, 2808.

124. Hoz, S. Acta Chemica Scandinavica, **1992**, *46*, 503.

125. Hoz, S. In Abstracts of the 77th Canadian Society for Chemistry Conference. Canadian Society for Chemistry, **1994**.

126. Jencks, W.P. and Gilchrist, M.J., *J. Am. Chem. Soc.*, **1962**, *84*, 2910.

127. Hupe, D.J. and Jencks, W.P., *J. Am. Chem. Soc.*, **1977**, *99*, 451.

128. Munoz de la Pena, A.; Ndou, T.T.; Zung, J.B.; Greene, K.L.; Live, D.H.; and Warner, I.M., *J. Am. Chem. Soc.* **1991**, *113*, 1572.

129. Schneider, H.-J.; Blatter, T.; and Simova, S., *J. Am. Chem. Soc.*, **1991**, *113*, 1996.

130. Hamai, S., *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 2721.

131. Catena, G.C. and Bright, F.V., *Anal Chem.*, **1989**, *61*, 905.

132. Barra, M. and de Rossi, R.H., *Can. J. Chem.*, **1991**, *69*, 1124.

133. Tee, O.S. and Gadosy, T.A., *J. Chem. Soc. Perkin Trans. 2.*, **1994**, 2307.

134. Gadosy, T.A. and Tee, O.S., *J. Chem. Soc. Perkin Trans. 2.*, **1995**, 71.

135. Blyth, C.A. and Knowles, J.R., *J. Am. Chem. Soc.*, **1971**, *93*, 3017.

136. Blyth, C.A. and Knowles, J.R., *J. Am. Chem. Soc.*, **1971**, *93*, 3021.

137. Jencks, W.P. and Carriuolo, J., *J. Am. Chem. Soc.*, **1960**, *82*, 1778.

138. Ballinger, P. and Long, F.A., *J. Am. Chem. Soc.*, **1960**, *82*, 795.

139. Giorgi, J.B.; Gadosy, T.A.; and Tee, O.S. *Manuscript in preparation.*

140. Dean, J.A., *Handbook of Organic Chemistry*, McGraw Hill, Montreal, **1987**.

141. Jencks, W.P. and Regenstein, J., in *Handbook of Biochemistry and Molecular Biology* (3rd edn.), G.D. Fassman Ed., CRC Press, Cleveland, Ohio, **1976**.

Appendix I

Table A1.1. Raw Data for the Hp-β-CD-assisted Basic Cleavage of *m*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_3	C_4
mM	EXPT# D-07	EXPT# D-11	EXPT# D-12
0	0.0502	0.0515	0.0293
1	0.163	0.155	0.106
2.5	0.283	0.267	0.172
5	0.432	0.383	0.225
10	0.585	0.482	0.265
15	0.672	0.540	0.282
20	0.717	0.576	0.286
	C_5	C_6	C_7
	EXPT# D-13	EXPT# D-15	EXPT# D-16
0	0.0316	0.0306	-[b]
1	0.107	0.103	0.0910
2.5	0.164	0.143	0.116
5	0.199	0.166	0.135

(continued...)

10	0.219	0.180	0.153
15	0.224	0.188	0.164
20	0.227	0.191	0.178

	C_8[b]	C_9[b]	C_{10}[b]
	EXPT# D-17	EXPT# D-36	EXPT# D-37
1	0.0867		
2.5	0.111		
5	0.135	0.146	0.157
10	0.169	0.203	0.245
15	0.198	0.253	0.292
20	0.223	0.283	0.348

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.40 at 25.0 ± 0.1 °C.

[b] Point for [CD] = 0 mM taken to be the same as that of the C_6 ester.

Table A1.2. Raw Data for the Hp-β-CD-assisted Basic Cleavage of *p*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_2	C_2
mM	EXPT# M-01	EXPT# M-80	EXPT# D-01
0	0.0664	0.0631	0.0665
1	0.0921	0.0968	0.0970
2.5		0.135	0.134
5	0.162	0.172	0.174
10	0.205	0.221	0.226
15	0.233	0.258	0.254
20	0.244	0.270	0.267
	C_3	C_4	C_4
	EXPT# D-03	EXPT# D-04	EXPT# D-29
0	0.0660	0.0374	0.0429
1	0.0939	0.0651	0.0665
2.5	0.126	0.0847	0.0869
5	0.157	0.102	0.103

(continued...)

10	0.187	0.115	0.115
15	0.201	0.120	0.123
20	0.210	0.122	0.124

	C_5 EXPT# D-05	C_5 EXPT# D-26	C_6 EXPT# D-02
0	0.0429	0.0433	0.0449
1	0.0621	0.0644	0.0651
2.5	0.0759	0.0800	0.0748
5	0.0862	0.0894	0.0798
10	0.0929	0.0964	0.0855
15	0.0960	0.100	0.0876
20	0.0961	0.100	0.0897

	C_6 EXPT# D-28	C_7 EXPT# D-27	C_8 EXPT# D-32
0	0.0438	-[b]	-[b]
1	0.0624	0.0631	0.0736
2.5	0.0739	0.0707	0.0832
5	0.0816	0.0775	0.0915

(continued...)

10	0.0867	0.0855	0.105
15	0.0896	0.0901	0.117
20	0.0910	0.0978	0.126

	C_9[b]	C_{10}[b]
	EXPT# D-31	EXPT# D-20
1	0.0863	0.0935
2.5	0.0953	0.109
5	0.107	0.126
10	0.130	0.150
15	0.143	0.169
20	0.157	0.180

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.40 at 25.0 ± 0.1 °C.

[b] Point for [CD] = 0 mM taken to be the same as that of the C_6 ester.

Table A1.3. Raw Data for the γ-CD-assisted Basic Cleavage of *m*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_4	C_6
mM	EXPT# D-208	EXPT# D-166	EXPT# D-168
0	0.0805	0.0401	0.0414
1	0.129	0.0609	0.0619
2.5	0.217	0.0936	0.112
5	0.317	0.140	0.153
7.5	0.374		
10	0.496	0.218	0.200[b]
12.5	0.536		
15	0.610	0.267	0.268
20		0.306	0.296

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C.

[b] Point omitted from analysis.

Table A1.4. Raw Data for the γ-CD-assisted Basic Cleavage of *p*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_3	C_4
mM	EXPT# D-202	EXPT# D-207	EXPT# D-204
0	0.104	0.105	0.0589
1	0.145	0.143	0.0826
2.5	0.203	0.187	0.128
5	0.292	0.263	0.181
7.5	0.367	0.327	0.221
10	0.434	0.386	0.232
12.5	0.495	0.436	0.270
15	0.554	0.477	0.301

	C_5	C_6	C_7
	EXPT# D-205	EXPT# D-201	EXPT# D-206
0	0.0594	0.0660	0.0558
1	0.0847	0.0958	0.0827

(continued...)

2.5	0.120	0.141	0.126
5	0.180	0.187	0.167
7.5	0.218	0.217	0.199
10	0.239	0.243	0.247
12.5	0.273	0.266	0.208[b]
15	0.288	0.287	0.267

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C.

[b] Point omitted from analysis.

Table A1.5. Raw Data for the DiMe-β-CD-assisted Basic Cleavage of *m*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_3	C_4
mM	EXPT# D-213	EXPT# D-191	EXPT# D-192
0	0.0807	0.0812	0.0504
1	0.0741	0.0655	0.0349
2.5	0.0680	0.0533	0.0257
5	0.0616	0.0433	0.0194
10	0.0538	0.0337	0.0152
15	0.0503	0.0310	0.0130
20	0.0486	0.0288	0.0119
	C_5	C_6	C_7
	EXPT# D-194	EXPT# D-198	EXPT# D-197
0	0.0491	0.0497	0.0503
0.10	0.0454	0.0415	0.0386
0.25	0.0398	0.0358	0.0300
0.50	0.0344	0.0302	0.0239

(continued...)

1.0	0.0270	0.0231	0.0197
1.5	0.0230	0.0191	0.0142
2.0	0.0204	0.0177	0.0137
5.0	0.0131		

	C_6
	EXPT# D-196
0	0.0489
0.10	0.0358
0.25	0.0292
0.50	0.0213
1.0	0.0165
1.5	0.0154
2.0	0.0134

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.40 at 25.0 ± 0.1 °C.

Table A1.6. Raw Data for the DiMe-β-CD-assisted Basic Cleavage of *p*-Nitrophenyl Alkanoates.[a]

	k_{obs}, s^{-1}		
[CD]	C_2	C_3	C_4
mM	EXPT# D-175	EXPT# D-181	EXPT# D-177
0	0.103	0.0823	0.0586
1	0.0945	0.0719	0.0452
2.5	0.0864	0.0612	0.0360
5	0.0784	0.0541	0.0286
10	0.0710	0.0456	0.0226
15	0.0662	0.0404	0.0209
20	0.0650	0.0378	0.0197

	C_5
	EXPT# D-182
0	0.0496
1	0.0321
2.5	0.0232

(continued...)

5	0.0178
10	0.0137
15	0.0118
20	0.0105

	C_6	C_7	C_8
	EXPT# D-185	EXPT# D-184	EXPT# D-178
0	0.0516	0.0487	0.0505
0.10	0.0478	0.0402	0.0358
0.25	0.0410	0.0345	0.0304
0.50	0.0354	0.0283	0.0238
1.0	0.0289	0.0218	0.0188
1.5	0.0247	0.0197	0.0183
2.0	0.0221	0.0179	0.0161

	C_9	C_{10}	C_8
	EXPT# D-183	EXPT# D-180	EXPT# D-178
0	0.0505	0.0406	
0.10	0.0449	0.0319	
0.25	0.0290	0.0264	

(continued...)

0.50	0.0230	0.0221
1.0	0.0200	0.0190
1.5	0.0187	0.0181
2.0	0.0171	0.0157[b]

[a] In aqueous solution, 0.2 M phosphate buffer, pH 11.40 at 25.0 ± 0.1 °C.

[b] Point omitted from analysis.

Appendix II

Table A2.1. Raw Data for the Cleavage of *p*-Nitrophenyl Acetate in the Presence of "Hydroxypropyl-β-cyclodextrin" and Various Alcohols.[a]

PI = 1-propanol					EXPT# D-56
$[PI]_o$	k_{obs}	k_{scal}	$[CD]^b$	$[PI]^c$	k_{corr}
mM	s^{-1}	s^{-1}	mM	mM	s^{-1}
0.0	0.288	0.262	20.0	0.00	0.343
100	0.302	0.275	15.4	95.4	0.387
200	0.304	0.277	12.5	192	0.416
300	0.311	0.284	10.5	290	0.454
400	0.313	0.285	9.01	389	0.485
500	0.316	0.288	7.91	488	0.519
PI = *iso*-propanol					EXPT # D-61
0.0	0.268	0.262	20.0	0.00	0.343
100	0.278	0.273	14.9	94.9	0.386
200	0.289	0.283	11.9	192	0.434
300	0.298	0.292	9.81	291	0.482
400	0.299	0.293	8.36	388	0.516

(continued...)

500	0.300	0.294	7.28	487	0.551

PI = 2-butanol					EXPT# D-71
0.0	0.261	0.262	20.0	0.00	0.343
60.0	0.268	0.269	12.3	52.3	0.404
120	0.264	0.265	8.69	109	0.453
180	0.259	0.260	6.68	167	0.498
240	0.256	0.257	5.41	225	0.547
300	0.251	0.252	4.54	285	0.589

PI = 1-butanol					EXPT# D-41
0.00	0.273	0.262	20.0	0.00	0.343
50.0	0.280	0.270	12.1	42.1	0.408
100	0.277	0.267	8.40	88.4	0.463
150	0.275	0.264	6.39	136	0.519
200	0.272	0.262	5.14	185	0.576
250	0.266	0.256	4.29	234	0.621

PI = 2-pentanol					EXPT# D-73
0.00	0.276	0.262	20.0	0.00	0.343
50.0	0.260	0.247	10.2	40.2	0.393
100	0.250	0.238	6.47	86.5	0.456

(continued...)

150	0.240	0.228	4.70	135	0.512
200	0.234	0.222	3.68	184	0.572
250	0.227	0.216	3.02	233	0.624

PI = *tert*-butanol EXPT# D-77

0.00	0.277	0.262	20.0	0.00	0.343
80.0	0.254	0.241	7.46	67.5	0.433
160	0.243	0.230	4.35	144	0.541
240	0.234	0.222	3.05	223	0.641
320	0.229	0.216	2.34	302	0.743
400	0.224	0.212	1.90	382	0.842

PI = *iso*-butanol EXPT# D-62

0.00	0.271	0.262	20.0	0.00	0.343
50.0	0.263	0.254	9.73	39.7	0.413
100	0.254	0.246	6.08	86.1	0.490
150	0.249	0.241	4.37	134	0.571
200	0.241	0.233	3.40	183	0.638
250	0.237	0.229	2.78	233	0.710

(continued...)

PI = 1-pentanol EXPT# D-44

0.00	0.283	0.262	20.0	0.00	0.343
10.0	0.275	0.255	15.2	5.23	0.357
20.0	0.268	0.248	11.7	11.7	0.375
30.0	0.262	0.243	9.28	19.3	0.399
40.0	0.256	0.237	7.55	27.6	0.424
50.0	0.252	0.234	6.30	36.3	0.452

PI = cyclopentanol EXPT# D-79

0.0	0.275	0.262	20.0	0.00	0.343
20.0	0.251	0.240	10.2	10.2	0.380
40.0	0.240	0.229	5.85	25.9	0.458
60.0	0.235	0.224	3.91	43.9	0.557
80.0	0.229	0.219	2.90	62.9	0.651
100	0.225	0.215	2.30	82.3	0.747

PI = *iso*-pentanol EXPT# D-65

0.00	0.254	0.262	20.0	0.00	0.343
5.00	0.250	0.258	16.8	1.78	0.352
10.0	0.247	0.255	14.0	3.99	0.366
15.0	0.244	0.252	11.7	6.65	0.383

(continued...)

20.0	0.244	0.253	9.75	9.75	0.410
25.0	0.240	0.248	8.24	13.2	0.430

PI = 1-hexanol EXPT# D-59

0.00	0.125	0.120	2.00	0.00	0.343
2.00	0.114	0.109	1.50	1.50	0.349
4.00	0.107	0.103	1.18	3.18	0.364
6.00	0.102	0.0975	0.958	4.96	0.372
8.00	0.0986	0.0947	0.803	6.80	0.394
10.0	0.0982	0.0943	0.688	8.69	0.439

PI = neopentanol EXPT# D-68

0.00	0.281	0.262	20.0	0.00	0.343
20.0	0.230	0.215	6.27	6.27	0.410
40.0	0.200	0.187	2.28	22.3	0.623
60.0	0.185	0.173	1.30	41.3	0.850
80.0	0.176	0.164	0.897	60.9	1.06
100	0.170	0.159	0.685	80.7	1.28

(continued...)

PI = cyclohexanol EXPT# D-83

0.0	0.269	0.262	20.0	0.00	0.343
15.0	0.229	0.223	8.17	3.17	0.381
30.0	0.199	0.194	2.90	12.9	0.558
45.0	0.186	0.182	1.53	26.5	0.805
60.0	0.179	0.175	1.01	41.0	1.06
75.0	0.174	0.170	0.756	55.8	1.30

PI = 2-hexanol[e] EXPT# D-75

0.00	0.128	0.120	2.00	0.00	0.343
4.09	0.118	0.111	1.57	3.66	0.349
8.17	0.110	0.104	1.28	7.45	0.350
12.3	0.104	0.0973	1.07	11.4	0.341
16.3	0.100	0.0940	0.928	15.2	0.347
20.4	0.0989	0.0929	0.813	19.2	0.370

[a] In 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Calculated using equation [53] (Chapter 3.5). [c] Calculated as $[PI]=[PI]_o-[CD]_o$ [CD]. [d] Calculated using equation [39] (Chapter 3.2). [e] 2-hexanol exhibited competitive inhibition (see text).

Table A2.2. Raw Data for the Cleavage of *p*-Nitrophenyl Hexanoate in the Presence of "Hydroxypropyl-β-cyclodextrin" and Various Alcohols.[a]

PI = 1-propanol					EXPT# D-58
$[PI]_o$	k_{obs}	k_{scal}	$[CD]^b$	$[PI]^c$	k_{corr}
mM	s^{-1}	s^{-1}	mM	mM	s^{-1}
0.00	0.0865	0.0894	20.0	0.00	0.0930
100	0.0947	0.0979	15.4	95.4	0.103
200	0.101	0.104	12.5	192	0.112
300	0.109	0.112	10.5	290	0.123
400	0.117	0.121	9.01	389	0.134
500	0.104	0.107	7.91	488	0.120
PI = *iso*-propanol					EXPT# D-49
0.00	0.0915	0.0894	20.0	0.00	0.0930
100	0.0962	0.0940	15.4	95.4	0.0992
200	0.101	0.0983	12.5	192	0.105
300	0.104	0.101	10.5	290	0.110

(continued...)

400	0.108	0.106	9.01	389	0.116
500	0.110	0.108	7.91	488	0.121

PI = 2-butanol EXPT# D-72

0.00	0.0850	0.0894	20.0	0.00	0.0930
60.0	0.0894	0.0941	12.3	52.3	0.101
120	0.0955	0.100	8.69	109	0.111
180	0.0948	0.0998	6.68	167	0.113
240	0.112	0.118	5.41	225	0.140
300	0.116	0.123	4.54	285	0.150

PI = 1-butanol EXPT# D-42

0.00	0.0900	0.0894	20.0	0.00	0.0930
50.0	0.0959	0.0953	12.1	42.1	0.102
100	0.104	0.104	8.40	88.4	0.115
150	0.111	0.110	6.39	136	0.126
200	0.115	0.115	5.14	185	0.136
250	0.121	0.120	4.29	234	0.148

(continued...)

PI = 2-pentanol EXPT# D-74

0.00	0.0865	0.0894	20.0	0.00	0.0930
50.0	0.0919	0.0950	10.2	40.2	0.103
100	0.0985	0.102	6.47	86.5	0.116
150	0.104	0.108	4.70	135	0.129
200	0.108	0.112	3.68	184	0.141
250	0.110	0.114	3.02	233	0.151

PI = *tert*-butanol EXPT# D-78

0.00	0.0858	0.0894	20.0	0.00	0.0930
80.0	0.0901	0.0939	7.46	67.5	0.104
160	0.0981	0.102	4.35	144	0.123
240	0.102	0.107	3.05	223	0.139
320	0.107	0.112	2.34	302	0.157
400	0.108	0.112	1.90	382	0.169

PI = *iso*-butanol EXPT# D-51

0.00	0.0883	0.0894	20.0	0.00	0.0930
50.0	0.0961	0.0974	9.73	39.7	0.106
100	0.107	0.108	6.08	86.1	0.125
150	0.112	0.114	4.37	134	0.139

(continued...)

200	0.118	0.119	3.40	183	0.154
250	0 124	0.125	2.78	233	0.172

PI = 1-pentanol EXPT# D-45

0.00	0.0874	0.0894	20.0	0.00	0.0930
10.0	0.0887	0.0907	15.2	5.23	0.0956
20.0	0.0912	0.0933	11.7	11.7	0.100
30.0	0.0944	0.0966	9.28	19.3	0.106
40.0	0.0966	0.0989	7.55	27.6	0.110
50.0	0.0996	0.102	6.30	36.3	0.116

PI = 2-hexanol EXPT# D-76

0.00	0.0682	0.0715	2.00	0.00	0.0930
4.09	0.0672	0.0705	1.58	3.67	0.0967
8.17	0.0670	0.0702	1.30	7.47	0.102
12.3	0.0660	0.0692	1.10	11.4	0.105
16.3	0.0650	0.0681	0.955	15.3	0.108
20.4	0.0654	0.0686	0.841	19.2	0.114

PI = cyclopentanol EXPT# D-80

0.00	0.0819	0.0894	20.0	0.00	0.0930

(continued...)

20.0	0.0806	0.0880	10.2	10.2	0.0948
40.0	0.0924	0.101	5.85	25.8	0.116
60.0	0.0995	0.109	3.91	43.9	0.135
80.0	0.107	0.117	2.90	62.9	0.156
100	0.112	0.122	2.30	82.3	0.176

PI = *iso*-pentanol EXPT# D-70

0.0	0.0820	0.0894	20.0	0.00	0.0930
20.0	0.0850	0.0927	9.75	9.75	0.101
40.0	0.0942	0.103	5.36	25.4	0.120
60.0	0.100	0.109	3.52	43.5	0.139
80.0	0.106	0.116	2.58	62.6	0.159
100	0.110	0.120	2.03	82.0	0.179

PI = 1-hexanol EXPT# D-105

0.00	0.0692	0.0715	2.00	0.00	0.0930
7.55	0.0626	0.0647	0.834	6.38	0.104
10.1	0.0613	0.0633	0.685	8.75	0.107
15.1	0.0592	0.0612	0.502	13.6	0.114

(continued...)

20.1	0.0572	0.0591	0.395	18.5	0.118
25.2	0.0507	0.0524	0.325	23.5	0.0914

PI = neopentanol EXPT# D-69

0.00	0.0842	0.0894	20.0	0.00	0.0930
20.0	0.0825	0.0877	6.27	6.27	0.0986
40.0	0.0900	0.0956	2.28	22.3	0.131
60.0	0.0965	0.103	1.30	41.3	0.174
80.0	0.100	0.106	0.897	60.9	0.216
100	0.101	0.108	0.685	80.7	0.255

PI = cyclohexanol EXPT# D-84

0.00	0.0842	0.0894	20.0	0.00	0.0930
15.0	0.0794	0.0874	8.17	3.17	0.0958
30.0	0.0891	0.0962	2.90	12.9	0.125
45.0	0.0995	0.104	1.53	26.5	0.166
60.0	0.104	0.110	1.01	41.0	0.213
75.0	0.106	0.113	0.756	55.8	0.258

[a] In 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Calculated using equation [53] (Chapter 3.5). [c] Calculated as $[PI]=[PI]_o-[CD]_o\,[CD]$. [d] Calculated using equation [39] (Chapter 3.2).

Appendix III

Table A3.1. Raw data for the Hydroxypropyl-β-cyclodextrin-assisted Basic Cleavage of *m*-Nitrophenyl Acetate in the Presence of Various Inhibitors.[a]

$[PI]_0$ mM	k_{obs} s^{-1}	k_{scal} s^{-1}	$[CD]^b$ mM	$[CD.PI]^c$ mM	$[PI]^d$ mM	K_I^e mM
Inhibitor = 2-methoxyethanol						EXPT# D-87
0.00	0.226	0.252	2.00	0.000	0.00	-
150	0.210	0.235	1.78	0.217	150	1230
200	0.206	0.229	1.72	0.281	200	1220
300	0.195	0.217	1.58	0.420	300	1130
400	0.196	0.218	1.59	0.413	400	[f]1540
500	0.194	0.217	1.57	0.428	500	[f]1830
Inhibitor = 3-pentanol						EXPT# D-93
0.00	0.224	0.252	2.00	0.000	0.00	-
5.01	0.206	0.231	1.75	0.254	4.75	32.7

(continued...)

10.0	0.190	0.214	1.54	0.462	9.56	31 8
15.0	0.178	0 201	1.39	0.607	14.4	33 1
20.0	0.166	0.187	1.24	0.757	19.3	31 7
25.1	0.158	0.178	1.14	0.857	24.2	32 2

Inhibitor = 1-heptanol EXPT# D-93

0.00	0.144	0.164	1.00	0.00	0.00	-
1.17	0.111	0.126	0.637	0.363	0.807	1.41
1.56	0.103	0.117	0.557	0.443	1.12	1.41
2.34	0.0926	0.105	0.451	0.549	1.79	1.47
3.12	0.0855	0.0971	0.381	0.619	2.50	1.54
3.90	0.0820	0.0931	0.347	0.653	3.25	1.73

Inhibitor = 1-butanesulphonate EXPT# D-89

0.00	0.227	0.252	2.00	0.000	0.00	-
37.5	0.189	0.210	1.49	0.510	37.0	108
50.0	0.181	0.201	1.39	0.606	49.4	1[illegible]
75.0	0.161	0.178	1.15	0.849	74.2	101
100.0	0.147	0.163	1.00	1.00	99.0	98.4
125.0	0.131	0.146	0.822	1.18	124	[illegible]86.4

(continued...)

Inhibitor = 1-pentanesulphonate EXPT# D-52

0.00	0.234	0.252	2.00	0.000	0.00	-
20.0	0.174	0.187	1 24	0.761	19.2	[f]31.3
40.0	0.140	0.150	0.867	1.13	38.9	[f]29.7
60.0	0.118	0.126	0.641	1.36	58.6	27.7
80.0	0.104	0.112	0.507	1.49	78.5	26.7
100	0.0937	0.101	0.412	1.59	98.4	25.6

Inhibitor = 1-hexanesulphonate EXPT# D-53

0.0	0.229	0.252	2.00	0.00	0.00	-
15.0	0.131	0.145	0.812	1.19	13.8	9.43
30.0	0.101	0.112	0.507	1.49	28.5	9.68
45.0	0.0872	0.0959	0.371	1.63	43.4	9.87
60.0	0.0771	0.0848	0.277	1.72	58.3	9.37
75.0	0.0715	0.0786	0.226	1.77	73.2	9.32

Inhibitor = 1-heptanesulphonate EXPT# D-54

0.00	0.230	0.252	2.00	0.00	0.00	-
6.00	0.128	0.140	0.768	1.23	4.77	2.98
12.0	0.0986	0.108	0.477	1.52	10.5	3.28

(continued...)

18.0	0.0858	0.0940	0.355	1.64	16.4	3.53
24.0	0.0782	0.0857	0.285	1.72	22.3	3.70
30.0	0.0705	0.0772	0.215	1.79	28.2	3.39

Inhibitor = 1-octanesulphonate EXPT# D-55

0.00	0.235	0.252	2.00	0.00	0.00	-
2.00	0.148	0.159	0.954	1.05	0.95	0.869
4.00	0.111	0.119	0.570	1.43	2.57	1.02
6.00	0.0922	0.0988	0.396	1.60	4.40	1.08
8.00	0.0837	0.0897	0.318	1.68	6.32	1.19
10.0	0.0759	0.0814	0.249	1.75	8.25	1.17

[a] In 0.2 M phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Calculated using equation [53] (Chapter 3.5). [c] Calculated using equation [58] (Chapter 4.2.2). [d] Calculated using equation [57] (Chapter 4.2.2). [e] Calculated using equation [59] (Chapter 4.2.2). [f] Point not included in the average.

Table A3.2. Fluorescence Data for 1-Anilino-8-naphthalenesulphonate in the Presence of Cyclodextrins.[a]

β-CD		EXPT# D-237
[CD], mM	F_{obs}	F_{rel}[b]
0.00	0.00215	1.00
0.500	0.00396	1.84
1.00	0.00583	2.71
2.00	0.00907	4.22
3.00	0.0120	5.58
5.00	0.0176	8.18
7.50	0.0234	10.9
10.0	0.0289	13.5
Hp-β-CD		EXPT# D-219
0.00	0.00259	1.00
0.400	0.202	78.2
1.00	0.405	157

(continued...)

2.00	0.599	232
3.00	0.719	278
4.00	0.789	305
5.00	0.835	323
6.00	0.867	335
8.00	0.916	354
10.0	0.940	363
12.0	0.962	372
16.0	0.999	386
20.0	1.03	399

[a] In 0.2 M aqueous phosphate buffer, pH 11.60 at 25.0 ± 0.1 °C. [b] Calculated as: $F_{rel} = F_{obs}/F_o$, where F_o is the observed fluorescence at 0 mM CD.

Table A3.3. Fluorescence Data for 1-Anilino-8-naphthalenesulphonate in the Presence of β-Cyclodextrin and Various Amines.[a]

n-propylamine					EXPT# D-264
$[RNH_2]_o$	F_{obs}	F_{rel}[b]	[CD.ANS][c]	[CD][d]	K_I[e]
mM		mM	mM	mM	mM
0.00	0.0310	13.4	0.0204	9.98	-
44.3	0.0250	10.8	0.0161	7.32	115
88.6	0.0208	8.99	0.0131	5.68	111
133	0.0250	10.8	0.0161	7.32	[f]358
177	0.0156	6.74	0.00943	3.85	107
199	0.0147	6.36	0.00879	3.56	107
221	0.0136	5.88	0.00801	3.20	101
n-butylamine					EXPT# D-265
0.00	0.0309	13.4	0.0204	9.98	-
40.0	0.0193	8.37	0.0121	5.15	37.5
80.0	0.0138	5.99	0.00818	3.28	35.8
120	0.0111	4.81	0.00626	2.44	36.3

(continued...)

160	0.00914	3.96	0.00487	1.86	34.7
180	0.00855	3.71	0.00445	1.69	34.9
200	0.00795	3.45	0.00402	1.52	34.3

n-pentylamine EXPT# D-266

0.00	0.0312	13.4	0.0204	9.98	-
21.1	0.0167	7.17	0.0101	4.19	11.0
42.2	0.0113	4.85	0.00633	2.47	11.4
63.3	0.00864	3.71	0.00445	1.69	11.2
84.4	0.00737	3.17	0.00355	1.33	11.7
95.0	0.00680	2.92	0.00315	1.18	11.5
106	0.00643	2.76	0.00289	1.07	11.6

n-hexylamine EXPT# D-239

0.00	0.0144	13.4	0.0136	9.99	-
2.03	0.0128	11.9	0.0119	8.40	2.33
4.06	0.0112	10.5	0.0104	7.00	2.52
6.09	0.00968	9.03	0.00879	5.72	2.42
8.11	0.00841	7.84	0.00750	4.73	2.55

(continued...)

9.13	0.00804	7.50	0.00712	4.45	2.87
10.1	0.00763	7.11	0.00670	4.14	3.04

n-heptylamine EXPT# D-258

0.00	0.0148	13.4	0.0136	10.0	---
1.53	0.0136	12.3	0.0124	8.83	[f]2.78
3.06	0.0116	10.5	0.0104	7.05	[f]0.265
4.60	0.0104	9.43	0.00922	6.06	1.01
6.13	0.00888	8.05	0.00771	4.89	0.967
6.89	0.00809	7.33	0.00693	4.31	0.910
7.66	0.00743	6.74	0.00628	3.85	0.937

cyclopentylamine EXPT# D-308

0.00	0.0300	13.4	0.0271	9.97	-
22.2	0.0129	5.76	0.0104	3.12	[f]6.95
44.4	0.00925	4.13	0.00685	1.97	[f]8.94
66.7	0.00795	3.55	0.00558	1.58	11.0
88.9	0.00730	3.26	0.00495	1.39	13.0
100	0.00710	3.17	0.00475	1.34	14.1
111	0.00712	3.18	0.00477	1.34	15.9

(continued...)

cyclohexylamine EXPT# D-307

0.00	0.0309	13.4	0.0271	9.97	-
3.94	0.0228	9.89	0.0194	6.47	[f]0.786
7.88	0.0160	6.94	0.0130	4.00	[f]1.27
11.8	0.0115	4.99	0.00872	2.56	1.52
15.8	0.00930	4.03	0.00664	1.90	1.81
17.7	0.00860	3.73	0.00597	1.70	1.94
19.7	0.00808	3.50	0.00548	1.55	2.07

[a] In aqueous solution, pH 11.60 at 25.0 ± 0.1 °C. Amine used as buffering species, except with *n*-hexylamine and *n*-heptylamine where a 0.2 M phosphate buffer was used. [b] Calculated using equation [66] (Chapter 4.2.3). [c] Calculated using equation [67] (Chapter 4.2.3). [d] Calculated using equation [68] (Chapter 4.2.3). [e] Calculated using equation [59] (Chapter 4.2.2) with $[CD.RNH_2] = [CD]_o - [CD] - [CD.ANS]$ and $[RNH_2] = [CD]_o - [CD.RNH_2]$. [f] Point not included in the average.

Table A3.4. Fluorescence Data for 1-Anilino-8-naphthalenesulphonate in the Presence of "Hydroxypropyl-β-Cyclodextrin" and Various Amines.[a]

n-propylamine EXPT# D-263

$[RNH_2]_o$ mM	F_{obs}	F_{rel}[b] mM	[CD.ANS][c] mM	[CD][d] mM	K_I[e] mM
0.00	0.147	255	0.0148	2.48	-
40.4	0.131	228	0.0133	1.93	137
80.7	0.120	208	0.0121	1.59	142
121	0.110	192	0.0111	1.37	146
161	0.101	176	0.0102	1.17	142
182	0.0964	168	0.00972	1.08	139
202	0.0929	161	0.00936	1.02	139

n-butylamine EXPT# D-267

0.00	0.140	255	0.0148	2.48	-
40.0	0.110	201	0.0117	1.49	[f]58.3
80.0	0.0862	157	0.00910	0.976	[f]50.6
120	0.0680	124	0.00717	0.685	44.8
160	0.0567	103	0.00597	0.534	43.1

(continued...)

180	0.0507	92.3	0.00533	0.462	40.4
200	0.0472	85.9	0.00496	0.422	40.3

n-pentylamine EXPT# D-268

0.00	0.137	255	0.0148	2.48	-
21.2	0.0934	174	0.0101	1.15	[f]17.1
42.3	0.0675	126	0.00727	0.699	[f]15.8
63.5	0.0514	95.6	0.00552	0.483	14.8
84.7	0.0400	74.4	0.00428	0.353	13.6
95.2	0.0366	68.1	0.00391	0.317	13.5
106	0.0326	60.7	0.00348	0.276	12.8

n-hexylamine EXPT# D-226

0.00	0.174	255	0.0148	2.49	-
1.78	0.158	232	0.0135	2.00	5.33
2.15	0.154	227	0.0132	1.90	5.05
2.68	0.149	219	0.0127	1.76	4.74
3.58	0.142	209	0.0121	1.60	4.87
5.36	0.127	187	0.0109	1.31	4.62
10.7	0.0938	138	0.00798	0.800	4.27

(continued...)

n-heptylamine EXPT# D-257

0.00	0.101	255	0.0148	2.48	-
1.53	0.0830	210	0.0122	1.62	1.23
3.06	0.0671	169	0.00983	1.10	1.34
4.60	0.0542	137	0.00793	0.792	1.35
6.13	0.0441	111	0.00644	0.592	1.32
6.89	0.0406	103	0.00592	0.530	1.33
7.66	0.0381	96.2	0.00556	0.487	1.37

n-octylamine EXPT# D-228

0.00	0.173	255	0.0148	2.49	-
1.04	0.148	218	0.0127	1.75	[a]0.708
2.08	0.115	169	0.00982	1.10	0.552
3.12	0.0840	124	0.00717	0.686	0.500
4.16	0.0641	94.6	0.00546	0.476	0.506
5.21	0.0513	75.7	0.00436	0.360	0.517

cyclopentylamine EXPT# D-311

0.00	0.149	255	0.0148	2.48	-
24.1	0.0969	166	0.00965	1.07	17.2
48.3	0.0711	122	0.00706	0.671	17.1

(continued...)

72.4	0.0538	92.4	0.00533	0.462	16.0
96.5	0.0439	75.4	0.00434	0.358	15.8
109	0.0381	65.4	0.00376	0.302	14.6
121	0.0374	64.2	0.00369	0.295	15.9

cyclohexylamine EXPT# D-310

0.00	0.145	255	0.0148	2.48	-
6.68	0.104	184	0.0107	1.27	5.68
13.4	0.0755	133	0.00771	0.760	5.10
20.0	0.0588	104	0.00599	0.537	4.96
26.7	0.0507	89.4	0.00516	0.443	5.33
30.1	0.0454	80.0	0.00461	0.386	5.11
33.4	0.0418	73.7	0.00424	0.348	5.07

[a] In aqueous solution, pH 11.60 at 25.0 ± 0.1 °C. Amine used as buffering species, except with *n*-hexylamine and *n*-heptylamine where a 0.2 M phosphate buffer was used. [b] Calculated using equation [66] (Chapter 4.2.3). [c] Calculated using equation [67] (Chapter 4.2.3). [d] Calculated using equation [68] (Chapter 4.2.3). [e] Calculated using equation [59] (Chapter 4.2.2) with $[CD.RNH_2] = [CD]_o - [CD] - [CD.ANS]$ and $[RNH_2] = [CD]_o - [CD.RNH_2]$. [f] Point not included in the average.

Appendix IV

Table A4.1. Raw Data for the Cleavage of *p*-Nitrophenyl Alkanoates by Various Non-binding Nucleophiles in the Presence of CDs.[a]

[CD] mM	[Nuc] mM	k_{obs} s^{-1}	[Nuc] mM	k_{obs} s^{-1}
CD	α		α	
Ester	pNPA		pNPH	
Nucleophile	Hydroxylamine		Hydroxylamine	
Experiment	D-114		D-115	
0.00	0.00	0.000430	1.00	0.000930
0.00	5.00	0.00912	5.00	0.00435
0.00	10.0	0.0200	10.0	0.00941
0.00	20.0	0.0408	20.0	0.0184
10.0	0.00	0.000600	1.00	0.000588
10.0	5.00	0.0129	5.00	0.00269

(continued...)

10.0	10.0	0.0267	10.0	0.00648
10.0	20.0	0.0535	20.0	0.0142

CD	α		α	
Ester	pNPA		pNPH	
Nucleophile	Imidazole		Imidazole	
Experiment	D-116		D-117	
0.00	25.0	0.0138	25.0	0.0109
0.00	50.0	0.0276	50.0	0.0214
0.00	75.0	0.0418	75.0	0.0317
0.00	100	0.0550	100	0.0430
10.0	25.0	0.0188	25.0	0.0125
10.0	50.0	0.0354	50.0	0.0236
10.0	75.0	0.0527	75.0	0.0358
10.0	100	0.0683	100	0.0446

(continued...)

CD	β		β	
Ester	pNPA		pNPH	
Nucleophile	Hydroxylamine		Hydroxylamine	
Experiment	D-140		D-141	
10.0	5.09	0.00811	5.09	0.00195
10.0	10.2	0.0164	10.2	0.00424
10.0	20.4	0.0345	20.4	0.00910
10.0	50.9	0.0900	50.9	0.0237

CD	β		β	
Ester	pNPA		pNPH	
Nucleophile	Imidazole		Imidazole	
Experiment	D-142		D-143	
10.0	25.2	0.0182	25.2	0.00686
10.0	50.4	0.0360	50.4	0.0131
10.0	75.6	0.0525	75.6	0.0195
10.0	101	0.0669	101	0.0260

(continued...)

CD	β		β	
Ester	pNPA		pNPB	
Nucleophile	β-Mercaptoethanol		β-Mercaptoethanol	
Experiment	D-127		D-129	
0.00	0.00	0.0816	0.00	0.0512
0.00	5.14	0.0145	5.72	0.0932
0.00	10.3	0.209	11.4	0.147
0.00	20.6	0.339	22.9	0.245
10.0	0.00	0.431	0.00	0.173
10.0	5.96	0 474	5.22	0.200
10.0	11.9	0.536	10.4	0.224
10.0	23.9	0.669	20.9	0.275

CD	β		β	
Ester	pNPH		pNPO	
Nucleophile	β-Mercaptoethanol		β-Mercaptoethanol	
Experiment	D-130		D-131	
0.0	0.00	0.0600	0.00	0.0452

(continued...)

0.0	5.72	0.0975	5.72	0.0789
0.0	11.4	0.145	11.4	0.114
0.0	22.9	0.243	22.9	0.142
10.0	0.00	0.142	0.00	0.202
10.0	5.22	0.154	5.22	0.208
10.0	10.4	0.167	10.4	0.230
10.0	20.9	0.200	20.9	0.261

CD	β		β	
Ester	pNPDec		pNPA	
Nucleophile	β-Mercaptoethanol		L-Histidine	
Experiment	D-128		D-169	
0.00	0.00	0.0497	25.1	0.00210
0.00	5.14	0.0922	50.3	0.00399
0.00	10.3	0.143	75.4	0.00580
0.00	20.6	0.307	101	0.00700
10.0	0.00	0.268	25.1	0.00214
10.0	5.96	0.340	50.2	0.00391

(continued...)

10.0	11.9	0.270	75.2	0.00548
10.0	23.9	0.633	100	0.00710

CD	β		β	
Ester	pNPA		pNPH	
Nucleophile	L-Alanine		L-Alanine	
Experiment	D-171		D-172	
0.00	25.0	0.00819	25.0	0.00347
0.00	50.0	0.0157	50.0	0.00656
0.00	75.0	0.0232	75.0	0.00957
0.00	100	0.0299	100	0.0129
10.0	25.1	0.0136	25.1	0.00357
10.0	50.1	0.0210	50.1	0.00480
10.0	75.2	0.0280	75.2	0.00582
10.0	100	0.0346	100	0.00684

(continued...)

CD	α		α	
Ester	pNPA		pNPPr	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-134		D-138	
10.0	0.00	0.165	0.00	0.113
10.0	6.98	0.233	5.17	0.151
10.0	14.0	0.308	10.3	0.196
10.0	27.9	0.455	20.7	0.271

CD	α		α	
Ester	pNPB		pNPPen	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-132		D-137	
10.0	0.00	0.0623	0.00	0.0793
10.0	5.39	0.0790	6.98	0.101
10.0	10.8	0.0948	14.0	0.123
10.0	21.6	0.133	27.9	0.171

(continued...)

CD	α		α	
Ester	pNPHep		pNPO	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-139		D-133	
10.0	0.00	0.149	0.00	0.124
10.0	5.17	0.154	6.98	0.138
10.0	10.3	0.173	14.0	0.154
10.0	20.7	0.201	27.9	0.188

CD	α		α	
Ester	pNPNon		pNPDec	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-151		D-152	
10.0	0.00	0.106	0.00	0.101
10.0	5.27	0.120	5.31	0.113
10.0	10.5	0.138	10.6	0.125
10.0	21.1	0.164	21.2	0.149

(continued...)

CD	β		β	
Ester	pNPA		pNPPr	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-108		D-121	
0.00	0.00	0.0895	0.00	0.0692
0.00	5.00	0.152	5.18	0.0977
0.00	10.0	0.217	10.4	0.193
0.00	20.0	0.342	20.7	0.305
10.0	0.00	0.493	0.00	0.300
10.0	5.00	0.552	5.18	0.340
10.0	10.0	0.607	10.4	0.375
10.0	20.0	0.722	20.7	0.467

CD	β		β	
Ester	pNPB		pNPPen	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-111		D-126	
0.00	0.00	0.0463	0.00	0.0514

(continued...)

0.00	5.00	0.0777	5.16	0.0850
0.00	10.0	0.110	10.3	0.122
0.00	20.0	0.174	20.6	0.196
10.0	0.00	0.180	0.00	0.142
10.0	5.00	0.197	6.66	0.160
10.0	10.0	0.213	13.3	0.179
10.0	20.0	0.240	26.6	0.219

CD	β		β	
Ester	pNPHep		pNPOct	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-123		D-110	
0.00	0.00	0.0470	0.00	0.0455
0.00	5.18	0.0624	5.00	0.0722
0.00	10.4	0.126	10.0	0.108
0.00	20.7	0.200	20.0	0.188
10.0	0.00	0.148	0.00	0.167
10.0	5.20	0.165	5.00	0.217

(continued...)

10.0	10.4	0.175	10.0	0.241
10.0	20.8	0.186	20.0	0.260

CD	β		β	
Ester	pNPNon		pNPDec	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-124		D-113	
0.00	0.00	0.0596	0.00	0.0382
0.00	5.16	0.0707	5.00	0.0662
0.00	10.3	0.113	10.0	0.132
0.00	20.7	0.166	20.0	0.211
10.0	0.00	0.311	0.00	0.256
10.0	5.46	0.336	5.00	0.256
10.0	10.9	0.360	10.0	0.356
10.0	21.8	0.402	20.0	0.395

(continued...)

CD	Hp-β		Hp-β	
Ester	pNPA		pNPPr	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-157		D-153	
10.0	0.00	0.336	0.00	0.260
10.0	5.31	0.391	5.31	0.292
10.0	10.6	0.446	10.6	0.330
10.0	21.2	0.543	21.2	0.406

CD	Hp-β		Hp-β	
Ester	pNPB		pNPPen	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-147		D-154	
10.0	0.00	0.150	0.00	0.132
10.0	5.27	0.167	5.31	0.142
10.0	10.5	0.183	10.6	0.154
10.0	21.1	0.217	21.2	0.183

(continued...)

CD	Hp-β		Hp-β	
Ester	pNPHep		pNPOct	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-155		D-150	
10.0	0.00	0.111	0.00	0.124
10.0	5.31	0.121	5.27	0.136
10.0	10.6	0.132	10.5	0.147
10.0	21.2	0.153	21.1	0.168

CD	Hp-β		Hp-β	
Ester	pNPNon		pNPDec	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-148		D-149	
10.0	0.00	0.145	0.00	0.172
10.0	5.27	0.164	5.27	0.187
10.0	10.5	0.177	10.5	0.204
10.0	21.1	0.206	21.1	0.237

(continued...)

CD	α		α	
Ester	pNPA		pNPH	
Nucleophile	β-Mercaptoethanol		β-Mercaptoethanol	
Experiment	D-118		D-119	
10.0	5.15	0.211	5.15	0.114
10.0	10.3	0.281	10.3	0.147
10.0	15.5	0.344	15.5	0.189
10.0	20.6	0.420	20.6	0.221

CD	α		Hp-β	
Ester	pNPH		pNPH	
Nucleophile	Trifluoroethanol		Trifluoroethanol	
Experiment	D-106		D-102	
0.00	0.00	0.0481	0.00	0.0499
0.00	5.00	0.0830	5.00	0.0868
0.00	10.0	0.118	10.0	0.124
0.00	20.0	0.185	20.0	0.189
2.00	0.00	0.0747	0.00	0.0757

(continued...)

2.00	5.00	0.0995	5.00	0.0924
2.00	10.0	0.124	10.0	0.110
2.00	20.0	0.172	20.0	0.141
4.00	0.00	0.0873	0.00	0.0841
4.00	5.00	0.107	5.00	0.0965
4.00	10.0	0.126	10.0	0.108
4.00	20.0	0.163	20.0	0.131
7.00	0.00	0.0995	0.00	0.0898
7.00	5.00	0.113	5.00	0.100
7.00	10.0	0.130	10.0	0.109
7.00	20.0	0.160	20.0	0.128
10.0	0.00	0.121	0.00	0.0903
10.0	5.00	0.138	5.00	0.0982
10.0	10.0	0.137	10.0	0.108
10.0	20.0	0.170	20.0	0.124

(continued...)

CD	β
Ester	pNPH
Nucleophile	Trifluoroethanol
Experiment	D-101

0.00	5.00	0.0738
0.00	10.0	0.106
0.00	20.0	0.168
4.00	5.00	0.127
4.00	10.0	0.140
4.00	20.0	0.167
8.00	5.00	0.135
8.00	10.0	0.148
8.00	20.0	0.170
10.0	5.00	0.137
10.0	10.0	0.148
10.0	20.0	0.171

[a] In aqueous solution pH 11.60 at 25.0 ± 0.1 °C. Buffers and pH as follows: hydroxylamine, 0.1 M phosphate, pH 8.00; imidazole, 0.1 M borate, pH 9.00; β-mercaptoethanol and trifluoroethanol, 0.2 M phosphate, pH 11.60; L-histidine, 0.1 M borate, pH 8.00; L-alanine, 0.1 M borate, pH 9.878.

Table A4.2. Raw Data for the Cleavage of *p*-Nitrophenyl Alkanoates by Various Alkylamines.[a]

[Nuc] mM	k_{obs} s^{-1}	[Nuc] mM	k_{obs} s^{-1}
Ester	pNPA		pNPA
Nucleophile	*n*-$PrNH_2$		*n*-$BuNH_2$
Experiment	D-278		D-292
0.0	0.0424	0.000	0.0403
50.0	0.588	21.1	0.316
100	1.12	42.1	0.598
188	2.07	78.9	1.08
250	2.70	105	1.41
Ester	pNPA		pNPA
Nucleophile	*n*-$PenNH_2$		*n*-$HexNH_2$
Experiment	D-286		D-247

(continued...)

0.00	0.0336	0.00	0.0991
5.24	0.103	2.50	0.138
10.5	0.174	5.00	0.181
19.6	0.295	7.51	0.221
26.2	0.385	10.0	0.256

Ester	pNPA	pNPA	
Nucleophile	*n*-$HepNH_2$	*c*-$PenNH_2$	
Experiment	D-269	D-312	
	0.000	0.0941	0.00	0.0353
	0.758	0.106	14.2	0.0739
	1.52	0.119	28.4	0.114
	2.84	0.141	53.3	0.180
	3.79	0.158	71.1	0.230

Ester	pNPA	pNPH	
Nucleophile	*c*-$HexNH_2$	*n*-$PrNH_2$	
Experiment	D-321	D-279	
	0.00	0.0328	0.0	0.0248

(continued..)

2.03	0.0361	50.0	0.282
4.07	0.0396	100	0.543
7.62	0.0458	188	0.985
10.2	0.0500	250	1.26

Ester	pNPH		pNPH	
Nucleophile	*n*-$BuNH_2$		*n*-$PenNH_2$	
Experiment	D-293		D-287	
	0.00	0.0238	0.00	0.0197
	21.1	0.154	5.24	0.0524
	42.1	0.288	10.5	0.0869
	78.9	0.552	19.6	0.145
	105	0.718	26.2	0.188

Ester	pNPH		pNPH	
Nucleophile	*n*-$HexNH_2$		*n*-$HepNH_2$	
Experiment	D-246		D-270	
	0.00	0.0593	0.000	0.0586
	2.50	0.0798	0.758	0.0641

(continued...)

	5.00	0.102	1.52	0.0688
	7.51	0.122	2.84	0.0788
	10.0	0.144	3.79	0.0852

Ester	pNPH		pNPH	
Nucleophile	*c*-PenNH_2		*c*-HexNH_2	
Experiment	D-315		D-318	
	0.00	0.0216	0.00	0.0190
	14.2	0.0408	2.03	0.0211
	28.4	0.0601	4.07	0.0227
	53.3	0.0924	7.62	0.0262
	71.1	0.114	10.2	0.0288

Ester	pNPPr		pNPBu	
Nucleophile	*n*-HepNH_2		*n*-HepNH_2	
Experiment	D-301		D-298	
	0.00	0.102	0.00	0.0616
	1.22	0.116	1.30	0.0710
	2.45	0.131	2.60	0.0809

(continued...)

4.59	0.157	4.88	0.0979
6.12	0.175	6.51	0.110

Ester	pNPPen
Nucleophile	*n*-HepNH_2
Experiment	D-304

0.00	0.0657
1.22	0.0758
2.45	0.0852
4.59	0.102
6.12	0.114

[a] In an aqueous buffer made from the amine set, to pH 11.60 at 25.0 ± 0.1 °C, except for *n*-HexNH_2 and *n*-HepNH_2 which were done in a 0.2 M phosphate buffer at pH 11.60.

Table A4.3. Raw Data for the Cleavage of *p*-Nitrophenyl Alkanoates by various Alkylamines in the Presence of CDs.[a]

	[Nuc] mM	k_{obs} s^{-1}	[Nuc] mM	k_{obs} s^{-1}
CD	β, 2.00 mM		β, 2.00 mM	
Ester	pNPA		pNPA	
Nucleophile	*n*-$PrNH_2$		*n*-$BuNH_2$	
Experiment	D-281		D-295	
	0.0	0.108	0.00	0.112
	12.5	0.232	5.26	0.162
	25.0	0.363	10.5	0.222
	50.0	0.649	21.1	0.341
	100	1.18	42.1	0.644
	188	2.13	78.9	1.11
	250	2.76	105	1.43

(continued...)

CD	β, 2.01 mM		β, 2.00 mM	
Ester	pNPA		pNPA	
Nucleophile	*n*-PenNH_2		*n*-HexNH_2	
Experiment	D-289		D-252	
	0.00	0.0975	0.000	0.305
	1.31	0.111	0.195	0.304
	2.62	0.124	0.488	0.302
	5.24	0.152	0.976	0.301
	10.5	0.214	1.95	0.299
	19.6	0.325	4.88	0.316
	26.2	0.410	9.76	0.368

CD	β, 2.00 mM		β, 5.02 mM	
Ester	pNPA		pNPA	
Nucleophile	*n*-HepNH_2		*n*-HepNH_2	
Experiment	D-274		D-275	
	0.000	0.263	0.000	0.422
	0.291	0.255	0.291	0.417

(continued...)

	0.582	0.249	0.582	0.412
	1.16	0.238	1.16	0.402
	2.33	0.227	2.33	0.386
	4.36	0.229	4.36	0.356
	5.82	0.239	5.82	0.345

CD	β, 7.50 mM	β, 10.0 mM
Ester	pNPA	pNPA
Nucleophile	*n*-HepNH_2	*n*-HepNH_2
Experiment	D-276	D-277

0.000	0.502	0.000	0.555
0.291	0.499	0.290	0.557
0.582	0.499	0.582	0.555
1.16	0.491	1.16	0.552
2.33	0.478	2.33	0.541
4.36	0.455	4.36	0.525
5.82	0.441	5.82	0.515

(continued...)

CD	β, 2.00 mM		β, 2.00 mM	
Ester	pNPA		pNPA	
Nucleophile	*c*-PenNH_2		*c*-HexNH_2	
Experiment	D-313		D-323	
	0.00	0.102	0.000	0.0927
	3.55	0.101	0.509	0.0875
	7.11	0.103	1.02	0.0821
	14.2	0.116	2.03	0.0744
	28.4	0.148	4.07	0.0673
	53.3	0.214	7.62	0.0666
	71.1	0.259	10.2	0.0673

CD	β, 2.00 mM		β, 2.00 mM	
Ester	pNPH		pNPH	
Nucleophile	*n*-PrNH_2		*n*-BuNH_2	
Experiment	D-280		D-294	
	0.0	0.0616	0.00	0.0571
	12.5	0.0999	5.26	0.0790

(continued...)

25.0	0.139	10.5	0.103
50.0	0.224	21.1	0.162
100	0.394	42.1	0.277
188	0.786	78.9	0.495
250	1.07	105	0.646

CD	β, 2.01 mM	β, 2.00 mM
Ester	pNPH	pNPH
Nucleophile	*n*-PenNH_2	*n*-HexNH_2
Experiment	D-288	D-253

0.00	0.0536	0.000	0.151
1.31	0.0595	0.195	0.154
2.62	0.0652	0.488	0.155
5.24	0.0787	0.976	0.166
10.5	0.106	1.95	0.172
19.6	0.160	4.88	0.192
26.2	0.201	9.76	0.231

(continued...)

CD	β, 2.00 mM		β, 2.00 mM	
Ester	pNPH		pNPH	
Nucleophile	*n*-$HepNH_2$		*c*-$PenNH_2$	
Experiment	D-273		D-316	
	0.000	0.133	0.00	0.0566
	0.256	0.136	3.55	0.0597
	0.511	0.136	7.11	0.0636
	1.02	0.136	14.2	0.0721
	2.04	0.141	28.4	0.0900
	3.83	0.145	53.3	0.120
	5.11	0.155	71.1	0.142

CD	β, 2.00 mM		Hp-β, 2.00 mM	
Ester	pNPH		pNPA	
Nucleophile	*c*-$HexNH_2$		*n*-$PrNH_2$	
Experiment	D-320		D-282	
	0.000	0.0500	0.0	0.102
	0.509	0.0492	12.6	0.239

(continued...)

	1.02	0.0481	25.2	0.374
	2.03	0.0483	50.5	0.595
	4.07	0.0463	101	1.10
	7.62	0.0466	189	1.98
	10.2	0.0479	252	2.63

CD	Hp-β, 2.00 mM		Hp-β, 2.01 mM	
Ester	pNPA		pNPA	
Nucleophile	*n*-$BuNH_2$		*n*-$PenNH_2$	
Experiment	D-297		D-290	
	0.00	0.0610	0.00	0.0573
	5.26	0.119	1.31	0.0720
	10.5	0.178	2.62	0.0862
	21.1	0.294	5.24	0.113
	42.1	0.577	10.5	0.174
	78.9	1.03	19.6	0.282
	105	1.37	26.2	0.360

(continued...)

CD	Hp-β, 2.00 mM		Hp-β, 2.01 mM	
Ester	pNPA		pNPA	
Nucleophile	*n*-HexNH_2		*n*-HepNH_2	
Experiment	D-255		D-271	
	0.000	0.231	0.000	0.192
	0.195	0.227	0.256	0.187
	0.488	0.225	0.511	0.184
	0.976	0.226	1.02	0.181
	1.95	0.231	2.04	0.179
	4.88	0.256	3.83	0.183
	9.76	0.319	5.11	0.192

CD	Hp-β, 2.00 mM		Hp-β, 2.00 mM	
Ester	pNPA		pNPA	
Nucleophile	*c*-PenNH_2		*c*-HexNH_2	
Experiment	D-314		D-322	
	0.00	0.0589	0.000	0.0541
	3.55	0.0637	0.509	0.0525

(continued...)

7.11	0.0710	1.02	0.0512
14.2	0.0893	2.03	0.0497
28.4	0.123	4.07	0.0486
53.3	0.190	7.62	0.0509
71.1	0.237	10.2	0.0553

CD	Hp-β, 2.00 mM	Hp-β, 2.00 mM
Ester	pNPH	pNPH
Nucleophile	*n*-$PrNH_2$	*n*-$BuNH_2$
Experiment	D-283	D-296

0.0	0.0438	0.00	0.0348
12.6	0.0797	5.26	0.0533
25.2	0.116	10.5	0.0728
50.5	0.217	21.1	0.114
101	0.391	42.1	0.204
189	0.692	78.9	0.370
252	0.905	105	0.548

(continued...)

CD	Hp-β, 2.01 mM		Hp-β, 2.00 mM	
Ester	pNPH		pNPH	
Nucleophile	*n*-PenNH_2		*n*-HexNH_2	
Experiment	D-291		D-254	
	0.00	0.0331	0.000	0.116
	1.31	0.0380	0.195	0.119
	2.62	0.0431	0.488	0.119
	5.24	0.0537	0.976	0.121
	10.5	0.0751	1.95	0.124
	19.6	0.120	4.88	0.136
	26.2	0.154	9.76	0.165

CD	Hp-β, 2.01 mM		Hp-β, 2.00 mM	
Ester	pNPH		pNPH	
Nucleophile	*n*-HepNH_2		*c*-PenNH_2	
Experiment	D-272		D-317	
	0.000	0.0979	0.00	0.0348
	0.256	0.0979	3.55	0.0371

(continued...)

	0.511	0.0987	7.11	0.0399
	1.02	0.0983	14.2	0.0460
	2.04	0.0986	28.4	0.0598
	3.83	0.101	53.3	0.0875
	5.11	0.106	71.1	0.108

CD	Hp-β, 2.00 mM		Hp-β, 5.00 mM	
Ester	pNPH		pNPH	
Nucleophile	*c*-HexNH_2		*n*-OctNH_2	
Experiment	D-319		D-329	
	0.000	0.0321	0.000	0.109
	0.509	0.0312	0.200	0.109
	1.02	0.0309	0.398	0.110
	2.03	0.0302	0.796	0.110
	4.07	0.0298	1.59	0.111
	7.62	0.0308	2.39	0.110
	10.2	0.0312	3.18	0.108
			3.98	0.106

(continued...)

CD	Hp-β, 5.00 mM		β, 5.00 mM	
Ester	pNPA		pNPH	
Nucleophile	*n*-OctNH_2		*n*-OctNH_2	
Experiment	D-330		D-331	
	0.00	0.286	0.000	0.132
	0.200	0.282	0.402	0.141
	0.398	0.280	0.804	0.148
	0.796	0.272	1.61	0.157
	1.59	0.261	2.41	0.165
	2.39	0.249	3.22	0.175
	3.18	0.239	4.02	0.184
	3.98	0.229		

CD	β, 5.00 mM		β, 2.00 mM	
Ester	pNPA		pNPPr	
Nucleophile	*n*-OctNH_2		*n*-HepNH_2	
Experiment	D-332		D-302	
	0.000	0.444	0.00	0.229

(continued...)

	0.402	0.437	0.306	0.222
	0.804	0.430	0.612	0.218
	1.61	0.417	1.22	0.207
	2.41	0.404	2.45	0.199
	3.22	0.390	4.59	0.201
	4.02	0.372	6.12	0.210

CD	β, 2.00 mM		β, 2.00 mM	
Ester	pNPBu		pNPPen	
Nucleophile	*n*-HepNH_2		*n*-HepNH_2	
Experiment	D-299		D-305	
	0.000	0.144	0.000	0.143
	0.325	0.142	0.306	0.142
	0.651	0.141	0.612	0.141
	1.30	0.138	1.22	0.142
	2.60	0.135	2.45	0.143
	4.88	0.139	4.59	0.145
	6.51	0.146	6.12	0.152

(continued...)

CD	Hp-β, 2.01 mM		Hp-β, 2.01 mM	
Ester	pNPPr		pNPBu	
Nucleophile	*n*-HepNH_2		*n*-HepNH_2	
Experiment	D-303		D-300	
	0.000	0.167	0.000	0.108
	0.306	0.164	0.325	0.107
	0.612	0.163	0.651	0.105
	1.22	0.160	1.30	0.101
	2.45	0.155	2.60	0.104
	4.59	0.166	4.88	0.107
	6.12	0.177	6.51	0.114

CD	Hp-β, 2.01 mM	
Ester	pNPPen	
Nucleophile	*n*-HepNH_2	
Experiment	D-306	
	0.000	0.104
	0.306	0.102
	0.612	0.102
	1.22	0.105

2.45	0.105
4.59	0.112
6.12	0.120

[a] In an aqueous buffer made from the amine set to pH 11.60 at 25.0 ± 0.1 °C, except for *n*-$HexNH_2$, *n*-$HepNH_2$, and *n*-$OctNH_2$ which were done in a 0.2 M phosphate buffer at pH 11.60.