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Aminolysis of the 4-Acetoxybenzoate Anion catalysed by Cyclodextrins

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ABSTRACT

Aminolysis of the 4-Acetoxybenzoate Anion catalysed by Cyclodextrins

Delna Ghadiali

From the kinetic investigation of the cleavage of the 4-acetoxybenzoate anion (4-ABA) in the presence of three cyclodextrins (α-CD, β-CD, and γ-CD) in basic aqueous solution, the results indicate that 4-ABA binds to α-CD fairly weakly while no binding is observed between 4-ABA and β-CD or γ-CD.

The effects of the three CDs on the kinetics of the aminolysis of 4-ABA by primary amines (*n*-butyl to *n*-octyl, *iso*-butyl, *iso*-pentyl, cyclopentyl, and cyclohexyl) have also been investigated. Rate constants for the nucleophilic attack by free and CD-bound amine (k_N and k_{Nc}) are obtained from analyses of the raw data. For α-CD, values of the reactivity ratios (k_{Nc}/k_N) for the linear amines are low (< 1) implying deceleration of the aminolysis reaction due to binding to the CD. In the case of β-CD, the reactivity ratios are slightly larger (> 1 for both *n*-alkyl and branched amines), implying modest catalysis. Yet, for the cyclic amines with this CD, these ratios are much larger suggesting stronger catalysis in the presence of β-CD. As for γ-CD, the values of the reactivity ratios are > 2, which also signifies modest catalysis. For all three CDs, transition state binding (pK_{TS}) strongly correlates to amine binding (pK_N), with slopes close to 1.00, demonstrating that the CD-bound amine reacts with free ester.

Geometric and/or hydrophobic factors appear to be the origin of catalysis. With respect to geometry, α-CD holds the alkylamino chain tightly, which decreases the ease of accessing the transition state, whereas with β- and γ-CD, the cavity sizes are larger facilitating the transformation to the transition state. Both β- and γ-CD demonstrate strong transition state binding ($pK_{TS} > pK_N$) which can be taken to mean that in this state, there is a lesser amount of hydrophobic surface area that is exposed to the aqueous medium (from the complex) than the initial state (the amine and ester are separate entities). In the case of α-CD, $pK_{TS} < pK_N$ indicating that this hydrophobic effect is slightly less in the transition state, resulting in a slight deceleration.

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I am most grateful to my family for all of their support that I have received during these two years. Thank-you for the encouragement, the love, and the opportunity you have given to me, to have pursued and achieved this level. I hope that I have made you proud.

This thesis is dedicated to my father,

Jimmy Homi Ghadiali

" Research is to see what everybody else has seen,

And to think what nobody else has thought. "

Albert Szent-Gyorgyi

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List of Abbreviations

CD	Cyclodextrin
S	Substrate
P	Product
TS	Transition state
C	Catalyst
TFE	2,2,2-trifluoroethanol
2-ME	2-mercaptoethanol
p-NPA	*para*-nitrophenyl acetate
p-NPH	*para*-nitrophenyl hexanoate
1-NA	1-naphthyl acetate
4-ABA	4-acetoxybenzoate anion

1. INTRODUCTION

1.1 Host-Guest Chemistry

In the last thirty years, supramolecular chemistry has rapidly expanded as chemists have explored more complex systems and interactions. In contrast to molecular chemistry, which is predominantly based on the covalent bonding of atoms, supramolecular chemistry is based on the association of two or more building blocks, which are held together by non-covalent forces.[1] These intermolecular interactions are the foundation of numerous chemical and biological processes, such as the binding of substrates by enzymes or receptors, the decoding of the genetic code, and the formation of protein complexes.[1]

One aspect of supramolecular chemistry is host-guest chemistry, which involves the formation of "a highly structured molecular complex" commonly referred to as a supermolecule, from "two or more molecules or ions held together in unique structural relationships by electrostatic forces."[2] These non-covalent, intermolecular forces include hydrogen bonding, ion pairing, van der Waals interactions, and donor-acceptor interactions. The substrate, or guest molecule, will interact with the receptor, or host molecule, as long as the two species can complement each other in terms of size, shape and binding sites.[3] It is essential that the host molecule's binding sites be oriented in a converging manner, whereas the guest molecule's binding sites are divergent. As depicted in Figure 1.1, upon the association of these converging and diverging binding sites, the supermolecule is formed.[2,4] This association is an extension of Emil Fischer's famous 1894 "lock and key" steric fit concept[5] whereby the host conformationally reorganizes the guest upon complexation.[6]

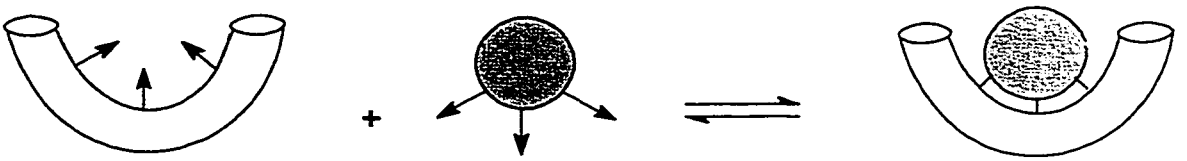


Figure 1.1 Complexation between Host molecule with converging binding sites and Guest molecule with diverging binding sites

From the evolution of supramolecular chemistry, three basic functional properties of the supermolecule have been depicted (Figure 1.2).[3]

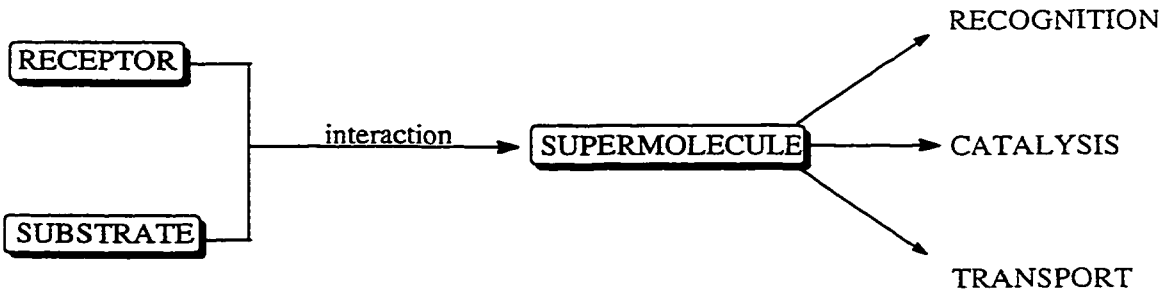


Figure 1.2 Evolution of Supramolecular Chemistry[3]

Molecular recognition involves selective binding of a specific substrate to its receptor yielding the supermolecule.[1] Selectivity is dependent on the presence and location of the binding sites of the substrate (guest) and the receptor (host) so as to achieve optimal interaction. Accordingly, there must be a large contact area between the two species so as to initiate multiple, non-covalent interaction sites.

In addition to binding sites, the receptor may also bear reactive sites, which may effect a chemical transformation on the bound substrate.[1,3] Thus, the receptor may act as a catalyst or a supramolecular reagent, binding to the substrate (formation of the supermolecule), transforming the bound species into products, and then releasing the

products along with the rejuvenated catalyst.

The field of supramolecular chemistry has over the last thirty years steadily expanded, and with it, new and exciting complexation chemistry has developed. From the point of view of the receptor, or host molecule, there has been a substantial increase in articles and books published involving coronands (crown ethers), cryptands, podands, and more recently, calixarene compounds. However, among all the potential hosts, it is the naturally occurring cyclodextrins that were first utilized as receptor molecules whose binding properties towards organic molecules were recognized and extensively studied,[1] yielding a host molecule that is able to undergo all three functional processes.

1.2 Cyclodextrins

Although cyclodextrins (CDs) were first referenced by Villers, in 1891, it wasn't until 1904, that cyclodextrins were isolated by Schardinger.[1,4,7,8] Cyclodextrins, also referred to as cycloamyloses, Schardinger dextrins, or cycloglucans, are naturally occurring cyclic oligosaccarharides. These bottomless bucket-shaped molecules are produced by the enzymatic degradation of starch by cyclodextrin glucosyl transferase, which can be obtained from *Bacillus macerans*, as well as *Klebsiella oxytoca*, *Bacillus circulans*, and *Alkalophylic bacillus*.[7]

Cyclodextrins have received much support and recognition, not only from the academic community but, from industry, as well. In the last thirty years, over 15,000 CD-related publications, including papers, patents, and abstracts, have been published.[9] Some aspects of cyclodextrins that make this class of compounds important are their ability to be

produced from a renewable natural material, thus production costs of these compounds for industrial purposes, have substantially decreased. These compounds are toxicologically harmless and as a result, cyclodextrins have been used as ingredients in drugs, foods, and cosmetics.[7]

1.2.1 Structural, Physical, and Chemical Properties

Cyclodextrins are truncated doughnut-shaped molecules consisting of a number of D(+)-glucopyranose units, that are joined by α-(1,4)-glycosidic type linkages (Figure 1.3). Natural cyclodextrins consist mainly of three common forms: α-CD, which comprises six glucopyranose units, is the smallest in size; β-CD comprises seven glucopyranose units; and γ-CD, which comprises eight such units, is the largest in size. Table 1.1 indicates some physical properties of these common cyclodextrins. Although higher homologues of increasing number of glucopyranose units exist (nine is δ-CD, ten is ε-CD, eleven is ζ-CD, and twelve is η-CD),[4,8-10] there is no indication of cyclodextrins that contain less than six glucopyranose units which may be due to potential steric hindrance.[4,8,11]

All glucopyranose units that form the cyclodextrin molecule are of the C1 chair conformation and therefore the CD resembles a truncated cone. Consequently, the wider rim of the CD is lined with secondary hydroxy groups (carbon-2 and 3 of the glucopyranose unit) while primary hydroxy groups line the narrow rim of the CD molecule (carbon 6),[8,10] as depicted in Figure 1.4.

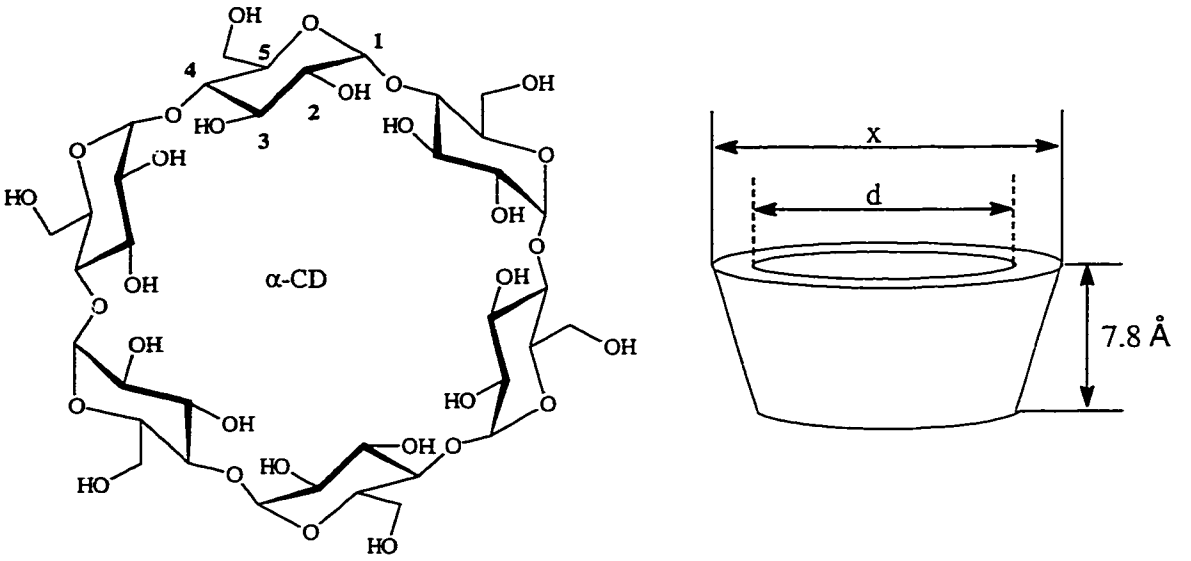


Figure 1.3 Doughnut-shaped α-CD containing six glucopyranose units joined by α-(1,4)-glycosidic type linkages

Table 1.1 Physical Properties of the Common Cyclodextrins[7]

Cyclodextrin	No. of glucose units	Molecular Weight (g/mol)	d (Å)	x (Å)	Solubility (g/100 ml H_2O)	Vol. of Cavity (Å)
α-CD	6	972	5.7	13.7	14.5	174
β-CD	7	1135	7.8	15.3	1.85	262
γ-CD	8	1297	9.5	16.9	23.2	427

The interior of the CD cavity is surrounded by glycosidic ether type linkages along with methine (C-H) groups, resulting in a macromolecule that is relatively hydrophilic on the exterior and yet, relatively hydrophobic in the interior.

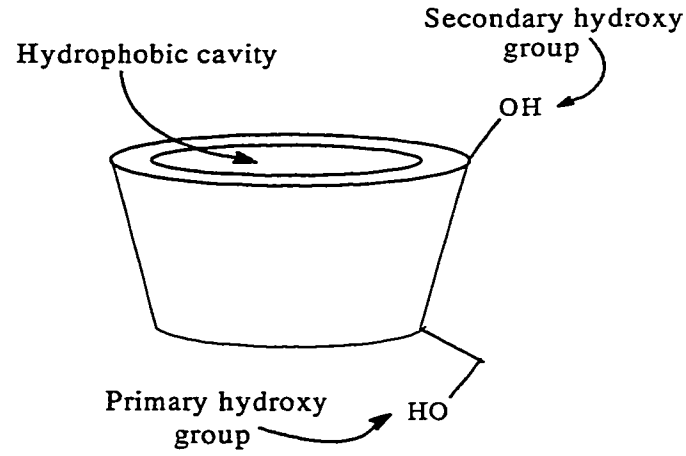


Figure 1.4 Location of Primary and Secondary Hydroxy Groups on a CD Molecule

One striking characteristic of the three common cyclodextrins is their different solubilities. As the ring size of the CDs increase, higher flexibility exists and so, in aqueous solution, as the CD's apolar cavity increases in size, it can accommodate an increasing number of water molecules. However, as the volume increases, these "bound" water molecules will vary energetically from the bulk water solution, much less. Therefore, γ-CD is more water soluble than α-CD. As well, in one glucopyranose unit, the hydroxy group of carbon-2 can hydrogen bond to the hydroxy group of carbon-3 of the adjacent glucose unit. This hydrogen bonding forms a belt around the β-CD molecule, thus making it a relatively rigid structure. Also, apparently the CD molecules are strongly bonded together so that β-CD is poorly soluble in comparison to the other two CDs. With α-CD, only four out of six possible hydrogen bonds are formed, due to one distorted glucopyranose unit. Thus, α-CD in the crystalline state is relatively flexible and so it demonstrates greater solubility in comparison to β-CD. On the other hand, because it is larger, γ-CD is more flexible and since

it has the largest cavity, it is the most soluble of the three common cyclodextrins.[10]

1.2.2 Inclusion Complexes

A fascinating property of cyclodextrins is their ability to incorporate other organic compounds into their cavity, both in the solid state and in solution.[1] The dissociation-association equilibrium that is established in solution between the cyclodextrin and its guest. appropriately reflect the name, inclusion complex. Guest compounds may range from reagents such as acids, amines, alcohols, small ions such as ClO_4^-, SCN^-, and halide anions to aliphatic and aromatic hydrocarbons, as well as noble gases, and aromatic dyes.[4,8,12] Obviously, the one main requirement for these guest molecules in aqueous solution, is their ability to fit into the cyclodextrin cavity, either partially or fully, since the common cyclodextrins can accommodate molecules of varying sizes. If the guest is too large, it will be unable to fit into the cavity and thus, complexation will not occur. It is however, possible for a large guest's side chains or groups to be included in the cyclodextrin cavity. If the guest is too small, then it will either weakly bind to the cavity or, it simply will not bind at all.

Inclusion complex formation can be detected by various spectroscopic methods including nuclear magnetic resonance, absorption, fluorescence, and optical rotation.[8] Examples of geometric compatibility between the guests and various cyclodextrins, can be seen in Table 1.2.

Table 1.2 The Ability of Cyclodextrins to Complex with Various Guests.[a,10]

Guest molecule	α-CD	β-CD	γ-CD
Propionic Acid	+	-	-
Butyric Acid	+	+	-
Biphenyl	+	+	+
Cyclohexane	+	+	+
Naphthalene	-	+	+
Anthracene	-	-	+
Cl_2	+	-	-
Br_2	+	+	-
I_2	+	+	+

[a] The positive sign indicates complexation, while the negative sign indicates no complexation.

Cyclodextrin inclusion complexes differ greatly in the crystalline state and in solution. In the crystalline structure, guest molecules can not only reside in the cavity, but may also be located in the intermolecular cavities of the crystal lattice.[10] In aqueous solution, cyclodextrins encapsulate the guest molecule while the surrounding environment is immersed in water molecules. The enclosed guest molecule orients itself so as to achieve maximum contact between the hydrophobic part of the guest and the apolar CD cavity, while the hydrophilic part of the guest situates itself as close to the exterior, as possible.[10] Therefore, there is maximum contact between the solvent and the guest's polar region. The schematic representation in Figure 1.5 depicts the formation of a CD-inclusion complex. The small circles represent the water molecules while the larger ones, are of the CD cavity. The exterior of the CD is hydrated by the water molecules while the interior of the apolar cavity is filled with energetically unfavourable water molecules. The hydrophilic part of the

potential guest molecule is surrounded by water molecules while the hydrophobic end repels them. Upon the penetration of the hydrophobic end of the guest into the CD cavity, an energetically favourable non-polar non-polar interaction occurs, associated with the displacement of "high energy"water molecules from the CD cavity. The hydrophilic end remains close to the exterior aqueous environment.

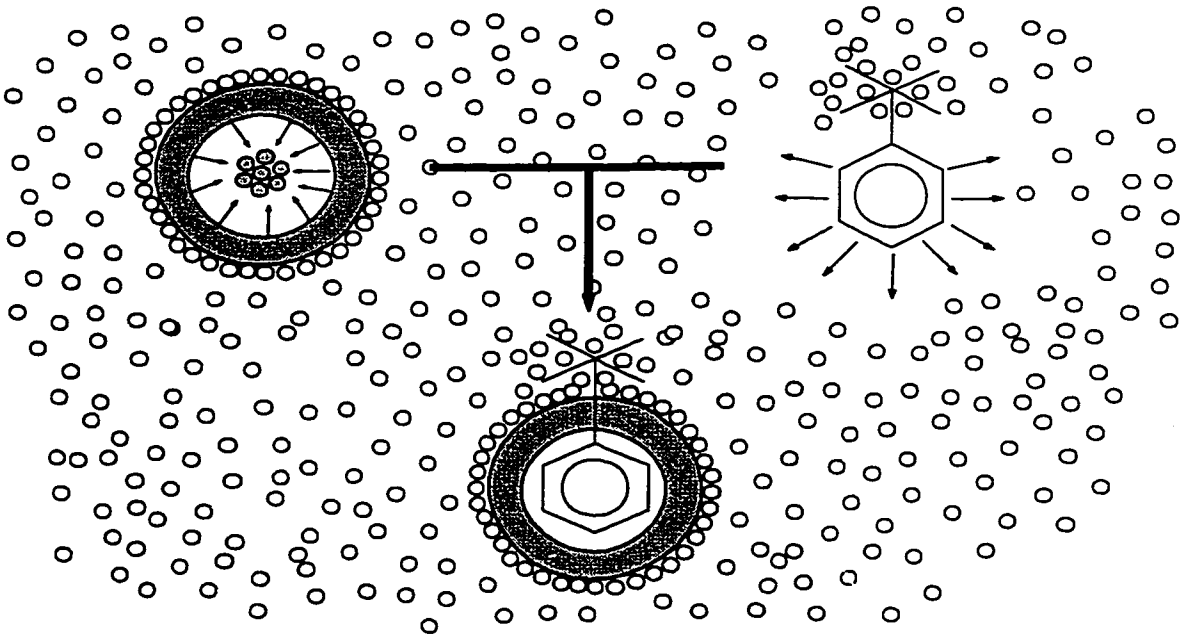


Figure 1.5 A Schematic Representation of the Formation of Cyclodextrin Inclusion Complexes [4]

In most cases, one cyclodextrin molecule binds to a single organic guest molecule to form a complex of the ratio 1:1, however, there have been reports[4,13-16] of formation of 2:1 (CD:guest) complexes, in which the guest molecule is of the appropriate polarity but it is too large to fit into one CD cavity only. A 1:2 complex involves one cyclodextrin molecule and two identical guest molecules, while a 1:1:1 complex consists of one cyclodextrin molecule and two different guest molecules. Such stoichiometries are known.[4,13-16]

The driving force and thus, stabilization of these inclusion complexes arises from

various and simultaneous intermolecular interactions which may include the release of "high energy" water molecules from the CD cavity, hydrogen bonding, London dispersion forces, relief of cyclodextrin ring strain energy, and van der Waals interactions.[1,4,8-10,12,15,18]

"High energy" water molecules refer to the water molecules that occupy the CD cavity and that are unable to form hydrogen bonding interactions as in the bulk water. Upon the entry of a guest molecule, these "high energy" water molecules are replaced into the bulk water, which is thought to contribute to the stabilization of the cyclodextrin complex. Although the exact number of water molecules situated inside the CD cavity remains uncertain, there appears to be a consensus that for α-CD, there are two to three water molecules that are located in the cavity before the appearance of any guest molecule.[1,9]

Entropy effects should also be considered when referring to inclusion complexes. Upon the inclusion of a guest molecule and the exclusion of the enthalpy-rich water molecules, an increase in the amount of favourable interactions should result, along with an increase in the degree of disorder of the system. Thus, as seen in over 2000 cyclodextrin complexes, this process involves a favourable enthalpy change (ΔH° is negative) and a favourable entropy change (ΔS° is positive).[9] As well, upon the exchange of guests, a conformational change of the cyclodextrin occurs which may also play a role in the stabilization of these complexes.

1.2.3 Practical Applications and Uses

Cyclodextrins and their inclusion complexes have numerous applications in the pharmaceutical industry, the food industry, cosmetics and toiletries, and in the agriculture industry. These applications arose from CDs' absence of toxicological side effects which therefore permits their consumption by humans.[10]

1.2.3.1 Pharmaceutical Industry

According to Szejtli,[7] in 1996, approximately 25% of all cyclodextrin-related publications were dedicated to the pharmaceutical application of cyclodextrins. This large percentage is due to cyclodextrins' many abilities such as complexation to drugs and, acting as auxillary additives including carriers, diluents, solubilizers and tablet ingredients.[10] Because most drug molecules are poorly water soluble, and may be sensitive to light, oxidation, and other ingredients in the drug formulation, they are ideal complex-forming partners for CDs. However, these drug molecules must be of the appropriate polarity, and structure to be included in the CD cavity.[10] Currently, more than a dozen drugs have already been approved and marketed in CD-complexed form and the various methodologies of administration include orally, through the rectum (suppositories), and dermatologically (ointments, creams, injections and eye-drops). Some countries that have approved the utilization of drugs that are formulated with CDs, include Germany (Prostavasin, Xund, and Tegra), Italy (Brexin, and Cicladol), Japan (Prostavasin, Prostarmon, Ulgut, Lonmiel, and Mena-Gargle), and Hungary (Allidex).[17] As well, upon formation of CD-inclusion complexes, bad odours or tastes of drugs are masked. For example, the antibacterial and

antifungal compound, allicin, which is the unpleasant odour ingredient of garlic. can form a CD-complex in which its smell is concealed.[18] Other examples of improved odour or taste include chloral hydrate, prostaglandins, amines, non-steroidal anti-inflammatory drugs (NSAIDs), thymol, and chloramphenicol.[17] Some substances of natural origin (eg. camomile oil) are unable to be stored for long periods of time, even in sealed containers due to their inevitable decomposition and polymerization of essential compounds (eg. terpenes). However, upon their inclusion in CD cavities, their lifetime is prolonged.[18]

Other advantages of forming CD-inclusion complexes include enhancement of the drug's solubility. For example, the solubilities of prostaglandins, NSAIDs, digitalis, steroid hormones, barbiturates, phenytoin, benzodiazepines, coumarin anticoagulants, and diuretics are all augmented.[17] Hydrolysis of substances encapsulated in CD-complexes, eg. aspirin, atropine, procaine, digitalis, and prostacycline, are inhibited, as is the photodecomposition of phenothiazines, ubiquinones, and vitamins.[17] Certain substances, such as oil-soluble vitamins A, D, and K, phenols, benzaldehyde, nitroglycerine, methyl salicylate, and essential oils, can be modified from liquid drugs to powders, upon their inclusion in CD cavities, and other substances such as L-menthol, D-camphor and salicylic acid no longer undergo evaporation upon formation of CD-inclusion complexes.[17]

For smokers, nicotine can form a crystalline complex with β-CD which can be directly used in cigarette filters. The addition of β-CD to cellulose filters removes a large proportion of the nicotine and tar from the filtered smoke.[10] As well, the gradual loss of tobacco aromas due to processing and storage can be eliminated upon their complexation to β-CD. These aromas remain unchanged until they are liberated by the action of burning

tobacco.[18]

Thus, cyclodextrin's employment in the pharmaceutical industry will be long-lasting. Future endeavours in this field will be intense as more and more companies make use of the bioavailability of drugs through their CD-inclusion complexes.

1.2.3.2 Food Industry

Cyclodextrins are also extremely important in the food industry due to the many substances that can be included as guest molecules in the cyclodextrin cavity. The main advantages of utilizing CD-included complexes are the stabilization of food flavours, the elimination of undesirable tastes and odours, and the improvement of quality, stability and storability of foods.

Flavouring and aroma substances, sweeteners, and, oils and fats, can all form inclusion complexes with CDs. Their stabilization is enhanced and can provide longer lasting odours and tastes. For example, formation of a peppermint-CD complex, which can be blended with chewing gum, yields an aroma that remains longer along with a longer-lasting intensive taste.[4] As well, upon the addition of β-CD complexed to various flavourings of tea leaves (lemon, bergamot, or peppermint), tea of poor quality is significantly enhanced.[18] Biscuits containing butter flavour.β-CD complex can still retain their flavouring even after two months of storage.[18]

Therefore, the numerous applications of CDs in the food industry are certainly becoming increasingly important. At the moment, Japan is the only country where CD applications in food products is unlimited. Denmark has allowed the use of β-CD as a

flavour carrier in chewing gum. One company in Hungary has been marketing spice extracts that are complexed with β-CD, and another company in France utilizes β-CD in the extraction process of cholesterol from butter oil.[17] Although there are only a handful of countries utilizing CDs in the food industry now, in due time, as the growing number of articles published on this topic increase, other countries may develop a need for CD complexed food products.

1.2.3.3 Cosmetics and Toiletries

Cyclodextrins are used favourably in cosmetics and toiletries. Upon the use of cyclodextrins in cosmetics, substances such as essential oils, fatty acids, ascorbic acid, lysozyme and colorants increase their stability.[17] Perfumes and fungicide substances can sustain their effects over long periods of time upon the addition of CDs. Cyclodextrins are also able to mask the disagreeable smells of mercaptan and ammonium glycolate (used in hair sprays), and iodine (used in antiseptics).[10,17]

Examples of cyclodextrin-used applications in cosmetics and toiletries are numerous. γ-Cyclodextrin can be added to self-tanning creams so as to mask the horrible odour produced. Cyclodextrins can also complex with menthol in shampoos, in order to reduce the strong menthol scent, and are also included in oral deodorizers in the form of powders, tablets, mouthwashes and toothpaste in order to reduce bad breath.[17] Cyclodextrin-complexed fragrances are utilized in solid perfumes, fragrant candles, incenses, and detergents. Cyclodextrins are able to form complexes with detergent molecules and act as defoaming agents. For example, on the addition of CDs to the rinsing water in a laundry,

small amounts of detergents that may have remained are complexed to the CD. Therefore, this defoaming effect can reduce the amount of water used in laundries.[18]

The utilization of CDs in cosmetics and toiletries is expected to grow rapidly considering the approval process for CD-containing products is quite simple and very rapid. This particular field appears to demonstrate unlimited versatility of CD use without any toxicological limitations and will therefore advance in the years to come.

1.2.3.4 Agriculture Industry

The effects that CDs have on complexing with pesticides are similar to those of formulating drugs. Cyclodextrin inclusion of insecticides, fungicides, herbicides, and defoliants, can stabilize these substances from rapid decomposition by light, oxygen, and heat, and are much safer to handle, transport, and use.

Ethylene is an effective hormone-like agent in the plant kingdom that can accelerate the ripening process of fruits (eg. tomatoes), and improving the yields of vegetables (eg. celery and lettuce). Also, ethylene binds to CDs and CD-treated plants demonstrating a retarded growth for the first few days, but later on, developing stronger and more quickly than the controls.[10]

1.3 Catalysis and Transition State Stabilization

Before discussing the contribution that cyclodextrins can make to catalysis, it would be beneficial to discuss catalysis in general. Enzymes and enzyme mimics are known to catalyse reactions by binding and thus stabilizing, selectively, the transition state of a particular reaction.[19-21] A method that was developed by Kurz,[22] and further elaborated by Wolfenden[23] and Kraut,[24] utilizes the Transition State Theory[25,26] to quantitate transition state stabilization by catalysts.

Using Transition State Theory, one takes into consideration two typical reactions: an uncatalysed reaction ((1), overleaf) where a substrate, S, undergoes reaction to form products, P, and a catalysed reaction (2) where C represents the catalyst forming a S.C complex, which further undergoes conversion to products. The rate constant, k_c, is the limiting rate constant for the reaction of the S.C complex, while K_S is the dissociation constant of the S.C complex. The second order rate constant, k_2, which characterises the substrate and catalyst converting to products, is equal to k_c/K_S. The rate constants of these processes are expressed by equations (3) and (4), where the term ν ($= k_BT/h$) represents the frequency over the energy barrier. At this point, two main assumptions are introduced. For mathematical purposes, it is considered that TS.C is the transition state of the uncatalysed reaction bound to the CD. Also, the frequency, ν, is assumed to be the same for both the uncatalysed and catalysed processes, and the transmission coefficients are assumed to be equal.[24] Given these assumptions, equation (3) can be divided by equation (4), leading to equation (5), which describes K_{TS}, as an apparent dissociation constant of the TS.C into the TS and the catalyst (6).

$$S \xrightarrow{k_u} P \qquad (1)$$

$$S + C \underset{K_S}{\rightleftharpoons} S.C \xrightarrow{k_c} P \qquad (2)$$

$$k_u = \nu \frac{[TS]}{[S]} \qquad (3)$$

$$k_2 = \nu \frac{[TS.C]}{[S][C]} = \frac{k_c}{K_S} \qquad (4)$$

$$K_{TS} = \frac{[TS][C]}{[TS.C]} = \frac{k_u}{k_2} = \frac{k_u K_S}{k_c} \qquad (5)$$

$$TS.C \underset{}{\overset{K_{TS}}{\rightleftharpoons}} TS + C \qquad (6)$$

Although equation (6) represents a reversible reaction, it is highly unlikely that the TS.C complex, once-formed, will dissociate reversibly into the transition state and the catalyst and so K_{TS} is considered only to be a *quasi*-equilibrium constant. However, K_{TS} can be used through equation (7), to provide information about the relative energies of the transition states of the uncatalysed and catalysed reactions.

$$\Delta G^\circ_{TS} = -RT\ln K_{TS} \qquad (7)$$

Since no assumptions are made about the mechanisms of the uncatalysed and catalysed reactions, K_{TS} and its variations with structure can be used to probe the transition state structure of various reactions.[30]

1.4 Cyclodextrins as Catalysts[8,27-30]

Although the ability of cyclodextrins to act as catalysts was recognized by Cramer.[4] in 1953, it was Tee,[29,30] who first utilized the Kurz method to estimate transition state stabilization of reactions mediated by cyclodextrins. Before discussing this aspect, it is necessary to outline the sort of kinetics observed in CD-mediated reactions.

In the absence of CD, a substrate can undergo an uncatalysed reaction, as expressed in equation (8a). For the CD-catalysed reaction, equation (8b) can be used where the substrate forms a 1:1 complex with the cyclodextrin before converting into products. For these two processes together, equation (8c) can be obtained, derived as shown in Appendix I, where the observed rate constant (k_{obs}) varies with the [CD] in a non-linear fashion.

$$S \xrightarrow{k_u} P \qquad (8a)$$

$$S + CD \underset{K_S}{\rightleftharpoons} S.CD \xrightarrow{k_c} P \qquad (8b)$$

$$k_{obs} = \frac{k_u K_S + k_c[CD]}{K_S + [CD]} \qquad (8c)$$

Equation (8c) corresponds to saturation kinetics, similar to Michaelis-Menten kinetics. As long as [S.CD] << [CD], then one can take [CD] = $[CD]_o$. The rate constant, k_u, can be directly measured at zero [CD] and the values for k_c and K_S can be graphically determined through various methods such as the Lineweaver-Burk approach and the Eadie-Hofstee method. However, it is now more appropriate to use a direct non-linear least-squares fitting to determine the values for k_c and K_S.[30]

In the case where the substrate weakly binds to the CD, then K_S >> [CD] and

equation (8c) can be reduced to (9). Accordingly, a plot of k_{obs} versus [CD] gives a straight line of slope k_2 (= k_c/K_S) and, with an intercept equalling k_u.

$$k_{obs} = k_u + k_2 [CD] \quad (9)$$

As mentioned previously, transition state stabilization can be quantitated by equation (7). This important aspect is best appreciated by reference to the Gibbs free energy diagram in Figure 1.6 where it is seen that $\Delta G°_{TS} = -RT\ln K_{TS}$ is the energy difference between the barriers of the catalysed and uncatalysed pathways.

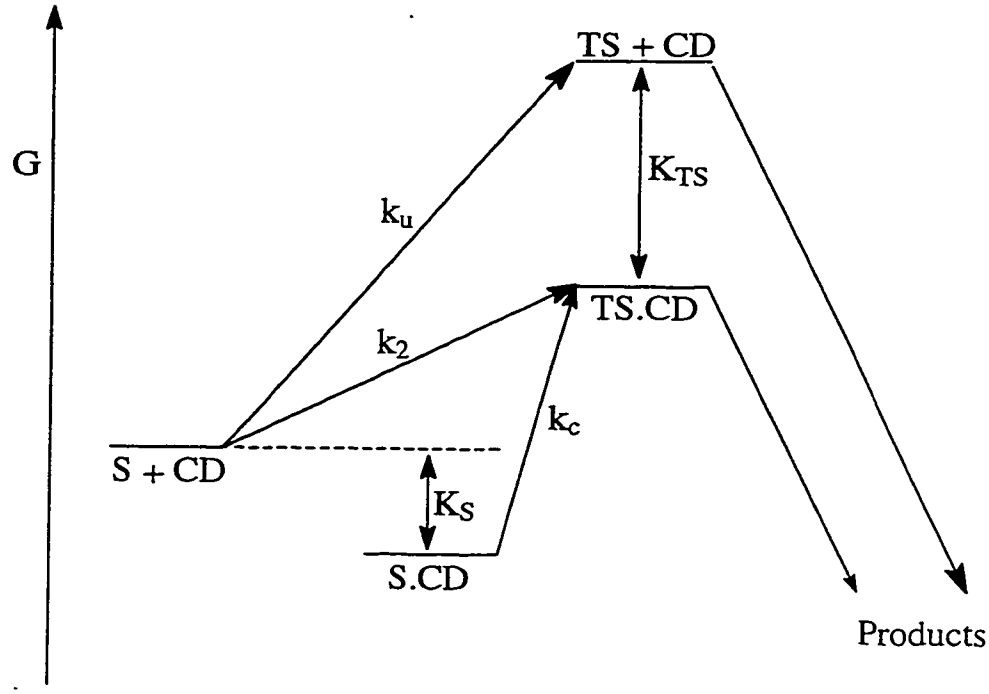


Figure 1.6: Gibbs Free Energy Diagram of Uncatalysed and CD-catalysed Reactions. Each free energy difference (ΔG) is directly related to the rate constants k_u, k_c, and k_2, or the equilibrium constants, K_S, and K_{TS} as shown.

According to Figure 1.6, upon complexation of the CD with the transition state, there is a lowering of the free energy resulting in a rate enhancement. Yet, if the substrate forms

a strong (stable) complex with the cyclodextrin, then retardation or inhibition of the reaction may arise. This decrease in rate would be due to the larger amount of energy required to overcome the transition state barrier for the CD-mediated reaction (the k_c process) than is required for the uncatalysed reaction (the k_u process).

Rate enhancement or deceleration may also be viewed in the form of equation (10), which is derived from eq. (5). According to equation (10), the acceleration (or retardation) of a CD-mediated reaction (k_c/k_u) is dependent on the strength of substrate binding relative to that of transition state binding. Hence, a cyclodextrin will catalyse a reaction if it binds more strongly to the transition state than to the substrate, and it will inhibit a reaction if it binds to the substrate more tightly.[19,29,30]

$$\frac{k_c}{k_u} = \frac{K_S}{K_{TS}} \qquad (10)$$

The kinetic parameters, k_u, k_c, and K_S (or k_u and k_2) are all measurable quantities, and provided these quantities are all measured under the same conditions, the pseudo-equilibrium constant, K_{TS} (and its logarithm), can be estimated. Values of K_{TS} may provide insight into differentiating between the different types of catalysis.[29]

As discussed in detail in the next section, CD-mediated reactions occur by either non-covalent catalysis or by covalent catalysis. Examples of many CD-catalysed reactions can be viewed in Table 1.3.

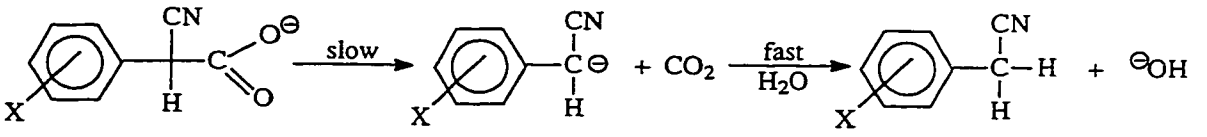
Table 1.3: An Overview of Cyclodextrin-catalysed Reactions[8,28]

Reaction	Substrate	k_c/k_u	Catalysis Type
Hydrolysis	Phenyl Esters	300	Covalent
	Penicillin	89	Covalent
	Aryl Carbonates	7	Covalent
	Aryl Sulfates	19	Non-covalent
Decarboxylation	Cyanoacetate ion	44	Non-covalent
Oxidation	Hydroxyketones	3	Non-covalent

1.4.1 Non-covalent Catalysis

In non-covalent catalysis, an inclusion complex is formed between the substrate and the cyclodextrin, where the latter's cavity is apolar and conformationally restricted so as to serve only as a reaction medium. Both microsolvent effects and conformational effects may play a role in the non-covalent catalysis by cyclodextrins.

An example of the microsolvent effect displayed by cyclodextrins is the decarboxylation of phenylcyanoacetate ions.[28,30,31] This type of reaction involves a rate-determining step in which there is heterolytic cleavage of the carbon-carboxyl carbon bond, as shown in Scheme 1.1.



Scheme 1.1: Decarboxylation of Phenylcyanoacetate Anions due to Microsolvent Effect

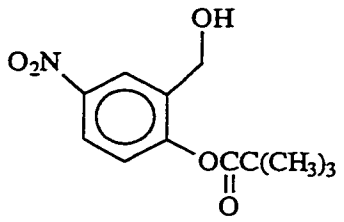
β-Cyclodextrin accelerates the first step of this reaction since the interior of its cavity is apolar in nature. As seen in Table 1.4, the maximum rate accelerations (k_c/k_u) do not vary largely (12-23) even though the K_S values alter with changing position and size of the substituent on the phenyl ring. Consequently, from a plot of pK_{TS} (= -log K_{TS}) versus pK_S (= -log K_S), the pK_{TS} values will vary in parallel with those of pK_S, strongly suggesting that transition state binding in the CD cavity is similar to that of substrate binding.[30]

Table 1.4: Decarboxylation of Phenylcyanoacetate Anions Catalysed by β-CD[31]

Phenyl Substituent	k_c/k_u	K_S (mM)	K_{TS} (mM)
4-MeO	15.9	17.6	1.11
4-Me	12.7	15.7	1.24
3-Me	15.8	37.3	2.36
2-Me	12.0	67.8	5.65
4-Br	16.6	8.54	0.514
4-Cl	23.3	17.6	0.755
2-Cl	19.8	29.8	1.51
H	18.7	39.5	2.11

From the study of the intramolecular acyl transfer of 2-hydroxymethyl-4-nitrophenyl trimethylacetate, **1**, to **2**, conformational effects may explain the seven-fold acceleration exhibited by α-CD, and the five-fold inhibitory effect displayed by β-CD.[4,8,30] From the constants in Table 1.5, the variation between the two cyclodextrins rests only on the binding of the substrate (K_S), and not of the transition state (K_{TS}). The K_{TS} values for both cyclodextrins are similar indicating that the strength of transition state binding does not change appreciably from α-CD to β-CD. However, in the initial state, there is stronger

binding of the substrate to β-CD, which leads to rate retardation where $k_c/k_u < 1$. This situation arises, presumably, due to the unreactive conformation of the substrate, **1**, which sits more deeply and more tightly in the larger β-CD cavity, thus disfavouring the geometry of the transition state. For α-CD, the substrate prefers the conformation as seen in **1'** which therefore results in catalysis.



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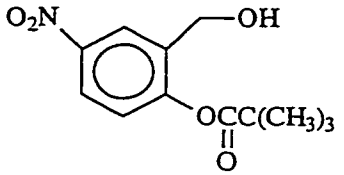

1'

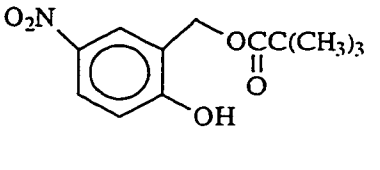

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Table 1.5: Kinetic Parameters for Intramolecular Acyl Transfer of **1** to **2**[32]

Cyclodextrin	k_c/k_u	K_S (mM)	K_{TS} (mM)
α-CD	7.3	47.8	6.52
β-CD	0.19	0.960	5.11

1.4.2 Covalent Catalysis

Covalent catalysis involves the formation of covalent bonds between a functional group of the cyclodextrin and the substrate during the rate-limiting step of the reaction. The first step of covalent catalysis is the formation of an inclusion complex between the cyclodextrin and the substrate, followed by covalency changes involving the CD. Numerous reactions occur via this type of catalysis including enolization, and the hydrolysis of amides and esters.

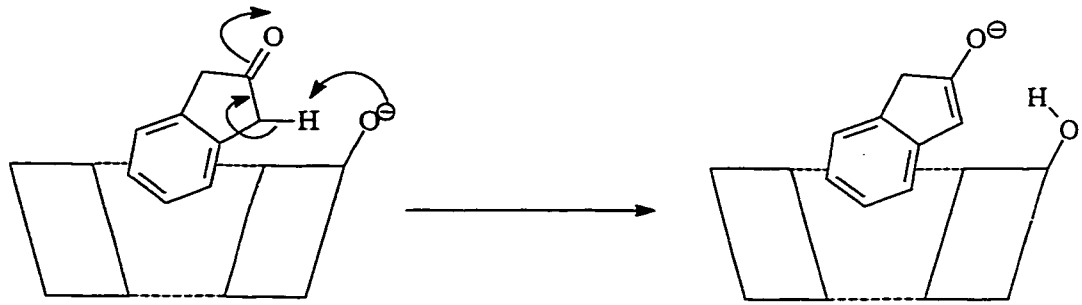
An example of covalent catalysis is the enolization of 2-indanone in basic solution,

which is modestly catalysed by α-, β-, and γ-CD.[33] In this reaction, a covalent bond is established between the enolizable proton on the substrate and the ionized secondary hydroxy group situated on the rim of the cyclodextrin. From Table 1.6, the maximum rate acceleration, k_c/k_u, is observed with α-CD which is a result of a large difference between the strengths of substrate binding, K_S, and, transition state binding, K_{TS}.

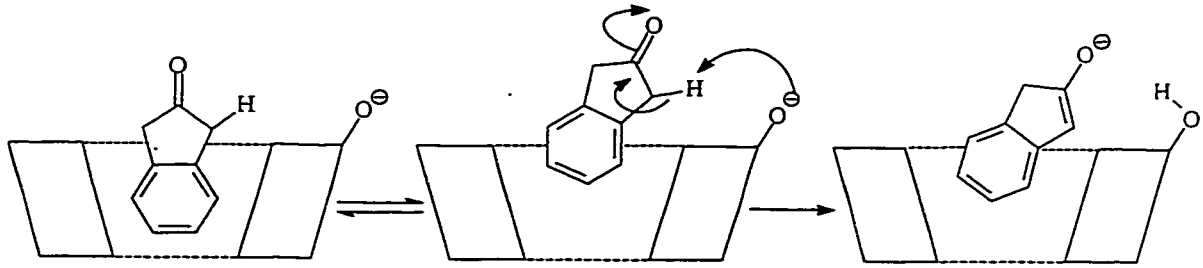
Table 1.6: Kinetic Parameters for the Enolization of 2-Indanone[33]

Cyclodextrin	k_c/k_u	K_S (mM)	K_{TS} (mM)
α-CD	22.0	59.5	2.7
β-CD	4.95	6.49	1.3
γ-CD	8.32	60.5	7.3

The reason for α-CD's large acceleration can be understood by looking at the reaction mechanism shown in Scheme 1.2. Since 2-indanone is too large to fit inside the α-CD cavity, it remains perched, possibly in a tilted conformation. This orientation is, in fact, ideal for catalysis since the enolizable proton of 2-indanone is in close proximity to a secondary hydroxy group of α-CD. In the case of β- and γ-CD, as seen in Scheme 1.3, both cyclodextrins consist of larger cavity sizes and thus, 2-indanone can easily slip into their respective cavities. However, this places the enolizable proton at a less favourable position for attack by an ionized secondary hydroxy group, and so the rate accelerations by β-CD and γ-CD are low compared to that by α-CD.

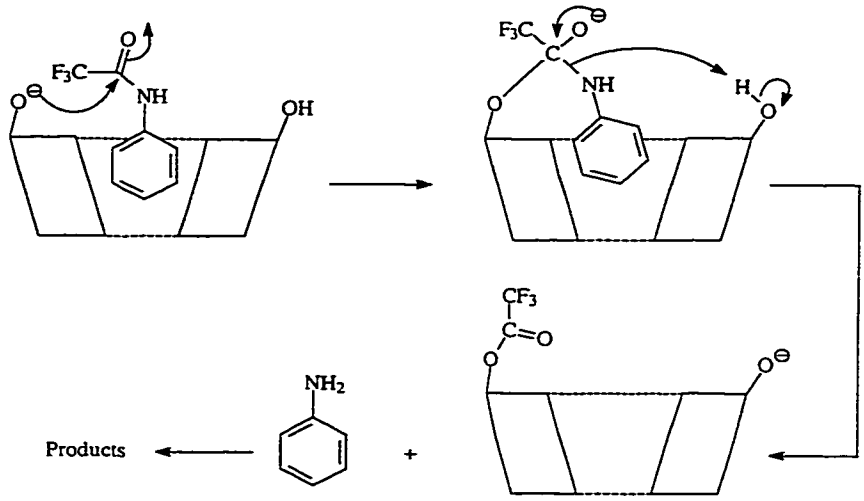


Scheme 1.2: Mechanism of the Enolization of 2-Indanone Catalysed by α-CD



Scheme 1.3: Mechanism of the Enolization of 2-Indanone Catalysed by β- and γ-CD

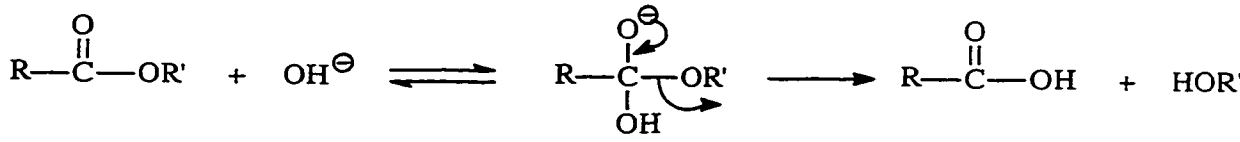
Cyclodextrins can covalently catalyse the cleavage of amides, as was demonstrated with the substrate *p*-nitrofluoroacetanilide reacting with α-CD.[8,34] As seen in Scheme 1.4, the mechanism involves nucleophilic attack by an ionized secondary hydroxy group of α-CD on the carbonyl carbon of the substrate. Formation of a tetrahedral intermediate results where the phenyl ring remains inside the cyclodextrin cavity. General acid catalysis is then afforded by proton transfer from a second hydroxy group of α-CD, to the anilide nitrogen, yielding aniline and a trifluoroacetylated cyclodextrin which undergoes further hydrolysis to regenerate the cyclodextrin. While this particular substrate is catalysed by α-CD, by a factor of 16, a less activated substrate, *p*-nitroacetanilide, is in fact, inhibited by α-CD.



Scheme 1.4: Mechanism of the Cleavage of *p*-Nitrofluoroacetanilide Catalysed by α-CD

1.5 Cleavage of Esters

Of all the reactions that cyclodextrin can covalently catalyse, the cleavage of esters has been the most extensively studied.[4,8,11,29,30,35,36] The general mechanism for the basic cleavage of esters follows Scheme 1.5, in which the nucleophile, OH^- attacks the carbonyl carbon to form a tetrahedral intermediate, followed by decomposition to products. As with the amide cleavage (above), the overall process is one of Acyl Transfer.



Scheme 1.5: Mechanism of the Cleavage of a Carboxylic Ester by a Nucleophile.[37]

In the case where cyclodextrin acts as a nucleophile, hydrolysis of the acylated cyclodextrin to form the regenerated CD is relatively slow, and therefore, the overall cleavage process by cyclodextrin is not truly catalysed. In fact, the strong covalent interaction between the ester

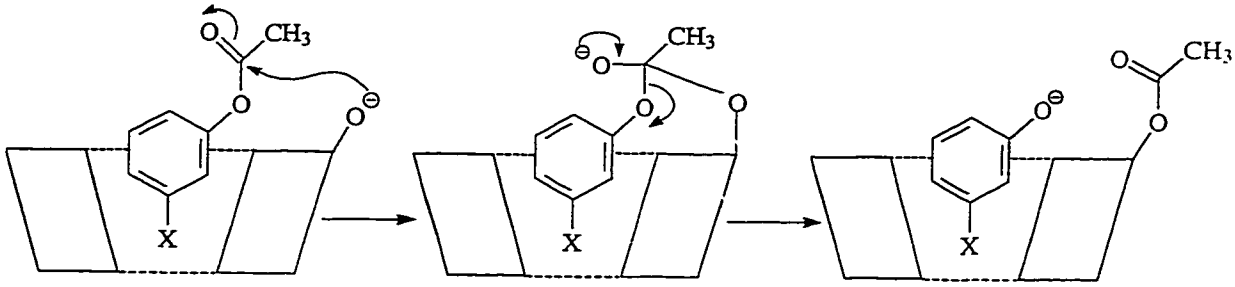
and the CD in the transition state has been duly reported by Tee [29,30] where K_{TS} values are quite low for aryl esters. Moreover, these low values demonstrate the strong dependence on the position and size of the substituents on the ester[34] as seen in Table 1.7.

Table 1.7: Kinetic Parameters for the Cleavage of Phenyl Acetate by α-CD[35]

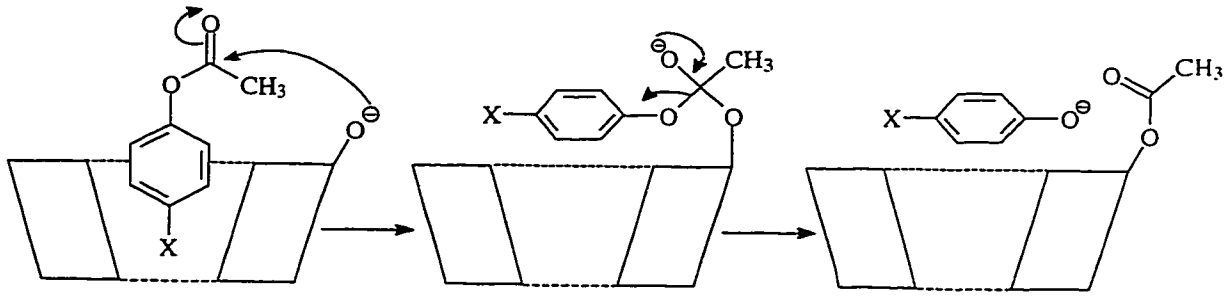
Substituent	k_c/k_u	K_S (mM)	K_{TS} (mM)
H	27	22	0.81
p-Me	3.3	11	3.3
m-Me	95	17	0.18
p-NO_2	3.4	12	3.5
m-NO_2	300	19	0.063
p-*t*-Bu	1.1	6.5	5.9
m-*t*-Bu	260	2.0	0.0077
p-CO_2^-	5.3	150	28
m-CO_2^-	68	105	1.5

From these results, it can be concluded that *m*-substituted phenyl acetates serve as better substrates for ester cleavage than their *p*-substituted counterparts.[8,29,30,35] The values of k_c/k_u and K_{TS} support this view because there are large accelerations for the *m*-substituted phenyl acetates (k_c/k_u ranges from 68 to 300), as opposed to the *para* compounds (k_c/k_u ranges only from 1 to 5). The values of substrate binding for each pair of isomers (K_S) seem to be similar, and thus, the difference in magnitude of the acceleration must be attributed largely to the variation in transition state binding. For the *m*-substituted compounds, the values of K_{TS} are lower, indicating stronger transition state binding. Hence, these kinetic parameters support the view that for the *meta* substituents, the carbonyl group of the ester

is located outside the CD cavity, close to the ionized hydroxy groups, which facilitates nucleophilic attack (Scheme 1.6). In contrast, with the *para* substituents, binding of the cyclodextrin in the transition state can only occur by the partial removal of the phenyl ring from the CD cavity (Scheme 1.7).



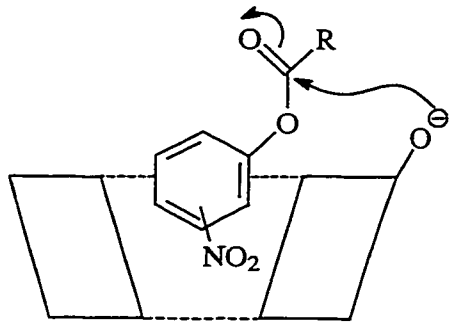
Scheme 1.6: Mechanism of the Cleavage of *m*-substituted Phenyl Acetates by Cyclodextrins



Scheme 1.7: Mechanism of the Cleavage of *p*-substituted Phenyl Acetates by Cyclodextrins

The main characteristics that support the conclusions for *m*- and *p*- substituted esters with α-CD are also observed with β-CD, but variation in kinetic parameters for β-CD, is generally not as distinct as for α-CD. This difference arises because β-CD's cavity is slightly larger than that of α-CD. Thus, the substituted phenyl groups located in β-CD have more room and must be held less rigidly. Consequently, *m*-substituted esters in β-CD are not cleaved as well as in α-CD, while *p*-substituted esters are cleaved better in β-CD.[30]

Recently, various studies have been concerned with the variation in chain length of phenyl esters.[38-42] A study by Bonora et al.[38] looked at the effects of acyl chain length on the basic cleavage of *p*-nitrophenyl alkanoates (C_2, C_4, C_6, C_8, and C_{12}) by both α- and β-CD. It was found that regardless of the cyclodextrin used, the K_S values markedly decreased as the acyl chain length increased, implying a switch in the binding mode of the substrate from aryl group inclusion, **3**, to alkyl group inclusion, **4**. The K_{TS} values also decreased with an increase in acyl chain length, however, this decrease was much more pronounced for the octanoate and the dodecanoate. The K_{TS} trend subsequently implied that in the transition state, a change in binding occurred from aryl group inclusion to alkyl group inclusion.



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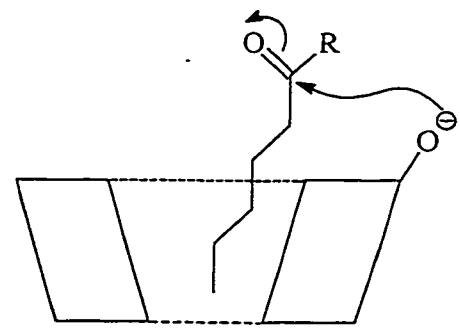

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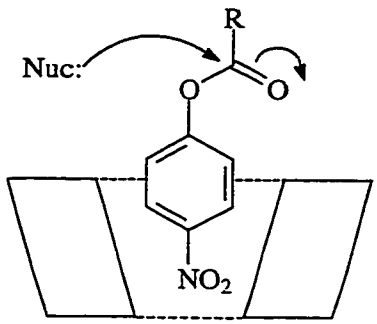
The effects of acyl chain length on the basic cleavage of *m*- and *p*-nitrophenyl alkanoates by α- and β-CD was studied by Tee et al.[39] Chain lengths ranging from acetate to hexanoate were examined. The results for the *para* series were comparable to the earlier report.[38] For the *meta* and *para* series, after acetate, there is a switch in the substrate binding mode from aryl inclusion, **3**, to alkyl inclusion, **4**. However, transition state binding for the *p*-nitrophenyl alkanoates is dominated by the inclusion of the alkyl group, **4**, whereas cleavage by aryl group inclusion, **3**, is preferred for the *m*-nitrophenyl alkanoates.

1.6 Aminolysis of Esters

Cyclodextrins can act as nucleophiles towards esters, through their ionized secondary hydroxy groups, but other nucleophiles may react with esters bound to cyclodextrins. How the ester will react towards the nucleophile when bound to the cyclodextrin, and how its reactivity will vary with the structure of the ester, the cyclodextrin, and the nucleophile are concerns that have prompted recent investigation.

Nucleophiles can be categorized into two groups: non-binding nucleophiles and binding nucleophiles. Examples of the former group are hydroxylamine, imidazole, and the anions of 2,2,2-trifluoroethanol (TFE), and 2-mercaptoethanol (2-ME), and the kinetics of their reactions with both *p*-nitrophenyl acetate (*p*-NPA) and *p*-nitrophenyl hexanoate (*p*-NPH) in the presence of cyclodextrins have been studied. These particular reactions were examined to understand how the reactivity of ester substrates can be affected by binding to CDs. From this initial study,[43] results indicated that the reactivities of CD-bound esters are equivalent or slightly reduced in comparison to that of the free esters, which suggests that the carbonyl carbon of the ester is located outside the cavity, accessible to the attacking nucleophiles in the medium. However, the mode of substrate binding in the transition state was not evident. Based on previous studies of ester.CD binding,[39,42,44,45] it was suggested that *p*-NPA probably reacts with non-binding nucleophiles as shown in **5**, whereas *p*-NPH could react with acyl inclusion, as in **6**.[43]



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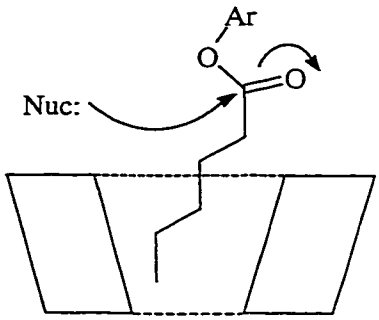

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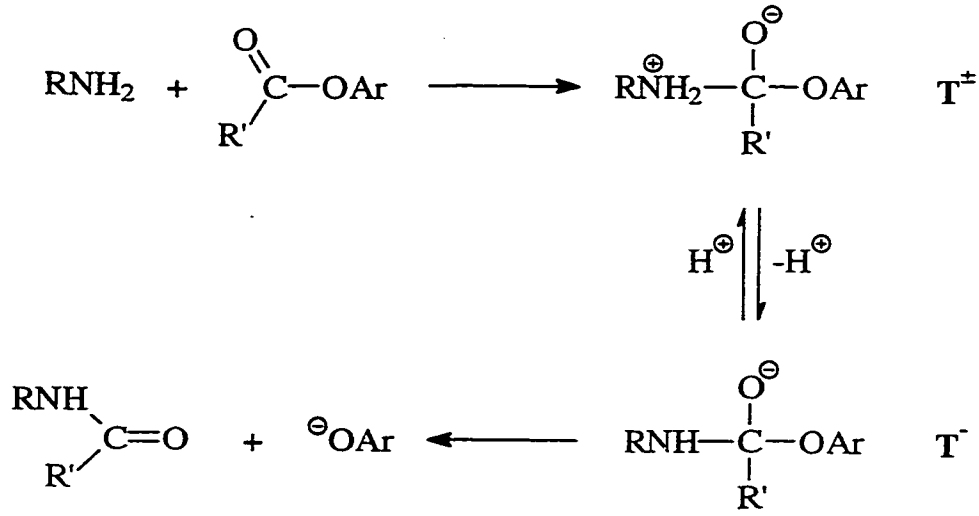
Subsequently, kinetic studies of the attack of TFE anion and 2-ME anion on a series of *p*-nitrophenyl alkanoates (C_2-C_{10}) in the presence of cyclodextrins afforded details as to the particular mode of transition state binding.[46] It was found that for short esters kinetic parameters are insensitive to the acyl chain length but they are appreciably sensitive for the long esters. Thus, from these parameters, it was determined that short esters that bind with aryl group inclusion do, in fact, react through this mode, as seen in **5**. However, the short esters that bind through acyl group inclusion, will have to first isomerize to a less stable, aryl group inclusion configuration, **5**, before undergoing reaction. In the case of long esters, the acyl groups are included in the cyclodextrin cavity while the carbonyl carbon is exposed to the basic medium, **6**, so that nucleophilic attack can occur.

Recently, binding nucleophiles, specifically primary amines, have been investigated.[42,47,48] Mechanistically, the aminolysis of aryl esters is depicted in Scheme 1.8, where the amine first attacks the carbonyl carbon of the ester. The zwitterionic intermediate $\mathbf{T}^{\pm}$ is formed, which at high pH will undergo fast deprotonation to form the anionic tetrahedral intermediate, $\mathbf{T}^{-}$, followed by the loss of the aryloxide ion to produce an amide. The rate-limiting transition state resembles $\mathbf{T}^{\pm}$ or $\mathbf{T}^{-}$ and so stabilization of these

intermediates should lower the overall activation barrier and increase the rate of reaction.



Scheme 1.8: Mechanism of the Aminolysis of Aryl Esters

For the aminolysis of aryl esters in the presence of cyclodextrins, four kinetic processes must be considered. As previously seen, in basic medium, equation (8a) depicts the uncatalysed hydrolysis of an ester substrate, while equation (8b) is the catalysed hydrolysis of the ester, via the formation of a S.CD complex. Equation (11) represents the reaction of the free (unbound) ester with the nucleophile (Nuc) while equation (12) depicts the reaction of the CD-bound ester complex with the free nucleophile. From these four competitive processes, the expected variation of k_{obs} with both [Nuc] and [CD] is displayed in equation (13), where the condition, [S.CD] << [CD] must be met.

$$S + Nuc \xrightarrow{k_N} P \qquad (11)$$

$$S + CD + Nuc \underset{K_S}{\rightleftharpoons} S.CD + Nuc \xrightarrow{k_{cN}} P \qquad (12)$$

$$k_{obs} = \frac{(k_u K_S + k_c[CD]) + (k_N K_S + k_{cN}[CD])\,[Nuc]}{(K_S + [CD])} \qquad (13)$$

Although, equation (13) is of an appropriate form, it is difficult to utilize due to its non-linear form and its dependency on both the concentration of the nucleophile and of the cyclodextrin. Consequently, conversion of this equation to a linear form, (14), is made by dividing this equation by $f_s = K_S/(K_S + [CD])$, which is simply the fraction of free substrate.

$$\frac{k_{obs}}{f_s} = k_u + k_N[Nuc] + \frac{k_c[CD]}{K_S} + \frac{k_{cN}[Nuc][CD]}{K_S} \qquad (14)$$

Analysis of kinetic data in terms of equation (13) was used to find rate constants k_{cN}.[42,47,48] The rate constant, k_N, was easily determined from the slope of the linear variations of k_{obs} versus [Nuc] [eq. (15)] in the absence of CD.

$$k_{obs} = k_u + k_N[Nuc] \qquad (15)$$

Although equations (13) and (14) include the substrate binding to the cyclodextrin, the possibility of nucleophile binding to the cyclodextrin must also be taken into account when amines are the nucleophile, as seen in eq. (16). Hence, both the [Nuc] and the [CD] must be corrected for the cyclodextrin-nucleophile binding. (Details of this calculation are given later in the Experimental section.)

$$CD + Nuc \underset{K_N}{\rightleftharpoons} CD.Nuc \qquad (16)$$

There are three kinetically equivalent processes of CD-mediated aminolysis that should be taken into account. Equation (12), which involves the S.CD complex reacting with the nucleophile, is kinetically equivalent to (17) and (18), both of which also involve ester, amine, and a CD. From the equivalence of the processes in equations (12) and (17), $k_3 = k_{cN}/K_S$. Likewise, because of the equivalence of equations (17) and (18), $k_3 = k_{Nc}/K_N$ (or $k_{Nc} = k_3K_N$). So, while the data analysis affords k_{cN}, both k_3 and k_{Nc} are easily obtained.

$$S + CD + Nuc \xrightarrow{k_3} P \qquad (17)$$

$$S + CD + Nuc \underset{K_N}{\rightleftharpoons} S + Nuc.CD \xrightarrow{k_{Nc}} P \qquad (18)$$

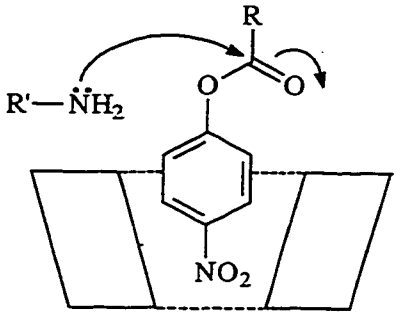
With respect to Transition State Stabilization, K_{TS} is defined as the apparent dissociation constant of the transition state of the CD-mediated aminolysis (TS.CD) [equations (12), (17), or (18)] into the transition state of normal aminolysis (TS) and the cyclodextrin [eq. (11)], as in equation (19).

$$K_{TS} = \frac{[TS][CD]}{[TS.CD]} = \frac{k_N K_S}{k_{cN}} = \frac{k_N}{k_3} = \frac{k_N K_N}{k_{Nc}} \qquad (19)$$

The sensitivity of all of these kinetic parameters is dependent on the structures of the molecules that are involved: the aryl ester, the amine, and the cyclodextrin. Although numerous plausible reaction modes can be suggested for the transition state and the initial state, only through understanding variations in the kinetic parameters can the correct binding modes be deduced. In other words, how are these parameters dependent on the chain lengths of the ester group and the amine, the binding ability of the substrate, and on the cavity size

of the cyclodextrin?

For example, a recent study by our lab, investigated the effect of four CDs (α-CD, β-CD, hydroxypropyl-β-CD,[a] and γ-CD) on the aminolysis of *p*-nitrophenyl alkanoates (acetate to heptanoate) by primary amines (*n*-propyl to *n*-octyl, *iso*-butyl, *iso*-pentyl, cyclopentyl, cyclohexyl, and benzyl) in aqueous solution.[47] From previous studies,[41-43] these esters show moderate binding ability to the four CD molecules, due to their relatively hydrophobic nature. Nonetheless, these esters can bind in two ways depending on the acyl chain length of the ester group. In the case of amines, they are known to bind to CDs and this binding is dependent on the chain length of the amine, as well as the size of the CD cavity.[46-50] At the onset of this investigation, the potential binding modes of the transition state in CD-mediated aminolysis were thought to be **7** to **11** since they are all kinetically equivalent.



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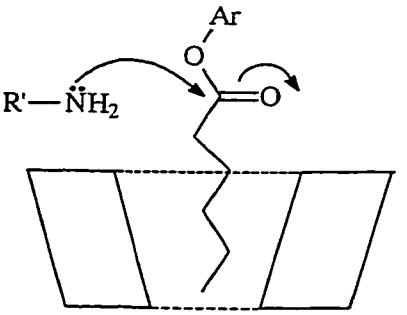
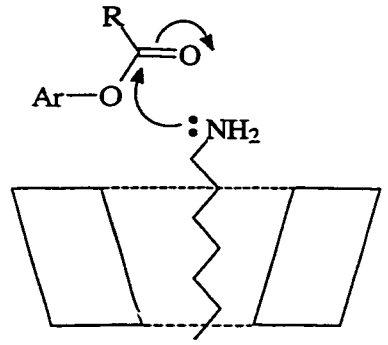


8

[a] Hydroxypropyl-β-CD (hp-β-CD) is a modified version of β-CD, in which six of the seven primary hydroxy groups are substituted with 2-hydroxypropyl groups. Therefore, the width of the cavity is similar to that of β-CD, but its depth may be varied.



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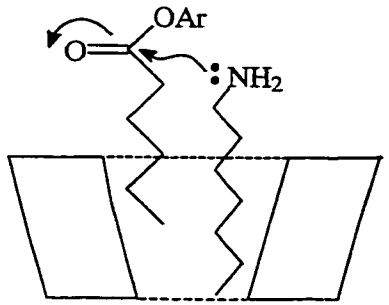

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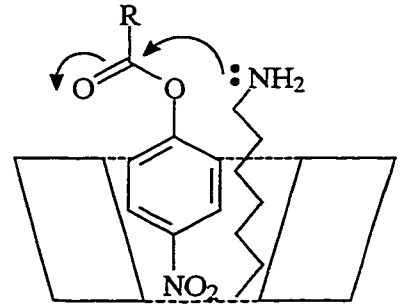


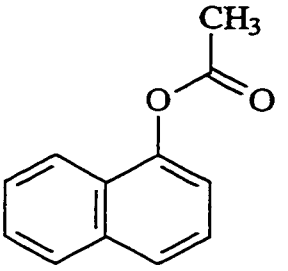
11

Analysis of the kinetic parameters is the only factor that can help distinguish between the varying modes and so, for the cleavage of *p*-nitrophenyl acetate (*p*-NPA) by *n*-alkylamines, the ratios k_{cN}/k_N and k_{Nc}/k_N were first analysed. The former ratio measures the relative reactivities of the CD-bound and free forms of the ester towards the amine, assuming that the CD-mediated reaction follows equation (11). The latter ratio measures the relative reactivities of the CD-bound and free forms of the amine towards the unbound ester, as in equation (18). The k_{cN}/k_N ratios were small and remained approximately constant for amines ranging from *n*-propyl to *n*-hexyl, but increased dramatically afterwards. This trend was viewed with all four cyclodextrins and as well, for the aminolysis of *p*-nitrophenyl hexanoate (*p*-NPH). Thus, this evidence clearly demonstrated that the short alkylamines react with CD-

bound ester (as in **7**) but upon reaching the longer amines, there is switch in binding mode to **9**, where it is the longer amines that are bound to the CD that react with the free ester. Concurrently, the trends for the k_{Nc}/k_N ratios display the exact opposite view where the ratios decrease for the short amines and are essentially constant for amines of longer chain lengths. Thus, this trend reflects the switch in binding mode from **7** to **9** as the amine chain lengthens.

The kinetic parameter, k_3, remains constant from *n*-propyl to *n*-hexylamine, upon which, it increases substantially by a factor of 10. This trend also gives a strong indication that there is a change in the mode of transition state binding from aryl group inclusion (**7**) for short amines, to alkylamino group inclusion for long amines (**9**). The other kinetic parameter, K_{TS}, is also in accordance with the switch in binding modes, whereby the large values initially for *n*-propyl to *n*-hexylamine indicate minimal binding of the amine to CD. Yet, upon reaching *n*-heptylamine, the apparent dissociation constant suddenly decreases complementing the view of a switch in binding to **9**, in which binding of the amine to the CD occurs. Interestingly, upon the completion of this study in which all the kinetic parameters were obtained, it was unequivocally determined that transition state binding did not occur between acyl inclusion of the ester substrate and the free nucleophile as in **8**.

Another investigation by Tee and Boyd[48] looked at the effects of the aminolysis of 1-naphthyl acetate (1-NA) and 2-naphthyl acetate (2-NA) in aqueous solution, in the presence of β-CD by primary amines (*n*-propyl to *n*-heptyl, cyclopentyl, and cyclohexyl). These two substrates as seen in **12** and **13**, respectively, are appropriate candidates for cyclodextrin binding due to their hydrophobic nature and it was shown earlier that they bind quite strongly to CDs.[49]



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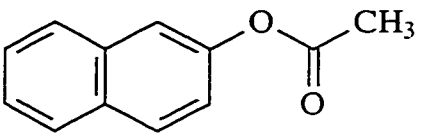


13

From the k_{cN}/k_N ratios, the values for all of the amines except two, were greater than unity with both substrates, demonstrating a significant increase in catalysis as the alkylamino chain increased. This suggests that the reaction must occur by ester binding [eq. (12)]or amine binding [eq. (18)]. However, for both the cyclic amines, the ratios were rather large suggesting a sensitivity of the amine binding to β-CD, which hindered the possibility of substrate binding. In the case of the reaction between the free ester and the CD-bound amine, the ratio, k_{Nc}/k_N, for all amines was greater than one and these values only modestly increased with increasing alkylamino chain length, irrespective of the substrate isomer used. This tell-tale sign suggested that the reaction proceeded as in equation (18) where transition state binding involves inclusion of the alkylamino group (**9**).

From the third-order rate constant, k_3, it appeared that for both substrates reacting with the primary *n*-alkylamines in the presence of β-CD, there is an appreciable increase with increasing alkylamino chain length. For the cyclic amines, these values are also comparatively large and are thus consistent with binding of the alkylamino group in the transition state, as in **9**.

The apparent transition state dissociation constants, K_{TS}, for both 1-NA and 2-NA decrease with increasing chain length, indicating that as the alkylamino group increases, the

strength of transition state binding also increases, consistent with the alkylamino group being located in the cyclodextrin cavity (**9**). However, there is some ester interaction as well because K_{TS} values for naphthyl acetates are lower than those for *p*-NPH and *p*-NPA for any given amine.[48]

1.7 Objective

In both of the two aminolysis studies[47,48] just discussed, the esters used were hydrophobic in nature, and could therefore participate in binding to the cyclodextrin. One can then predict what would happen to the same reaction if an ester with weak binding abilities was used. In 1967, VanEtten et al.[35] looked at the basic cleavage of such a substrate: the *p*-carboxyphenyl acetate anion, or as IUPAC and our lab refer to it as, 4-acetoxybenzoate anion, (4-ABA), as seen in **14**. This substrate is not expected to bind to CDs since in basic media it is hydrophilic due to the carboxylate anion that it carries and also because of its short acyl chain.

14 **15**

From their study of the cleavage of 4-ABA by α-CD, in a carbonate buffer at pH 10.60, VanEtten et al. demonstrated that the ester binds only weakly to the CD cavity (K_S = 150 ± 90 mM), and displays minimal catalysis.

Two previous studies dealing with *p*-NPA and 1-NA demonstrated transition state binding modes involving the amines, but they were complicated by ester substrate binding.

In order to completely understand how amines interact specifically with CDs, further investigation was required in which ester binding to the CD was not significant. From the results obtained by VanEtten, the ester anion, 4-ABA, appeared to be a suitable candidate to further understand the characteristics of amine binding in the transition state. Since this ester has demonstrated minimal participation in substrate binding, it is presumed that the CD-mediated aminolysis of 4-ABA by primary amines can be further investigated as to the transition state binding modes. As well, by varying the size of the CD cavity, a greater understanding can be gained as to what effects amine binding in the transition state, if anything does, and as well, what are the limits to the amine's binding capacity. Thus, the kinetics of the aminolysis of 4-ABA by primary amines (*n*-propyl to *n*-octyl, *iso*-butyl, *iso*-pentyl, cyclopentyl, and cyclohexyl) in basic aqueous solution and in the presence of α-, β-, and γ-CD, were investigated, following an initial study of ester cleavage in basic solution to probe the binding of 4-ABA to CDs.

2. RESULTS AND DISCUSSION

2.1 Cleavage of 4-ABA in the presence of Cyclodextrins: Comparison to Other Substrates

The kinetics for the basic cleavage of 4-ABA in the presence of α-, β-, and γ-CD were measured, utilizing a stopped-flow spectrophotometer. The pseudo first-order rate constants k_{obs}, were collected over a range of CD concentrations by following the appearance of the 4-carboxyphenoxide dianion (**15**) at 278 nm. The raw kinetic data obtained, are collected in Appendix II.

As seen in Figure 2.1, 4-ABA with α-CD displays saturation-type kinetics and hence, equations (8a) and (8b) can be applied to afford the relationship between k_{obs} and [CD] as described in equation (8c). This equation and the measured k_{obs} values were used to obtain K_S and k_c by non-linear least squares analysis. From these kinetic parameters given in Table 2.1, the curved graph in Figure 2.1 was calculated and plotted. By contrast, 4-ABA with both β- and γ-CD showed linear plots, as displayed in Figure 2.1. This linearity is indicative of very weak or no binding at all between the ester substrate and the CD ($K_S >> [CD]$). Therefore, the linear equation (9) was fitted to the data, where k_u is the y-intercept, and k_2 is the slope. These kinetic parameters, along with K_{TS} values derived from equation (5) are also presented in Table 2.1.

For comparative purposes, the cleavage of 4-ABA with TFE was also investigated. Since the pK_a of TFE is 12.4[51] which is close to that of CDs (12.2, 12.3),[52] a comparison can be made between the nucleophilicities of the CD anions and the trifluoroethoxide anion.[49] The results for this reaction appeared to follow those of β- and γ-CD where, as Table 2.1 and

Figure 2.1 demonstrate, no saturation kinetics was obtained and so, no binding was established between the substrate and the anion.

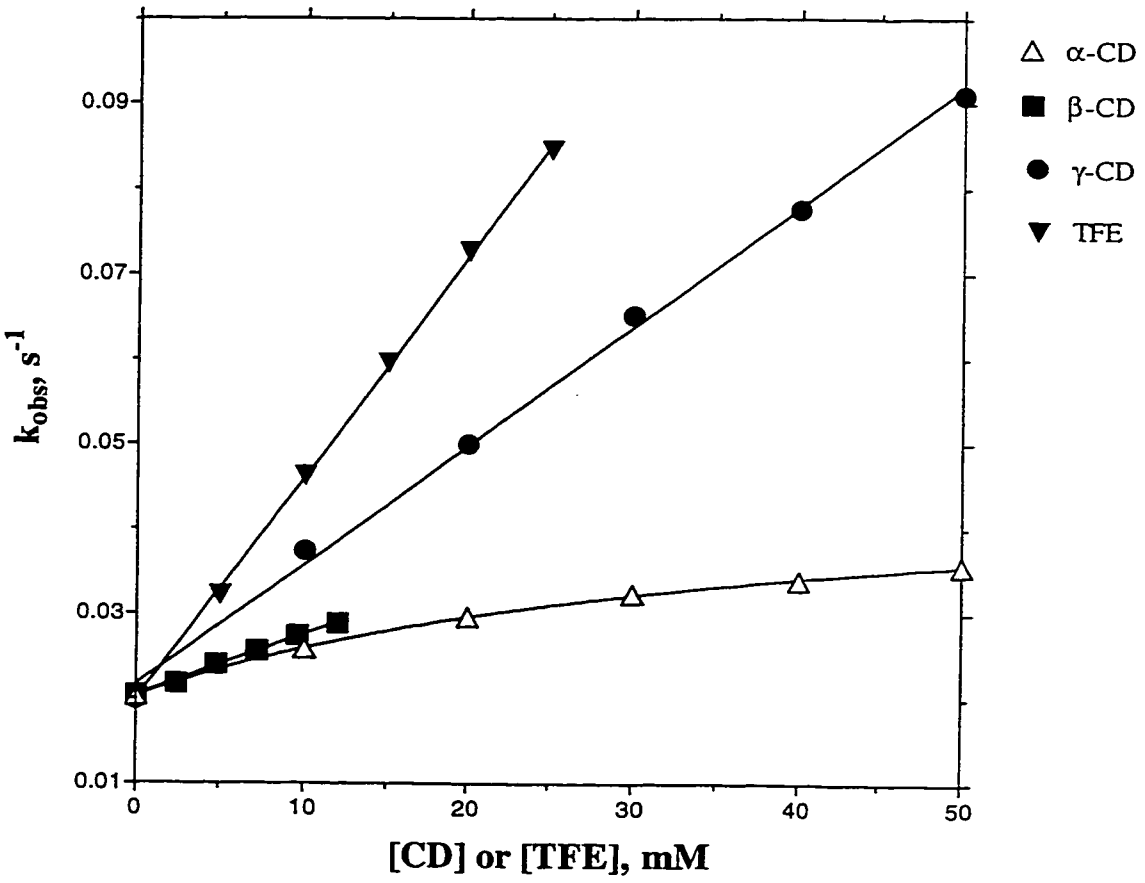


Figure 2.1: Plot of k_{obs} versus [CD] or [TFE] for the Cleavage of 4-ABA by CDs or TFE

Table 2.1: Constants for the Cleavage of 4-ABA by Cyclodextrins and TFE[a]

	k_u (s^{-1})	K_S (mM)	k_c (s^{-1})	k_c/k_u	k_2 ($M^{-1}\ s^{-1}$)	K_{TS} (mM)
α-CD	0.0203	38.2 ± 1.9	0.0473 ± 0.0007	2.53	1.24	16.4
β-CD	0.0204	[b]			0.721 ± 0.023	28.3
γ-CD	0.0198	[b]			1.40 ± 0.04	14.1
TFE	0.0203	[b]			2.60 ± 0.03	7.78

[a] At 25 ± 0.1 °C, in 0.2 M phosphate buffer at pH 11.60
[b] Saturation kinetics not observed

As can be seen from the values in Table 2.1, α-CD is the only CD that is able to bind, albeit weakly, to the anionic, hydrophilic ester substrate, with a dissociation constant, K_S, of moderate value and a maximum acceleration, k_c/k_u, value larger than 1. From the linear plots in Figure 2.1, both β- and γ-CD demonstrate no binding to 4-ABA and therefore no K_S or k_c values are ascertained. These results are in accordance with the relative sizes of the CD cavities. Since there is weak binding between the ester and α-CD, but no binding at all for β- and γ-CD, this demonstrates that 4-ABA must be bound very loosely to α-CD to even allow some sort of binding to occur. However, as the CD cavity size increases with β- and γ-CD, 4-ABA's small size, short acyl group, and unattractive anionic nature disallows appropriate binding to these latter CDs. Hence, no ester binding occurs for these CDs. Moreover, the K_{TS} values are all relatively large, indicative of weak binding in the transition state.

In comparison, for the reaction of 4-ABA with TFE, a linear plot is also displayed identical to that of both β- and γ-CD. This similarity essentially demonstrates that the anions

of the two CDs have similar nucleophilicity towards 4-ABA as an oxyanion of the same basicity, but the k_2 values are smaller for the CDs probably for steric reasons.

To further appreciate the results obtained with 4-ABA, a comparison can be made to other substrates that bind more tightly, such as *p*-NPA and 1-NA. The results for the cleavage of *p*-NPA with α- and β-CD[39], and γ-CD[41] can be viewed in Table 2.2 while those for the cleavage of 1-NA with the same CDs[48] can be viewed in Table 2.3.

Table 2.2: Constants for the Cleavage of *p*-NPA by Cyclodextrins[39-41]

CD	k_u (s^{-1})	k_c (s^{-1})	k_c/k_u	K_S (mM)	k_2 ($M^{-1}\,s^{-1}$)	K_{TS} (mM)
α-CD[c]	0.096	0.27	2.8	10	27	3.6
β-CD[c]	0.096	0.78	8.1	7.8	100	0.96
γ-CD[a]	0.104	1.61	15	35.6	45	2.4

[a] At 25 ± 0.1 °C, in 0.2 M phosphate buffer at pH 11.60
[c] At 25 ± 0.1 °C, in 0.2 M phosphate buffer at pH 11.70

Table 2.3: Constants for the Cleavage of 1-NA by Cyclodextrins[a,48]

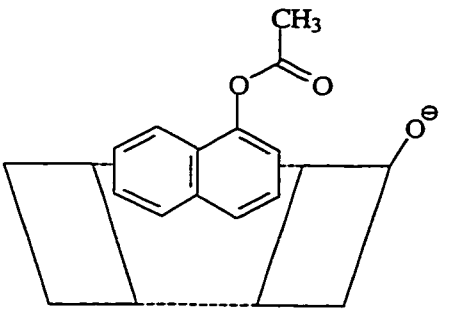
CD	k_u (s^{-1})	k_c (s^{-1})	k_c/k_u	K_S (mM)	k_2 ($M^{-1}\,s^{-1}$)	K_{TS} (mM)
α-CD	0.015	0.44	30	39	12	1.3
β-CD	0.016	0.503	31	6.3	80	0.20
γ-CD	0.014	0.122	8.8	10.7	11	1.2

[a] At 25 ± 0.1 °C, in 0.2 phosphate buffer at pH 11.60

For both *p*-NPA and 1-NA reacting with all three CDs, saturation kinetics was displayed, and so in all of these cases, ester substrate binding occurred (as seen from the relatively low K_S values). These results were expected for these substrates, due to their hydrophobic nature, in comparison to 4-ABA's hydrophilic, anionic nature.

Some notable trends relating to the substrate binding constant, K_S, that should be mentioned are as follows. The K_S value for the binding of *p*-NPA to γ-CD is relatively large, exhibiting weak substrate binding, while the values for α- and β-CD are both small, demonstrating rather modest binding to *p*-NPA. This situation arises due to γ-CD's larger cavity size in comparison to those of α- and β-CD. *p*-NPA must be held very loosely by γ-CD, and so the K_S value of 35.6 mM is obtained for this CD. In the case of 1-NA, the weakest substrate binding is seen with α-CD with a K_S value of 39 mM, while the tightest binding is seen with β-CD because its larger cavity is more able to accommodate 1-NA. A plausible explanation for this observation comes from the fact that 1-NA appears to bind to β-CD in an 'equatorial' manner as viewed in **16**. However, in order to react with the ionized hydroxy group of the CD, the substrate must come out of the cavity and position itself in more of an 'axial' position, as seen in **17**. By comparison, α-CD's cavity is smaller than that of β-CD's, and so most likely 1-NA is bound to α-CD in a perched, 'axial' position, as seen in **17**. This mode of binding may explain the relatively large K_S value found with α-CD.

The ester anion, 4-ABA, is able to bind to α-CD, albeit weakly (Table 2.1). However, with β- and γ-CD, the binding of 4-ABA is weaker (or non-existent), presumably due to the larger sizes of these two CDs.



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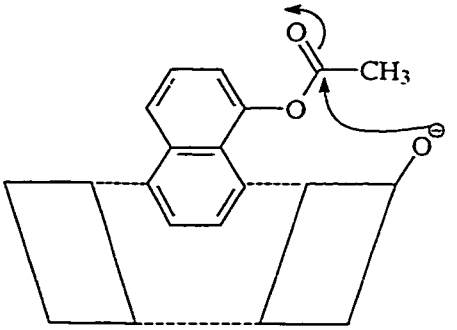


17

The maximal rate accelerations, k_c/k_u, for *p*-NPA and 1-NA with their respective CDs, are all greater than 1 indicative of acceleration. Interestingly, for the cleavage of *p*-NPA, a trend is viewed in which k_c/k_u increases with increasing cavity size while the cleavage of 1-NA is modestly accelerated by both α- and β-CD. In comparison, 4-ABA binds only to α-CD, and consequently, no k_c nor k_c/k_u values are obtained for both β- and γ-CD. However, 4-ABA with α-CD demonstrates acceleration, although it is minimal.

From the low values of the dissociation constant, K_{TS}, transition state binding appears to be quite strong for both *p*-NPA and 1-NA by all CDs. In the case of 4-ABA, transition state binding is much weaker as demonstrated from the higher K_{TS} values. Moreover, of the three CDs, β-CD affords the strongest transition state binding for *p*-NPA and 1-NA while this CD displays the weakest binding to 4-ABA. The strong binding between β-CD and *p*-NPA and 1-NA demonstrate that their aryl rings fit in the cavity very well, as opposed to that of 4-ABA. In comparison, because the K_{TS} values for the cleavage of 4-ABA by CDs are all very large, this is a good indication that in the transition state, there is no binding occurring between 4-ABA and any of the CDs.

2.2 Aminolysis of 4-ABA

In order to understand how CDs effect the aminolysis of 4-ABA, two sets of experiments were performed. The first set investigated the kinetics of aminolysis by primary amines in the absence of CDs while the second set looked at the same reaction in the presence of CDs. The raw kinetic data for both of these sets appears in Appendix II. For experiments with no CD present, analysis of the results in terms of $k_{obs} = k_u + k_N$ [Amine] (eq. (15)) was carried out. A plot of k_{obs} versus [Amine] as seen in Figure 2.2, gives a linear slope equal to k_N, which measures the reactivity of 4-ABA towards the amine nucleophile. All of these plots demonstrate good linearity in which the correlation coefficient, r, is ≥ 0.998. The values of k_N are displayed in Table 2.4, along with the dissociation constants (K_N) for the amine.CD complexes which were determined previously.[42,47]

From both the graph and the table, it is seen that the k_N values do not vary greatly. For the *n*-alkylamines, there is little variation, however, for the cyclic amines, the k_N values are lower indicating less reactivity. This latter trend is expected due to their bulky cyclic group nearby the reacting amino group.[53] In the case of the branched amines, *iso*-pentylamine appears to have approximately the same reactivity towards 4-ABA as the other *n*-alkylamines because the branching is located sufficiently away from the amino group so as not to decrease the reactivity. With respect to *iso*-butylamine, there is a marked decrease in reactivity towards 4-ABA, possibly due to the closeness of the branch to the nucleophilic amino group.

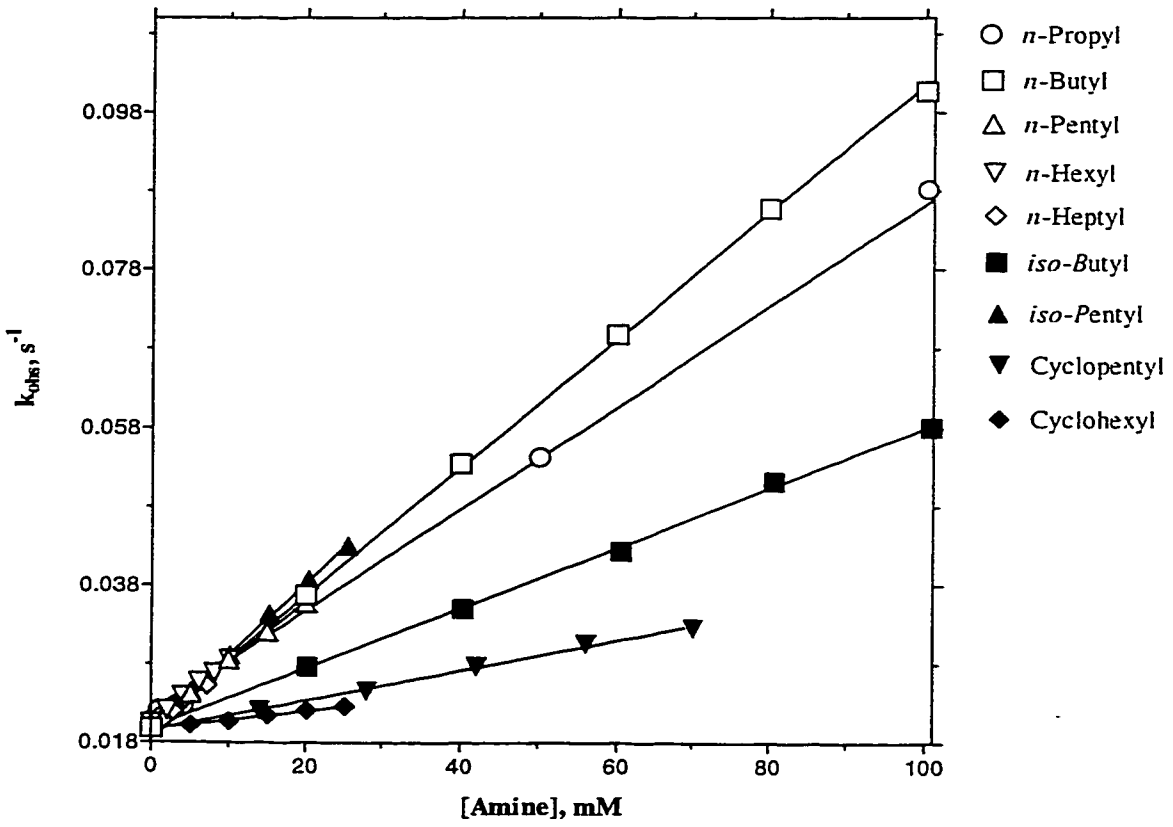


Figure 2.2: Plot of k_{obs} versus [Amine] for the Aminolysis of 4-ABA in the Absence of CD

To understand the criteria for binding of amines to the CDs, the dissociation constants (K_N, Table 2.4) must be examined. These values have been shown to be dependent on the hydrophobicity and size of the bound guests.[42,47] For the *n*-alkyl amines, both of these properties increase linearly with the number of carbons (N) in the alkyl chain and so a plot of pK_N (= -log K_N) as a function of the chain length (N) yields a straight line for each CD.[42]

Table 2.4: Rate Constants for the Reaction of Alkylamines with 4-ABA and Dissociation Constants of Amine.CD Complexes[a]

Amine	k_N (M^{-1} s^{-1})	K_N, (mM)		
		α-CD	β-CD	γ-CD
n-Propyl	0.643 ± 0.007	46.6	108	149
n-Butyl	0.811 ± 0.008	13.3	35.6	65.6
n-Pentyl	0.779 ± 0.018	3.08	11.4	19.5
n-Hexyl	0.798 ± 0.023	1.6	2.62	9.29
n-Heptyl	0.823 ± 0.038	0.431	0.955	2.84
n-Octyl	0.823[b]	0.134[c]	0.273[c]	1.12[c]
iso-Butyl	0.381 ± 0.005		16.2	
iso-Pentyl	0.915 ± 0.014		5.81	
Cyclopentyl	0.191 ± 0.005		13.5	
Cyclohexyl	0.116 ± 0.006		1.83	

[a] At 25 ± 0.1 °C at pH 11.60. At this pH, the amines are at least 90% in their free base forms. k_u = 0.0200 ± 0.0002 s^{-1}. K_N values taken from Refs. 42, 47.
[b] Difficult to measure accurately, and so assumed to be the same as for *n*-heptylamine.
[c] Obtained by extrapolation of the linear relationship between $pK_N = -\log K_N$ and chain length (Refs. 42, 47).

This plot can be treated as a linear free energy relationship (LFER) in which the slopes represent a dependence of amine binding to structural changes. This can be taken to mean that one can vary the chain length in order to probe alkylamine inclusion for aminolysis in the transition state.[47,48] Moreover, from the values of K_N in Table 2.4, it can be seen that the alkylamines fit quite tightly in α-CD, less tightly in β-CD, and even less so in γ-CD, as is appropriate to the CD sizes.[42,47] As well, from the linear variation of pK_N with N, the dissociation constants for *n*-octylamine can be estimated via extrapolation of the line for all three CDs. This estimation was necessary because of the low solubility of *n*-octylamine, and

its complexes, in water.

2.3 Aminolysis of 4-ABA in the presence of CDs

The second set of experiments, referred to above, involves the aminolysis of 4-ABA by primary amines in the presence of CDs. Although there are several ways of looking at this reaction, the best way of understanding it, is to utilize the rate constant, k_3 from equation (17), which is a third order process that gives an unbiased view of the CD-mediated reaction. As previously stated, k_3, can be looked at from two different points of view. Since this rate constant is equal to k_{cN}/K_S (from eq. (12)) as well as k_{Nc}/K_N (from eq. (18)), any variation in k_3 with respect to the structures of the reactants (substrate, CD, and amine) can provide information on the possible structures of the transition states.[30]

Previous studies have made use of equation (14) in order to obtain kinetic parameters such as k_c/K_S, k_N, and k_{cN}/K_S, from which a value for the rate constant, k_{cN} was extracted.[47]

$$\frac{k_{obs}}{f_s} = k_u + k_N[Nuc] + \frac{k_c[CD]}{K_S} + \frac{k_{cN}[Nuc][CD]}{K_S} \qquad (= 14)$$

However, due to a possible disparity between the contributions of the two aminolysis terms (equations (11) and (12)) with respect to k_{obs}, equation (14) was adapted so as to circumvent this problem. The four terms in equation (14) can be separated in two ways depending on how strongly the substrate binds to the CD.

2.3.1 Aminolysis of 4-ABA in the presence of α-CD

If the substrate does bind significantly, then separation of the four terms of equation

(14) is made possible by first subtracting the first two terms, since both k_u and k_N can be obtained independently from the reaction involving the ester with only the nucleophile (eq. (15)). The processes involving the CD (equations (8b) and (12)) are thus isolated. transforming equation (14) to (20) where k_{corr} varies linearly with [Nuc].

$$k_{corr} = \frac{(k_{obs}/f_s - k_u - k_N[Nuc])\, K_S}{[CD]}$$

$$= k_c + k_{cN}[Nuc] \qquad (20)$$

From the study of basic ester cleavage by CDs (Section 2.1), there is appreciable binding of the substrate to α-CD and therefore, $f_s < 1$, unlike the case with β-CD and γ-CD. By plotting k_{corr} versus varying [Amine], at constant [CD] in accord with equation (20). a linear slope can be obtained which estimates k_{cN}, while the y-intercept is simply k_c. Examples of such plots can be seen in Figure 2.3 which involves the aminolysis of 4-ABA by *n*-alkylamines in the presence of α-CD.

As noticed in Figure 2.3, the k_c values do not deviate much, nor should they, although from the slopes, there is a marked increase in the k_{cN} values as the alkylamino chain lengthens. Correspondingly, the third order rate constant, k_3, (= k_{cN}/K_S) also increases appreciably because K_S is constant. These results strongly imply that for α-CD, the third order reaction occurs by alkylamino group inclusion in the CD, and with the ester substrate located outside the CD cavity, as in **18** → **19** (overleaf).

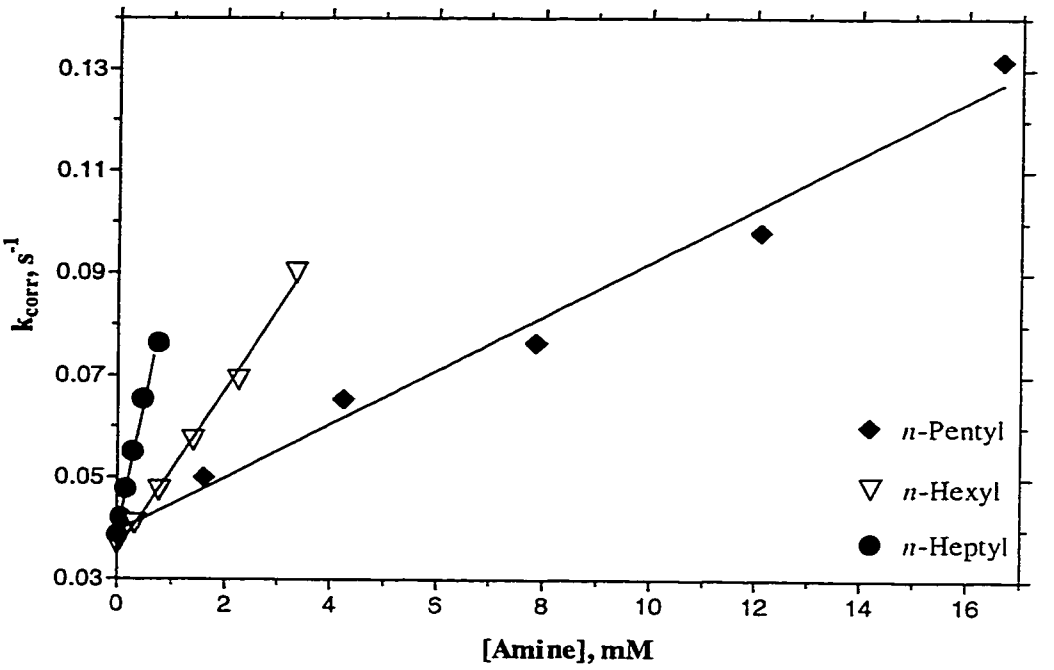
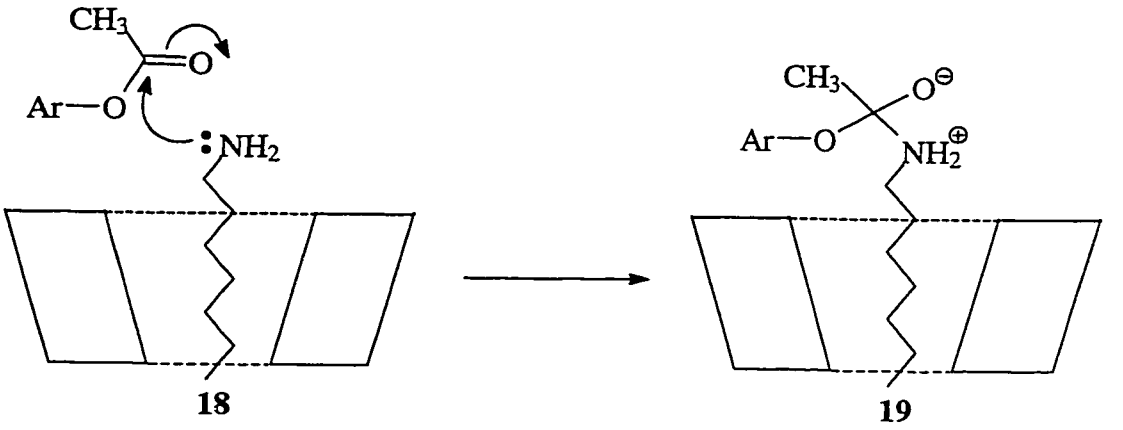


Figure 2.3: Plots of k_{corr} versus [Amine] for the Aminolysis of 4-ABA in the Presence of α-CD. The [Amine] has been corrected for binding of the amine to the CD and the plots are according to eq. (20).



Two other kinetic parameters that aid in differentiating between binding modes, are the reactivity ratios, k_{cN}/k_N and k_{Nc}/k_N. As previously stated, the ratio k_{cN}/k_N measures the relative reactivities of the CD-bound and free forms of the ester substrate towards the nucleophile assuming that the CD-mediated reaction involves the bound ester reacting with

the free nucleophile as in equation (12). The other ratio k_{Nc}/k_N measures the relative reactivities of the bound and free forms of the amine nucleophile, towards the substrate assuming that the CD-mediated reaction involves the bound amine reacting with the free ester, as in equation (18).

Table 2.5: Kinetic Parameters for the Aminolysis of 4-ABA by Alkylamines in the Presence of α-CD

Amine	k_{cN} (M^{-1} s^{-1})	k_{cN}/k_N	k_3 (M^{-2} s^{-1})
n-Butyl	1.93 ± 0.18	2.38	50.5
n-Pentyl	5.26 ± 0.32	6.75	138
n-Hexyl	15.8 ± 0.5	19.8	414
n-Heptyl	51.6 ± 2.4	62.6	1350
n-Octyl	178 ± 40	216	4650

In Table 2.5, the k_{cN} values for the aminolysis of 4-ABA by alkylamines in the presence of α-CD are displayed, along with the k_{cN}/k_N ratios and k_3 values. The k_{cN}/k_N ratios are all greater than one, and as the alkylamino chain lengthens, there is a dramatic increase in their magnitude. This trend displays a dependency of amine chain length, suggesting that these amines are bound to the CD in the transition state. Verification of this suggestion can be obtained by observing the ratio, k_{Nc}/k_N which will be discussed next.

Table 2.6: Kinetic Parameters for the Aminolysis of 4-ABA in the Presence of α-CD[a,b]

Amine	k_3 (M^{-2} s^{-1})	k_{Nc} (M^{-1} s^{-1})	k_{Nc}/k_N	K_{TS} (mM)
n-Butyl	50.5 ± 2.2	0.673 ± 0.030	0.83	16.1
n-Pentyl	138 ± 2	0.424 ± 0.005	0.54	5.66
n-Hexyl	414 ± 7	0.662 ± 0.011	0.83	1.93
n-Heptyl	1350 ± 5	0.582 ± 0.002	0.71	0.61
n-Octyl	4650 ± 815	0.623 ± 0.109	0.76	0.177

[a] As described in the text, values of k_3 were determined from the analysis of the variation of k_{obs} with [amine]. for reaction in the presence of 10 mM of the CD, in a 0.2 M phosphate buffer at pH 11.60, and at 25 °C.
[b] Experiments with *n*-propylamine, and to a lesser extent with *n*-butylamine, were unsuccessful because the contribution from CD-mediated aminolysis is too small.

Table 2.6 contains other kinetic parameters relating to the aminolysis reactions mediated by α-CD, namely, k_3, k_{Nc}, and the dissociation constant, K_{TS}. As seen there, the values of the ratio k_{Nc}/k_N are very small and are approximately constant, as the alkylamino chain increases. These results are a good indication that the CD-mediated reaction proceeds as shown in **18** → **19**, with the amine bound to the CD reacting with the free ester substrate. As well, with respect to catalysis, because the values of the k_{Nc}/k_N ratio are all *less than one*, it is understood that α-CD decelerates the aminolysis of 4-ABA by primary amines, rather than catalysing it. This is not unexpected since it is believed that α-CD holds the alkylamino group rigidly, and perhaps too rigidly to allow aminolysis to take place with ease.[47]

In order to understand transition state binding further, the dissociation constants (K_{TS}) can also be analysed. For reaction in the presence of α-CD, the K_{TS} values in Table 2.6, all decrease with increasing amine chain length, which means that as the chain of the amine is lengthened, transition state binding of the alkylamino chain to this CD becomes

progressively stronger.

2.3.2 Aminolysis of 4-ABA in the presence of β-CD

In the case where the ester substrate binds minimally to the CD, $K_S >> [CD]$, and so $f_s = K_S / (K_S + [CD])$ which is equal to 1. This situation allows equation (14) to be transformed into equation (21), where $k_2 = k_c/K_S$ (Section 1.4) and $k_3 = k_{cN}/K_S$ (Section 1.6). For the purposes of analysis, equation (21) is rearranged to form equation (22), where k_{corr} also varies linearly with [Nuc].

$$k_{obs} = k_u + k_N[Nuc] + k_2[CD] + k_3[Nuc][CD] \qquad (21)$$

$$k_{corr} = \frac{(k_{obs} - k_u - k_N[Nuc])}{[CD]}$$

$$= k_2 + k_3[Nuc] \qquad (22)$$

From Section 2.1, it was shown that 4-ABA does not bind to β-CD and so, equation (22) must be used to determine the necessary kinetic parameters. Examples of plots based on equation (22) are seen in Figures 2.4 and 2.5 for the aminolysis of 4-ABA by alkylamines. in the presence of β-CD. Later on, in Figure 2.6, similar plots are given for aminolysis in the presence of γ-CD.

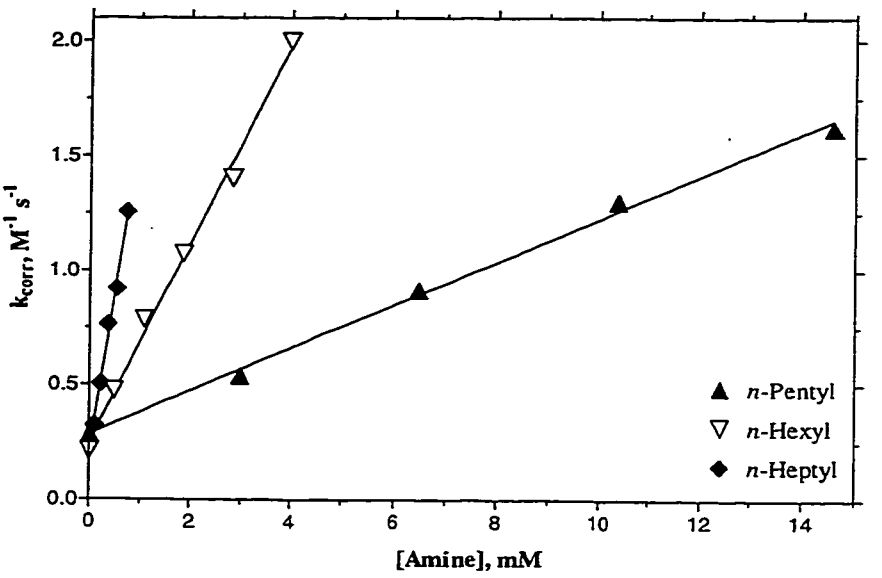


Figure 2.4: Plots of k_{corr} versus [Amine] for the Aminolysis of 4-ABA by *n*-alkylamines in the Presence of β-CD. The [Amine] has been corrected for binding of the amine to the [CD] and the plots are according to eq. (22).

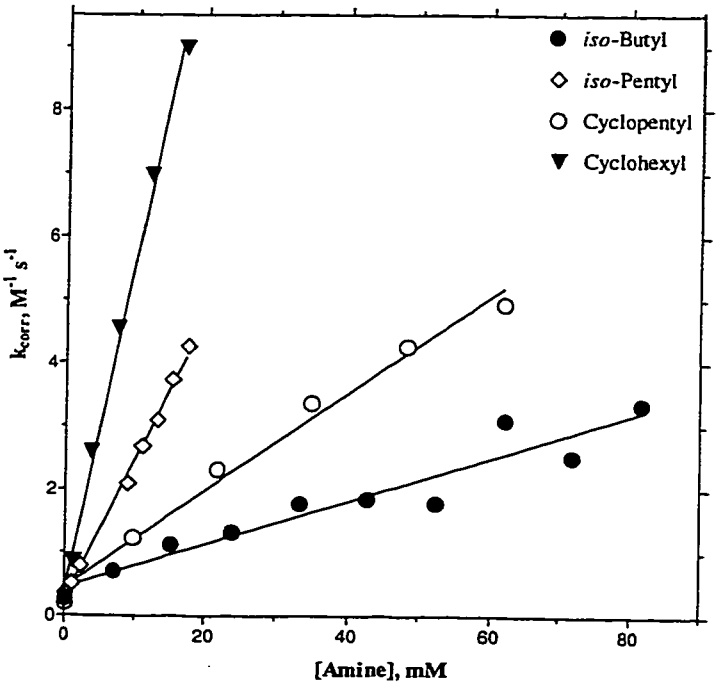


Figure 2.5: Plots of k_{corr} versus [Amine] for the Aminolysis of 4-ABA by non-linear amines in the Presence of β-CD. The [Amine] has been corrected for binding of the amine to the [CD] and the plots are according to eq. (22).

As noticed with Figures 2.4 and 2.5, the slopes of the graphs (equalling k_3) steadily increase as the amine chain lengthens. These consistent trends again provide strong evidence that the alkylamino group is located in the β-CD cavity and that the ester substrate is located outside the CD, again as in structures **18** → **19**.

Utilizing the rate constant, k_3, the equation $k_{Nc} = k_3 \cdot K_N$ can be used to determine the rate constant, k_{Nc}, which measures the rate of reaction between the Amine.CD complex with the free ester substrate. Values of k_3 and k_{Nc} for β-CD are collected in Table 2.7, along with the values for the k_{Nc}/k_N ratio and dissociation constant, K_{TS}.

Table 2.7: Kinetic Parameters for the Aminolysis of 4-ABA in the Presence of β-CD[a,b]

Amine	k_3 (M^{-2} s^{-1})	k_{Nc} (M^{-1} s^{-1})	k_{Nc}/k_N	K_{TS} (mM)
n-Butyl	-[b]			
n-Pentyl	93.9 ± 3.0	1.07 ± 0.03	1.37	8.3
n-Hexyl	432 ± 16	1.13 ± 0.04	1.42	1.85
n-Heptyl	1430 ± 60	1.36 ± 0.06	1.66	0.577
n-Octyl	-[c]			
iso-Butyl	33.9 ± 3.6	0.549 ± 0.059	1.44	11.2
iso-Pentyl	232 ± 14	1.35 ± 0.08	1.47	3.95
Cyclopentyl	76.8 ± 4.6	1.04 ± 0.06	5.4	2.49
Cyclohexyl	532 ± 20	0.974 ± 0.037	8.4	0.219

[a] As described in the text, values of k_3 were determined from the analysis of the variation of k_{obs} with [amine], for reaction in the presence of 10 mM of the CD, in a 0.2 M phosphate buffer at pH 11.60, and at 25 °C.
[b] Experiments with *n*-propylamine, and to a lesser extent with *n*-butylamine, were unsuccessful because the contribution from CD-mediated aminolysis is too small to be determined accurately.
[c] CD-mediated aminolysis was barely detectable at the amine concentrations accessible.

For the aminolysis of 4-ABA by *n*-alkylamines in the presence of β-CD, the k_{Nc}/k_N ratios are all slightly above one which indicates some catalysis by the CD. Also, the values remain constant, as the amine chain length increases, which reflects the mode of binding stated earlier: regardless of the alkylamino chain length, the amine will bind to the CD and react with the free ester substrate (**18**). For the branched amines, both *iso*-butyl and *iso*-pentyl appear to follow the same trend as the *n*-alkylamines in which the k_{Nc}/k_N values are constant and collectively, the *n*-alkyl and *iso*-alkylamines have an average ratio of 1.47 ± 0.11 indicating catalysis and the same mode of binding. For the cyclic amines, the k_{Nc}/k_N values are 5 to 8 fold larger than the other amines, suggesting that catalysis is much greater and transition state binding is much stronger to β-CD, as is reflected in the K_{TS} values.

In the presence of β-CD, the K_{TS} values all decrease with increasing chain length. For the branched amines, *iso*-butylamine binds approximately as strongly to the CD in the transition state as *n*-pentylamine, while *iso*-pentylamine binds as strongly as *n*-hexylamine. For the cyclic amines, these amines tend to bind more strongly to β-CD in comparison to *n*-pentylamine which is understandable since the bulkiness of the amine must influence the stability of the transition state structure. Comparison of K_{TS} (and pK_{TS}) values for the different CDs is deferred to Section 2.3.4.

2.3.3 Aminolysis of 4-ABA in the presence of γ-CD

In Section 2.1, it was determined that 4-ABA does not bind to γ-CD, and so, as with β-CD, equation (22) must be applied to determine the desired kinetic parameters. Examples of appropriate plots are seen in Figure 2.6.

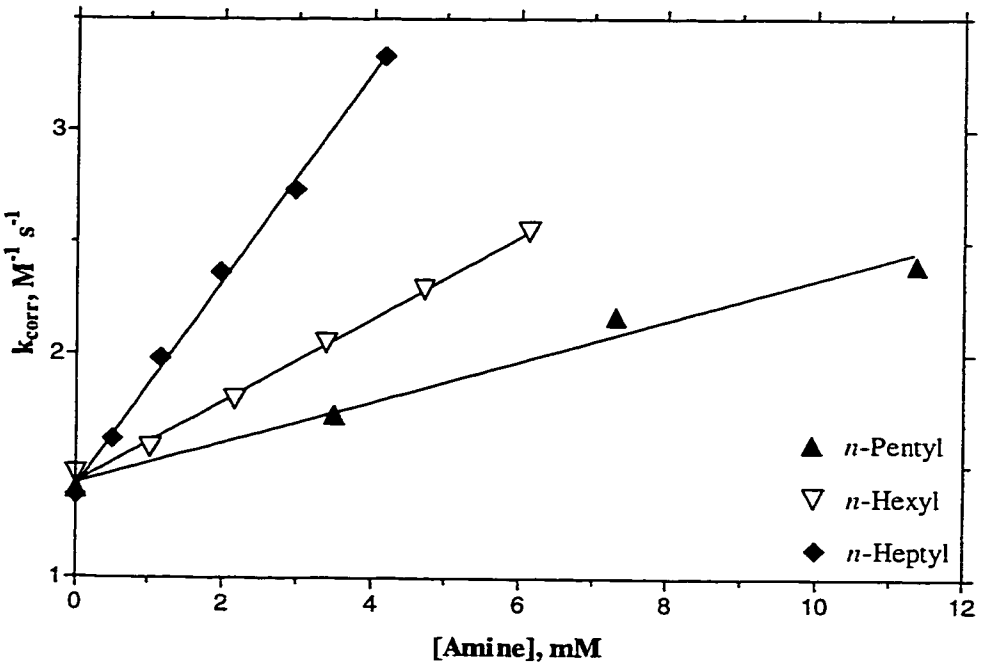


Figure 2.6: Plot of k_{corr} versus [Amine] for the Aminolysis of 4-ABA by *n*-alkylamines in the Presence of γ-CD. The [Amine] has been corrected for binding of the amine to the [CD] and the plots are according to eq. (22).

As seen earlier in Figures 2.4 and 2.5 for β-CD, Figure 2.6 for γ-CD shows that the slopes of the graphs (equalling k_3) steadily increase as the amine chain lengthens. These consistent trends yet again provide strong evidence that the alkylamino group is located in the cavity of γ-CD as in structures **18** → **19**.

Utilizing the third order rate constant, k_3, the Amine.CD rate constant, k_{Nc} can be determined as seen in Table 2.8. From these values, the ratio, k_{Nc}/k_N which measures the reactivity of the bound and free forms of the amine towards the substrate, can then be derived as can the dissociation constant, K_{TS}, [eq. (19)].

Table 2.8: Kinetic Parameters for the Aminolysis of 4-ABA in the Presence of γ-CD[a,b]

Amine	k_3 (M^{-2} s^{-1})	k_{Nc} (M^{-1} s^{-1})	k_{Nc}/k_N	K_{TS} (mM)
n-Butyl	-[b]			
n-Pentyl	91.1 ± 8.7	1.78 ± 0.17	2.28	8.56
n-Hexyl	183 ± 5	1.70 ± 0.05	2.14	4.35
n-Heptyl	466 ± 13	1.32 ± 0.04	1.61	1.77
n-Octyl	1670 ± 60	1.87 ± 0.06	2.27	0.494

[a] As described in the text, values of k_3 were determined from the analysis of the variation of k_{obs} with [amine]. for reaction in the presence of 10 mM of the CD, in a 0.2 M phosphate buffer at pH 11.60, and at 25 °C.
[b] Experiments with *n*-propylamine, and to a lesser extent with *n*-butylamine, were unsuccessful because the contribution from CD-mediated aminolysis is too small.

For the aminolysis of 4-ABA by *n*-alkylamines in the presence of γ-CD, the values of the reactivity ratio, k_{Nc}/k_N, are larger than those of β-CD by approximately a factor of 2, indicating that as the CD cavity increases in size, catalysis becomes even stronger. These values, however, remain constant with increasing amine chain length demonstrating that the amine will be bound to the CD, regardless of the number of carbons, N.

For transition state binding in the presence of γ-CD, the K_{TS} values also decrease with increasing amine chain length. This strongly suggests that tighter binding in the transition state occurs as the alkylamino group increases in size.

2.3.4 Aminolysis of 4-ABA: Comparison between α-, β-, and γ-CD

Further appreciation for the results involving the three CDs can be afforded by viewing the effects that the CDs have on one particular amine. As examples, plots are presented in Figures 2.7 to 2.9 for the aminolysis of 4-ABA by *n*-pentylamine, *n*-hexylamine

and *n*-heptylamine in the absence and presence of 10 mM of each of the three CDs, where k_{obs} versus $[Amine]_o$ displays how CDs effect the rate of ester cleavage.

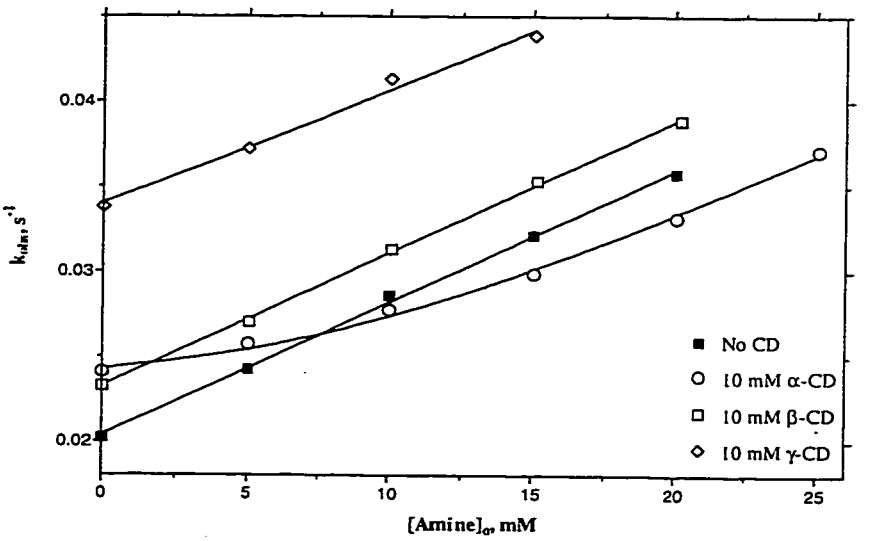


Figure 2.7: Plots of k_{obs} versus $[Amine]_o$ for the Aminolysis of 4-ABA by *n*-Pentylamine in the Absence and in the Presence of α-, β-, and γ-CD

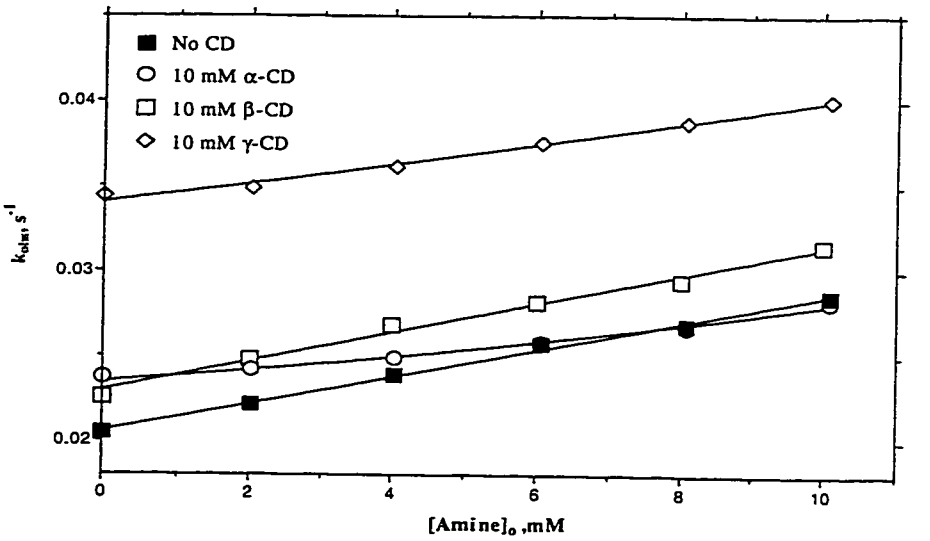


Figure 2.8: Plots of k_{obs} versus $[Amine]_o$ for the Aminolysis of 4-ABA by *n*-Hexylamine in the Absence and in the Presence of α-, β-, and γ-CD

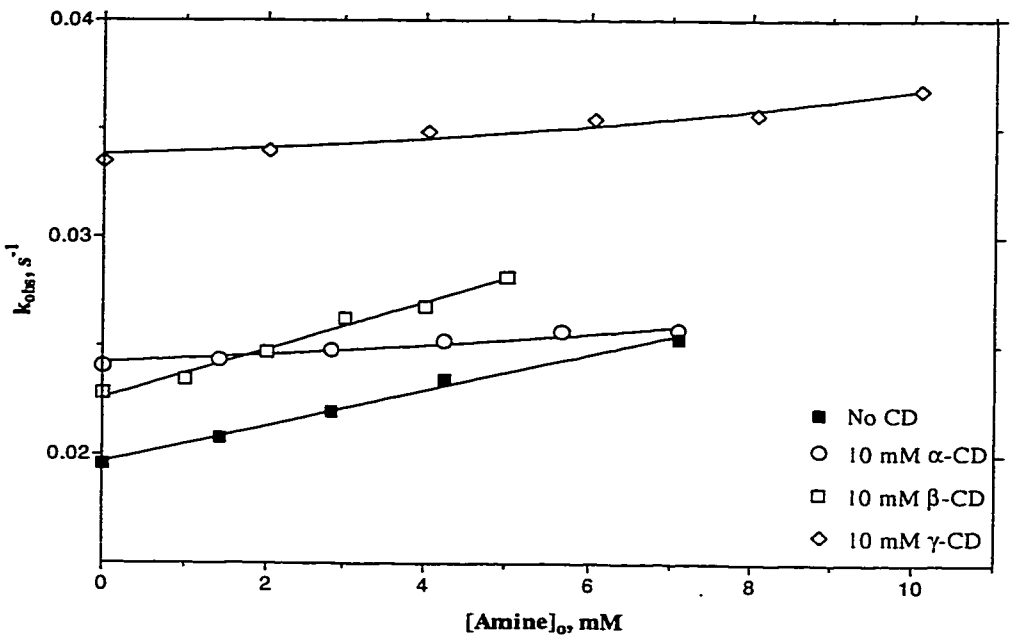


Figure 2.9: Plots of k_{obs} versus $[Amine]_o$ for the Aminolysis of 4-ABA by *n*-Heptylamine in the Absence and in the Presence of α-, β-, and γ-CD

From Figures 2.7 to 2.9, several issues can be addressed. Although most of these plots look linear, most are in fact, slightly curved. In the absence of CD, the plots are linear for the uncatalysed aminolysis reaction. In the presence of both β-CD and γ-CD, only binding of the amine to these CDs is involved, [eq. (21)], resulting in slightly curved plots. However, in the presence of α-CD, the binding of the ester to the CD, as well as the binding of the amine, must be taken into consideration [eq. (20)]. Therefore, plots involving α-CD demonstrate more pronounced curves. At zero [Amine], the different y-intercepts reflect the reactivity of the ester with the CD (Figure 2.1). The differences between the curved plots for the three CDs are only partially due to the modest catalysis by both β- and γ-CD, and the slight retardation by α-CD.

To emphasize further the origin of the curves in Figures 2.7 to 2.9, a figure has been constructed for the aminolysis reaction of 4-ABA reacting with *n*-pentylamine in the presence of α-CD, showing the contributions of the four cleavage processes set out in equations (8a), (8b), (11), and (12) (Figure 2.10). These contributions have been calculated by taking the rate constants k_u and k_N from the variation of k_{obs} with [Amine] with no CD (Figure 2.2), while k_c and k_{cN} are from the linear dependence of k_{corr} on [Amine] (Figure 2.3). plotted according to eq. (20).

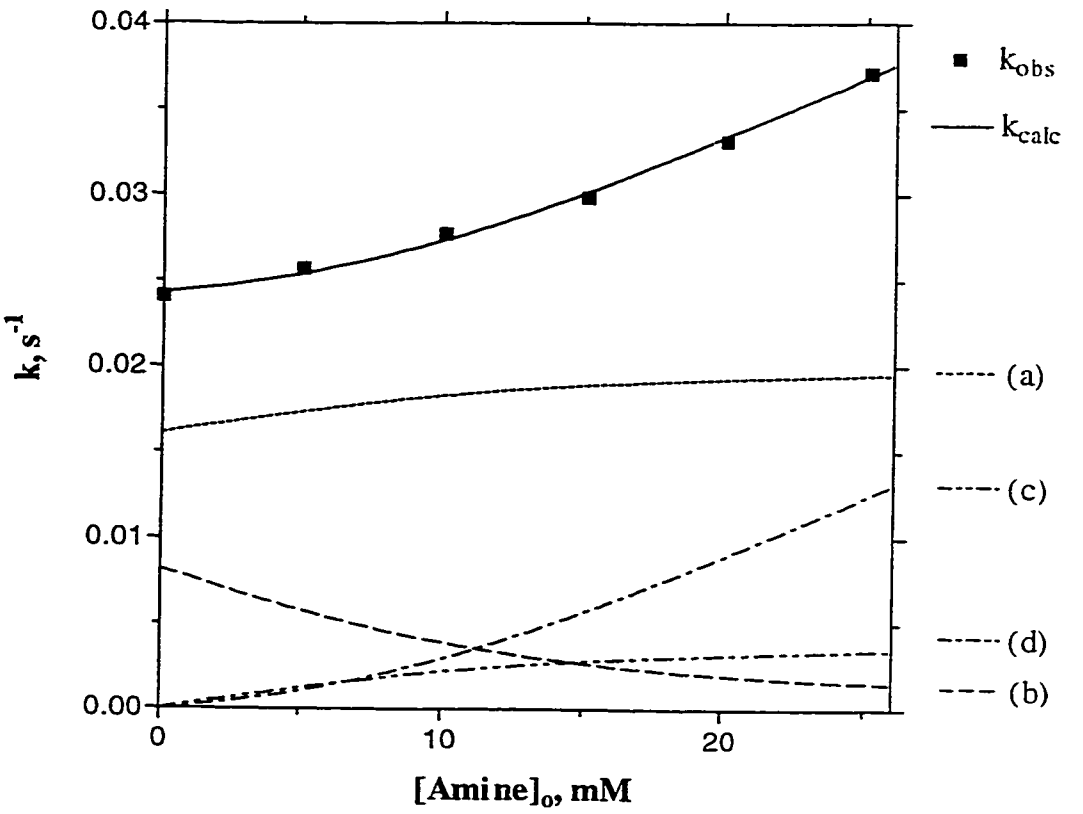


Figure 2.10: Variation of the First-order Contributions of Four Cleavage Pathways of 4-ABA reacting with *n*-Pentylamine in the Presence of α-CD. (a) refers to the uncatalysed cleavage [eq. (8a)]; (b) CD-mediated cleavage [eq. (8b)]; (c) uncatalysed aminolysis [eq. (11)]; and (d) CD-mediated aminolysis [eq. (12)].

As seen from the decidedly curved plots in Figure 2.10, with an increase in $[Amine]_o$, the amount of free CD decreases due to amine-CD binding and so does the amount of CD-mediated cleavage of 4-ABA (b). At the same time, the background hydrolysis of 4-ABA increases modestly, (a), because less 4-ABA is bound. Concurrently, there is an increase in the contributions of the two aminolysis processes, (c) and (d), as the $[Amine]_o$ increases. At high $[Amine]_o$, the CD-mediated aminolysis reaction (d) levels off as the CD becomes saturated with the amine. Overall, k_{obs} curves upwards with increasing $[Amine]_o$, due to the increases in the contributions of both aminolysis pathways.

For a more complete view on transition state binding, pK_{TS} (= - log K_{TS}) is used to measure the stability of the transition state that is afforded by the CD and so any variations of it with respect to the structure, can give information on transition state binding.[30] Figures 2.11 to 2.13 display plots of pK_{TS} versus pK_N (= -log K_N) for the aminolysis of 4-ABA in the presence of the three CDs, for which the relevant data are collected in Table 2.9.

Table 2.9: pK_{TS} and pK_N values for the Aminolysis of 4-ABA in the Presence of CDs[a,b]

Amine	pK_N	pK_{TS}
	(a) α-Cyclodextrin	
n-Butyl	1.88	1.80
n-Pentyl	2.51	2.25
n-Hexyl	2.80	2.72
n-Heptyl	3.37	3.22
n-Octyl	3.87	3.75

	(b) β-Cyclodextrin	
n-Butyl	-[b]	-[b]
n-Pentyl	1.94	2.08
n-Hexyl	2.58	2.73
n-Heptyl	3.02	3.24
n-Octyl	-[c]	-[c]
iso-Butyl	1.79	1.95
iso-Pentyl	2.24	2.40
Cyclopentyl	1.87	2.64
Cyclohexyl	2.74	3.66
	(c) γ-Cyclodextrin	
n-Butyl	-[b]	-[b]
n-Pentyl	1.71	2.07
n-Hexyl	2.03	2.36
n-Heptyl	2.55	2.75
n-Octyl	2.95	3.31

[a] The values of K_N are given in Table 2.4 and those of K_{TS} are in Tables 2.6 to 2.8.
[b] Experiments with *n*-propylamine, and to a lesser extent with *n*-butylamine, were unsuccessful because the contribution from CD-mediated aminolysis is too small.
[c] CD-mediated aminolysis was barely detectable at the amine concentrations accessible.

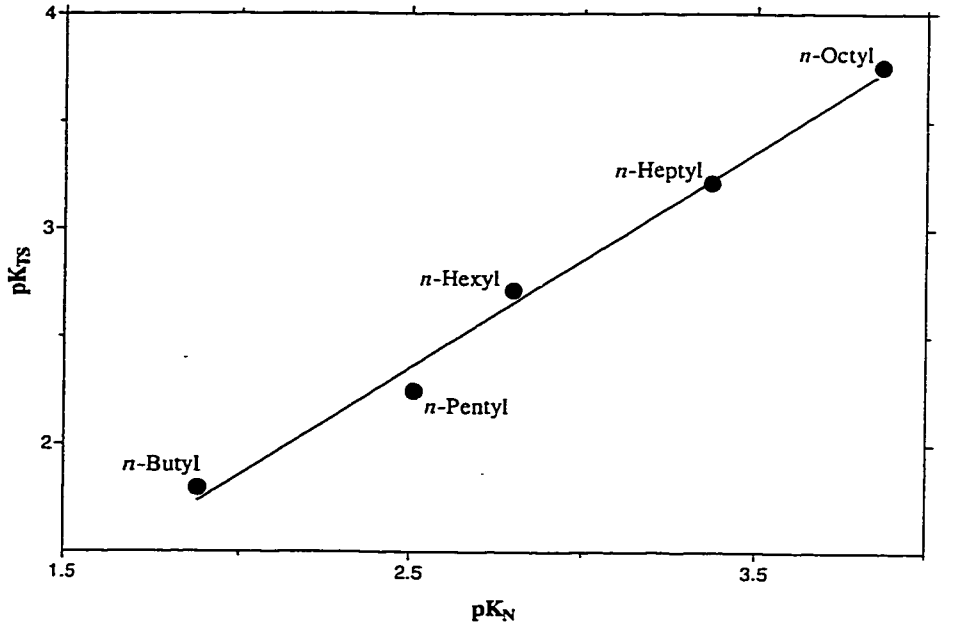


Figure 2.11: Plot of pK_{TS} versus pK_N for the Aminolysis of 4-ABA in the Presence of α-CD

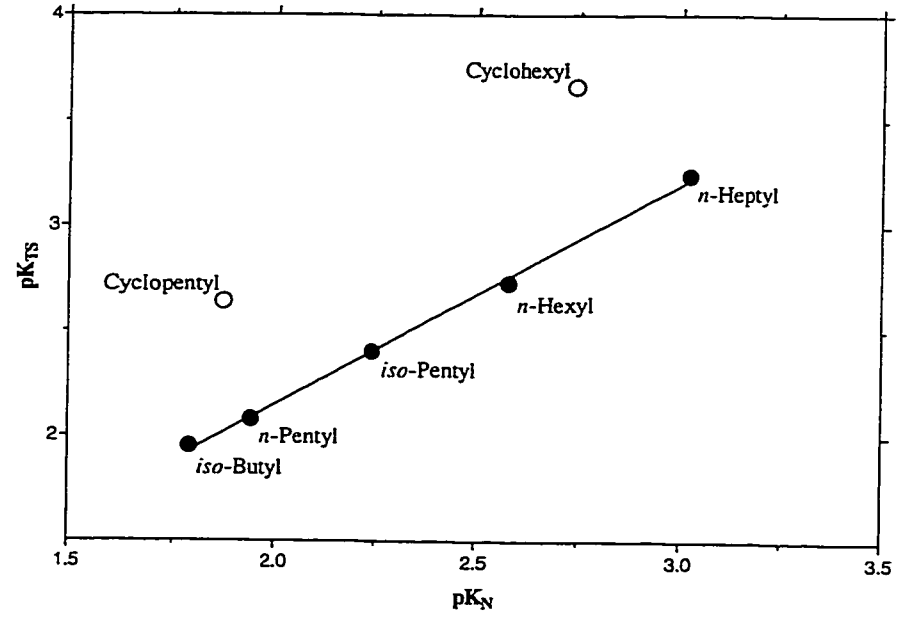


Figure 2.12: Plot of pK_{TS} versus pK_N for the Aminolysis of 4-ABA in the Presence of β-CD

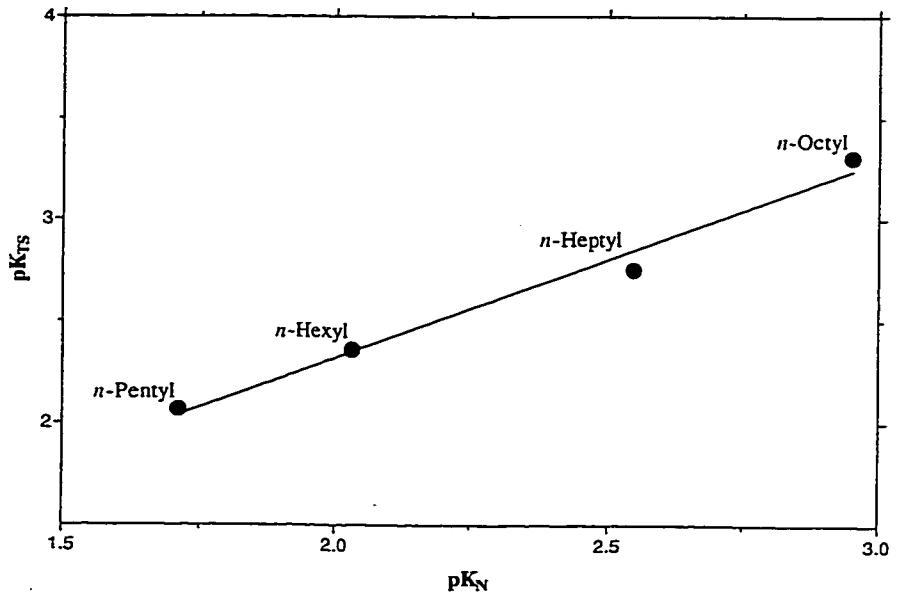


Figure 2.13: Plot of pK_{TS} versus pK_N for the Aminolysis of 4-ABA in the Presence of γ-CD

From a cursory view of these three plots, with the exception of the cyclic amines, pK_{TS} increases *linearly* with pK_N for all the CDs, and with slopes of *one* (Table 2.10). These slopes demonstrate a strong dependence of pK_{TS} on the binding ability of the amine (pK_N) for the aminolysis of 4-ABA. In the case of β-CD and the branched amines, pK_{TS} values are just as sensitive to the pK_N values which is quite reasonable since the branching of these amines are far enough away from the nucleophilic nitrogen for them to react like linear amines. The strong correlations ($r > 0.99$) between transition state binding and initial state binding of the amine, with slopes of one, are excellent evidence that regardless of the size of the amine and the CD, the CD-bound amine is reacting with the free ester, as depicted in structures **18** → **19** (p. 52).

Table 2.10: Correlation between Transition State Binding and Initial State (Amine) Binding for CD-mediated Aminolysis [a]

	α-CD	β-CD	γ-CD
Slope	1.00 ± 0.06	1.05 ± 0.02	0.97 ± 0.09
Intercept	-0.14 ± 0.17	0.06 ± 0.05	0.39 ± 0.21
Correlation Coefficient (r)	0.9952	0.9993	0.9915
No. of Points (n)	5	5[b]	4

[a] From the linear regression of pK_{TS} (= - log K_{TS}) against pK_N (= -log K_N). The values of K_N are given in Table 2.4 and those of K_{TS} are in Tables 2.6 to 2.8.
[b] Includes the two branched amines.

To see how the alkylamino chains are held with respect to CDs in the transition state, Figure 2.14 is a combination of Figures 2.3 to 2.6 (non-cyclic amines only) which shows the differences and similarities between the CDs. All three CDs display slopes near one reflecting the correlation between transition state binding and amine binding. Both β- and γ-CD show moderate catalysis, as their reactivity ratios (k_{Nc}/k_N, Tables 2.7 and 2.8), are all greater than one. However, with α-CD, the ratios are slightly less than one which corresponds to modest retardation. These differences may be ascribed to the different sizes of the CD cavities. With α-CD, the alkylamino chain will be held tightly which may make the transition state harder to reach. The two larger CDs will hold the chain less rigidly, so that access to the transition state is easier.

Another factor is probably important, also: hydrophobicity.[54-56] Binding of alkyl chains to CDs is driven by the hydrophobic effect.[39,42,43,50] The fact that $pK_{TS} > pK_N$ for β-CD and γ-CD could mean that the aminolysis transition state is slightly more hydrophobic than

the corresponding alkylamine, separately, because it displaces more water molecules. In the case of α-CD, the effect may be slightly less so that $pK_{TS} < pK_N$. In all probability, both geometric and hydrophobic factors are important.

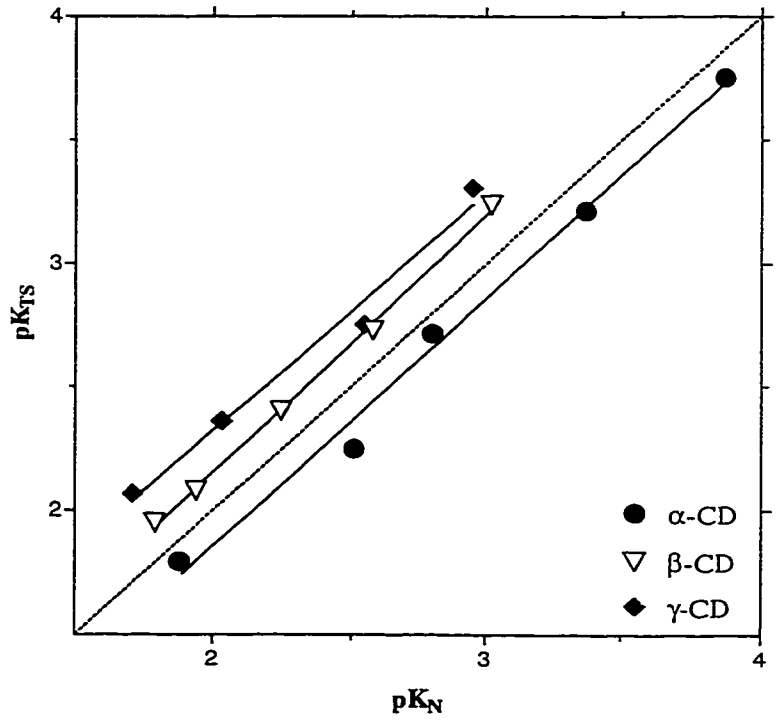


Figure 2.14: Correlation of Transition State Binding with Amine Binding for all CDs. The dashed line represents a slope of 1.00.

With respect to the cyclic amines, they show stronger transition state binding with β-CD in comparison to the branched and linear amines. Again, the origin may be due to geometric and/or hydrophobic factors. Both cyclopentylamine and cyclohexylamine are likely to be held fairly rigidly in β-CD, but this could still give rise to catalysis if the CD-bound aminolysis transition state excludes more water than the amine. In other words, the bound transition state has less total hydrophobic surface area exposed to the aqueous medium than the bound amine.

2.4 Aminolysis of 4-ABA in the presence of CDs: Comparison to Other Substrates

From studying the aminolysis of 4-ABA in the presence of different CDs, transition state binding for this reaction has been looked at. However, by keeping the CD constant while changing the ester, the effects of transition state binding with respect to the substrates can be analysed. From previous studies by our lab,[47,48] ester substrates that have already been looked at and are considered to be good candidates are *p*-NPA and 1-NA.

The data for the aminolysis of *p*-NPA and 1-NA in the presence of β-CD have been collected in Table 2.11, and plots of pK_{TS} versus pK_N of these two ester substrates, along with 4-ABA are presented in Figure 2.15. For both these esters, pK_{TS} varies linearly with pK_N, and slopes are near one, except for *p*-NPA with *n*-propylamine and *n*-butylamine.

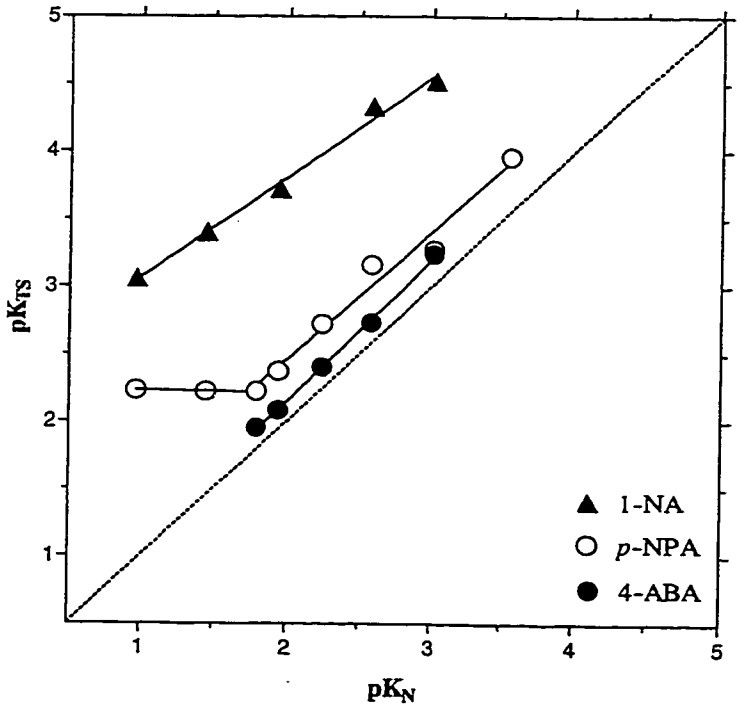


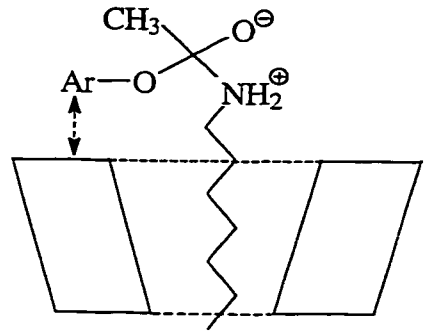
Figure 2.15: Correlation of Transition State Binding with Amine Binding for β-CD. The dashed line represents a slope of 1.00.

Table 2.11: pK_{TS} and pK_N values for the Aminolysis of *p*-NPA and 1-NA by β-CD[47,48]

Amine	pK_N	pK_{TS}
	(a) *p*-Nitrophenyl Acetate	
n-Propyl	0.967	2.23
n-Butyl	1.45	2.22
iso-Butyl	1.79	2.22
n-Pentyl	1.94	2.37
iso-Pentyl	2.24	2.72
n-Hexyl	2.58	3.16
n-Heptyl	3.02	3.27
n-Octyl	3.56	3.96
	(b) 1-Naphthyl Acetate	
n-Propyl	0.967	3.06
n-Butyl	1.45	3.40
n-Pentyl	1.94	3.72
n-Hexyl	2.58	4.33
n-Heptyl	3.02	4.52

As seen from both the Table and the Figure, pK_{TS} for 1-NA is appreciably larger than for the other ester substrates, because it shows stronger catalysis of aminolysis. For any given amine, the order of strength of transition state binding (and catalysis) is: 1-NA > *p*-NPA > 4-ABA. These differences must be due to some attractive interaction involving the ester aryl group and the CD which is present in the transition state but not in the initial state. Again, the interaction is most probably hydrophobic.[54-56] In the case of 1-naphthyl acetate, it has two hydrophobic surfaces exposed to the aqueous solution in the initial state but if one

of them is shielded by the CD in the transition state (cf. **20**), then an additional hydrophobic interaction contributes to its stabilization and hence the higher pK_{TS}.



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For the aminolysis of the ester, *p*-NPA, a distinctly biphasic behaviour is observed, in which for the short amines (C_3 and C_4), pK_{TS} is insensitive to pK_N demonstrating that there is aryl inclusion in the transition state (see structure **7**, p. 35). Yet, upon reaching longer amines (C_5 and upwards), the values of pK_{TS} begin to rise steeply with pK_N due to a change in transition state binding to one in which the reaction takes place between the CD-bound amine and the free ester (structure **9**, p. 36). The branched amines follow the same pattern as the linear amines, with *iso*-butylamine being at the break point.

In comparison to these two esters, the aminolysis of 4-ABA in the presence of β-CD displays milder catalysis. In this case, 4-ABA binds very weakly to the CD which can be seen from the K_S value (> 100 mM) because it is relatively hydrophilic - it has an ionized carboxyl group. For the linear and branched amines, there is a strong correlation between transition state binding and amine binding, and modest catalysis results because of a slightly stronger hydrophobic interaction in the transition state than in the initial state.

3. CONCLUSIONS

Cleavage kinetics of 4-ABA in basic aqueous solution displays weak binding to α-CD while no binding was observed for the cleavage of 4-ABA with either β- or γ-CD. These results are expected since 4-ABA is an anionic, hydrophilic compound as opposed to cyclodextrins which are internally hydrophobic. The weak binding of 4-ABA to α-CD is expected, presumably due to this CD holding the ester substrate loosely. As the size of the CD cavity increases as is the case of β- and γ-CD, binding becomes too weak to be observed. As such, transition state binding of 4-ABA during basic cleavage is weak for all three CDs. Comparison of these results to those of *p*-NPA and 1-NA demonstrate that these esters display saturation kinetics due to stronger substrate binding which is expected due to their hydrophobic nature.

From the aminolysis of 4-ABA in the absence of any CD, the rate constant, k_N, for each amine was determined. These values do not vary greatly for the linear amines (*n*-propyl to *n*-octyl). However, the cyclic amines (cyclopentyl and cyclohexyl), show lower reactivity due to the close proximity between the bulky cyclic group and the reacting amino group. As for the branched amines (*iso*-butyl and *iso*-pentyl), their reactivities are not very different from those of the linear amines.

Aminolysis of 4-ABA in the presence of CDs displayed varying results. In the case of α-CD, this reaction is slightly retarded while for β- and γ-CD, the aminolysis reaction is modestly catalysed. Therefore, catalysis increases as follows: α-CD < β-CD < γ-CD. For α-CD, there is some initial binding of both ester and amine to the CD independently. however, in the transition state, the mode of binding is one in which the amine is bound to

the CD reacting with the free ester (**18** → **19**, p. 52). In the case of β- and γ-CD, initial state binding of the ester is not important and transition state binding occurs between the CD-bound amine and the free ester (**18** → **19**), regardless of the amine chain length. Thus, transition state binding correlates strongly with amine binding for all three CDs (Figure 2.14).

With regards to the other acetate esters, 1-NA and *p*-NPA, aminolysis in the presence of β-CD, in comparison to 4-ABA, demonstrates that catalysis increases as follows: 4-ABA < *p*-NPA < 1-NA. The ester, 1-NA displays higher pK_{TS} values which can be accounted for by additional stabilizing interactions between the aryl group of the ester and the CD in the transition state. As for the ester, *p*-NPA, a biphasic behaviour is observed in which there is a switch in the binding mode from aryl group inclusion for the short amines (**7**, p. 35), to alkylamino group inclusion for the longer amines (**9**, p. 36). With respect to 4-ABA, there is no such switch because this ester is more hydrophilic and it does not bind well to β-CD.

Finally, this study was carried out to provide support for the main conclusions of earlier studies,[47] and it has done so. There seems little doubt that 4-ABA reacts with amines as in **18** → **19**, and in larger measure *p*-nitrophenyl alkanoates appear to react likewise.

4. EXPERIMENTAL

4.1 Materials

α-, β-, and γ-Cyclodextrins were purchased from Wacker Chemie (Munich, Germany). The ester substrate, 4-acetoxybenzoic acid, the primary alkylamines, acetonitrile, and sodium phosphate (dibasic, heptahydrate) were all purchased from Aldrich Chemical Company, and were of the highest grade available. Standard 1.00 M NaOH and 1.00 M HCl were purchased from A & C American Chemicals Ltd. (Montréal).

4.2 Solutions

For the hydrolysis reactions, two sets of solutions were made. In the cases involving α-CD, γ-CD, and TFE, the first set consisted of varying concentrations of CD or TFE in a 0.4 M phosphate buffer set to pH 11.60. The second set consisted of a 0.1 M stock solution of 4-acetoxybenzoic acid (in acetonitrile), diluted with distilled water to 100 μM. For the experiment involving β-CD, the first set consisted of varying concentrations of CD in the same phosphate buffer. The second set consisted of 100 μM of the ester, in exact amounts of CD (as in the first set). For these cleavage experiments, the concentrations of α- and γ-CD ranged from 0 to 50 mM, β-CD ranged from 0 to 12 mM, and TFE ranged from 0 to 25 mM (Figure 2.1), all upon mixing in the reaction cell.

For experiments involving the aminolysis reaction in the absence of CD, two sets of solutions were prepared. The first set consisted of varying concentrations of the amine (=Nuc) in a 0.4 M phosphate buffer set to pH 11.60, while the second set consisted of a 100 μM solution of the ester in water.

Two sets of solutions were prepared for the aminolysis reactions in the presence of α- or γ-CD. The first set contained varying concentrations of the amine in the 0.4 M phosphate buffer set to pH 11.60, while the second set of solutions was an aqueous solution of the ester and 20 mM of α- or γ-CD.

In the case of β-CD, because of its low solubility in water, both reactant solutions contained β-CD. In the first set, the solutions contained varying concentrations of the amine, *with* 10 mM of β-CD (in a phosphate buffer set at 11.60) diluted with buffer set at the same pH. The second set contained aqueous solutions of the ester and 10 mM of β-CD.

For all aminolysis experiments, the range of amine concentrations used after mixing in the reaction cell can be viewed in Table 4.1. For the aminolysis experiments in the presence of a fixed concentration of CD, the concentrations of the three CDs was 10 mM upon mixing, with the same amine concentration ranges.

Table 4.1: Amine Concentrations for Experiments on the Aminolysis of 4-ABA

Amine	Concentration Range, mM
n-Butyl	0 - 100
n-Pentyl	0 - 25
n-Hexyl	0 - 10
n-Heptyl	0 - 5.0
n-Octyl	0 - 4.0
iso-Butyl	0 - 100
iso-Pentyl	0 - 25
Cyclopentyl	0 - 70
Cyclohexyl	0 - 25

4.3 Kinetic Measurements

The kinetics of the hydrolysis and the aminolysis of 4-ABA were monitored by an Applied Photophysics SX17MV Single Beam Stopped-flow Spectrophotometer. Formation of the product, 4-carboxyphenoxide dianion (**15**) was monitored at 278 nm. A temperature of 25.0 ± 0.1 °C was maintained in the observation cell via circulation from a thermostatically-controlled water bath. The two reactant solutions were injected from syringes and upon mixing, the concentrations decreased by a factor of 2, due to a 1:1 mixing in the cell. From 5 to 10 absorbance traces, each one composed of 400 points, an average trace was taken. By non-linear least squares fitting of a first-order exponential, $A = A_\infty + (A_\circ - A_\infty)\exp(-k_{obs}t)$ to the average trace, a first order rate constant, k_{obs}, was obtained.

4.4 Data Analysis

Kinetic data for the cleavage of 4-acetoxybenzoic acid by α-CD, was analysed in terms of saturation kinetics [eq. (8c)], from which the binding constant, K_S, was determined. For the cleavage of 4-acetoxybenzoic acid by β-CD, γ-CD, and TFE, the kinetic data was based simply on the linear equation (9).

For the aminolysis in the absence of CD, the simple linear equation (15) was applied to the kinetic data. For the aminolysis reactions in the presence of α-CD, data analysis was carried out in terms of equation (20), from which the rate constant, k_{cN} was obtained, and the third-order rate constant, k_3, was then calculated. For reaction in the presence of β- and γ-CD, the analysis used equation (22), from which k_3 was obtained. All analyses were performed using Lotus 1-2-3™ spreadsheets. An example is given overleaf.

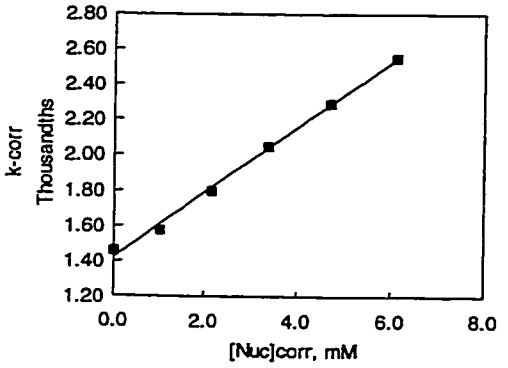
EXPT:	DELM_73		NO SUBSTRATE BINDING							
DATE:	Sept. 9, 1999									
ESTER:	4-ABA	KN =	9.29	mM	ku =	0.01983	1/s			
CAT.	Gamma-CD									
NU:	n-Hexylamine	[CD]o =	10.00	mM	kN =	0.7979	1/M.s			

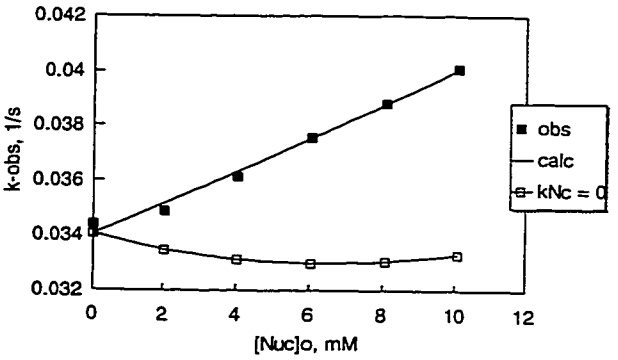
[CD]o (mM)	[Nuc]o (mM)	k(obs) (1/s)	k(calc) (1/s)	%-diff	[CD] (mM)	[Nuc] (mM)	k(corr) Obs	k(corr) Calc	If kNc = 0	% diff
10.00	0.00	0.03440	0.03405	1.0	10.000	0.000	1.457E-03	1.422E-03	0.03405	0.0
10.00	2.02	0.03487	0.03515	-0.8	9.006	1.026	1.579E-03	1.610E-03	0.03346	5.1
10.00	4.04	0.03614	0.03630	-0.4	8.116	2.156	1.798E-03	1.818E-03	0.03309	9.7
10.00	6.05	0.03755	0.03750	0.1	7.331	3.381	2.049E-03	2.042E-03	0.03295	13.8
10.00	8.07	0.03879	0.03875	0.1	6.637	4.707	2.291E-03	2.285E-03	0.03303	17.3
10.00	10.09	0.04008	0.04005	0.1	6.029	6.119	2.549E-03	2.544E-03	0.03329	20.3

Regression Output:

Constant		1.422E-03	= k2
Std Err of Y Est		2.587E-05	
R Squared		0.99699	r = 0.99849
No. of Observations		6	
Degrees of Freedom		4	
X Coefficient(s)	1.834E-04		
Std Err of Coef.	5.040E-06		

k3 =	183.40	+/-	5.04	1/M^2.s
kNc =	1.704	+/-	0.047	1/Ms
kNc/kN =	2.135	+/-	0.059	
Kts =	4.351			mM
pKts =	2.361			





4.5 Corrections to [CD] and [Amine]

Analysis in terms of equation (14) or equation (22) require values of [CD] and [Amine] that are corrected for the formation of the Amine.CD complex [eq. (16)].

$$CD + Nuc \underset{K_N}{\rightleftharpoons} Nuc.CD \qquad (=16)$$

In order to determine these values, the definition of K_N and the two equations for mass balance must be used as follows:

$$K_N = \frac{[CD][Nuc]}{[Nuc.CD]}$$

$$[Nuc]_o = [Nuc] + [Nuc.CD]$$

$$[CD]_o = [CD] + [Nuc.CD]$$

Combination of these three equations leads to:

$$K_N = \frac{[CD]([Nuc]_o - [CD]_o + [CD])}{([CD]_o - [CD])}$$

which upon expansion, gives a quadratic equation in [CD]. Solution of this quadratic equation is:

$$[CD] = \frac{-b + (b^2 + 4K_N[CD]_o)^{1/2}}{2}$$

where $b = (K_N - [CD]_o + [Nuc]_o)$.

Then, by difference,

$$[Nuc] = [Nuc]_o - [Nuc.CD] = [Nuc]_o - ([CD]_o - [CD])$$

This approach has been used successfully in three previous studies.[47,48,50]

REFERENCES

1. Vögtle, F., *Supramolecular Chemistry: An Introduction*, John Wiley & Sons, New York, 1991.
2. Cram, D.J., *Angew. Chem. Int. Ed. Engl.*, 1988, **27**, 1009.
3. Lehn, J.-M., *Science*, 1985, **227**, 849.
4. Szejtli, J., *Cyclodextrins and their Inclusion Complexes*, Akademial Kiado, Budapest, 1988.
5. Fischer, E., *Ber. Deutsch. Chem. Ges.*, 1894, **27**, 2985. (cited by Ref. 21)
6. *Host Guest Complex Chemistry: Macrocycles*, Vögtle, F., Weber, E., (Eds), Springer-Verlag, New York, 1985.
7. Szejtli, J., *Chem. Rev.*, 1998, **98**, 1743.
8. Bender, M.L., Komiyama, M., *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978.
9. Easton, C.J., Lincoln, S.F., *Modified Cyclodextrins*, Imperial College Press, London, 1999.
10. Szejtli, J., *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, 1988.
11. Sundararajan, P.R., Rao, V.S., *Carbohydr. Res.*, 1970, **13**, 351.
12. Saenger, W., in *Inclusion Compounds*, Vol. 2, Atwood, J.L., Davies, J.E.D., MacNicol, D.D., (Eds), Chap. 8, Academic Press, London, 1984.
13. Giorgi, J.B., Tee, O.S., *J. Am. Chem. Soc.*, 1995, **117**, 3633.
14. Wenz, G., *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 803.

15. Rekharsky, M.V., Inoue, Y., *Chem. Rev.*, 1998, **98**, 1875.

16. Connors, K.A., *Chem. Rev.*, 1997, **97**, 1325.

17. Brochure by Wacker-Chemie GmbH, Hanns-Seidel-Platz 4, D-8000 Munich 83.

18. Szejtli, J., in *Inclusion Compounds*, Vol. 3, Atwood, J.L., Davies, J.E.D., MacNicol, D.D., (Eds), Chap. 11, Academic Press, London, 1984.

19. Kirby, A.J., *Angew. Chem. Int. Ed. Engl.*, 1996, **35**, 707.

20. Jencks, W.P., *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York, 1969.

21. Fersht, A., *Enzyme Structure and Mechanism*, 2nd Edn., W.H. Freeman and Company, New York, 1985.

22. Kurz, J.L., *J. Am. Chem. Soc.*, 1962, **85**, 987.

23. Wolfenden, R., *Acc. Chem. Res.*, 1972, **5**, 10.

24. Kraut, J., *Science*, 1988, **242**, 533.

25. March, J., *Advanced Organic Chemistry: Reactions, Mechanism, and Structure*, 4th Edn., John Wiley & Sons, New York, 1992.

26. Laidler, K.J., *Chemical Kinetics*, 3rd Edn., Harper & Row Publishers, New York, 1987.

27. Bender, M.L., in *Enzyme Mechanisms*, Page, M.I., Williams, A., (Eds), Chap. 4, Royal Society of Chemistry, London, 1987.

28. Komiyama, M., Bender, M.L., in *The Chemistry of Enzyme Action*, Page, M.I., (Ed), Chap. 14, Elsevier, New York, 1984.

29. Tee, O.S., *Carbohydr. Res.*, 1989, **192**, 181.

30. Tee, O.S., *Adv. Phys. Org. Chem.*, 1994, **29**, 1.

31. Straub, T.S., Bender, M.L., *J. Am. Chem. Soc.*, 1972, **94**, 8875.

32. Griffiths, D.W., Bender, M.L., *J. Am. Chem. Soc.*, 1973, **95**, 1679.

33. Tee, O.S., Donga, R.A., *J. Chem. Soc., Perkin Trans. 2*, 1996, 2763.

34. Komiyama, M., Bender, M.L., *J. Am. Chem. Soc.*, 1977, **99**, 8021.

35. VanEtten, R.L., Sebastian, J.F., Clowes, G.A., Bender, M.L., *J. Am. Chem. Soc.*, 1967, **89**, 3242.

36. VanEtten, R.L., Clowes, G.A., Sebastian, J.F., Bender, M.L., *J. Am. Chem. Soc.*, 1967, **89**, 3253.

37. Fendler, J.H., Fendler, E.J., *Catalysis in Micellar and Macromolecular Systems*, Academic Press, New York, 1975.

38. Bonora, G.M., Fornasier, R., Scrimin, P., Tonellato, U., *J. Chem. Soc., Perkin Trans. 2*, 1985, 367.

39. Tee, O.S., Mazza, C., Du, X-x., *J. Org. Chem.*, 1990, **5**, 3603.

40. Tee, O.S., Gadosy, T.A., Giorgi, J.B., *J. Chem. Soc., Perkin Trans. 2*, 1993, 1705.

41. Tee, O.S., Gadosy, T.A., *J. Chem. Soc., Perkin Trans. 2*, 1994, 2191.

42. Tee, O.S., Gadosy, T.A., Giorgi, J.B., *Can. J. Chem.*, 1996, **74**, 736.

43. Tee, O.S., Gadosy, T.A., *J. Chem. Soc., Perkin Trans. 2*, 1994, 2307.

44. Gadosy, T.A., Tee, O.S., *J. Chem. Soc., Perkin Trans. 2*, 1994, 715.

45. Gadosy, T.A., Tee, O.S., *J. Chem. Soc., Perkin Trans. 2*, 1994, 2069.

46. Gadosy, T.A., Tee, O.S., *J. Chem. Soc., Perkin Trans. 2*, 1995, 71.

47. Gadosy, T.A., Boyd, M.J., Tee, O.S., *J. Org. Chem.*, 2000, **65**, in press.

48. Tee, O.S., Boyd, M.J., *Can. J. Chem.*, 1999, **77**, 950.

49. Tee, O.S., Boyd, M.J., *J. Chem. Soc. Perkin Trans. 2*, 1995, 1237.

50. Tee, O.S., Gadosy, T.A., Giorgi, J.B., *Can. J. Chem.*, 1997, **75**, 83.

51. Jencks, W.P., Gilchrist, M., *J. Am. Chem. Soc.*, 1962, **84**, 2911.

52. Gelb, R.I., Schwartz, L.M., Bradshaw, J.J., Laufer, D.A., *Bioorg. Chem.*, 1980, **9**, 200.

53. Williams, A., in *The Chemistry of Enzyme Action*, Page, M.I., (Ed), Chap. 5. Elsevier, New York, 1984.

54. Tanford, C., *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd Edn, John Wiley & Sons, New York, 1980.

55. Blokzijl, W., Engberts, J.B.F.N., *Angew. Chem. Int. Ed. Engl.*, 1993, **32**, 1545.

56. Abraham, M.H., *J. Am. Chem. Soc.*, 1982, **104**, 2085; *J. Chem. Soc. Faraday Trans. 1*, 1984, **80**, 153.

APPENDIX I

APPENDIX I

Derivation of the Rate Equation for Substrate Cleavage in CD

Utilizing equations (8a) and (8b) from p. 18,

$$S \xrightarrow{k_u} P \tag{8a}$$

$$S + CD \underset{K_S}{\rightleftharpoons} S.CD \xrightarrow{k_c} P \tag{8b}$$

the overall rate is:

$$v = k_u [S] + k_c [S.CD] \tag{1}$$

$$v = k_{obs} [S]_o \text{ where } [S]_o = [S] + [S.CD] \tag{2}$$

From equation (1) and (2),

$$k_{obs} [S]_o = k_u [S] + k_c [S.CD] \tag{3}$$

$$k_{obs} = \frac{k_u [S] + k_c [S.CD]}{[S]_o} \tag{4}$$

$$k_{obs} = \frac{k_u [S]}{[S]_o} + \frac{k_c [S.CD]}{[S]_o} \tag{5}$$

Since, $$K_S = \frac{[S][CD]}{[S.CD]} \tag{6}$$

Then using the first term in equation (5) and equation (2) gives,

$$\frac{[S]_o}{[S]} = \frac{[S] + [S.CD]}{[S]} = 1 + \frac{[S.CD]}{[S]} = 1 + \frac{[CD]}{K_S} \tag{7}$$

$$\frac{[S]}{[S]_o} = \frac{K_S}{K_S + [CD]} \tag{8}$$

As well, using the second term in equation (5) and equation (2) gives,

$$\frac{[S]_o}{[S.CD]} = \frac{[S] + [S.CD]}{[S.CD]} = \frac{[S]}{[S.CD]} + 1 = \frac{K_S}{[CD]} + 1 \tag{9}$$

$$\frac{[S.CD]}{[S]_o} = \frac{[CD]}{K_S + [CD]} \tag{10}$$

Substituting equations (8) and (10) into equation (5) yields,

$$k_{obs} = \frac{k_u K_S}{K_S + [CD]} + \frac{k_c [CD]}{K_S + [CD]} \tag{11}$$

$$k_{obs} = \frac{k_u K_S + k_c [CD]}{K_S + [CD]} \tag{12}$$

This equation, which is the same as eq. (8c) in Section 1.4, describes saturation kinetics,[30] such as seen in Figure 2.1 for the cleavage of 4-ABA by α-CD.

APPENDIX II

APPENDIX II

All solutions were prepared at 25°C in 0.2 M phosphate buffer, pH 11.60 (after mixing).

Table A.1: Raw Data for the Cleavage of 4-ABA by α-CD

$[CD]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0203
10.0	0.0258
20.0	0.0296
30.0	0.0323
40.0	0.0339
50.0	0.0356

Table A.2: Raw Data for the Cleavage of 4-ABA by β-CD

$[CD]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0204
2.40	0.0218
4.80	0.0240
7.20	0.0256
9.60	0.0274
12.0	0.0288

Table A.3: Raw Data for the Cleavage of 4-ABA by γ-CD

$[CD]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0198
10.0	0.0373
20.0	0.0499
30.0	0.0652
40.0	0.0776
50.0	0.0910

Table A.4: Raw Data for the Cleavage of 4-ABA by TFE

[TFE] (mM)	k_{obs} (s^{-1})
0.0	0.0203
5.00	0.0322
10.0	0.0462
15.0	0.0595
20.0	0.0726
25.0	0.0845

Table A.5: Raw Data for the Aminolysis of 4-ABA by *n*-Propylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0203
50.1	0.0543
100	0.0881
150	0.120
201	0.151
251	0.182

Table A.6: Raw Data for the Aminolysis of 4-ABA by *n*-Butylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0198
20.0	0.0368
40.0	0.0535
60.0	0.0698
80.0	0.0857
100	0.101

Table A.7: Raw Data for the Aminolysis of 4-ABA by *n*-Pentylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0202
5.01	0.0243
10.0	0.0286
15.0	0.0321
20.0	0.0358
25.0	0.0362[a]

[a] Point omitted from analysis

Table A.8: Raw Data for the Aminolysis of 4-ABA by *n*-Hexylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0205
2.02	0.0221
4.04	0.0239
6.06	0.0257
8.08	0.0268
10.1	0.0286

Table A.9: Raw Data for the Aminolysis of 4-ABA by *n*-Heptylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0196
1.42	0.0208
2.84	0.0220
4.25	0.0235
5.67	0.0251[a]
7.09	0.0253

[a] Point omitted from analysis

Table A.10: Raw Data for the Aminolysis of 4-ABA by *iso*-Butylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0200
20.2	0.0277
40.3	0.0351
60.5	0.0424
80.6	0.0512
101	0.0581

Table A.11: Raw Data for the Aminolysis of 4-ABA by *iso*-Pentylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0200
5.10	0.0245
10.2	0.0292
15.3	0.0344
20.4	0.0388
25.5	0.0430

Table A.12: Raw Data for the Aminolysis of 4-ABA by Cyclopentylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0196
14.0	0.0221
28.0	0.0246
42.1	0.0278
56.1	0.0307
70.1	0.0326

Table A.13: Raw Data for the Aminolysis of 4-ABA by Cyclohexylamine

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0198
5.03	0.0202
10.1	0.0207
15.1	0.0215
20.1	0.0221
25.1	0.0226

Table A.14: Raw Data for the Aminolysis of 4-ABA by *n*-Butylamine in the Presence of α-CD

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0240
10.0	0.0289
20.0	0.0354
30.0	0.0437
40.0	0.0500
50.1	0.0587

Table A.15: Raw Data for the Aminolysis of 4-ABA by *n*-Pentylamine in the Presence of CDs

$[Amine]_o$ (mM)	k_{obs}, (s^{-1})		
	α-CD	β-CD	γ-CD
0.0	0.0241	0.0233	0.0338
5.01	0.0257		0.0372
5.05		0.0270	
10.0	0.0277		0.0413
10.1		0.0313	
15.0			0.0439
15.1	0.0299	0.0353	
20.1	0.0332		0.0437[a]
20.2		0.0389	
25.1	0.0371		0.0467[a]
25.2		0.0410[a]	

[a] Point omitted from analysis

Table A.16: Raw Data for the Aminolysis of 4-ABA by *n*-Hexylamine in the Presence of CDs

$[Amine]_o$ (mM)	k_{obs}, (s^{-1})		
	α-CD	β-CD	γ-CD
0.0	0.0237	0.0226	0.0344
2.00		0.0248	
2.02	0.0242		0.0349
4.00		0.0268	
4.04	0.0249		0.0361
6.00		0.0282	
6.05			0.0376
6.06	0.0258		
8.00		0.0294	
8.07			0.0388
8.08	0.0267		
10.0		0.0315	
10.1	0.0282		0.0401

Table A.17: Raw Data for the Aminolysis of 4-ABA by *n*-Heptylamine in the Presence of CDs

$[Amine]_o$ (mM)	k_{obs}, (s^{-1})		
	α-CD	β-CD	γ-CD
0.0	0.0241	0.0228	0.0335
1.00		0.0235	
1.42	0.0244		
2.00		0.0247	
2.02			0.0340
2.84	0.0248		
3.01		0.0263	
4.01		0.0268	
4.04			0.0349
4.25	0.0252		
5.01		0.02817	
5.67	0.0257		
6.05			0.0354
7.09	0.0258		
8.07			0.0357
10.09			0.0368

Table A.18: Raw Data for the Aminolysis of 4-ABA by *n*-Octylamine in the Presence of α-CD and γ-CD

$[Amine]_o$ (mM)	k_{obs}, (s^{-1})	
	α-CD	γ-CD
0.0	0.0251	0.0347
0.52	0.0232	
1.03	0.0239	
1.05		0.0346
1.55	0.0242	
1.58		0.0349
2.58	0.0242	
2.62		0.0356
3.61	0.0248	
3.67		0.0359
4.12	0.0251	
4.19		0.0365

Table A.19: Raw Data for the Aminolysis of 4-ABA by *iso*-Butylamine in the Presence of β-CD

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0231
10.0	0.0280
20.0	0.0320
30.0	0.0349
40.0	0.0389
50.0	0.0418
60.0	0.0446
70.0	0.0505
80.0	0.0524
90.0	0.0571
100	0.0582[a]

[a] Point omitted from analysis

Table A.20: Raw Data for the Aminolysis of 4-ABA by *iso*-Pentylamine in the Presence of β-CD

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0240
2.50	0.0257
5.00	0.0282
7.50	0.0284
10.0	0.0289
12.5	0.0335
15.0	0.0369
17.5	0.0397
20.0	0.0419
22.5	0.0447
25.0	0.0471

Table A.21: Raw Data for the Aminolysis of 4-ABA by Cyclopentylamine in the Presence of β-CD

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0224
14.0	0.0294
28.0	0.0334
42.0	0.0364
56.0	0.0390
70.1	0.0411

Table A.22: Raw Data for the Aminolysis of 4-ABA by Cyclohexylamine in the Presence of β-CD

$[Amine]_o$ (mM)	k_{obs} (s^{-1})
0.0	0.0221
5.15	0.0257
10.3	0.0295
15.4	0.0303
20.6	0.0311
25.7	0.0312