6^A-ω-Aminoalkylamino-Cyclodextrins: Their Preparation and Studies of Their Self-inclusion Complexes and Catalytic Nature.

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Errata.

Page 22: The diagram is incorrectly labelled as "Figure 1.4.2.3", it should read "Figure 1.4.2.2".

Page 23 - Line 6: The figure referred to in the text should read "Figure 1.4.2.4" not "1.4.3.3"

Re: Page 32-33 and Scheme 2.1.2.1: The specific rate constant, k_C, refers to the rate determining step of the "over-all" rate of de-acylation of the guest ester. While the total de-acylation process is indeed a multi-step process involving the formation of a zwitterion intermediate, we are only concerned with the rate limiting step of this process. The kinetic argument related to Scheme 2.1.2.1 is formulated with this point in mind.

Pages 35, 36, 40, 44, 45, 46, 47, 48, 49, 50, 51, 101 and 107: References are made and the structure is given for *p*-nitrophenylpropanoate. The ester employed was in fact p-nitrophenylbutanoate.

Page 45 lines 9 and 10: The sentence should read "It would seem likely however, that as the pK_a's of organic acids have been reported to increase upon addition of MeCN,[13] that the concentrations of the non-reactive β-CDenH^+ and β-CDenH_2^{2+} are increased upon addition of MeCN."

Page 65 Figure 3.4.2.1 and Page 67 Scheme 3.4.2.1: All the compounds containing the norbornyl moiety are the *exo*-isomer. Figure 3.4.2.1 and Scheme 3.4.2.1 may give the impression that the compound isomerises to the *endo*-isomer during reaction, which is impossible.

Page 101: The physical properties of the synthetically prepared *p*-nitrophenylbutanoate and *p*-nitrophenylbenzoate esters were consistent with those of the commercially available products from Sigma-Aldrich. The physical properties of these compounds were not in themselves relevant to the project other than for the identification of each compound.

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Extra special thanks to Jen Weeks whose knowledge, help and support was a crucial ingredient in the production of this thesis and for her friendship and counselling service on matters unrelated to chemistry ☺.

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STATEMENT.

To the best of my knowledge and belief, this thesis contains no material that has been submitted for any other degree or diploma in any University, nor any material previously published or written by another author except where due reference is made in the text.

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Signed

Michael J. Field.
March 2000

ABSTRACT.

This thesis describes the synthesis and characterisation of some 6^A-amino-substituted β-cyclodextrins and studies of their inclusion complexes. Particular attention is given to the role of the host/guest complexes in the de-esterification of various *p*-nitrophenyl esters by 6^A-(2-aminoethylamino)-6^A-deoxy-β-Cyclodextrin (β-CDen). The reaction kinetics for the reactions between β-CDen and the two esters *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate were investigated in both 100% water solution and a mixed solvent system of 70:30 H_2O: Acetonitrile. Reaction rates for the de-esterification of both esters show a direct concentration dependence on the host β-CDen. No instances of Michaelis-Menten kinetics where detected and the presence of an alternative reaction pathway to the reaction products was believed to be present. The addition of acetonitrile to the reaction system saw a marked reduction in overall reaction rates due to the influence of acetonitrile on the transition state solvation and diminution of the cyclodextrin "hydrophobic effect" that facilitates the formation of host-guest complexes in aqueous solution.

The synthesis and characterisation of potential "molecular knot" modified β-cyclodextrins was also investigated. The reactions of 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin with the esters *p*-Nitrophenyl noradamantane-3-carboxylate and *p*-Nitrophenyl norbornan-2-acetate lead to the formation of the corresponding 6-aminohexylamino substituted cyclodextrins. The substituents of each of these derivatives are complexed within the annulus of the modified β-cyclodextrin. Addition of adamantane-1-carboxylate to solutions of these modified cyclodextrins causes the noradamantyl and norbornyl substituents to compete for complexation within the annulus with adamantane-1-carboxylate

The reaction of1,4-bis(*p*-nitrophenoxycarbonyl)-2,3-dimethyl cubane with 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin gives a cyclodextrin dimer. The cubanyl group is complexed within the annulus f one of the cyclodextrin entities giving a product that is asymmetric on the NMR time-scale. The addition of two equivalents of adamantane-1-carboxylate to the dimer generates a symmetric 1:2 host-guest complex where the cubanyl group has been displaced from the annulus and each cyclodextrin entity has complexed a molecule of adamantane-1-carboxylate. The pK_as of these

modified 6-aminohexylamino-β-cyclodextrins were determined by potentiometric titrations.

The synthesis of modified β-cyclodextrins mono-substituted with a multi-dentate metal ion binding substituent was also attempted. Synthetic attachment of acetic acid pendant "arms" to the primary and secondary nitrogens of 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin (β-CDen) and 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin (β-CDpn) to afford the modified cyclodextrins β-CD-ED3A and β-CDPD3A. These attempts were unsuccessful due to the formation of a cyclic piperazine intermediate compound (in the former case) that barred the addition of the acetic acid pendant arm to the nitrogen attached to the $C6^A$ carbon of the β-cyclodextrin. This compound was unsuitable for metal binding and alternative methods of arm attachment resulted in similar products.

The nitrogens attached to the $C6^A$ carbon of β-CDen and β-CDpn were non-nucleophillic and no evidence was ascertained to suggest that substitution of an acetic acid arm was substituted onto the $C6^A$ attached nitrogen in either the β-CDen or β-CDpn system. Mass spectra of the isolated modified β-cyclodextrin products from each of these systems suggested that the major products of the substitution reactions between α-chloroacetic acid and β-CDen and β-CDpn were not the tri-substituted multi-dentate systems required.

ABBREVIATIONS.

[X]	Concentration of species X (mol dm^{-3})
Å	angström (10^{-10} m)
amu	Atomic Mass Units
DMF	*N*, *N* dimethyl formamide
en	Ethylene diamine.
FAB-MS	Fast Atom Bombardment Mass spectrometry.
GPU	D-(+)-Glucopyranose unit.
K	Degrees Kelvin
K_a	Acid dissociation constant
m/z	Mass per charge
MW	Molecular Weight.
$NaClO_4$	Sodium perchlorate
NaOH	Sodium Hydroxide
NMP	*N*-methyl pyrolidin-2-one.
NMR	Nuclear Magnetic Resonance
P_2O_5	Phosphorus pentoxide
pK_a	$-\log_{10}[K_a]$
pn	1,6-Diamono propane
ppm	Parts per million
TLC	Thin Layer Chromatography
Tosyl	*p*-Toluene sulphonate.
Tosylate	*p*-toluene sulphonate
tren	6^A-(2-(bis(2-aminoethyl)amino)ethylamino)-6^A-deoxy-β-cyclodextrin.
Ts	Tosyl (*p*-toluene sulphonyl)
TsCl	*p*-Toluene sulphonyl chloride
β-CD	β-Cyclodextrin.
β–CD-ED3A	6^A-(2-aminoethylamino-*N, N', N'*-triacetic acid)-6^A-deoxy-β-cyclodextrin.
β–CD-PD3A	6^A-(3-aminopropylamino-*N, N', N'*-triacetic acid)-6^A-deoxy-β-cyclodextrin.

Chapter 1: Introduction

1.1 – General Structural Properties of Cyclodextrins

The homochiral oligosaccharides known as cyclodextrins[1] are produced by the action of the amylase of *Bacillus Macerans* on starch related compounds. The cyclodextrins (α, β and γ) (Figure 1.1) consist of either 6, 7 or 8 D-(+)-glucopyranose units joined together by α-(1,4)-linkages. Crystallographic X-ray studies of cyclodextrin hydrates show that each glucopyranose unit is held rigid in a $^4C_1$ chair conformation such that each molecule exists in an annular structure resembling a shallow truncated cone.[2] The narrow end of the cone is delineated by the primary hydroxyl groups on C6 and the wider end by the secondary hydroxyl groups on C2 and C3. The interior of

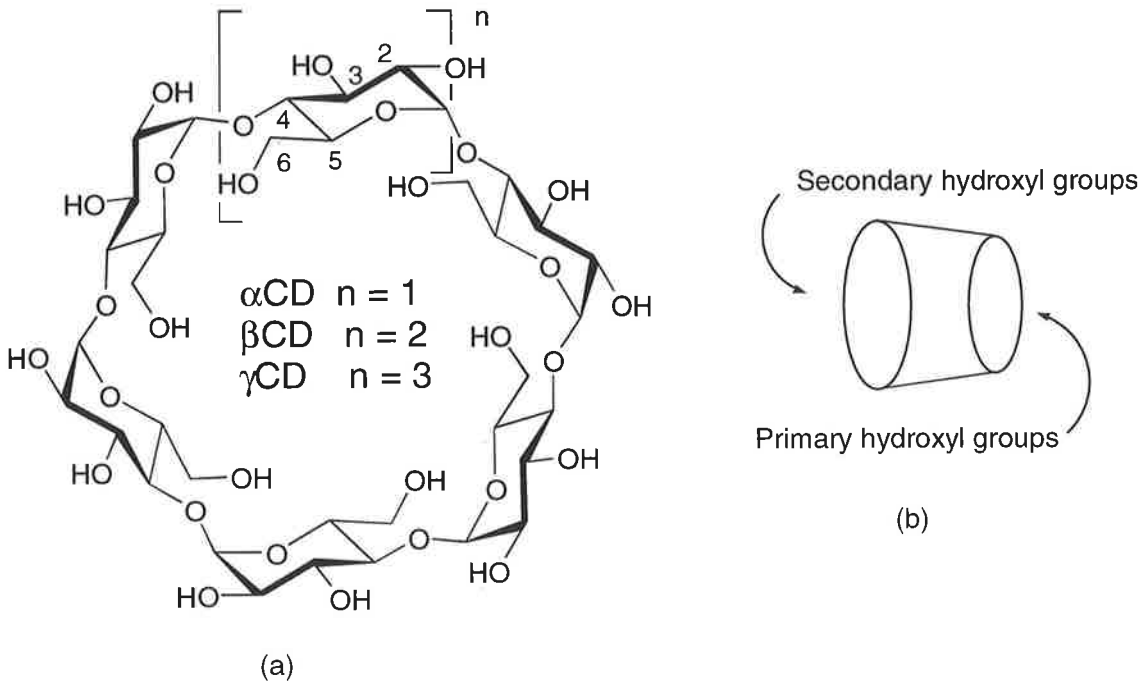


Figure 1.1: (**a**) The Cyclodextrins (α, β and γ) consist of 6, 7 or 8 D-(+)-glucopyranose units joined together by α-(1,4)-linkages. (**b**) Cyclodextrins are usually depicted as a truncated cone with the narrow end depicting primary hydroxyl groups attached at the C6 positions and the secondary hydroxyl groups attached to the C2 and C3 position as the wider end. When a group is joined by a single line to either the narrow or the wide end of the annulus this indicates substitution at C6 and at C2 or C3, respectively.

the annulus consists of a ring of glucosidic oxygens flanked on each side by methine groups, which direct their hydrogens inside the annulus thus creating a hydrophobic surface. It is the hydrophobic nature of the annulus that facilitates the formation of secondary bonded host-guest complexes between cyclodextrins and many organic molecules in aqueous solution. The ability to form such complexes has generated the large amount of interest in the chemistry of the cyclodextrins.

The physical data of the more common α-, β- and γ-cyclodextrin are given in Table 1.1. [1-3] The annulus depth is uniform for each of the cyclodextrins, being determined by the width of the glucose molecule (~8 Å), while the internal diameter of the annulus is determined by the number of glucopyranose units in the cyclodextrin. The diameter of the annulus is responsible for the selectivity in complex formation based on the size of the guest. The smaller annulus of α-cyclodextrin tightly encapsulates benzene analogues while β-cyclodextrin forms tight complexes with napthalenes. The larger γ-cyclodextrin annulus either encapsulates larger guests or sometimes two guests.

Table 1.1: Properties of α-, β- and γ-cyclodextrin

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Molecular Weight	972	1135	1297
H_2O Solubility(g/100 ml)	14.5	1.85	23.2
Specific rotation $[\alpha]^{D}_{25}$	150.5	162.5	177.4
Internal Diameter (Å)	~4.5	~7.0	~8.5
Cavity Depth (Å)	8.0	8.0	8.0

1.1.1 – Nomenclature

Viewing the primary hydroxyl end of β-cyclodextrin, each glucopyranosyl residue is labelled clockwise from A to G. The 'A' residue is determined by the substitution, which takes priority. Hence, each substitution is assigned a prefix indicating the number of the carbon in the glucose sub-unit to which the substituent is attached and the letter of the glucopyranose residue (Figure 1.1.1.1).

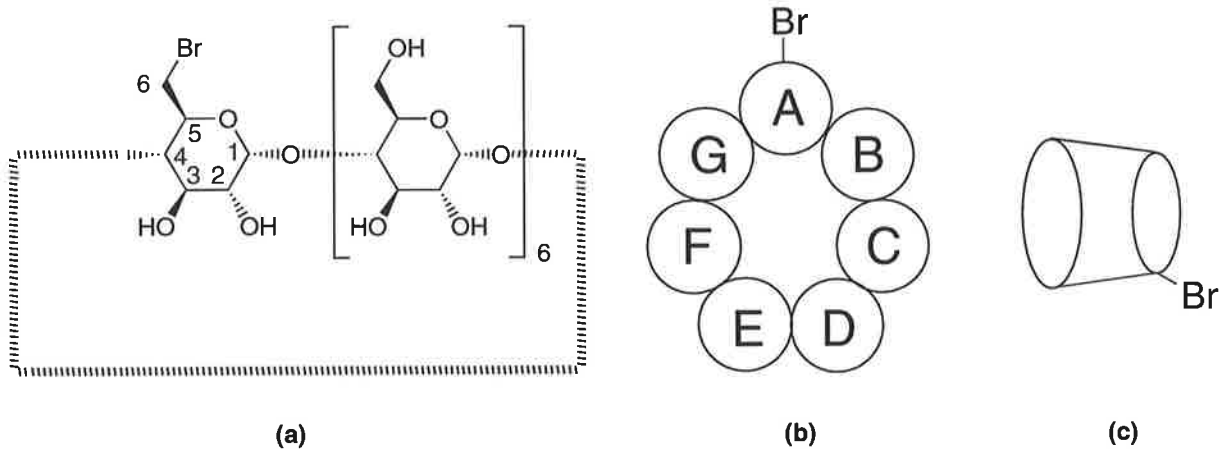
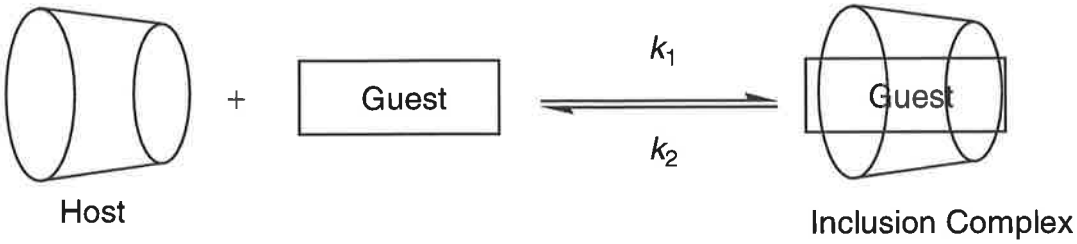


Figure 1.1.1.1: Schematic representation of the structure of 6^A-bromo-6^A-deoxy-β-cyclodextrin showing (**a**) the atom labelling of an individual glucose residue, (**b**) a view of the modified cyclodextrin from above the primary face showing the labelling of the glucose residues and (**c**) the truncated cone representation of the modified cyclodextrin that is used throughout this thesis.

When naming the cyclodextrins described in detail in Chapter 3, the following abbreviation system was employed. Firstly, the parent cyclodextrin is named (ie. β-cyclodextrin) followed by the abbreviated parent "blocking / terminal group" (ie. Norbornane = Norb, Noradamantane = Norad etc) followed by the number of carbons separating the two nitrogens of the ω-aminoalkylamino substituent (eg. six for the 6-aminohexylamino substituent). Eg. 6^A-(6-N-(2-bicyclo[2.2.1]hept-2-ylacetyl)-aminohexyl)amino-6^A-deoxy-β-cyclodextrin is abbreviated to β-CDNorb6.

1.2 – Cyclodextrin Host-Guest Complexes

The most interesting and exploitable characteristic of the cyclodextrins is their hydrophobic annulus. The annulus can encapsulate a guest molecule to form a host-guest complex (Scheme 1.2.1). These are usually 1:1 host-guest complexes but this is dependent on the shape and geometry of the guest and the cyclodextrin involved.[4] Many different guest molecules complex in the cavities of cyclodextrins and generally these guests incorporate a hydrophobic entity that complexes in the hydrophobic region of the cyclodextrin's annulus.[5, 6]



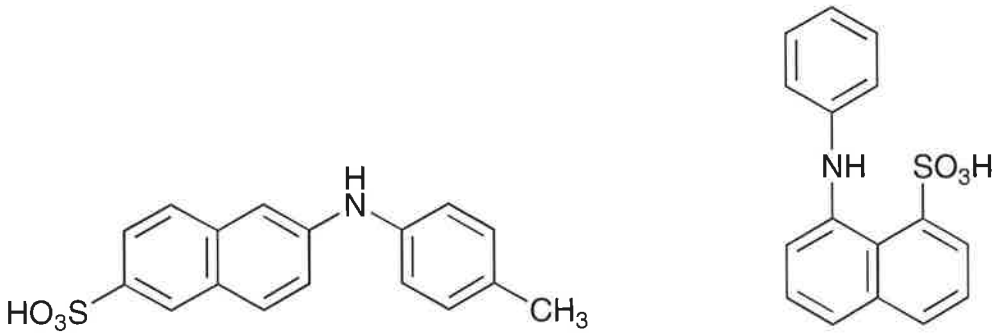
Scheme 1.2.1: Schematic representation of the complexation of a guest into the cyclodextrin annulus with a stability constant, $K = k_1/k_2$.

Several different hypotheses have been proposed to account for the formation of host-guest complexes with cyclodextrins. These hypotheses include (1) the release of "high energy" water from within the cavity on complexation of a guest;[7] (2) the relief of conformational strain energy of the uncomplexed cyclodextrin (especially α-cyclodextrin);[8] (3) the hydrophobic interactions between host and guest[9] and (4) the electrostatic interactions such as dipole-induced dipole, dipole-dipole, London dispersion force and hydrogen bonding.[10] A recent review discusses each of these effects and concludes that the main driving force for complexation of an organic molecule within the cyclodextrin annulus is the hydrophobic effect.[11] Recently, molecular modelling studies have shown that there is a linear relationship between the logarithm of the stability constant, *K*, and the maximum change (decrease) in the exposed hydrophobic surface area of the host as it is overlapped by the hydrophobic surface of a guest.[12] Structures of the host-guest complexes predicted by this model are consistent with the reported crystal structures of these complexes.

The variation in the size of the annuli of the cyclodextrins (α-, β- and γ-cyclodextrin) allows for discrimination in the complexation process based on size and shape of the guest. The cyclodextrins are also homochiral molecules. The chirality of the cyclodextrins leads to the formation of diastereomeric complexes with racemic guests. The diastereomeric complexes that are formed sometimes have different stabilities leading to chiral discrimination. However, in practise this discrimination is usually small due to the inherent symmetry of the cyclodextrin host.[2]

1.2.1 – Detecting Host-Guest Complexes

Detecting a host-guest complex may be achieved by a variety of analytical methods. Ultraviolet visible spectroscopy is extensively used in the study of host-guest complex formation.[13-16] Certain guest molecules exhibit changes in their UV/VIS spectra when complexed in the annuli of cyclodextrins. Stability constants can therefore be derived from measuring the variation of the guest absorption spectrum at a standard guest concentration and cyclodextrin concentration is varied. Some guest molecules also show fluorescence in the presence of cyclodextrins in aqueous media. The change in intensity of the guest fluorescence is directly proportional to the extent of host-guest complexation. Napthalenesulfonic acids such as 6-(4-toluidino)-2 -napthalenesulfonic acid (Figure 1.2.1.1(a)) and 1-anilino-8-napthalenesulfonic acid (Figure 1.2.1.1(b)) both fluoresce in organic solvents but in water the fluorescence is quenched. Addition of a cyclodextrin able to form a host-guest complex with 6-(4-toluidino)-2 -napthalenesulfonic acid and 1-anilino-8-napthalenesulfonic acid causes these acids to fluoresce in aqueous solution.[17, 18]



(a) (b)

Figure 1.2.1.1: Organic napthalene based sulphonic acids (a) 6-(4-toluidino)-2 -napthalenesulfonic acid and (b) 1-anilino-8-napthalenesulfonic acid which fluoresce in aqueous solution when complexed inside the annulus of β-cyclodextrin.

Potentiometric titrations are used to detect complexation between cyclodextrins and organic acids.[1, 19] The pK_as of organic acids such as phenols and carboxylic acids change on host-guest complexation with a cyclodextrin.[19] For example, 4-hydroxybenzoic acid has a pK_a of 4.53, but when titrated in the presence of α-cyclodextrin the apparent pK_a is 1.25 units higher. The reverse is seen for 4-nitrophenol, which has a pK_a of 7.09, but in the presence of α-cyclodextrin, the apparent pK_a is one unit lower.[19]

Optical rotation was utilised by Breslow *et al*,[20] to determine the stability constants of a series of host-guest complexes in a solution of 5 mM β-cyclodextrin in DMSO. The stability constants were determined from the variation of optical rotation as a function of added guest concentration.

High performance liquid chromatography (HPLC) is often used to study the complexation of guest molecules by cyclodextrins.[21] A steady state concentration of the guest is passed through an appropriate HPLC column. On injection of the cyclodextrin onto the column, a host-guest complex is formed between the cyclodextrin and guest. A complexed guest is more rapidly eluted than a non-complexed guest and is detected at a shorter elution time. This procedure is repeated for various concentrations of guest to afford a relationship between the number of moles of guest bound per mole of host present (r) and the concentration of guest ([L]). The stability constants can be found from the intercept of the r/[L] axis when a plot of r/[L] versus [L] is constructed.[21] This determination depends on the host-guest complex being labile.

Nuclear magnetic resonance (NMR) spectroscopy is a widely used analytical technique for detecting host-guest complexes.[22] Upon complexation with a cyclodextrin, the chemical shifts of the guest may change. If this occurs, measurement of the change in chemical shifts between the ^{1}H and ^{13}C signals of complexed and non-complexed guests vary with the total concentration of cyclodextrin. The stability of the associated host-guest complex may be determined from these chemical shift variations. Recent NMR studies[23] investigated the complex formation of various benzoic acids with α-CD. The chemical shifts of protons attached to the C3 and C5 carbons of the glucose units (which are orientated such that they point into the annulus) move substantially upfield upon addition of the guest compound. This effect is attributed to their proximity to the aromatic ring of the guest. This is consistent with the aromatic ring shielding the C3 and

C5 protons. The shielding effect on the chemical shifts of the C1, C2 and C4 is minimal due to their orientation away from the annulus interior (Figure 1.2.1).

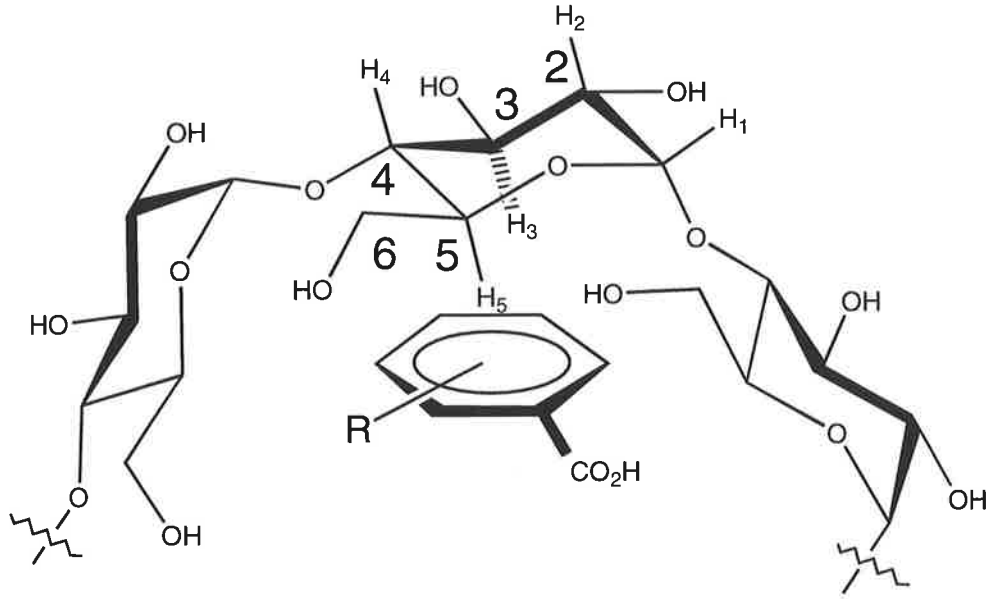


Figure 1.2.1: Schematic representation of the shielding effect visible by NMR spectroscopy between C5 and C3 protons of the cyclodextrin with complexed guest. The H3 and H5 protons move substantially upfield on complexation.[23]

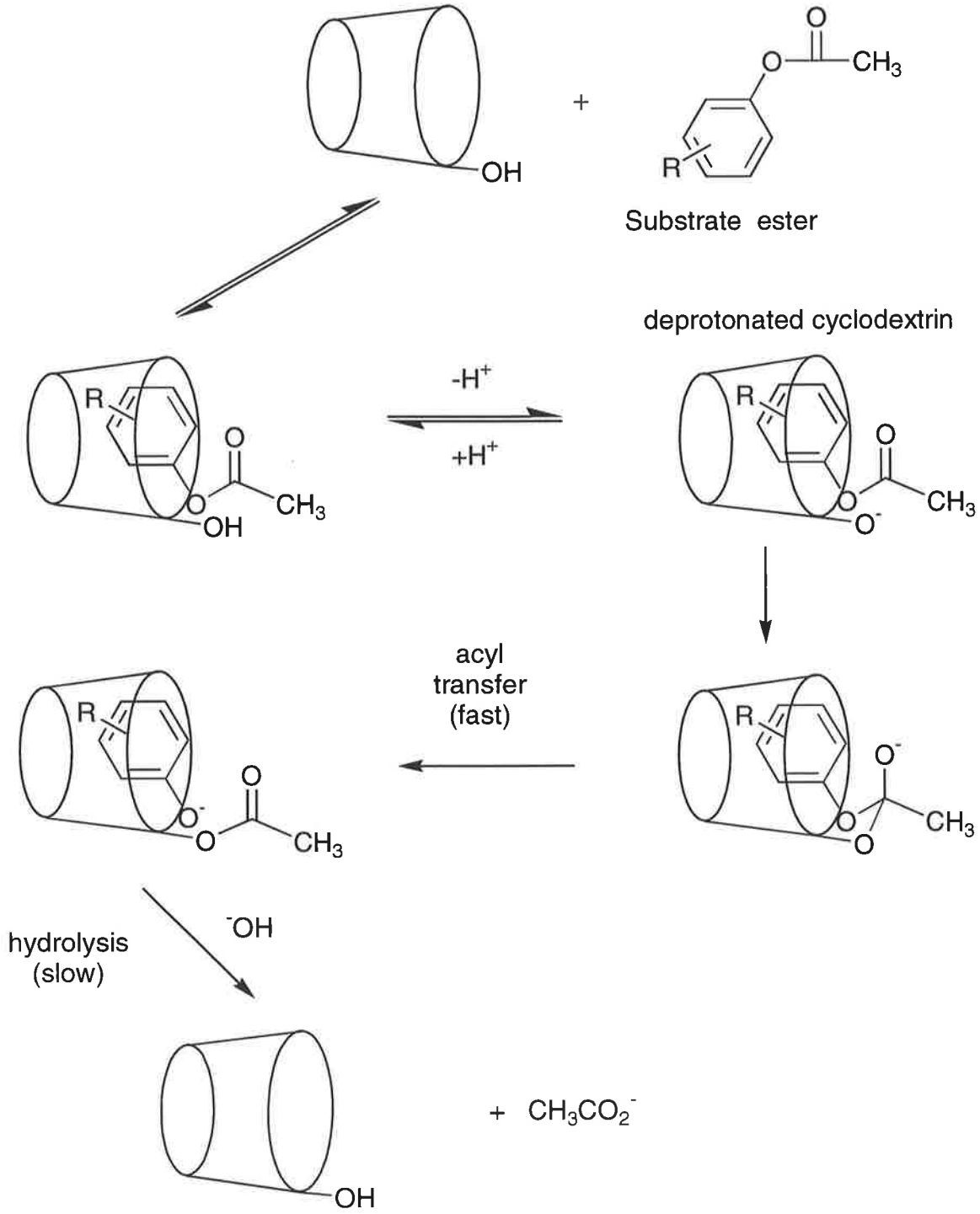
NMR spectroscopy is also used to determine the orientation of the guest within the cyclodextrin annulus.[22] Techniques such as NOE, HSQC, HMBC and HETCOR experiments can be used to determine the locations of guest atoms within the annulus. The most useful technique, and the one used extensively in the work described in this thesis, is the ^{1}H 2D ROESY NMR experiment. ROESY spectra identify through-space dipolar interactions between the protons of a guest molecule inside the cyclodextrin annulus and the protons of the annulus interior. Thus, from this data, the position of the guest within the annulus can be determined.

1.3 - Cyclodextrins as Enzyme Mimics

1.3.1- Host-Guest Complexation and Catalysis of Nitrophenyl Esters

Cyclodextrins are well known for their ability to catalyse the reactions of guest molecules through host-guest complexes. For this reason, they have been widely studied as models for enzyme binding and catalysis.[7, 24] Features of enzyme-catalysed reactions such as kinetic saturation, substrate specificity, substrate-enzyme complexation and competitive inhibition have all been mimicked by cyclodextrins.[25-27] The catalytic action in enzymes proceeds through either covalent or non-covalent catalysis and numerous examples of cyclodextrins accelerating or catalysing such reactions exist.

Cyclodextrins catalyse the base hydrolysis of phenyl esters through the formation of a transient *O*-acyl cyclodextrin (Scheme 1.3.1.1). Bender *et al* extensively studied the de-esterification of phenyl acetates by cyclodextrins throughout the 1960's and this pioneering work established the mechanism for this catalysis.[1, 16, 24, 27] A deprotonated C2 hydroxyl group ($pK_a \approx 12$) makes a nucleophilic attack on the substrate ester, previously complexed within the annulus of the cyclodextrin, to give an *O*-acyl cyclodextrin and a phenoxide. The reaction conditions (usually pH $\geq$ 10.5) are such that there is a slow hydrolysis of the *O*-acyl cyclodextrin to regenerate the cyclodextrin catalyst. This mechanism resembles that of the hydrolysis reactions catalysed by the enzyme chymotrypsin. Chymotrypsin has a binding pocket adjacent to its active site capable of binding aryl groups such as phenylalanine and tyrosine and a hydroxyl group of a serine residue that is activated as a nucleophile through a charge relay network.[28]



Scheme 1.3.1.1: Schematic representation of the catalytic de-esterification of nitrophenyl acetates by β-cyclodextrin. A C2 hydroxyl group is deprotonated ($pK_a \approx 12$) to generate the active catalyst.

Generally, the greatest acceleration effects of de-esterification occur when α-cyclodextrin is the catalyst and the guest is a *m*-substituted phenyl ester (Table 1.3.1).[29, 30] However, the de-esterification of *m*- and *p*-substituted phenyl esters are both accelerated in the presence of α-, β- and γ-cyclodextrin. The magnitude of the rate enhancements and the difference between the reactivities of *m*- and *p*-substituted phenyl esters decrease as the size of the cyclodextrin annulus increases. The difference in reactivity of the different cyclodextrins and their guests has been correlated with differences in transition state binding.[31] Recent modelling studies utilising a molecular dynamics methodology gave results that were in agreement with the experimental data and support this observation.[32, 33]

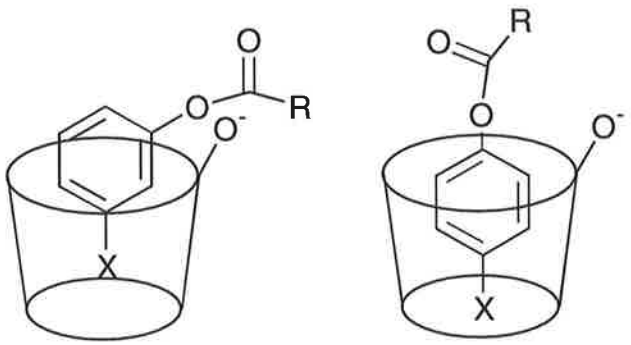
Table 1.3.1: Selected rate acceleration data for phenyl acetates in the presence of cyclodextrins.[27] (k_{un} refers to the rate of de-esterification in the absence of a cyclodextrin, while k_{obs} refers to the rate of de-esterification in the presence of a cyclodextrin.)

		α-CD		β-CD		γ-CD	
Ester	k_{un} 10^{-4} s^{-1}	$k_{obs}10^{-2}$ s^{-1}	k_{obs}/k_{un}	$k_{obs}10^{-2}$ s^{-1}	k_{obs}/k_{un}	$k_{obs}10^{-2}$ s^{-1}	k_{obs}/k_{un}
m-tolyl	6.96	2.70	**39.0**	1.140	16.0		
p-tolyl	6.64	0.187	2.8	0.443	6.7		
m-*t*-butylphenyl	4.90	11.10	**227.0**	12.100	**247.0**	2.65	54.0
p-*t*-butylphenyl	6.07	0.102	1.7	0.135	2.2	2.51	41.0
m-chlorophenyl	19.10	21.50	**113.0**	3.49	18.0	1.48	7.7
p-chlorophenyl	15.20	0.453	3.0	1.55	10.0	1.34	8.8
m-nitrophenyl	46.40	47.90	**103.0**	25.00	**54.0**	4.65	10.0
p-nitrophenyl	69.40	1.79	2.6	4.66	6.7	4.33	6.2

1.3.2 - Geometric Requirements of Host-Guest Complexation

The cyclodextrin annulus possesses a geometry that facilitates the de-esterification of *m*-substituted phenyl esters. For *m*-substituted phenyl esters, very little molecular movement is needed for reaction to occur as the esters are bound within the annulus such that the ester functionality is positioned in close proximity to the ionised secondary hydroxyl group (Figure 1.3.2.1(a)). The *p*-substituted phenyl esters, once bound in the annulus, are positioned further away from the active hydroxyl group

(Figure 1.3.2.1(b)) and some movement within the host-guest complex is needed for de-esterification to occur. This leads to a greater enhancement of the rate of reaction for *m*-substituted phenyl esters relative to that of the *p*-substituted analogues in the presence of cyclodextrins.



(a) (b)

Figure 1.3.2.1: Representation the cyclodextrin host-guest complexes of the (a) *m*- and (b) *p*-substituted phenyl esters showing the geometric orientation of the ester functionality in relation to the ionised secondary hydroxyl group.

1.3.3 – Chain Length Effects on the Host-Guest Complexes of Phenyl Esters With Cyclodextrins

The formation of a host-guest complex between a cyclodextrin and a guest ester is dependent on the hydrophobicity of the guest. Small phenyl esters form tight host-guest complexes with the cyclodextrin and exhibit large de-esterification accelerations due to the geometrical positioning of the reactive groups in such host-guest complexes. Recent studies[30, 34] of the kinetics of de-esterification of a range of long chain (six plus carbons) aryl *m*- and *p*-substituted phenyl esters (Figure 1.3.3.1) by α- and β-cyclodextrin emphasise the importance of the hydrophobicity of the substrate ester. The *m*- and *p*-substituted phenyl esters have different initial host-guest complexation and transition states depending on the length of the alkyl sub-unit of the guest esters. For *m*-substituted phenyl esters, an initial host-guest complex with β-cyclodextrin in which the aryloxy moiety is complexed in the β-cyclodextrin annulus (Figure 1.3.3.2(a)) is reported. Due to this orientation being very efficient, de-esterification occurs through a similar transition state. For *p*-substituted phenyl esters, the kinetics of de-esterification vary significantly with the length of the alkyl chain in a way that suggested both the initial complexation and the transition state both proceed through alkyl group

complexation (Figure 1.3.3.2(b)). The de-esterification of the *m*-substituted phenyl esters had little or no dependence on the length of the alkyl chain (at least to six carbons in length) consistent with the complexation of the aryl group being preferred over alkyl complexation. Thus, the rates of de-esterification of substituted phenyl esters

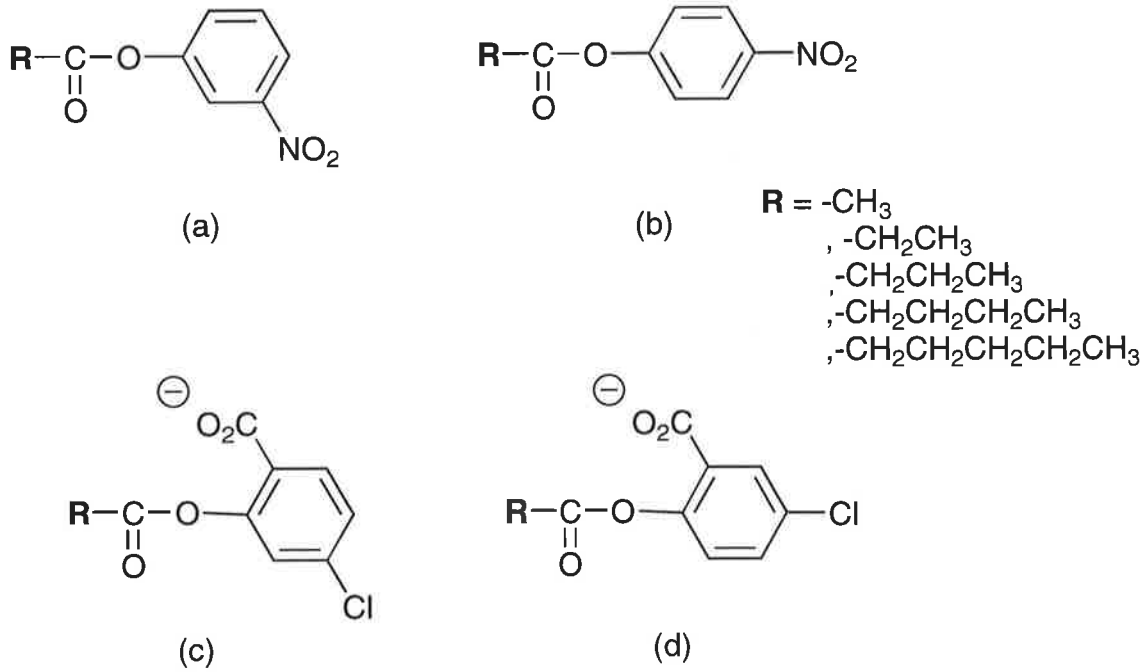


Figure 1.3.3.1: *m*- and *p*-substituted long chain phenyl esters used to investigate the effects of lengthening the alkyl portion of the ester on the rate of de-esterification and host-guest complex formation.[30, 34]

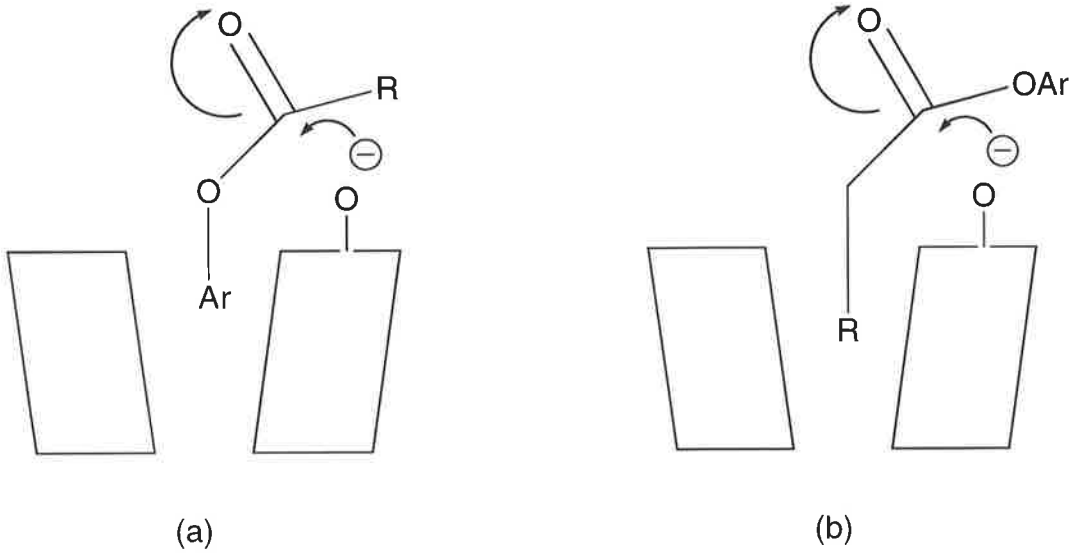
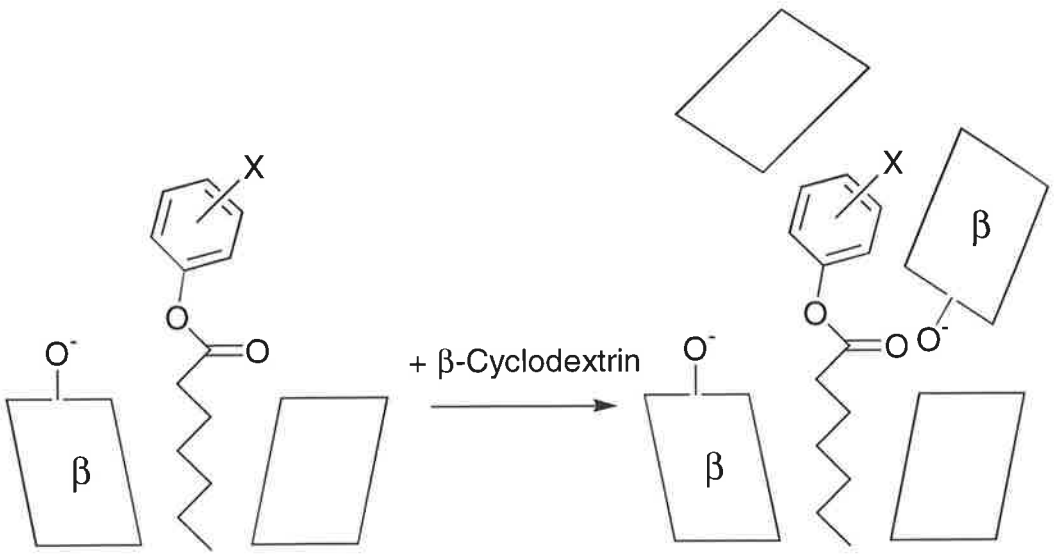


Figure 1.3.3.2: The different modes of complexation of *m*- and *p*-nitrophenyl esters. (**a**) Represents esters with short alkyl chains, which form host-guest complexes through aryl complexation and (**b**) represents esters with long alkyl chains which host-guest complexes through alkyl complexation.

are accelerated for the *m*-substituted phenyl esters to a greater extent than those of the *p*-substituted analogues. For *p*-substituted phenyl esters de-esterification rates reflect the length of the alkyl chain and its associated hydrophobicity which increases with the length. Esters based on (c) and (d) in figure 1.3.3.1 show little dependence on the alkyl chain length. This is consistent with this family of esters complexing in the cyclodextrin annulus through acyl insertion only which emphasises the efficiency of this mode of complexation.

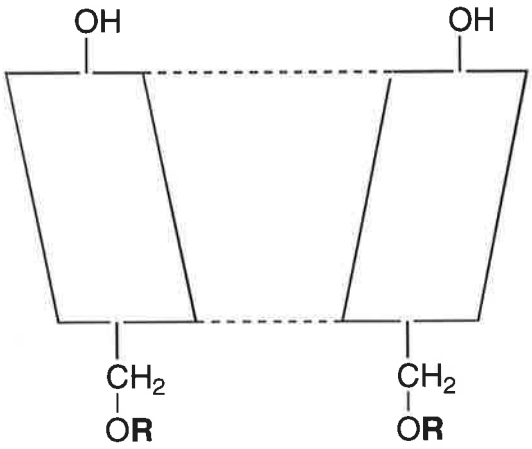
Long (6-12 carbons) alkyl chain phenyl esters have a secondary de-esterification pathway through the formation of a 2:1 host-guest complex (Figure 1.3.3.3). If the alkyl portion of the ester is sufficiently long and hydrophobic it will complex in the cyclodextrin annulus in preference to the aromatic portion of the ester. Such an orientation of the guest positions the aryl portion of the ester outside the annulus of the primary cyclodextrin and away from the ionised secondary hydroxyl reactive centre. This allows the encapsulation of the aryl portion of the ester by a second cyclodextrin and leads to the subsequent de-esterification of the guest ester.



1:1 host-guest complex 2:1 host-guest complex.

Figure 1.3.3.3: The 2:1 β-cyclodextrin:ester complex that also leads to de-esterification of the guest at high concentration of β-cyclodextrin. As shown, the alkyl complexation allows a second β-cyclodextrin to encapsulate the aryl group if this group protrudes far enough out of the first β-cyclodextrins annulus.

The de-esterification of long chain alkyl substituted phenyl esters in the presence of hydroxypropyl-β-cyclodextrin (Hp-β-CD, Figure 1.3.3.4) is an example of this. The formation of a 2:1 host-guest complex is responsible for the increased rate of de-esterification seen in the experimental data (Table 1.3.3.1) for *p*-substituted phenyl esters with eight or more carbons in the alkyl chain.



Hp-β-CD, **R** = $CH_2CH(OH)CH_3$

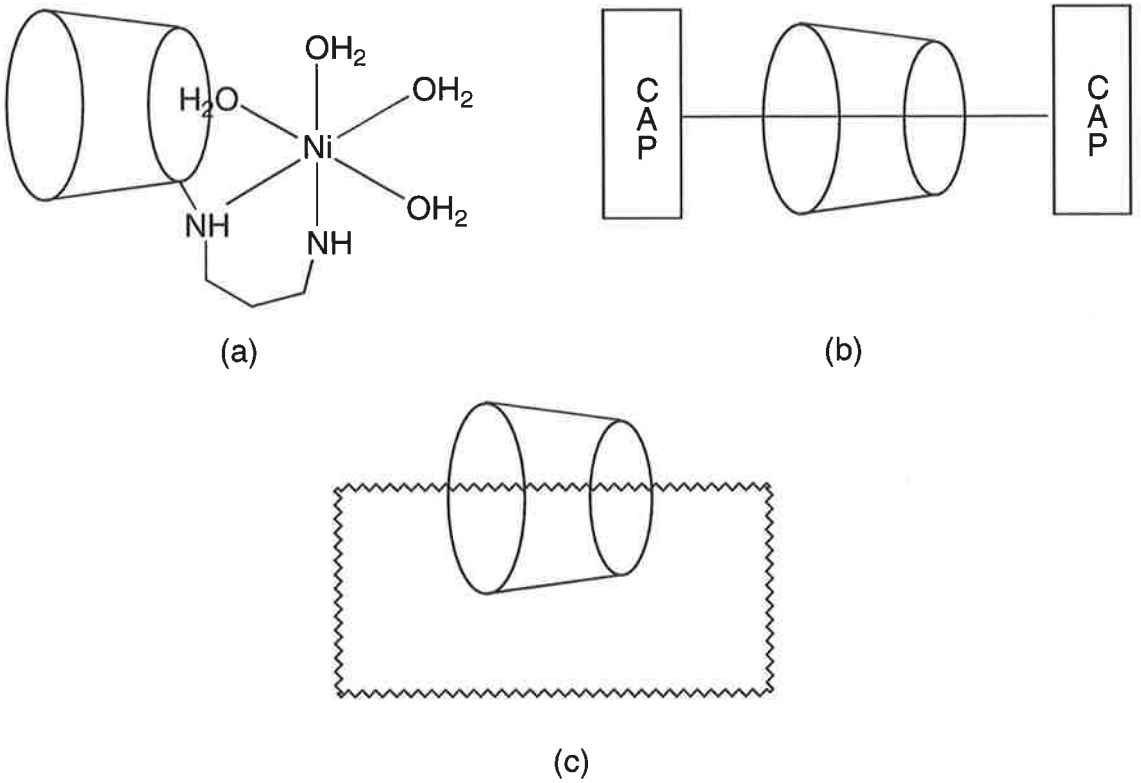
Figure 1.3.3.4: Hydroxypropyl-β-cyclodextrin (Hp-β-CD, where each primary hydroxyl group is replaced by a 2-hydroxypropyl group), which was shown to produce a productive 2:1 host-guest complex on addition of guest esters with extremely long alkyl carbon chains.[30]

Table 1.3.3.1: Rate accelerations for the cleavage of *m*-nitrophenyl and *p*-nitrophenyl alkanoates by Hp-*β*-CD. C_x refers to the number of consecutive, saturated carbons in the straight chain alkoxy portion of the nitrophenyl ester (ie C_2 = -O-CH_2-CH_3). k_c = The rate constant for de-esterification in the presence of Hp-β-CD. k_u = The rate constant for de-esterification in the absence of Hp-β-CD.

Ester	k_c/k_u	Ester	k_c/k_u
m-nitrophenyl esters		*p*-nitrophenyl esters	
C_2	19.0	C_2	5.25
C_3	13.7	C_3	3.74
C_4	11.1	C_4	3.36
C_5	8.07	C_5	2.44
C_6	6.60	C_6	2.10
C_7	4.67	C_7	1.75
C_8	3.66	C_8	1.95
C_9	2.36	C_9	2.23
C_{10}	1.62	C_{10}	2.10

1.4- Modified Cyclodextrins.

While many types of enzymatic behaviour have been studied and mimicked using unmodified cyclodextrins, their inherent symmetry and lack of specific complexing groups limit their usefulness as enzyme mimics. Modified cyclodextrins may be tailored to complex selected guests. Modification also provides avenues into new areas of supramolecular chemistry.[2] Using now standard synthetic techniques, the production of metallocyclodextrins,[35] rotaxanes and catenanes[36] (Scheme 1.4.1) and surface monolayers of modified cyclodextrins[37] is possible.

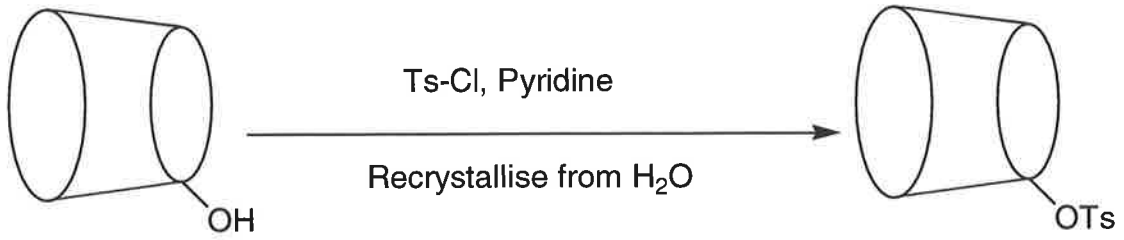


Scheme 1.4.1: It is possible to synthesise a wide range of supramolecular compounds from the cyclodextrins such as (a) metallocyclodextrins, (b) rotaxanes, where "CAP" is a group too large to pass through the cyclodextrin annulus and (c) catenanes, where the "squiggly" square represents another macrocycle.

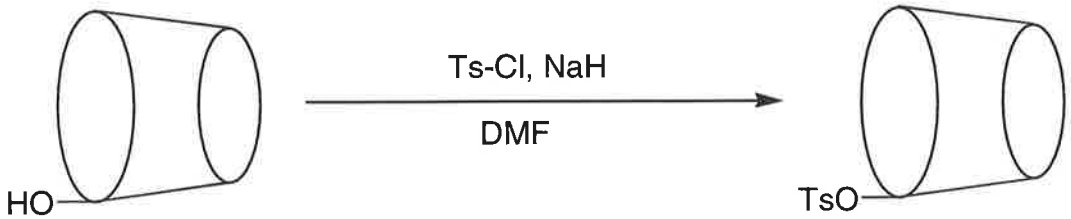
The cyclodextrins provide convenient templates onto which functional groups and various other substituents can be assembled with controlled geometry. This results in improved molecular recognition and procedures for chemical separation through complexation of guest molecules. The introduction of a range of substituents into modified cyclodextrins has been the basis of extensive catalytic studies.[38, 39] Modification also facilitates the study of the photochemistry of cyclodextrin complexes, through which the enhancement of guest reactivity occurs.[40] Light harvesting assemblies[40-42] and photochemically active complexes have also been constructed.[40]

1.4.1 – Modifying the Hydroxyl Groups of Cyclodextrins

Modification of cyclodextrins may be achieved through manipulation of the hydroxyl groups. Substitution of either a primary (Scheme 1.4.1.1(a)) or secondary hydroxyl (scheme 1.4.1.1(b)) group greatly extends the range of reactions of the cyclodextrins. This is exemplified by the tosylation of a hydroxyl group on the primary face of the cyclodextrin (Scheme 1.4.1.1).[43] This is one of the most important aspects of modifying the cyclodextrins and hence there have been several different methods reported to achieve the same result.[43-47]



(a) primary hydroxyl substitution.



(b) secondary hydroxyl substitution.

Scheme 1.4.1.1: Mono-tosylation reactions of hydroxyl groups of β-cyclodextrin. The shown examples are methods for (a) mono-tosylation of a primary hydroxyl group[43, 48] and (b) mono-tosylation of a secondary hydroxyl group.[49]

Synthetic manipulation of the cyclodextrin-tosylates allows the addition of other groups to the edge of the cyclodextrin annulus through standard nucleophilic substitution of the tosyl group.[50] The mono-tosylated cyclodextrins, through reactions with nucleophiles, afford the corresponding de-oxy cyclodextrins, such as halides (when treated with sodium halide), azides (sodium azide), amines (ammonia solution) and polyamines (by reaction with polyamines). Most functional groups have been attached

to cyclodextrins to afford specialised reactivity. The introduction of any basic residue onto the cyclodextrin makes the isolation and purification of modified cyclodextrins a great deal easier through chromatography. When the hydroxyl groups of the cyclodextrin are replaced with an easily protonated group (such as an amine) ion exchange chromatography is commonly exploited to isolate the modified cyclodextrin.

The substitution of the tosyl group for a substituted (or non-substituted) nitrogen-containing group is a standard way of increasing the reactivity of the cyclodextrins. By replacing the tosyl group with an amino group (Figure 1.4.1.2), the advantages of modifying the cyclodextrins are apparent. The aqueous solubilities of the conjugate acids of Figure 1.4.1.2(a) and Figure 1.4.1.2(b) are approximately 70 g/100 cm^3 in the

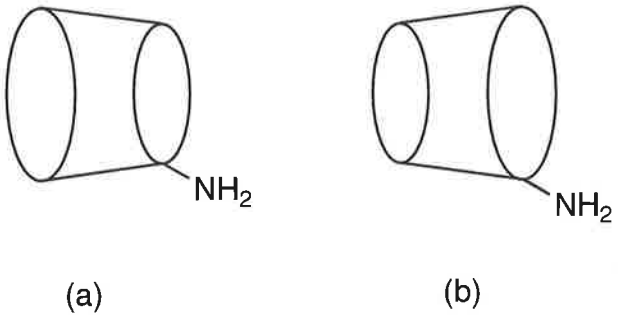


Figure 1.4.1.2: Replacing a hydroxyl group of β-cyclodextrin with an amine to give (a) 6^A-amino-6^A-deoxy-β-cyclodextrin and (b) 3^A-amino-3^A-deoxy-β-cyclodextrin.

former case and greater than 70 g/100 cm^3 in the latter case. Thus, the solubility of the cyclodextrin increases from 1.85 g/100 cm^3 for β-cyclodextrin to greater than 70 g/100 cm^3 with the change to only one hydroxyl group.[43] The pK_as of the conjugate acids of these amino-β-cyclodextrins in aqueous media are 8.7 and 7.5 respectively.

Previous studies [13, 14] found that the initial complexation of the guest esters and the formation of the transition state are distinct processes, and do not necessarily produce similar host-guest structures. Studies of the catalytic properties of 6^A-amino-6^A-deoxy-α-cyclodextrin and its β-cyclodextrin analogue (Figure 1.4.1.2(a))[51] in the de-esterification of *m*- and *p*-nitrophenyl acetate investigated the orientation of phenyl esters in the annuli of α- and β-cyclodextrin (Table 1.4.1).

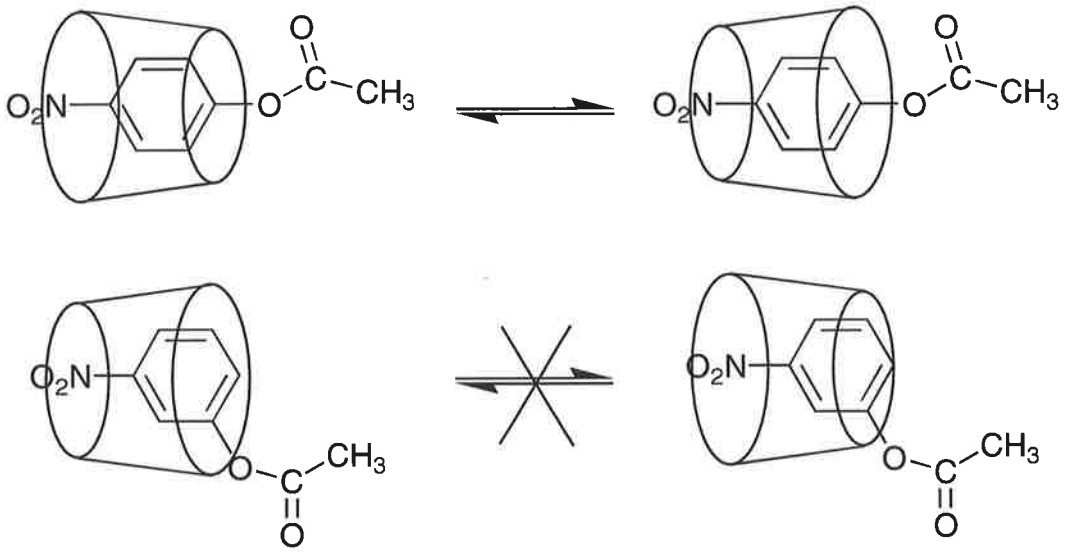


Figure 1.4.1.3: The different orientations of complexation for *m*- and *p*-nitrophenyl acetate. The *p*-substituted phenyl acetate complexation is able to equilibrate between the two host-guest orientations shown above, while the *m*-substituted phenyl acetate is not.

This study reported that the k_c values for reactions of *m*-nitrophenyl acetate complexed by the amino-substituted α- and β-cyclodextrins was approximately 30% less than those for the corresponding complexes of the unmodified cyclodextrins. This was contrary to the results that would be expected if the amino-substituents reacted directly with the complexed *m*-nitrophenyl acetate. These results were in accordance with the hypothesis, originally proposed by VanEtten *et al.*,[27, 52] that catalysis of the de-esterification of *m*-nitrophenyl acetate by either α- or β-cyclodextrin involves a host-guest complex in which the oxycarbonyl group of *m*-nitrophenyl acetate is in the vicinity of the wider end of the cyclodextrin cavity, where it reacts with a deprotonated secondary hydroxyl group of the cyclodextrin (Figure 1.3.3.2). The amino-substituents located at the other end of the annuli had little effect on the rate of reaction of complexed *m*-nitrophenyl acetate.

By contrast with the situation with *m*-nitrophenyl acetate, the amino-substituent of the modified β-cyclodextrin markedly increased the rate of reaction of the complexed *p*-nitrophenyl acetate. The k_c value was four times larger than was observed for the reaction of *p*-nitrophenyl acetate with unmodified β-cyclodextrin. The similarity between the dissociation constant, $K_{diss,}$ values of the complexes of *p*-nitrophenyl acetate with β-cyclodextrin and the corresponding amine indicated that the amino-substituent had little

effect on the complexation process. These results were consistent with one mode of inclusion of *p*-nitrophenyl acetate having the oxycarbonyl group positioned in the vicinity of the primary hydroxyl groups of β-cyclodextrin. While this complex of β-cyclodextrin is catalytically inactive and hitherto has remained undetected for that reason, in the analogous complex of the amino-substituted β-cyclodextrin, the nucleophilic amino group is in close proximity to the oxycarbonyl group of *p*-nitrophenyl acetate and reaction proceeds. Because the amino group of an amino-substituted cyclodextrin is unprotonated at a much lower pH than are the secondary hydroxyl groups of a cyclodextrin (eg 6^A-(1,2-diaminoethyl)-6^A-deoxy-β-cyclodextrin (β-CDen) has pK_as of 9.42 and 5.70, while β-cyclodextrins secondary hydroxyl groups have pK_as ≈ 12), nucleophilic attack by the amino group occurs at a much lower pH than does nucleophilic attack by a deprotonated secondary hydroxyl group.

Table 1.4.1: Dissociation constants (K_{diss}) for host-guest complex formation between modified cyclodextrins and *m*- and *p*-nitrophenyl acetate and rate constants (k_c) for the de-esterification of each by cyclodextrins.[51]

Cyclodextrin	*p*-nitrophenyl acetate		*m*- nitrophenyl acetate	
	k_o (s^{-1})		**k_o (s^{-1})**	
-	0.0023 [A]		0.0022 [A]	
	k_c (s^{-1})	**K_{diss} (mol dm^{-3})**	**k_c (s^{-1})**	**K_{diss} (mol dm^{-3})**
α-CD	undetected	undetected	0.17[A]	0.012[A]
α-CD-NH_2	0.009[A]	0.01[A]	0.12[A]	0.008[A]
β-CD	0.012[A]	0.009[A]	0.05[A]	0.006[A]
β-CD-NH_2	0.048[A]	0.008[A]	0.004[A]	0.003[A]

[A] In 0.1 mol dm^{-3} borate buffer at pH 10, 298.2 K.

1.4.2 – Metallocyclodextrins.

Complexes between a modified cyclodextrin and a metal ion are called binary metallocyclodextrins.[35] These binary metallocyclodextrins can catalyse reactions beyond that of modified cyclodextrins. Binary metallocyclodextrins (such as [Ni (β–CDpn)]$^{2+}$, Scheme 1.4.2.1) offer exciting prospects in the areas of chiral discrimination[40], metallo-enzyme mimicking[35] and as light harvesting assemblies.[41, 42] Binary metallocyclodextrins can complex guests which may also co-ordinate with the metal centre to form ternary metallocyclodextrins. This provides the opportunity to examine metal centre and cyclodextrin interactions in the formation of ternary metallocyclodextrins as is exemplified by the binary metallocyclodextrin [Ni(βCDpn).$(H_2O)_4$]$^{2+}$ (Figure 1.4.2.1) complexation of phenylalanine anion. The binary metallocyclodextrin showed a greater than six fold enantioselectivity for binding (S)-phenylalanine.

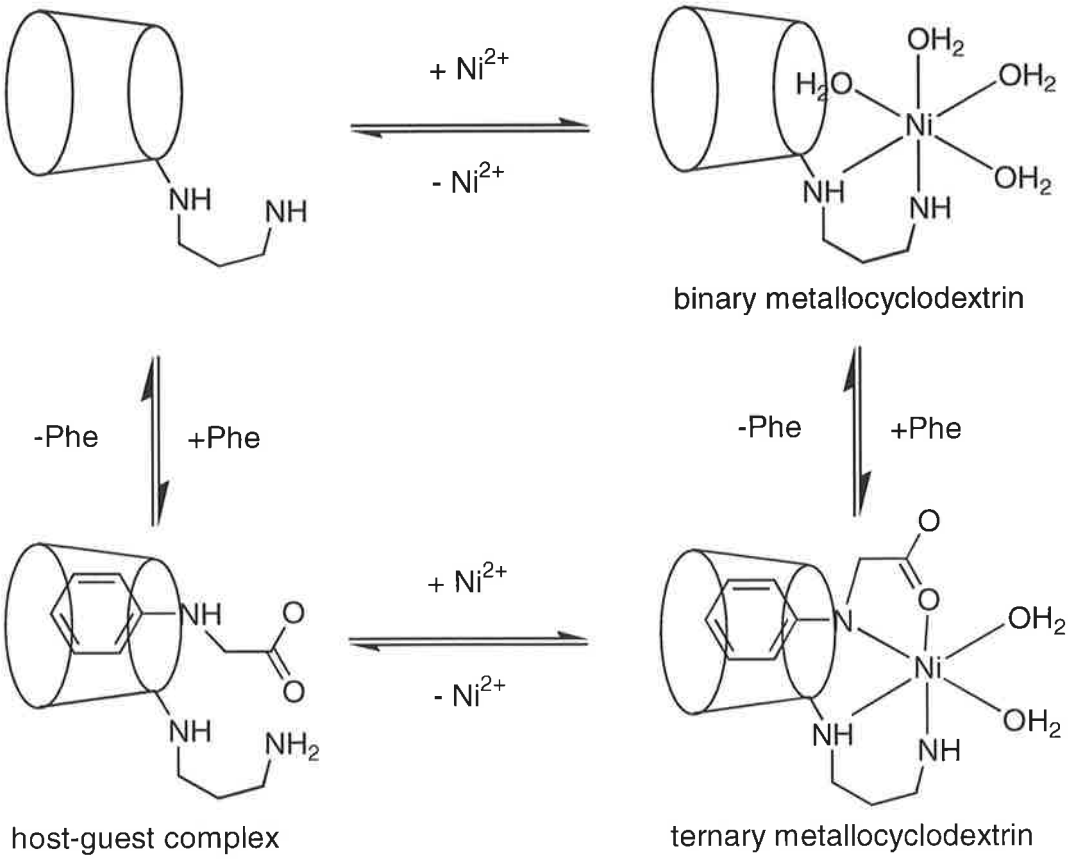


Figure 1.4.2.1: 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin (β-CDpn), the binary metallocyclodextrin tetraaqua-6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin-nickel(II) and the ternary metallocyclodextrin formed on complexation of phenylalanine anion (Phe^-).

Some ternary metallocyclodextrins are also very efficient light harvesting assemblies.[41] A light harvesting assembly is a molecular device that converts ultra-violet (UV) light into visible light. A light harvesting assembly usually consists of two major parts, the antenna (which must possess a chromophore) and the emission centre. The antenna's chromophore collects UV light energy, excitation energy is transferred to the metal centre, which by accepting this energy becomes excited itself. The excited metal centre undergoes emissive decay at a longer wavelength back to the ground state (Figure 1.4.2.2). An example of such a device is 6^A-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane)-β-cyclodextrin-europium(III) (Figure 1.4.2.3).[41, 53] In benzene solution, benzene complexes in the β-cyclodextrin annulus of 6^A-(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane)-β-cyclodextrin-europium(III) and acts as the antenna. When radiated with UV light, benzene becomes excited and transfers this excitation energy to the europium(III) ion which becomes excited itself and emits visible red light at λ_{ex} = 394 nm.

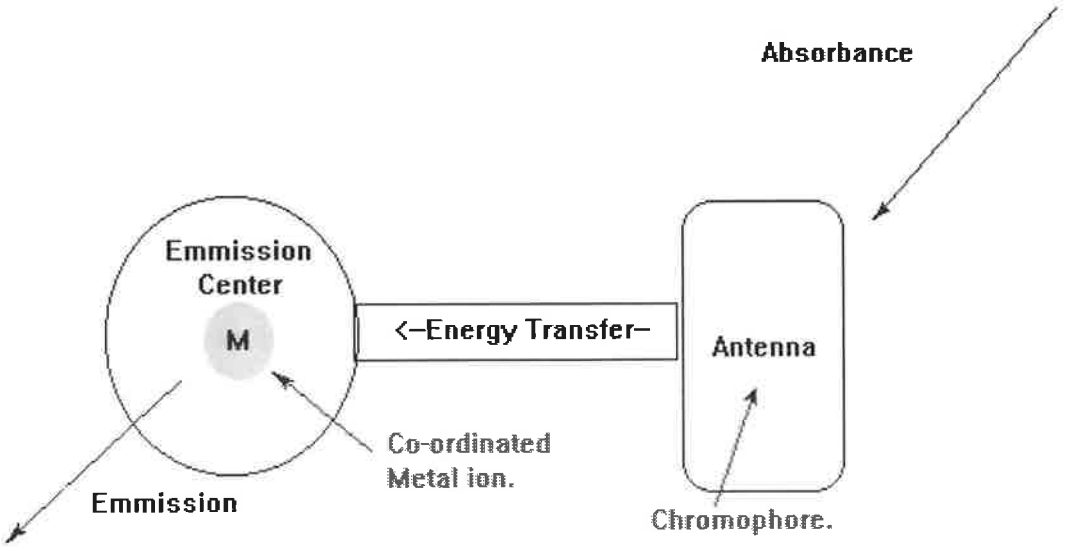


Figure 1.4.2.3: Schematic representation of a light harvesting assembly. Shown are all the major components such as the antenna, metal ion, and energy transmission pathways.

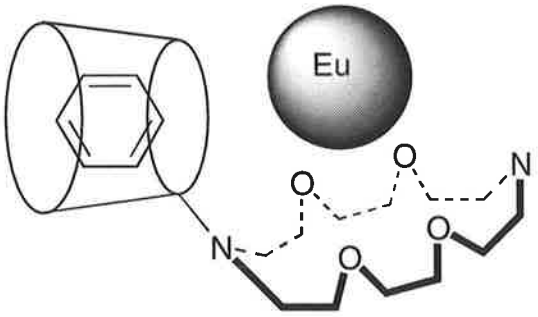


Figure 1.4.2.3: This ternary metallo-β-cyclodextrin has been shown to be a light harvesting assembly. Red luminescence is observed with europium at λ_{ex} = 394 nm in benzene.

Cyclodextrin dimers (Figure 1.4.2.4) have a dual annular system that can be exploited in order to achieve specific chemical goals.[2] Ogoshi *et al.*[54-56] developed a β-cyclodextrin dimer sandwiched metalloporphyrin to mimic the cytochrome P-450 function of mono-oxygenases (Figure 1.4.2.5) Ogoshi *et al*[54] prepared water-soluble porphyrins sandwiched with β-cyclodextrin skeletons. Two binding pockets are provided by the β-cyclodextrins, above and below a metalloporphyrin (Figure 1.4.3.3). This compound facilitated the epoxidation of hydrophobic alkenes in an aqueous phosphate buffer using iodosylbenzene as an oxygen source and the β-cyclodextrin sandwiched porphyrinatoiron(II) as the catalyst.[54] The epoxidation of cyclohexane was found to proceed effectively when this catalyst was used, whilst only a trace of the cyclohexene oxide was detected when the parent tetrakis (4-sulfonatophenyl)porphyrinatoiron(II) was used as the catalyst. The catalytic effect was attributed to the effective binding of an alkene in the β-cyclodextrin cavities and stabilization of the oxene generated in the aqueous media by bulky and hydrophobic β-cyclodextrin moieties. The β-cyclodextrin in Figure 1.4.2.5 exemplifies a varied range of such metallocyclodextrins.

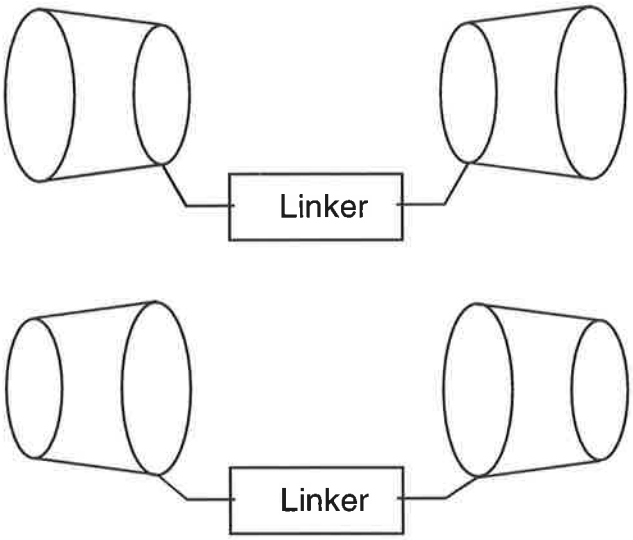


Figure 1.4.2.4: General cyclodextrin dimers. These compounds have a dual annular system, which can be exploited to achieve strong host-guest complexation. The "linker" is a group covalently attached to both cyclodextrins.

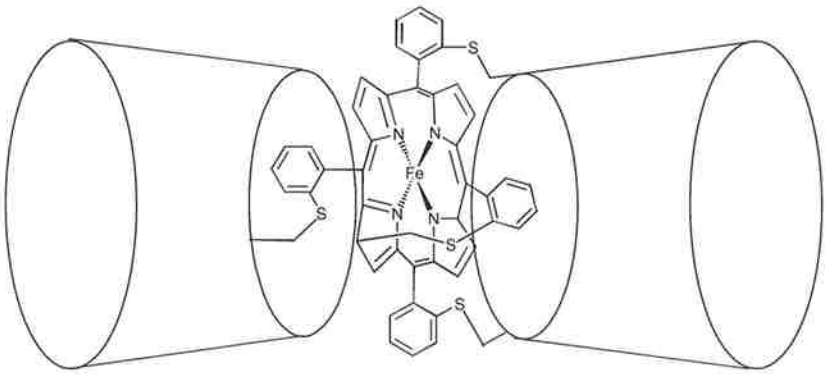


Figure 1.4.2.5: The β-cyclodextrin dimer that was shown to mimic certain cytochrome P-450-dependant mono-oxygenase functions. It consisted of a metallo-porphyrin sandwiched between two β-cyclodextrins.

1.4.3 – Other Aspects of Cyclodextrins

β-Cyclodextrins are also used extensively in everyday household products. The reason behind the broad application of such β-cyclodextrin based compounds is that they pose no problems toxicologically or metabolically due to their natural sugar basis. β-Cyclodextrin is also cheap to produce in large amounts. The cyclodextrins are being increasingly introduced into biotechnological applications,[57] their introduction into the pharmaceutical industry saw roughly 1700 patents filed concerning cyclodextrin based compounds between 1980-1992.[58] Cyclodextrins where shown to enhance the

bioavailability and stabilisation of some drugs. They can also reduce side effects of certain drugs and mask unpleasant tastes and smells. The cyclodextrins are also effective drug carriers.

β-Cyclodextrin is widely used in the food, cosmetics and toiletries industries.[59] β-Cyclodextrin provides protection against oxidation and also against the harmful effects of heat, light and exposure to air (ie. used as preservatives) and thus extends the lifespan of foodstuffs. As in the pharmaceutical industry, β-cyclodextrin is used in taste modification and the reduction of unpleasant tastes and odours. The addition of β-cyclodextrin to foodstuffs can also favourably modify certain physical properties such as water retention, emulsion stability and texture.

Cyclodextrins also prove useful in analytical separation methods[60], due to their ability to discriminate between enantiomers. The stabilities and solubilities of host-guest complexes of cyclodextrins are very guest dependant and hence a mixture of compounds may be separated by fractionation of the host-guest complex according to their solubilities. Cyclodextrins are utilised as intensity enhancement agents in analytical fluorimetric measurements. Clearly, cyclodextrins have become an integral part of many chemical and technological processes due to their ability to be tailored to perform many varied roles.

This thesis explores the preparation of ω-aminoalkylamino modified β-cyclodextrins, the study of their catalytic behaviour and their solution structural aspects. The attempted synthesis of β-cyclodextrins mono-substituted with multidentate metal ion binding substituent is also discussed.

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Chapter 2: Catalysis Studies of Substituted β-Cyclodextrins

2.1 – Introduction

2.1.1 – Catalysis by Unmodified Cyclodextrins

As mentioned in the introductory chapter, cyclodextrins have long attracted attention in catalysis and as enzyme mimics due to the way in which they act as hosts to complex guest molecules and induce reactions of the complexed species. The natural cyclodextrins induce the alkaline de-esterification of esters and hydrolysis of other carboxylic acid derivatives. They may be classed as cyclodextrin-hydrolases, by analogy with enzymes which catalyse reactions of this type.

Preliminary work by Tee[1] and Bender[2, 3] explored the cyclodextrin mediation of de-esterification of phenyl acetates. They established that in basic aqueous solution α-cyclodextrin and β-cyclodextrin greatly accelerated the rates of de-esterification of a range of *m*-substituted phenyl acetates, while the de-esterification of *p*-substituted phenyl acetates were only slightly accelerated. (see Chapter 1.3.1). They also reported that specific requirements must be met in order for a host-guest complex to form and the acceleration of phenyl ester de-esterification to occur. These requirements include the initial binding of the ester within the host cyclodextrin annulus and positioning of the esters oxycarbonyl group near a deprotonated secondary hydroxyl group (the reaction centre) of the cyclodextrin.

The type of phenyl ester is also important. Substituted phenyl esters possessing a long alkyl chain are orientated within the annulus of α- or β-cyclodextrin such that complexation of the chain is favoured in preference to the ester phenyl moiety. This results in a reduction of the cyclodextrin acceleration effect with very long (C6-C10) alkyl chain esters. The reduction is caused partly by the increased distance between the reactive centres of the cyclodextrin host and phenyl ester guest together with the increased propensity for alkyl insertion of these phenyl esters. In the case of very long alkyl substituted phenyl esters (C10-C12), a productive 2:1 host-guest complex is formed at high concentrations of β-cyclodextrin, increasing the rate of de-esterification above that observed for the shorter alkyl substituted phenyl esters.

Bender *et al*,[2, 3] reported that the pseudo-first order rate constant for the de-esterification of *p*-nitrophenyl acetate by β-cyclodextrin was dependent on the concentration of β-cyclodextrin. The de-esterification acceleration by β-cyclodextrin approached a maximum, similar to the behaviour observed in enzyme kinetics (Figure 2.1.1.1). Michaelis-Menten kinetics characterises the relationship between values of k_c and K_{diss} (the reaction rate constant for the complexed *p*-nitrophenyl acetate and the dissociation constant for the Michaelis complex, respectively).

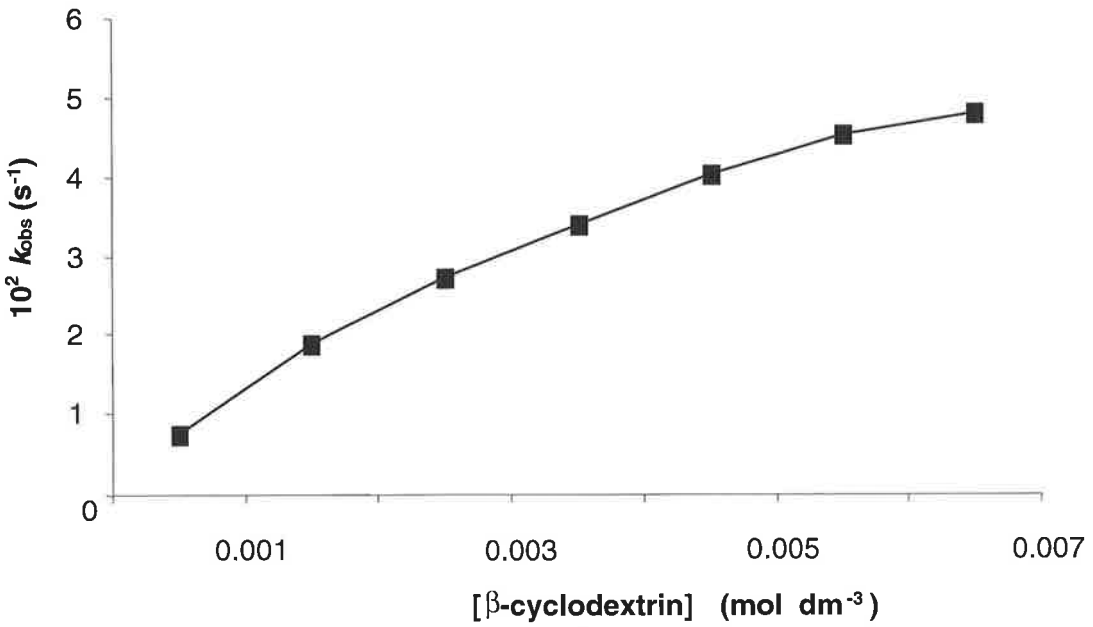


Figure 2.1.1.1: Rate data for β-cyclodextrin and *p*-nitrophenyl acetate at pH = 10.6 showing that the rate of de-esterification approaches a limiting rate at high concentrations of β-cyclodextrin.[3] (k_{obs} is the observed first order rate constant.)

Despite the interest in the catalytic qualities of unmodified cyclodextrins, their efficiency in de-esterification reactions is limited. Alkaline reaction conditions (pH = 10.6 or higher) are required to deprotonate the cyclodextrin secondary hydroxyl groups to generate a reactive cyclodextrin species. The resulting intermediate

acylated cyclodextrins are also generally slow to hydrolyse to regenerate the cyclodextrin for a further catalytic cycle.

2.1.2 – Catalysis Studies with Modified β-Cyclodextrins

Recent studies investigated the effects of a series of amino-substituted β-cyclodextrins (β–CDX, Figure 2.1.2.1) on the de-esterification of *p*-nitrophenyl acetate.[4] Mono-substitution of a primary hydroxyl group of β-cyclodextrin with a series of polyamine groups was undertaken to determine whether changing the distance of the reactive centre (the primary amine) from the oxycarbonyl group of *p*-nitrophenyl acetate affected the rate of de-esterification. This series of 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin derivatives formed host-guest complexes with *p*-nitrophenyl acetate and gave rates of de-esterification similar to those reported for the de-esterification of *p*-nitrophenyl acetate by β-cyclodextrin.

The kinetic measurements of the de-esterification of *p*-nitrophenyl acetate by the diamino substituted cyclodextrins shown in Figure 2.1.2.1 were carried out at pH 9.1 in 0.05 mol dm^{-3} borate buffer at 298.2 K. These conditions were utilised to make the mono protonated 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin the dominant species. The pK_as of the protonated modified cyclodextrins used are shown in Table 2.1.2.1 below.

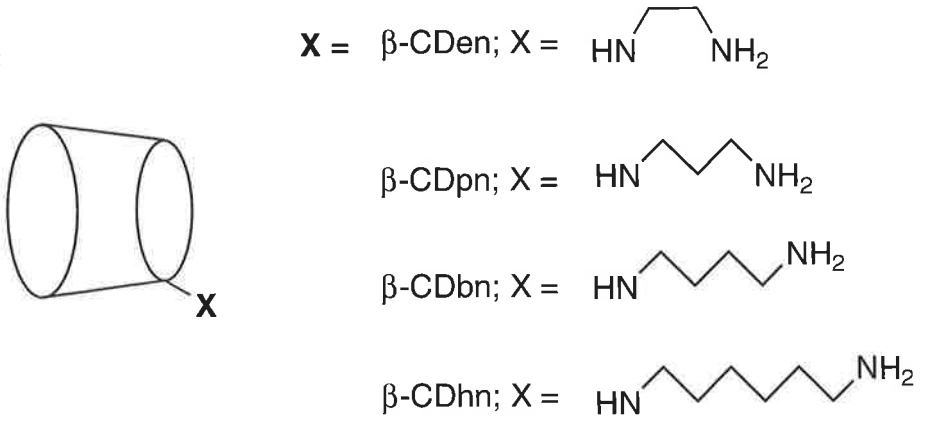
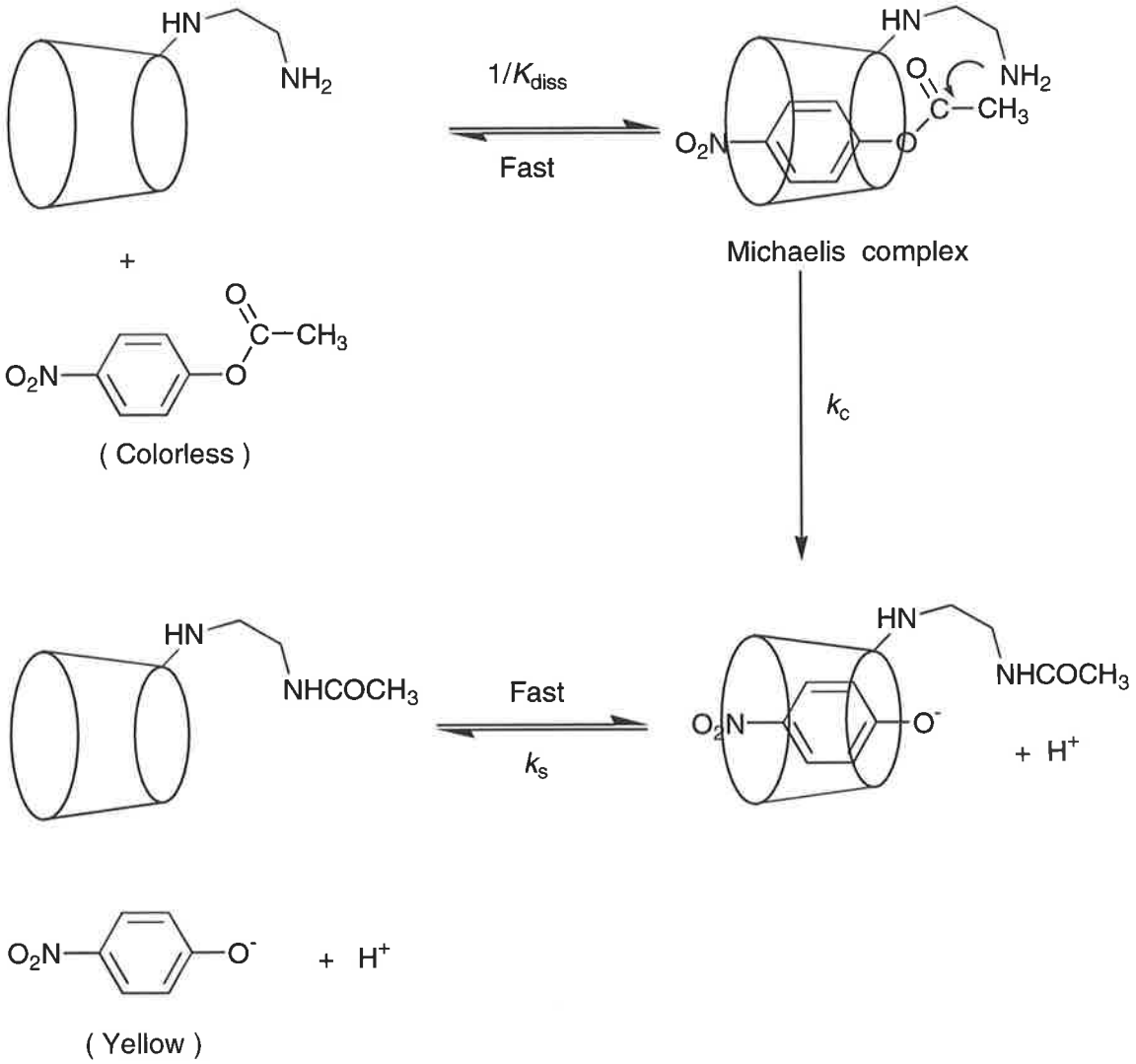


Figure 2.1.2.1: The series of 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins utilised to study the nature of their reactions with *p*-nitrophenyl acetate.[4] The nomenclature en, pn, bn and hn denote the ω-aminoalkylamino substituents attached to the β-cyclodextrin. For example en = 1,2-diaminoethane, pn = 1,3-diaminopropane and bn = 1,4-diaminobutane.

Table 2.1.2.1: The pK_as of the protonated primary and secondary amines of the 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins and the percentage of each species present at pH = 9.1 and 298.2 K.[4]

[β-CDX]	pK_{a1}	pK_{a2}	% β-CDX	% β-CDXH$^+$	% β-CDXH$_2^{2+}$
β-CDen	9.42 ± 0.01	5.70 ± 0.01	27.54	72.43	0.04
β-CDpn	9.90 ± 0.10	7.39 ± 0.01	10.94	86.92	2.13
β-CDbn	10.26 ± 0.02	8.06 ± 0.01	4.70	85.49	9.82
β-CDhn	10.27 ± 0.03	8.72 ± 0.03	3.40	63.35	33.25

Saturation kinetics were not observed at the concentrations of modified cyclodextrins used in the study (Figure 2.1.2.2).[4] The overall rates of de-esterification were shown to decrease as the length of the ω-aminoalkylamino-substituents of the 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins increased. In each case, the acceleration effect (k_{obs}-k_{un}) (where k_{obs} is the observed first order rate constant of de-esterification in the presence of the modified cyclodextrin and k_{un} is the rate constant for de-esterification in the absence of the modified cyclodextrin) varied linearly with the concentration of each 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin. These findings are consistent with a small proportion of the reactants existing as a host-guest complex consistent with equation 2.1 for Michaelis-Menten kinetics and with Scheme 2.1.2.1. The variation of k_{obs}-k_{un} with [β-CDX] is consistent with the limiting condition [β-CDX]/K_{diss}«1 and k_{obs}-k_{un} ≈ (k_c / K_{diss})[β-CDX] in Equation 2.1. The ratio of k_c / K_{diss} is dependent on the amount of each protonation state of the 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin species (ie. un-, mono- and di-protonated). As shown in Table 2.1.2.1 the dominant β-CDX species at pH = 9.1 is β-CDXH$^+$. Through pH dependence studies, the mono- and di-protonated species were determined to be insignificant contributors to the de-esterification process and the fully deprotonated species (β-CDX) were identified as the dominant nucleophiles. When the data shown in Figure 2.1.2.2 was corrected to reflect the total proportion of β-CDX in solution at pH = 9.1 the ratio k_c / K_{diss} for each β-CDX was shown to be comparable and was consistent with the de-esterification of *p*-nitrophenyl acetate proceeding through a host-guest complex.



Scheme 2.1.2.1: Schematic representation of the de-esterification reaction of *p*-nitrophenyl acetate by β-CDen. K_{diss} is the dissociation constant of the Michaelis complex, k_c is the rate of de-acylation of the guest ester and k_s is the rate of dissociation of the products.

$$k_{obs} - k_{un} = \frac{\frac{k_c}{K_{diss}}[\beta\ CD - X]}{1 + \frac{[\beta\ CD - X]}{K_{diss}}}$$

Equation 2.1

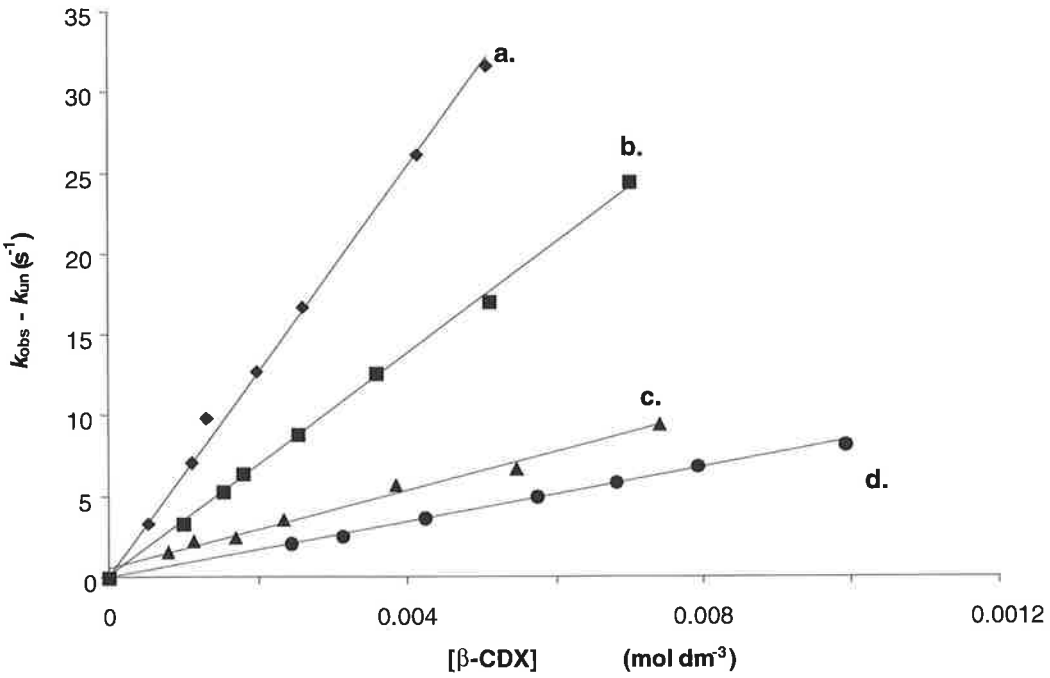


Figure 2.1.2.2: A comparison of k_{obs}-k_0 of de-esterification of *p*-nitrophenyl acetate by 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins at pH 9.1, 298.3 K where each point is an average of three values. (**a**) β-CDen, (**b**) β-CDpn, (**c**) β-CDbn, (**d**) β-CDhn.[4]

The de-esterification of *p*-nitrophenyl acetate by β-CDen (Figure 2.1.2.1) was not dependent on the concentration of either the mono- or di-protonated β-CDen species.[4]. It follows that the major reactive species is the unprotonated β-CDen species. Through product analysis studies, the primary amines were established as the major reactive centre for each of the β-CDX. However, for β-CDhn there was ≤25% of a second product isolated corresponding to de-esterification by a deprotonated secondary hydroxyl group of β-CDhn. The second product was attributed to a rapid preequilibrium between β-CDX and *p*-nitrophenyl acetate to form two reactive complexes that differed in their orientation of the guest inside the annulus of β-CDX. The first host:guest complex had the ester functionality positioned near the primary end of the annulus of β-CDX and led to de-esterification through nucleophillic attack by the ω-aminoalkylamino substituent. The second host:guest complex had the ester functionality positioned near the secondary end of the annulus of β-CDX and de-esterification could eventuate through reaction with a deprotonated

secondary hydroxyl group or with the ω-aminoalkylamino substituent should it be sufficiently long.

2.2 – Chain Length Effects In the De-esterification of *p*-Nitrophenyl Esters

The new chemistry described in this chapter is an extension of the studies mentioned above and that of Tee[1] and VanEtten.[2, 3] Here, the nature of the reactions between 6^A-(2-aminoethyl)amino-6^A-deoxy-β-cyclodextrin (β-CDen, Figure 2.1.2.1) and two *p*-substituted nitrophenyl ester guests are investigated.

The rate of de-esterification of *p*-nitrophenyl acetate is shown to increase upon addition of 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins. As the ω-aminoalkylamino substituent becomes longer and more flexible, the reactive primary amine is able to orientate itself for a more effective nucleophillic attack on the carbonyl group of *p*-nitrophenyl acetate.[4] The host-guest complex of *p*-nitrophenyl acetate and β-CDen have previously shown a large acceleration effect on the rate of de-esterification.[4] Therefore, β-CDen is chosen to maximise de-esterification acceleration effects of any host-guest complexes formed in solution.

The esters *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate (Figure 2.1.2.3) are chosen due to their differing acyl and aryl substituents which should allow for differing modes of complexation within the annuli of β-CDen. These esters are similar in size and should not protrude very far out of the annuli of β-CDen upon host-guest complexation. Thus, they should be positioned such that they are in close proximity to the primary amine of β-CDen and de-esterification should readily occur.

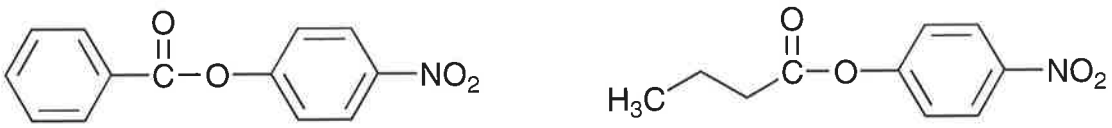
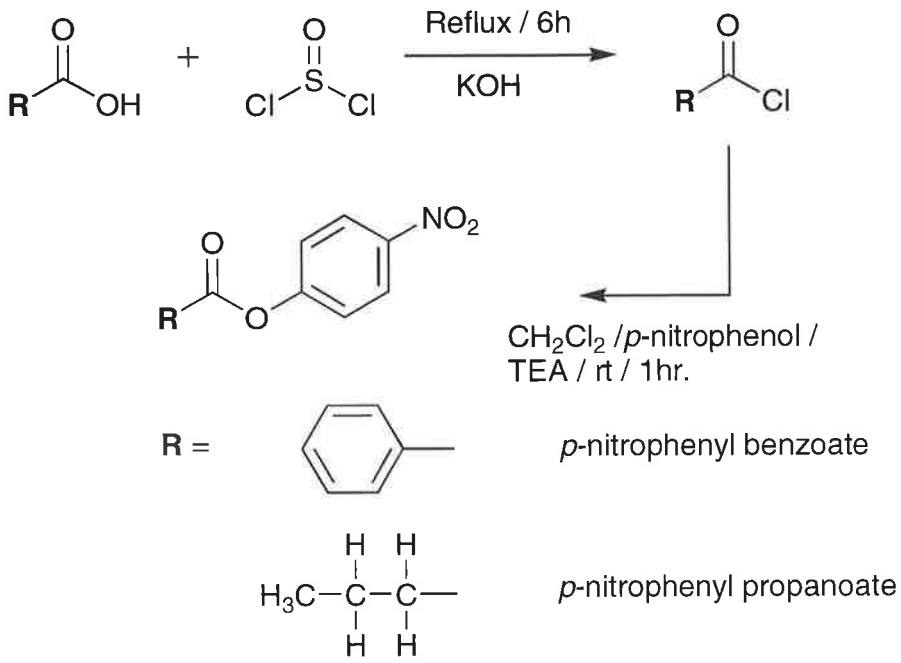


Figure 2.1.2.3: The guest esters *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate. Both should complex inside the annulus of β-CDen to form a Michaelis-like host:guest complex.

2.2.1 – Synthesis of *p*-Nitrophenyl Benzoate and *p*-Nitrophenyl Propanoate

The guest esters *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate were synthesised by conversion of their parent carboxylic acids (benzoic acid and butyric acid) to the corresponding acid chloride. Subsequent treatment with *p*-nitrophenol with slow addition of one equivalent of triethylamine (TEA) affords the crude esters, which were purified by silica gel chromatography and/or distillation (Scheme 2.2.1.1).

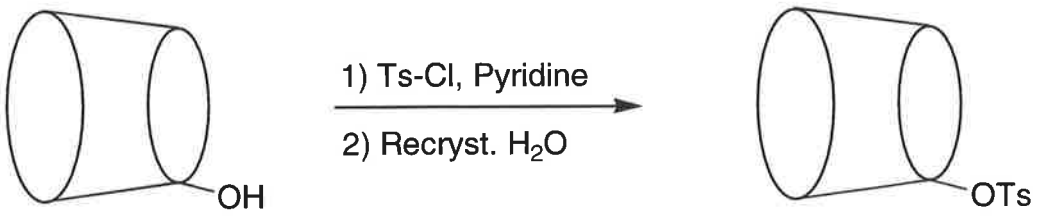
The pure *p*-nitrophenyl benzoate was obtained as a white crystalline solid in 75% yield and *p*-nitrophenyl propanoate was obtained as a golden brown oil in 60% yield. Both esters gave a single spot by TLC, which did not correspond to either the parent acids or *p*-nitrophenol, thus indicating purity. The ^{1}H NMR spectra of *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate were consistent with the Sigma-Aldrich NMR spectra.[5]



Scheme 2.2.1.1: Synthesis of *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate were achieved through the reaction of the parent alkyl acid chloride with *p*-nitrophenol.

2.2.2 - Synthesis of 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin and 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin

β-Cyclodextrin possesses seven primary hydroxyl groups, all with identical reactivity, so it is possible to induce multiple substitutions at the primary face of β-cyclodextrin. This outcome was not desirable and while the mono-tosylation of β-cyclodextrin has been reported previously,[6] these methods give products whose purity was insufficient for further studies and for this reason, an alternative method was employed.[7, 8] This alternative method of mono-tosylation reacts β-cyclodextrin with *p*-toluene sulfonyl chloride utilising pyridine as the solvent / base (Scheme 2.2.2.1). Limiting the availability of *p*-toluene sulfonyl chloride in the reaction vessel (ie by slow, constant addition) ensured a reduction of the di-tosylated products associated with the reaction. Reduction of the molar ratio of

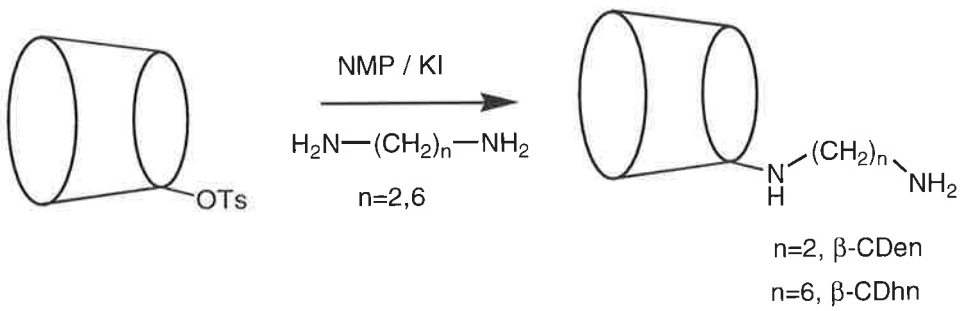


Scheme 2.2.2.1: Conversion of a primary hydroxyl group of β-cyclodextrin to the mono-tosylate is realised by reaction with *p*-toluene sulfonyl chloride in dry pyridine to give consistent yields of around 35%.

p-toluene sulfonyl chloride to β-cyclodextrin further reduced, but did not eliminate, the production of the di-tosylated product. The pure 6^A-(4-toluine sulfonyl)-6^A-deoxy-β-cyclodextrin (β-CD-OTs) was isolated by repeated recrystallisation from hot water to leave the pure β-CD-OTs and less than 5% unsubstituted cyclodextrin. It is most important to remove the poly-tosylated products because of the potential difficulties in removing the polyaminated-cyclodextrins of subsequent reactions. The above method gave consistent yields of up to 36% of the desired pure mono-tosylated product.

Pure β-CD-OTs was identified by it's ^{1}H NMR spectrum, which showed an AB-quartet at δ7.56 ppm corresponding to the 1,4-substituted benzene ring, and a singlet at δ2.5 ppm corresponding to the methyl resonance of the tosyl group. These two resonances appear in regions removed from the resonances of the parent β-cyclodextrin and are therefore very characteristic. β-CD-OTs had a higher R_f value (R_f = 0.55) than that seen for β-cyclodextrin (R_f = 0.45) on TLC under the standard conditions. Some hydrolysis of β-CD-OTs back to β-cyclodextrin occurs in all reactions due to the presence of water. This water remains associated with all cyclodextrin derivatives despite extensive drying over phosphorus pentoxide under vacuum.

6^A-(2-Aminoethylamino)-6^A-deoxy-β-cyclodextrin (β-CDen) and 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin (β-CDhn, see chapter 3) were prepared from β-CD-OTs using a modified version of a standard S_N2 tosylate substitution reaction (Scheme 2.2.2.2).[9] Nucleophillic displacement of the tosyl group by the ω-aminoalkylamino substituent, in dry *N*-methyl pyrolidinone (NMP) in the presence of a potassium iodide catalyst afforded β-CDen and β-CDhn in consistent yields of around 50% (NMP was used in place of the more commonly used DMF due to unwanted reactions between DMF and 1,2-diaminoethane). The iodide catalyst is more nucleophillic than the ω-aminoalkylamino compounds and readily replaces the tosyl group to form 6^A-iodo-6^A-deoxy-β-cyclodextrin as an intermediate which is readily converted to the corresponding 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin in solution.



Scheme 2.2.2.2: Schematic representation of the Synthesis of β-CDen and β-CDhn from the mono-tosylated β-cyclodextrin by S_N2 displacement of the tosyl group by iodine anion and subsequent displacement of the intermediate iodo-β-cylodextrin by the amino substituent.

6^A-Substituted amino cyclodextrins β-CDen and β-CDhn were isolated by precipitation with dry ethanol with subsequent purification by ion exchange chromatography. β-CDen was readily identified by its ^{13}C NMR spectrum that showed the ethylene bridge carbons at δ 40.9 ppm and δ 50.5 ppm. The peak at δ 50.5 ppm is assigned to the carbon closest to the β-cyclodextrin as the hydrogen bonding of the β-cyclodextrin primary hydroxyl groups de-shields the first carbon of the ethylene bridge causing it to resonate further downfield than the second carbon of the bridge (Figure 2.2.2.1). The 6-aminohexylamino arm of β-CDhn also showed a number of broad multiplets between δ 2.7 ppm and δ 1.4 ppm in its proton NMR spectrum. The methylene groups closest to the amino groups (Hn1 and Hn6) resonate further upfield (at δ 2.7 ppm and δ 2.6 ppm) than those of Hn2-Hn5 (δ 1.3 –1.6 ppm) methylene groups (Figure 2.2.2.1).

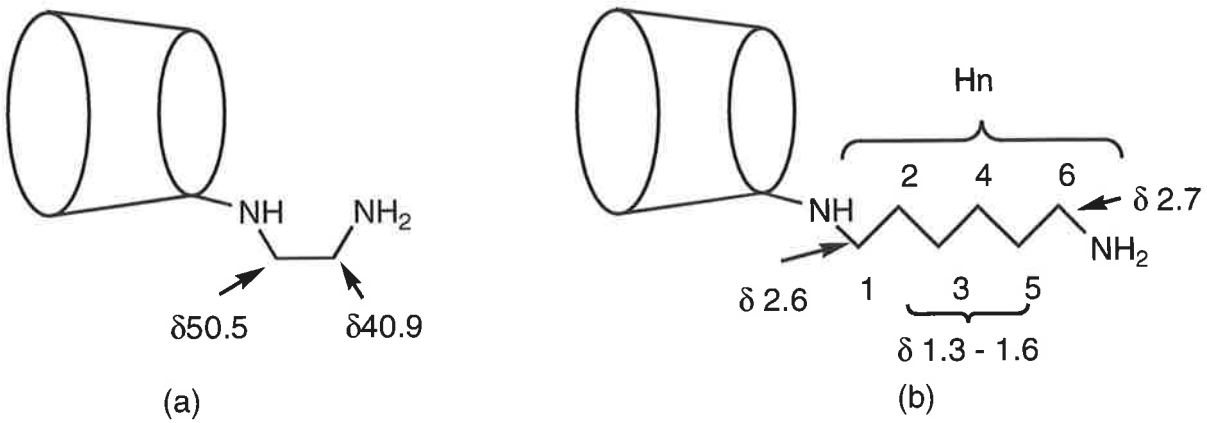


Figure 2.2.2.1: **(a)** The methylene carbons of β-CDen as seen in the ^{13}C NMR spectra. **(b)** The 6-aminohexylamino substituent of β-CDhn shows distinct resonances for the Hn1 and Hn6 methylene groups that are substantially up-field from those of the Hn2-5 methylene groups in the ^{1}H NMR spectrum.

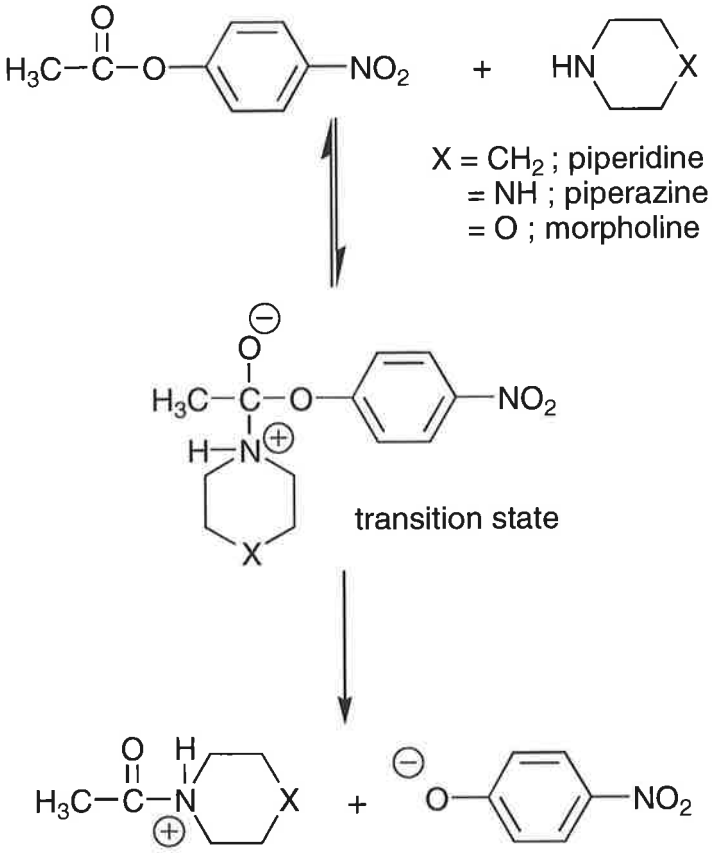
The natural symmetry of the cyclodextrin system is disrupted by the addition of a pendant arm. The C^{13} NMR spectra often show the carbons $C1^A$, $C4^A$, $C5^A$ and $C6^A$ of the substituted glucopyranose unit being well separated from those of the unsubstituted glucopyranose residues. The shifted $C6^A$ resonances of β-CDen and β-CDhn were visible at around δ 50 ppm, which is about 10 ppm upfield from the resonances of the other carbons C6. The shifted $C5^A$ resonances were observed at

around 2-5 ppm upfield from the rest of the C5 resonances (The chemical shifts of new resonances of ω-aminoalkylamino-β-cyclodextrins have been shown to be dependent on the pH at which the spectrum is recorded).[10] The shifted carbon $C4^A$ resonance is shifted about 4 ppm downfield from those of the other C4 carbons and the shifted $C1^A$ resonance is only shifted 1-2 ppm upfield from those of the rest of the C1 carbons. The introduction of a substituent arm also increases the polarity of the molecule (compared with β-cyclodextrin) and hence β-CDen (R_f = 0.17) and β-CDhn (R_f = 0.37) appeared substantially lower than β-cyclodextrin (R_f = 0.45) under standard TLC conditions.

2.3 – Solvent Effects On The Reactivity of *p*-Nitrophenyl Esters

The kinetic measurements of the de-esterification of *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate by β-CDen were carried out in two solvent systems. This was due to *p*-nitrophenyl benzoate being only partially soluble in 100% water and causing clouding in the reaction cell of the spectrophotometer. A solvent system of 70:30 water:acetonitrile (MeCN) was employed for the *p*-nitrophenyl benzoate system. For comparison, studies incorporating *p*-nitrophenyl propanoate were performed in both 100% water and 70:30 water:MeCN solvent systems.

The effects of solvent on the reactions of *p*-nitrophenyl acetate were investigated by Ik-Hwon Um *et al.*[11] Second order rate constants where determined for the reaction between *p*-nitrophenyl acetate with morpholine, piperazine and piperidine in varying compositions of MeCN:H_2O (Table 2.3.1). Addition of MeCN to an aqueous system caused the reactivity of the secondary amine systems studied



Scheme 2.3.1: Schematic representation of the transition state binding and mechanism of the reaction between *p*-nitrophenyl acetate and various secondary amine systems.[11]

to decrease (Table 2.3.1). Reactivity decreased by up to 30 - 40 mole percent MeCN and remained relatively constant for further increasing MeCN mole percentages. Such solvent effects on reaction rates can be explained by the Hughes-Ingold rules in a qualitative manner,[12] since a gradual increase of the mole percentage of MeCN is considered to change the hydrogen bonding structure of water and the microenvironment of the reactants and transition state. In such aminolysis reactions, it is generally accepted that the reaction proceeds through an addition-elimination pathway in which the rate-determining step depends on the basicity of the nucleophilic amines and the leaving aryloxides.[13, 14] The formation of the transition state in Scheme 2.3.1 is affected by the addition of MeCN as the dissociation energy needed to proceed to products is dependent on the transition state solvation. This is decreased by the addition of acetonitrile. It is therefore energetically less favorable for the transition state to dissociate and form products in the presence of MeCN and as a consequence the rate of the de-esterification reaction slows.

Table 2.3.1: Summary of second-order rate constants k_2 ($M^{-1}s^{-1}$) for the reactions of *p*-nitrophenyl acetate with piperidine, piperazine and morpholine in various MeCN:H_2O mixtures at temperatures of 288K, 298K and 308K.[12]

	k_2 ($M^{-1}s^{-1}$)								
	piperidine			piperazine			morpholine		
Mole % MeCN	288K	298K	308K	288K	298K	308K	288K	298K	308K
0	24.6	41.2	61.7	3.23	5.72	10.6	0.251	0.485	0.886
10	-	14.5	-	-	2.83	-	-	0.281	-
20	-	6.68	-	-	1.35	-	-	0.146	-
30	2.49	3.79	6.09	0.568	0.94	1.58	0.0577	0.104	0.176
40	-	2.73	-	-	0.78	-	-	0.086	-
50	-	2.14	-	-	0.70	-	-	0.079	-
60	1.23	1.95	2.69	0.419	0.66	1.08	0.0426	0.073	0.121
70	-	1.56	-	-	0.68	-	-	0.072	-
80	-	1.61	-	-	0.73	-	-	0.071	-
90	1.1	1.69	2.33	0.591	0.85	1.37	0.0463	0.070	0.103

This type of rate retardation is possible in the reaction between β-CDen and the esters *p*-nitrophenyl benzoate and propanoate. The difference between the pK_as of β-CDenH_2^{2+} in aqueous solution and in 70:30 H_2O:MeCN was of concern as the rate of de-esterification is dependent on the concentration of the fully deprotonated β-CDen species. Previous studies[11] reported that the pK_as of Brönsted acids in MeCN become much larger than they are in aqueous conditions (eg *p*-nitrophenol has a pK_a of 7.2 in H_2O and a pK_a of 20.7 in 100% MeCN). Potentiometric titrations were attempted to determine the pK_as of β-CDenH_2^{2+} in the 70:30 H_2O:MeCN solvent system. However, the titration curve for MeCN obscured the titration of β-CDenH_2^{2+} and prevented determination of its pK_as.

2.4 – De-esterification of *p*-Nitrophenyl Esters By 6^A-(ω-Aminoalkylamino)-6^A-deoxy-β-cyclodextrins

In the present study pH dependence of the de-esterification of *p*-nitrophenyl benzoate in the presence of β-CDen in a 70:30 H_2O:MeCN solvent system was examined. A series of borate buffered solutions were used to maintain constant pH during the course of the reaction as described in chapter 5. It is seen from Figure 2.4.1 that k_{obs} shows a significant increase above pH = 9.4. The pH dependence was not investigated above pH = 10 as above this value it was anticipated that deprotonation of the secondary hydroxyl groups of β-CDen would occur.

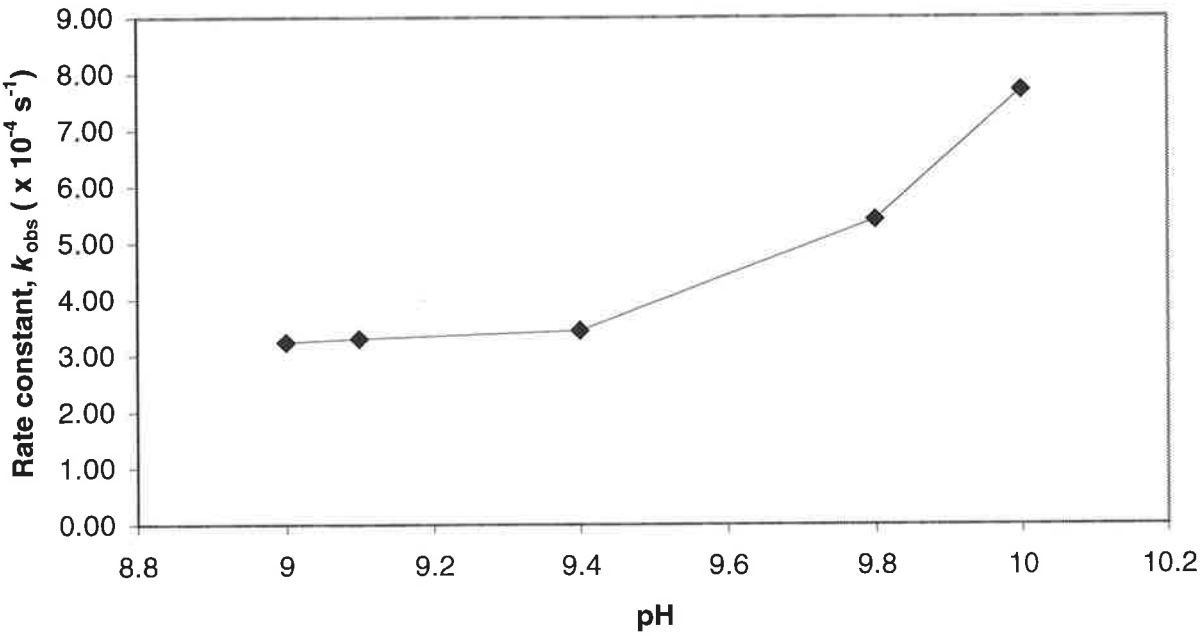


Figure 2.4.1: A plot of k_{obs} against pH for the de-esterification of *p*-nitrophenyl benzoate in the presence of β-CDen in borate buffered solution.([*p*-nitrophenyl benzoate] = 2 x 10^{-4} mol dm^{-3}, [β-CDen] =2 x 10^{-3} mol dm^{-3}).

The de-esterification of *p*-nitrophenyl benzoate was accelerated by the addition of β-CDen in 70:30 H_2O:MeCN (Figure 2.4.2(c)). The acceleration was directly proportional to the concentration of β-CDen present in solution, consistent with previous studies.[4] The possibility that the absence of kinetic saturation expected for a Michaelis-Menten mechanism was due to an insufficient concentration of

β-CDen (which may have been a limitation of previous studies) was investigated by employing concentrations of β-CDen up to 0.0043 mol dm^{-3}.

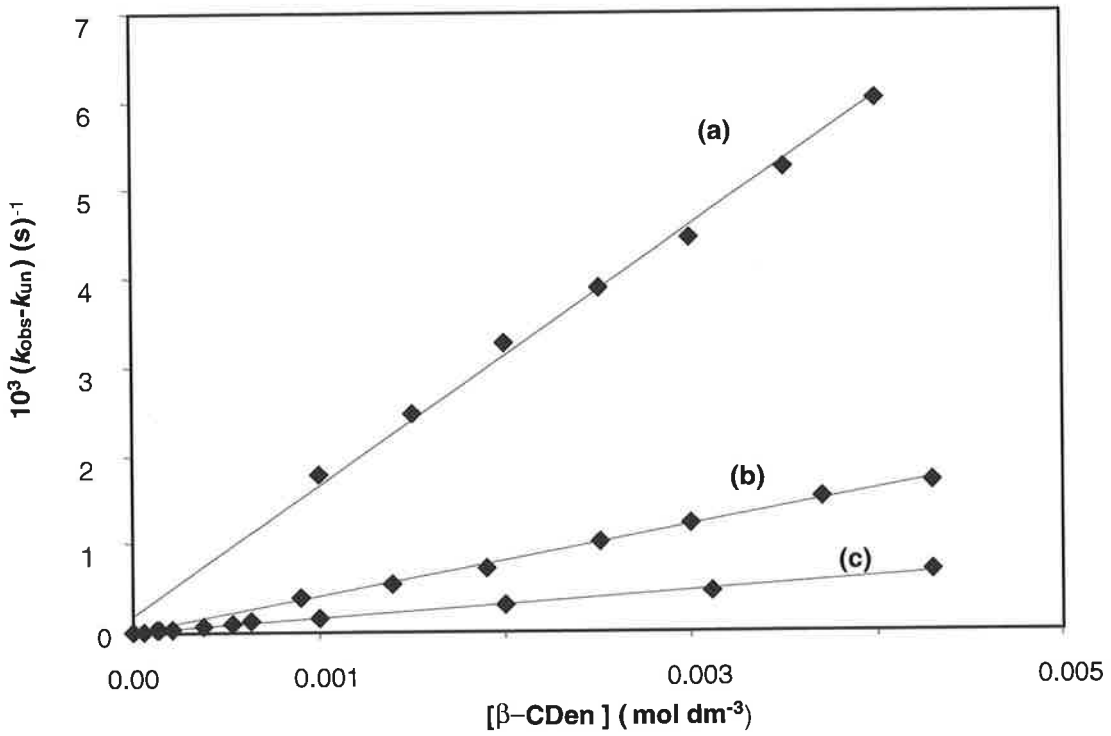


Figure 2.4.2: Comparison of observed de-esterification rates for (**a**) *p*-nitrophenyl propanoate in 100% Water (**b**) *p*-nitrophenyl propanoate in 70:30 H_2O:MeCN and (**c**) *p*-nitrophenyl benzoate in 70:30 H_2O:MeCN. ([Ester] = 2 x 10^{-4} mol dm^{-3}).

The de-esterification of *p*-nitrophenyl propanoate was also accelerated by the addition of β-CDen in 70:30 H_2O:MeCN (Figure 2.4.2(b)) and gave similar results as those seen above for *p*-nitrophenyl benzoate. The overall rates of de-esterification of *p*-nitrophenyl propanoate where faster than those of *p*-nitrophenyl benzoate in the same solvent system.

The de-esterification of *p*-nitrophenyl propanoate by β-CDen in aqueous solution (Figure 2.4.2(a)) was accelerated to a much larger extent than is observed for the same reaction in the 70:30 H_2O:MeCN solvent system. This acceleration was comparable to that seen previously for the de-esterification of *p*-nitrophenyl acetate by β-CDen.[4]

The rates of de-esterification in the 70:30 H_2O:MeCN solvent system may be affected in a number of ways by the presence of MeCN. Firstly, as shown previously

for 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins in aqueous solution,[4] the concentration of the fully deprotonated β-CDen species is the major reactive species and its concentration in solution will determine the rate of de-esterification of *p*-nitrophenyl esters. The presence of MeCN will affect the equilibrium between the un-, mono- and di-protonated β-CDen present in solution, due to the change in pK_a of the protonated primary and secondary nitrogens of β-CDenH_2^{2}. As the pK_as of β-CDenH_2^{2+} were not able to be determined in the 70:30 H_2O:MeCN solvent system, the exact amounts of β-CDen, β-CDenH^+ and β-CDenH_2^{2+} present at pH = 9 are unknown. It would seem likely however, that as the pK_as of in MeCN has been reported to increase the pK_as of organic acids,[13] that the concentrations of the non-reactive β-CDenH^+ and β-CDenH_2^{2+} are increased upon addition of MeCN. If the total concentration of the fully deprotonated β-CDen species is diminished in the presence of MeCN, then the rate of de-esterification will be decreased also, consistent with the results reported here.

The MeCN present in the reaction may also complex in the β-CDen annulus, expelling water molecules and removing or decreasing the hydrophobic interactions that facilitate the inclusion of hydrophobic guests. Should this eventuate, the formation of the Michaelis-like host:guest complex which leads to de-esterification of guest esters may be decreased, leading to a reduction in the rate of de-esterification. This scenario is likely to be only a contributing factor to the overall rate reduction seen for the de-esterification of *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate above in the 70:30 H_2O:MeCN solvent system, as there remains a large volume of water still present.

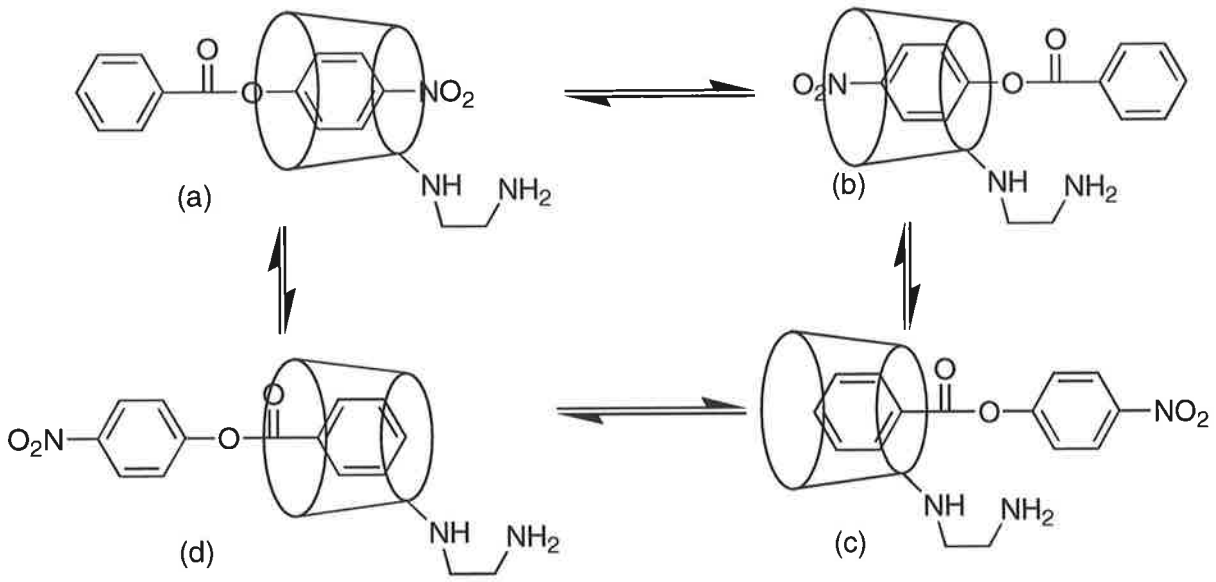
If it is assumed that the concentration of the Michaelis complex increases linearly with the increase in β-CDen concentration for the data in Figure 2.4.1 then the slopes $(k_{obs}-k_{un})/[\beta\text{-CDen}] = k_c/ K_{diss}$ when [β-CDen]/Kdiss ≈ 0 in Equation 2.1 as given in Table 2.4.1. The ratio k_c / K_{diss} can be found for each system by using the initial slope for low concentrations (Table 2.4.1). However, this does not take into account the concentrations of β-CDenH^+ and β-CDenH_2^{2+} in solution in the two solvent systems. The ratio k_c / K_{diss} can be compared for the de-esterification of *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate in the 70:30 H_2O:MeCN solvent system and shows that the de-esterification of *p*-nitrophenyl propanoate proceeds 2.5

times more rapidly than that of *p*-nitrophenyl benzoate. This may be due to a combination of differing inductive effects of the benzene ring of *p*-nitrophenyl benzoate and the propyl group of *p*-nitrophenyl propanoate and their ability to

Table 2.4.1: The variation in the ratio's of k_c / K_{diss} for the rates of de-esterification of the *p*-nitrophenyl benzoate and propanoate esters by β-CDen in borate buffer at pH = 9 and 298.3 K

Reaction (Host, guest, solvent)	K_c/ K_{diss}
β-CDen, *p*-nitrophenyl benzoate, H_2O	1.46 ± 0.03
β-CDen, *p*-nitrophenyl benzoate, 70:30 H_2O : MeCN	0.40 ± 0.01
β-CDen, *p*-nitrophenyl benzoate, 70:30 H_2O : MeCN	0.15 ± 0.02

influence the carbonyl groups susceptibility to nucleophillic attack and to the orientation of the esters in the host:guest complex. The *p*-nitrophenyl group of *p*-nitrophenyl propanoate should complex within the annulus of β-CDen in preference to the shorter and less hydrophobic propyl chain. This will enable the *p*-nitrophenyl propanoate to be rapidly de-esterified by β-CDen. *p*-Nitrophenyl benzoate may form different host:guest complexes depending on whether the *p*-nitrophenyl or the benzene group of the ester is complexed in the annulus (Scheme 2.4.1). Of the four possible orientations, only two ((b) and (c) in Scheme 2.4.1) have the ester functionality positioned in close proximity to the primary amine of β-CDen and would lead directly to rapid de-esterification of *p*-nitrophenyl benzoate. However, substantial movement of the ester within the annulus is required for the host:guest complexes (a) and (d) in Scheme 2.4.1 to achieve a favourable orientation for de-esterification (assuming that a productive 2:1 host:guest complex does not exist for this reaction). This would slow the observed acceleration effects of de-esterification over that seen for *p*-nitrophenyl propanoate in the same solvent system, consistent with the experimental data.



Scheme 2.4.1: Schematic representation of the possible complexation of the aryl moieties of *p*-nitrophenyl benzoate. Of these host:guest complexes, (b) and (c) would lead to the most rapid de-esterification.

Comparison of the ratio of k_c / K_{diss} for the de-esterification of *p*-nitrophenyl propanoate by β-CDen in aqueous solution (Figure 2.4.1(a)) with the ratio k_c / K_{diss} for the de-esterification of *p*-nitrophenyl acetate by β-CDen in aqueous solution,[4] shows a much larger difference. De-esterification of *p*-nitrophenyl acetate is 4.5 times faster than *p*-nitrophenyl propanoate by β-CDen at pH = 9.0. Previous studies reported the de-esterification of *p*-nitrophenyl acetate with unmodified β-cyclodextrin was also faster than the de-esterification of *p*-nitrophenyl propanoate under the same conditions.[1] The difference in reactivity of *p*-nitrophenyl acetate and *p*-nitrophenyl propanoate is attributed to steric hindrance of the ester carbonyl group[1] and is most probably the only factor involved in the rate reduction. Both esters should form similar Michaelis-like host:guest complexes which incorporated the complexation of the *p*-nitrophenyl group and position the carbonyl group near the primary face of the annulus of β-CDen in close proximity to the primary amine of the 2-aminoethylamino substituent.

2.5 - Inhibition Studies

The linear relationship between the acceleration effect (k_{obs}-k_{un}) and the concentration of β-CDen exists in both solvent systems discussed above. The formation of a Michaelis-like host:guest complex through which de-esterification of *p*-nitrophenyl esters is thought to occur is likely to be affected by the presence of MeCN. This suggests the existence of an alternate reaction pathway that is not dependent on the complexation of the guest ester in the annulus of β-CDen. The most plausible explanation is that the 2-aminoethylamino substituent of β-CDen is able to react as a sterically hindered diamine through a standard S_N2 reaction regardless of whether the ester is included in the β-CDen annulus.

In order to provide further information about the nature of the reactive complex, the competitive inhibition of the de-esterification of *p*-nitrophenyl propanoate by β-CDen in the presence of adamantane-1-carboxylate, was undertaken. Adamantane-1-carboxylate was chosen as a competitive inhibitor due to it providing reasonable inhibition in previous studies of unmodified cyclodextrins.[3, 4, 15]

Table 2.5.1:The rate constants for the de-esterification of *p*-nitrophenyl propanoate by β-CDen in the presence and absence of adamantane-1-carboxylate (AC).

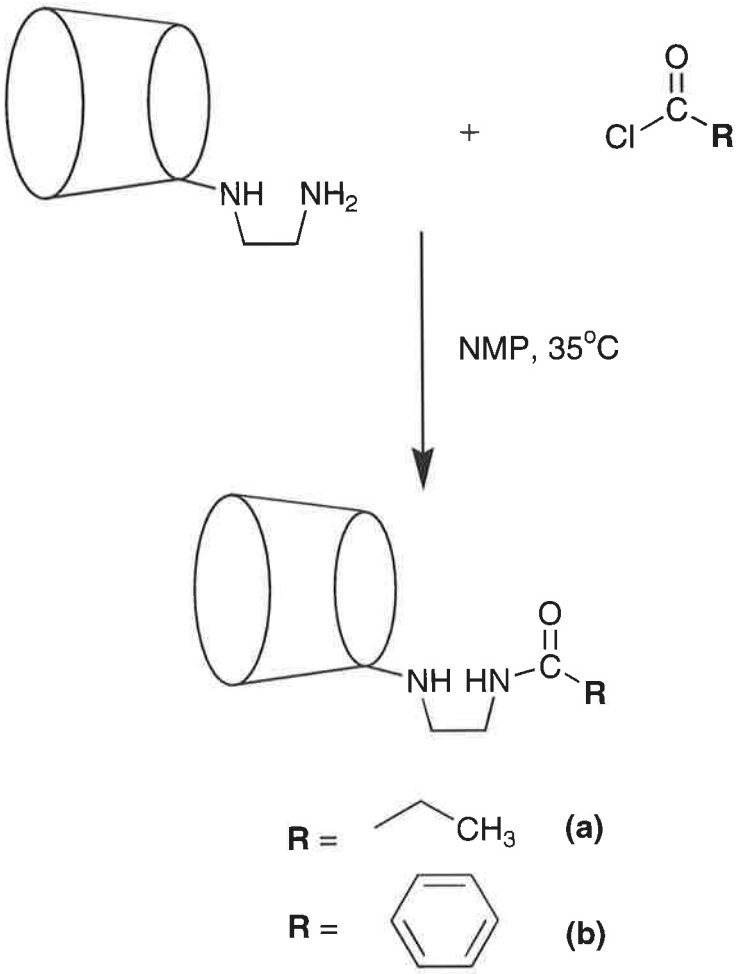
System. [β-CDen] = 2 x 10^{-3} mol dm^{-3} [*p*-nitrophenyl propanoate] = 2 x 10^{-4} mol dm^{-3}	k_{obs}-k_{un} (s^{-1}). x 10^{-3}
100% water.	3.27 ± 0.02
100% water. 0.5 eq. AC	1.96 ± 0.02
100% water. 0.75 eq. AC,	1.14 ± 0.02
70:30 H_2O:MeCN	3.04 ± 0.03
70:30 H_2O:MeCN, 0.5 eq. AC.	2.10 ± 0.02
70:30 H_2O:MeCN, 0.75 eq. AC.	1.86 ± 0.03

The stability of the host-guest complex between β-cyclodextrin and adamantane-1-carboxylate is quite high in aqueous solution (1.8×10^4 dm^3 mol^{-1} at pH 8.5). Adamantane-1-carboxylate is therefore expected to form a stable host-guest complex in the annulus of β-CDen. The rates of de-esterification were monitored as before, using a borate buffer medium at pH = 9, 298.2 K. The values of k_{obs}-k_{un} were determined in the presence and absence of adamantane-1-carboxylate (Table 2.3.2).

The addition of 0.5 equivalents of adamantane-1-carboxylate caused a 40% reduction in the rate of de-esterification of *p*-nitrophenyl propanoate by β-CDen in the 100% water solvent system and a 31% reduction in the 70:30 H_2O:MeCN solvent system. Addition of 0.75 equivalents of adamantane-1-carboxylate caused a 65% reduction in the 100% water solvent system and 39% in the 70:30 H_2O:MeCN solvent system. This is consistent with adamantane-1-carboxylate being complexed in the β-CDen annulus in solution and inhibiting the de-esterification of *p*-nitrophenyl propanoate through a Michaelis-like host:guest complex. However, the extent of inhibition is not what would be expected should the Michaelis-like host:guest complex be solely responsible for the de-esterification of the *p*-nitrophenyl esters studies here. The results reported in aqueous solution for the inhibition of the de-esterification of *p*-nitrophenyl propanoate are not quantitative and although still quite high, support the existence of an alternate de-esterification pathway. The inhibition of the de-esterification reactions in the 70:30 H_2O:MeCN solvent system also suggests that the formation of a Michaelis-like host:guest complex is not the sole pathway for de-esterification to occur. Any existing alternate de-esterification mechanism would most probably be an S_N2 reaction pathway existing between the 2-aminoethylamino substituent of β-CDen and the esters, similar to that seen for other ω-aminoalkylamino cyclodextrins.[4] If this alternate mechanism operates in the presence and absence of adamantane-1-carboxylate such that β-CDen functions as a sterically hindered diamine, then any decrease in rate due to the reduction of the Michaelis-like host:guest complex between β-CDen and *p*-nitrophenyl propanoate would be partially offset by such a reaction pathway. Support for this scenario is strengthened by the fact that upon addition of 0.75 equivalents of adamantane-1-carboxylate to the 70:30 H_2O:MeCN reaction system the rate of de-esterification is slowed to 60% of the rate in the absence of the inhibitor.

2.6 - Product analysis

The products of the de-esterification of *p*-nitrophenyl propanoate and *p*-nitrophenyl benzoate by β-CDen are *p*-nitrophenolate and a dominant modified β-cyclodextrin ((a) β-CD-NH-$(CH_2)_2$-$NHCOCH_2CH_2CH_3$ and (b) β-CD-NH-$(CH_2)_2$-NHCOPh respectively in Scheme 2.5.1). The amido-modified β-cyclodextrin intermediates are identifiable by TLC, each consisting of a single spot at R_f = 0.95 (for 2.5.1(a)) and R_f = 0.9 (for 2.5.1(b)) compared with β-CDen, R_f = 0.35 and β-CD, R_f = 0.55 in the standard TLC solvents. Both amido-modified β-cyclodextrins were seen to slowly hydrolyse to regenerate β-CDen when left in pH = 9 borate buffer solution. After 2-3 days neither were observed in their respective solutions (TLC).



Scheme 2.5.1: Synthesis of the amido-cyclodextrins **(a)** and **(b)** from β-CDen and the acid chlorides of benzoic and butyric acids.

Synthesis of the amido-modified β-cyclodextrins was achieved through reaction of β-CDen with the acid chlorides of benzoic acid and butyric acid (Scheme 2.5.1). The build up of the acid by product stopped the complete conversion due to protonation of the reactive primary amine of β-CDen. These reactions were carried out for comparison studies only and hence were not optimised for yield. The synthetic amido-modified β-cyclodextrins had identical R_f values to the amido-modified cyclodextrins isolated from the spectrophotometer cells. The synthetic amido-modified β-cyclodextrins are susceptible to hydrolysis under basic conditions and could not be purified by ion exchange chromatography due to substantial hydrolyses of the amides to regenerate β-CDen.

Conclusions:

The presence of MeCN reduces the rate of the de-esterification reaction between *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate and β-CDen. The MeCN may occupy the annulus of β-CDen and may substantially reduce any hydrophobic interactions that would normally facilitate the complexation of hydrophobic guests into the annulus. This decreases the rate acceleration effects due to a reduction in the formation of the Michaelis-like host:guest complexes through which de-esterification is thought to occur. The addition of MeCN may also cause a reduction in the amount of deprotonated β-CDen (the dominant nucleophile in the de-esterification process) and limit the formation of the Michaelis-like host:guest complex.

The linear relationship between the observed rates of de-esterification and β-CDen concentration and the low levels of inhibition observed upon addition of adamantane-1-carboxylate to the 70:30 H_2O:MeCN suggests the existence of a second reaction pathway. This pathway is likely to be a standard S_N2 reaction between the primary amine of β-CDen and the *p*-nitrophenyl esters.

The intermediate products of the de-esterification reactions between β-CDen and *p*-nitrophenyl benzoate and propanoate were seen to slowly regenerate β-CDen if left in alkaline solution (pH = 9 or above) for 2-3 days. While this means that the reaction is catalytic, the turnover is very slow.

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Chapter 3: Intramolecular Complexation in Modified β-Cyclodextrins

3.1 – Introduction

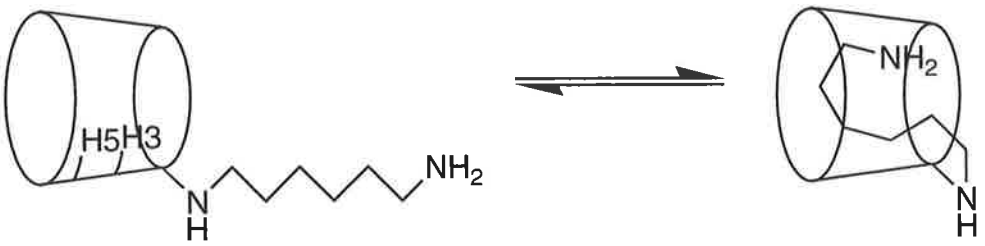
The addition of a hydrophobic substituent to the rim of β-cyclodextrin sometimes increases the stability of the host-guest complexes formed with small aromatic guests when compared with those of β-cyclodextrin.[1] Increasing the length of the hydrophobic substituent may facilitate the self-complexation of the substituent within the annulus of the modified β-cyclodextrin. Such self-complexations have been reported to limit the complexation of guests by the modified β-cyclodextrin.[2] Recently, attempts to limit the self-complexation of ω-aminoalkylamino substituents attached to β-cyclodextrin through *N*-substitution of the ω-nitrogen with bulky *t*-butoxycarbonyl (*t*-BOC) groups have been reported.[3] The resultant compounds exhibited a decreased propensity for self-complexation of the modified substituent relative to that of their parent ω-aminoalkylamino-β-cyclodextrin.

The attachment of a group that is larger than *tert*-butyl to the end of a hydrophobic substituent attached to β-cyclodextrin may stop the self-complexation of the substituent altogether. Alternatively, the attachment of a large group to the end of a self-complexed hydrophobic substituent raises the possibility of preparing novel derivatives of cyclodextrins that may be considered to be molecular knots.[4]

3.2 – Molecular Knots

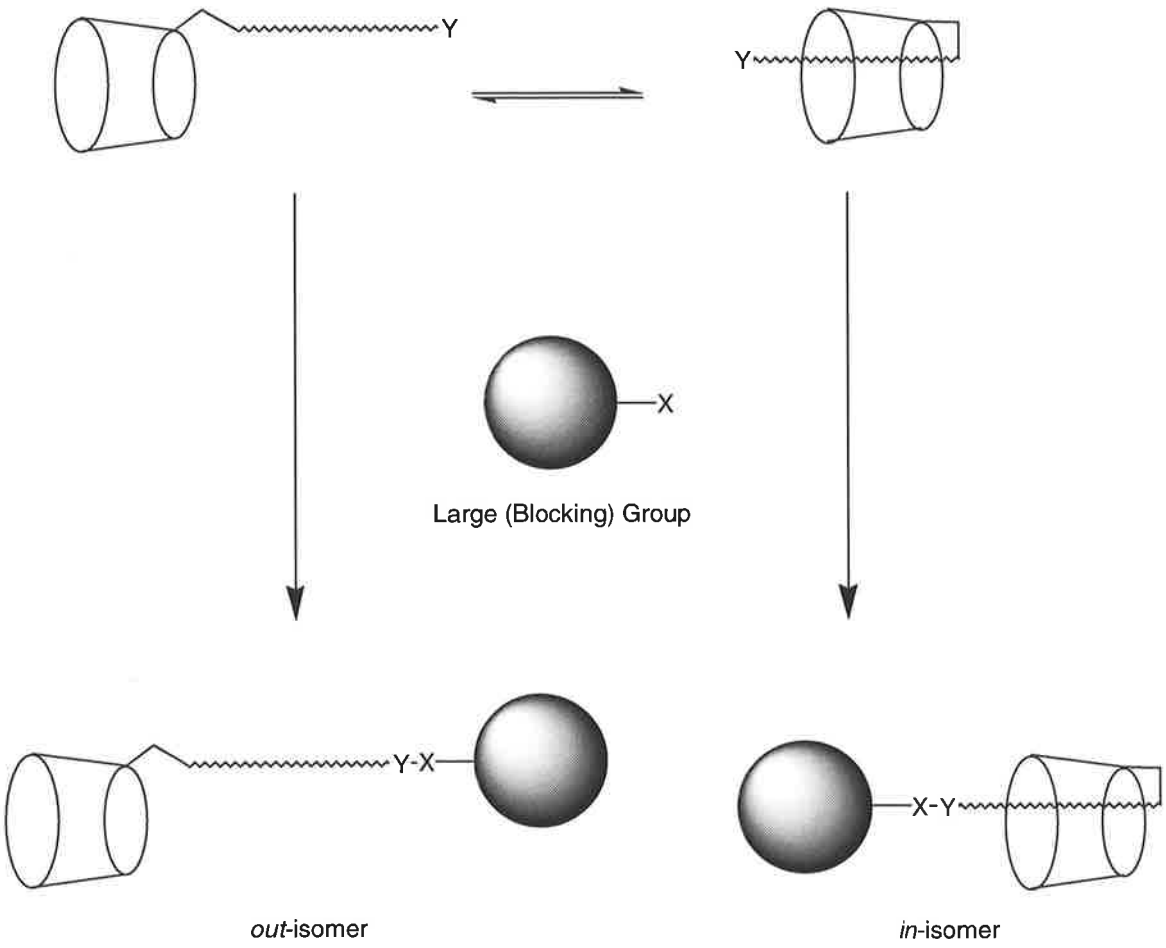
Previous studies of long chain 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins have shown that self-complexation of the ω-aminoalkylamino substituent is common as the length (and associated hydrophobicity) of the alkyl chain increases.[2, 5-10] Kean *et al.*[8] reported the self-complexation of 6-aminohexylamino arm of 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin (β-CDhn) into the modified β-cyclodextrin annulus (Scheme 3.2.1). The 2D ROESY NMR spectrum of this self-complexed β-CDhn exhibited cross-peaks arising from NOE interactions between the methylene protons of the 6-aminohexylamino substituent and the annular H3 and H5 of the modified β-cyclodextrin annulus,

consistent with the self-complexation of the hydrophobic arm residing inside the annulus of β-CDhn.



Scheme 3.2.1: Schematic representation of the self-complexation of the 6-aminohexylamino arm of β-CDhn at pH ≥ 11.5. Cross-peaks arising from NOE interactions between the annular H3 and H5 protons of the modified β-cyclodextrin and the 6-aminohexylamino substituent, consistent with its self-complexation into the annulus of the modified β-cyclodextrin, were visible in the associated 2D ROESY NMR spectra.[8]

If a sufficiently large (blocking) group is covalently attached to the terminus of such a self-complexed substituent, it may prevent the arm from leaving the annulus through its steric interactions with the secondary face of the cyclodextrin. The inability of the substituent to vacate the annulus will leave it "knotted" through the primary and secondary faces of the cyclodextrin (Scheme 3.2.2).



Scheme 3.2.2: Proposed formation of a molecular knot. X and Y represent functional groups which can react to form a covalent bond. If the blocking group is sufficiently large then the substituent chain of the *in*-isomer will be unable to pass through the annulus and the *in*-isomer will exist as a molecular knot.

If an equilibrium exists between the self-complexed and the uncomplexed forms of the substituted β-cyclodextrin, then two isomeric products exist. The *in*-isomer is produced when the substituent chain is self-complexed into the annulus of the β-cyclodextrin entity and the attachment of the blocking group to the terminus occurs at the secondary face of the annulus. The *out*-isomer is produced if the substituent chain is not self-complexed in the annulus when the attachment of the blocking group to the terminus occurs. If the blocking group is small enough to pass

through the annulus, then the isomers are interconvertable and the *in*-isomer would simply be a self-complexed isomer of the substituted β-cyclodextrin, but not a mechanically constrained molecular knot. It is therefore imperative that the blocking group is large enough to prevent its passage through the β-cyclodextrin annulus, if the modified β-cyclodextrin is to exist as a molecular knot.

3.2.1 – Detecting a "Molecular Knot"

^{1}H 2D-ROESY NMR spectroscopy can determine whether host-guest complexation within the annulus of β-cyclodextrin entity occurs. Examining the cross-peaks arising from NOE interactions between the annular H3 and H5 protons of β-cyclodextrin entity (which are orientated such that they point into the centre of the annulus, Scheme 3.2.1) and the protons of a guest provides information on the complexation of the guest. If specific cross-peaks are visible in the spectra between the annular protons of the cyclodextrin and the protons of a guest substituent, then these provide basic structural information about the host:guest complex.

The detection of cross-peaks alone does not conclusively identify a self-complexed β-cyclodextrin as a mechanically held knot. The self-complexation of the *out*-isomer (Scheme 3.2.2) will also show cross-peaks consistent with the complexation of the substituent in the annulus of the β-cyclodextrin entity (the *in*-isomer). These modes of complexation can be differentiated by the introduction of a competing guest molecule with a high affinity for the β-cyclodextrin annulus. The ability of this competing guest molecule to displace substituents from the β-cyclodextrin annulus may be used to distinguish between self-complexing modified β-cyclodextrins and molecular knots. If the molecule is unable to displace the substituent chain and large terminal group from the annulus, this may indicate the formation of a molecular knot.

A suitable competitive guest molecule for such use is adamantane-1-carboxylate. The effectiveness of adamantane-1-carboxylate as a competitive guest has been well documented and studies indicate that once adamantane-1-carboxylate occupies the annulus of β-cyclodextrin it leaves no room for the further complexation of additional molecules, including water.[11] (The formation constant, *K*, for the

complex between adamantane-1-carboxylate and β-cyclodextrin is 1.8×10^4 dm^3 mol^{-1} at pH 8.5). Adamantane-1-carboxylate has been used in these studies as a probe to determine whether a substituent can be displaced from the annulus of modified β-cyclodextrins.

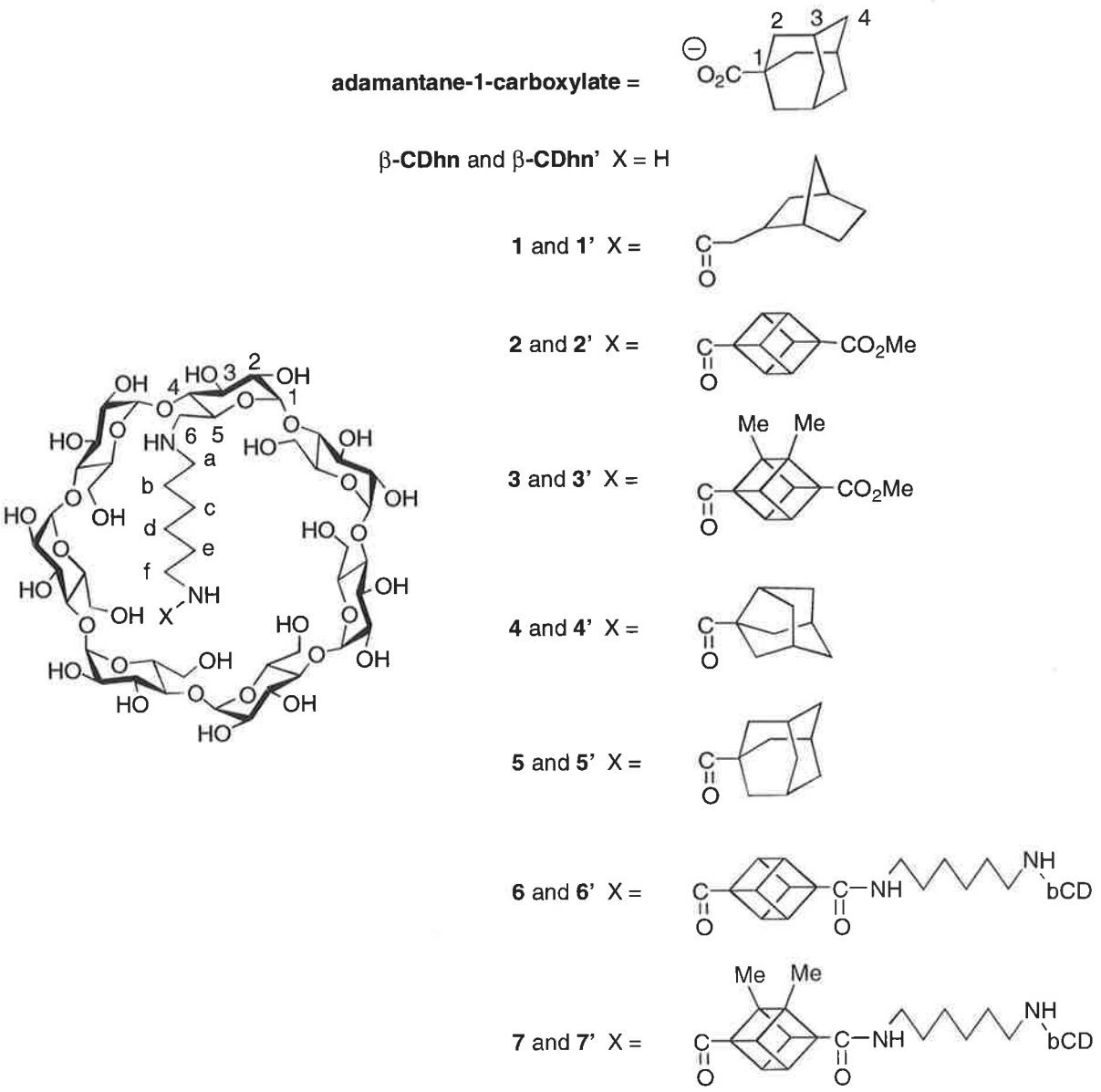


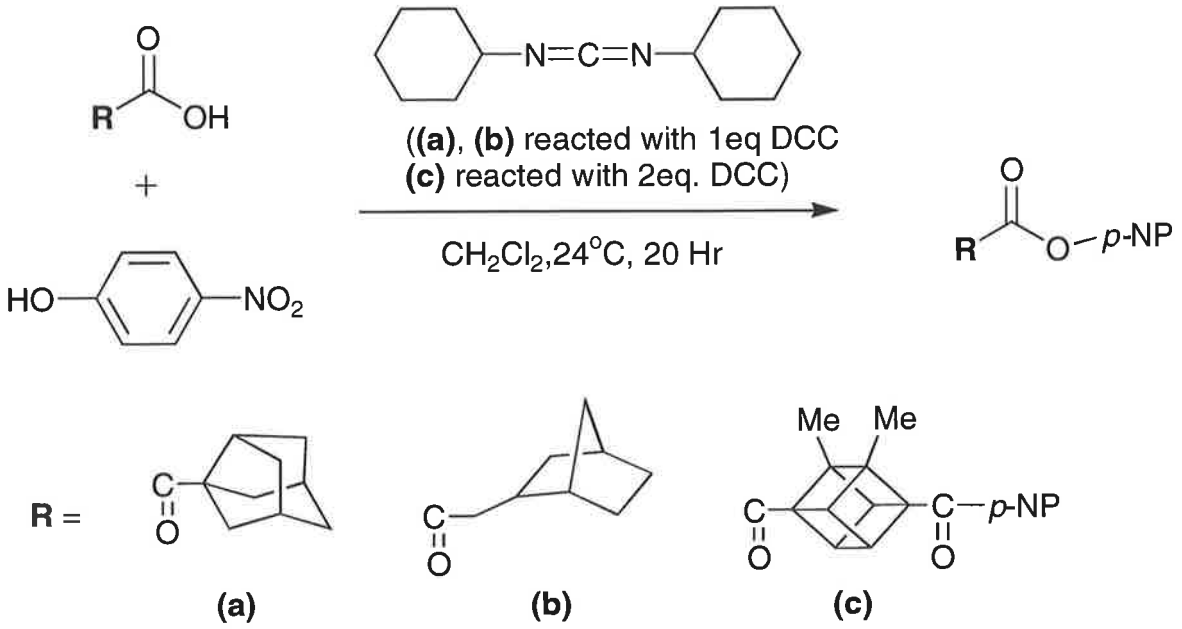
Figure 3.2.1.1: β-CDhn and the terminal blocking groups, **X**, used to construct potential molecular knot and dimers. **X'** denotes the isomer where the substituent **X** is complexed inside the annulus. Monomers **2**, **3**, **5** and dimer **6** have been reported previously[7] with **5** meeting all requirements to be classed as a molecular knot. The prefixes annular, hexyl, norbornyl, cubyl, noradamantyl and adamantyl are used as appropriate in referring to ^1H and ^{13}C resonances in the NMR spectra. Also shown is adamantane-1-carboxylate used as a competitive guest.

The **X** moieties **1-7** shown in Figure 3.2.1.1 can form the self-complexed **1'-7'** by encapsulating the cubanyl, adamantyl, noradamantyl or norbornyl groups in the annulus of the β-cyclodextrin entity. The ability of adamantane-1-carboxylate to dislodge these substituent groups from the annulus is an indication of the strength of self-complexation in **1'-7'**. If adamantane-1-carboxylate dislodges these substituent groups from the annulus of the β-cyclodextrin entity NOE interactions between the protons of adamantane-1-carboxylate and the annular protons H3 and H5 of the β-cyclodextrin entity should appear as cross-peaks in the associated 2D ROESY NMR spectra. There should also be a concurrent loss or diminution of the NOE interactions associated with the 6-aminohexylamino chain or terminal group and the annular H3 and H5 protons of the β-cyclodextrin entity. The absence of NOE interactions between the protons of adamantane-1-carboxylate and the annular protons of the β-cyclodextrin entity in the 2D ROESY spectrum indicates that the self-complexation of the substituent is more favourable than the complexation of adamantane-1-carboxylate and may indicate the formation of a "molecular knot".

3.3 – Synthesis of Potential "Knotted" Cyclodextrins

The work presented in this chapter required the synthesis of the esters *p*-nitrophenyl noradamantane-3-carboxylate, *p*-nitrophenyl norbornan-2-acetate and 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane. The reactions of β-CDhn with the appropriate *p*-nitrophenyl esters mentioned above leads into the synthesis of **1-7** in Figure 3.2.1.1.

The three *p*-nitrophenyl esters *p*-nitrophenyl noradamantane-3-carboxylate, *p*-Nitrophenyl norbornan-2-acetate and 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane were synthesised by a general method[12] utilising the addition of one equivalent each of dicyclohexylcarbodiimide (DCC) to a stirring solution of the parent carboxylic acid and *p*-nitrophenol (Scheme 3.3.1). The pure *p*-nitrophenyl esters *p*-nitrophenyl noradamantane-3-carboxylate, *p*-nitrophenyl norbornan-2-acetate and 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane esters were obtained in 93%, 83% and 67% yields, respectively, after a standard work-up followed by purification by either flash chromatography[13] or squat column chromatography.[14, 15]



Scheme 3.3.1.: Synthesis of *p*-nitrophenyl esters (a) *p*-nitrophenyl noradamantane-3-carboxylate, (b) *p*-nitrophenyl norbornan-2-acetate and (c) 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane were achieved through the reaction of the parent carboxylic acid with *p*-nitrophenol in the presence of dicyclohexylcarbodiimide (DCC).

The modified cyclodextrins **1** and **4** and the dimer **7** (Figure 3.2.1.1) were synthesised by reaction of one mole of 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin (β-CDhn) with one mole of either *p*-nitrophenyl noradamantane-3-carboxylate, *p*-nitrophenyl norbornan-2-acetate in dry DMF for the monomeric modified β-cyclodextrins and two moles of β-CDhn with one mole of 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane in dry DMF for the β-cyclodextrin dimer.

When β-CDhn was allowed to react with *p*-nitrophenyl norbornan-2-acetate the amido-modified β-cyclodextrin 6^A-(6-*N*-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin (β–CDNorb6, **1** in Figure 3.2.1.1) was obtained in 51% yield. The 600 MHz ^{1}H NMR spectrum of this product shows resonances for the norbornyl protons at δ 0.9 – 1.7 ppm overlapping with those of the protons H_{b-e} of the alkyl chain. The 75 MHz ^{13}C NMR shows a resonance at δ 176.7 ppm corresponding to the amide carbonyl group. The product gave a clean electro-spray mass spectrum with a molecular ion at m/z 1370

When β-CDhn was allowed to react with *p*-nitrophenyl noradamantane-3-carboxylate the amido-modified cyclodextrin 6^A-deoxy-6^A-(6-*N*-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)aminohexyl)amino-β-cyclodextrin (βCDNorad6, **4** in Figure 3.2.1.1) was obtained in 54% yield. The 600 MHz ^{1}H NMR spectrum of this product shows resonances for the noradamantyl protons between δ 1.7 – 2.0 ppm which were well removed from the resonances of the hexyl chain at δ 0.9 – 1.5 ppm. The 75 MHz ^{13}C NMR shows a resonance at δ 181.6 ppm corresponding to the amide carbonyl group. The product gave a clean electro-spray mass spectrum with a molecular ion at m/z 1382.

When β-CDhn was allowed to react with 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane the β-cyclodextrin dimer 1,4-bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocabonyl)-2,3-dimethylcubane (βCDhn$_2$CuMe$_2$, **7** in Figure 3.2.1.1) was obtained in 60% yield. The 600 MHz ^{1}H NMR spectrum of this product shows three multiplet resonances for the dimethylcubyl methine protons at δ 4.30, δ 4.10 and δ 3.80 ppm and resonances for the methyl protons overlapping with those of the hexyl chain at δ 1.0 – 1.5 ppm. The 75 MHz ^{13}C NMR spectrum shows three resonances for the amide carbonyl at δ 176.1, 175.7 and 174.4 ppm suggesting that the product is asymmetric.

3.4 – Self-Complexation Studies of 6^A-(6-Aminohexylamino)-6^A-deoxy-β-cyclodextrin

The observed intramolecular complexation of the 6-aminohexylamino arm in the β-cyclodextrin annulus of 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin (β-CDhn, Scheme 3.2.1.)[8] introduces the possibility of generating a range of self-complexing modified β–cyclodextrins through substitution of the primary nitrogen of β-CDhn with a variety of moieties, **X**. When the substituent **X** precursor has either one or two groups participating in this substitution, a modified β-cyclodextrin monomer and a linked β-cyclodextrin dimer are obtained, respectively (Figure 3.2.1.1). The monomers where **X** is a cubyl, dimethylcubyl or adamantyl group (**2**, **3** and **5** in Figure 3.2.1.1) and the dimer where **X** incorporates a cubyl group (**6** in

Figure 3.2.1.1) have been reported previously.[7] The **X** moieties of **1**, **4** and **7** are discussed in this thesis and were selected on the basis that they are hydrophobic and likely to enter the largely hydrophobic β–cyclodextrin annulus to form intramolecular complexes stabilised by a combination of secondary forces in aqueous solution.[9, 16-21] The preparation of the norbornyl and noradamantyl modified β-cyclodextrin monomers (**1** and **4** in Figure 3.2.1.1) and the dimethylcubyl β-cyclodextrin dimer (**7** in Figure 3.2.1.1) are discussed in Chapter 4.

3.4.1 – Self-Complexation of 6^A-(6-Aminohexylamino)-6^A-deoxy-β-cyclodextrin Modified With Terminal Groups Incorporating an Adamantyl, Cubyl or Dimethyl Cubyl Group and the Cubane Dimer

The previously reported systems **2**, **3**, **5** and **6** show that the cubyl and adamantyl groups of **2**, **3** and **5** complex in the β-cyclodextrin annulus in preference to the 6-aminohexylamino chain to give **2'**, **3'** and **5'**.[1, 7] The associated ^{1}H 2D-ROESY NMR spectra show significant cross-peaks arising from NOE interactions between the annular H3 and H5 protons of the β-cyclodextrin entity and the cubyl and adamantyl protons. In each case, no interactions were observed between the 6-aminohexylamino chain and the annular H3 and H5 protons of β-cyclodextrin, consistent with it not entering the annulus of the β-cyclodextrin entity.

The addition of one mole of adamantane-1-corboxylate to a solution of **2/2'** was shown to displace the cubyl group from the β-cyclodextrin annulus of **2'**, indicating that **2** is not a molecular knot. The increased size and hydrophobicity of the dimethyl cubyl entity of **3** enabled it to compete for complexation within the annulus upon the addition of adamantane-1-carboxylate. The self-complexed dimethyl cubane derivative, **3'**, shows NOE interactions existing between the cubane methyl groups and the β-cyclodextrin annular H5 protons and between the protons of adamantane-1-carboxylate and the annular H3 and H5 protons after the addition of one mole of adamantane-1-carboxylate. This is consistent with the dimethyl cubyl group competing for complexation within the annulus of **3/3'** with adamantane-1-carboxylate in a dynamic equilibrium.

The addition of two moles of adamantane-1-carboxylate to a solution of **5/5'** did not dislodge the adamantyl group of the substituent from the annulus of the β-cyclodextrin entity. The substituent adamantyl group has a similar hydrophobicity to that of the added adamantane-1-carboxylate and thus the added adamantane-1-carboxylate was unable to push the adamantyl substituent out of the β-cyclodextrin annulus. The covalent attachment of the adamantyl substituent gives it an entropic advantage for complexation within the annulus over that of the added adamantane-1-carboxylate, thus the self-complexation of the substituent is favoured over complexation of adamantane-1-carboxylate. It was reasoned that because adamantyl compounds form the most stable host:guest complexes with cyclodextrins[11] no other added molecule would be able to displace the substituent from the annulus of the β-cyclodextrin entity. While the adamantyl substituent of **5'** may be able to pass through the β-cyclodextrin annulus, it was not pushed out by adamantane-1-carboxylate and was therefore considered a molecular knot, held together mainly by non-covalent forces if the mechanical (steric) forces were not effective.

The ^{1}H 2D ROESY NMR spectra of the cubane dimer **6** showed cross-peaks associated with NOE interactions between the β-cyclodextrin annular H3 and H5 protons and those of the cubyl group. The interactions were not as pronounced as those seen for the monomers **2/2'** and **3/3'** and were consistent with only one β-cyclodextrin entity interacting with the cubyl group consistent with shallow complexation of the cubane near the end of the annulus of one of the β-cyclodextrin entities (Figure 3.4.1.1). The complete complexation of the cubyl group of **6/6'** into the annulus was not possible due to steric interactions between the linker chain and the β-cyclodextrin rim.

The asymmetric system **6'** became highly symmetric upon addition of two equivalents of adamantane-1-carboxylate. The ^{1}H 2D-ROESY NMR spectra show the complete expulsion of the cubyl group from the β-cyclodextrins annulus and the complexation of adamantane-1-carboxylate into both cyclodextrin annuli.[1]

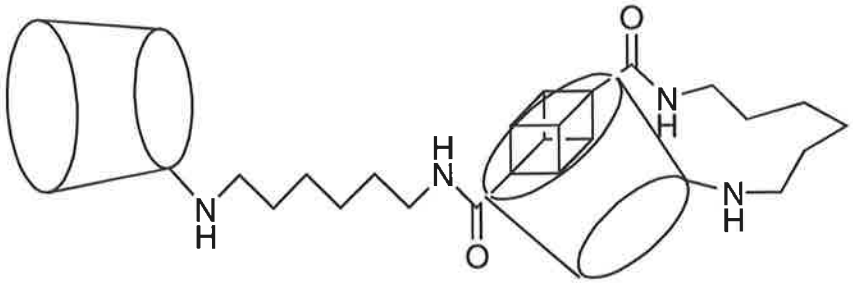


Figure 3.4.1.1: Representation of the complexation of the cubyl group within one of the cyclodextrin moieties of cyclodextrin **6** to give an asymmetric system **6'**. Partial complexation of the cubyl group maybe possible from both faces of the cyclodextrin.

3.4.2 – Self-Complexation of 6^A-(6-Aminohexylamino)-6^A-deoxy-β-cyclodextrin Modified With Terminal Groups Incorporating Norbornyl and Noradamantyl Terminal Groups

The adamantyl terminal group of **5/5'** was shown to form a very stable self-complex in the annulus of β-cyclodextrin, which may be a molecular knot.[1] The physical shape and "level of hydrophobicity" of the adamantyl group may be the driving force behind the formation of such molecular knots. In this study the norbornane and noradamantane based **X** moieties of **1** and **4** were chosen to test this hypothesis. These groups are larger and more hydrophobic than the cubyl blocking groups of **2**, **3** and **5** and comparable to the adamantyl substituent of **5** in terms of hydrophobicity and size. It is envisaged that **1/1'** and **4/4'** will compete for complexation in the β-cyclodextrin annulus more effectively than **2/2'** and **3/3'** and therefore show similar results to those obtained for the adamantyl moiety of **5/5'.**

The 600 MHz ^{1}H 2D-ROESY spectrum of a solution of **1** in D_2O at pD ≥ 12 shows significant cross-peaks arising from NOE interactions between the polycyclic norbornyl group and the H3 and H5 protons of the β-cyclodextrin annulus (Figure 3.4.2.1 and Table 3.4 (see page 78)). Usually, the annular H3 and H5 resonances occur at δ3.8 ppm and δ3.5 ppm, respectively, however in Figure 3.3.2.1, these resonances cannot be separately distinguished from each other nor from the resonances of H6 that lies on the outside of the β-cyclodextrin annulus. Hence, in the assignment of cross-peaks the annular H3 and H5 resonances are not distinguished between. However, as the variations in the ROESY spectra discussed here are consistent with intra- and intermolecular complexation in the β–cyclodextrin annulus,

it would seem unlikely that any of the cross-peaks arise from NOE interactions of the protons of complexed moieties with H6 on the outside of the β–cyclodextrin annulus. Cross-peaks also arise from interactions between protons which are at small through-bond separations from each other, these provide little information on complexation and are not further considered.

The two bridgehead protons H_i and H_I at δ1.95 and δ2.35 ppm both show strong cross-peaks associated with NOE interactions with the annular H3 and H5 protons of the cyclodextrin. There are no visible cross peaks associated with NOE interactions between the H3 and H5 protons and those of the 6-aminohexylamino chain. The complexation of the norbornyl substituent is most consistent with Figure 3.4.2.1, where the norbornyl and acetyl groups are complexed into the annulus while the hexyl chain is not complexed.

On addition of one equivalent of adamantane-1-carboxylate, cross peaks arising from NOE interactions between the norbornyl substituent of **1** and the annular H3 and H5 protons of the β-cyclodextrin entity (Figure 3.4.2.2 and Table 3.4) still exist. This is consistent with adamantane-1-carboxylate competing for binding within the annulus with the norbornyl substituent of **1**. However, the cross-peaks associated with the NOE interactions between the protons of adamantane-1-carboxylate and the annular protons of the β-cyclodextrin entity are much stronger than those associated with the norbornyl substituent. This is consistent with the norbornyl group competing with adamantane-1-carboxylate for complexation within the annulus in a dynamic equilibrium as shown in Scheme 3.4.2.1. The orientation of the carboxylate group of adamantane-1-carboxylate towards the secondary face of the β–cyclodextrin annuli of both **1** and **4** is consistent with modelling studies of intermolecular complexes formed by adamantane-1-carboxylate with β–cyclodextrin and β-CDhn **8**.[7, 22]

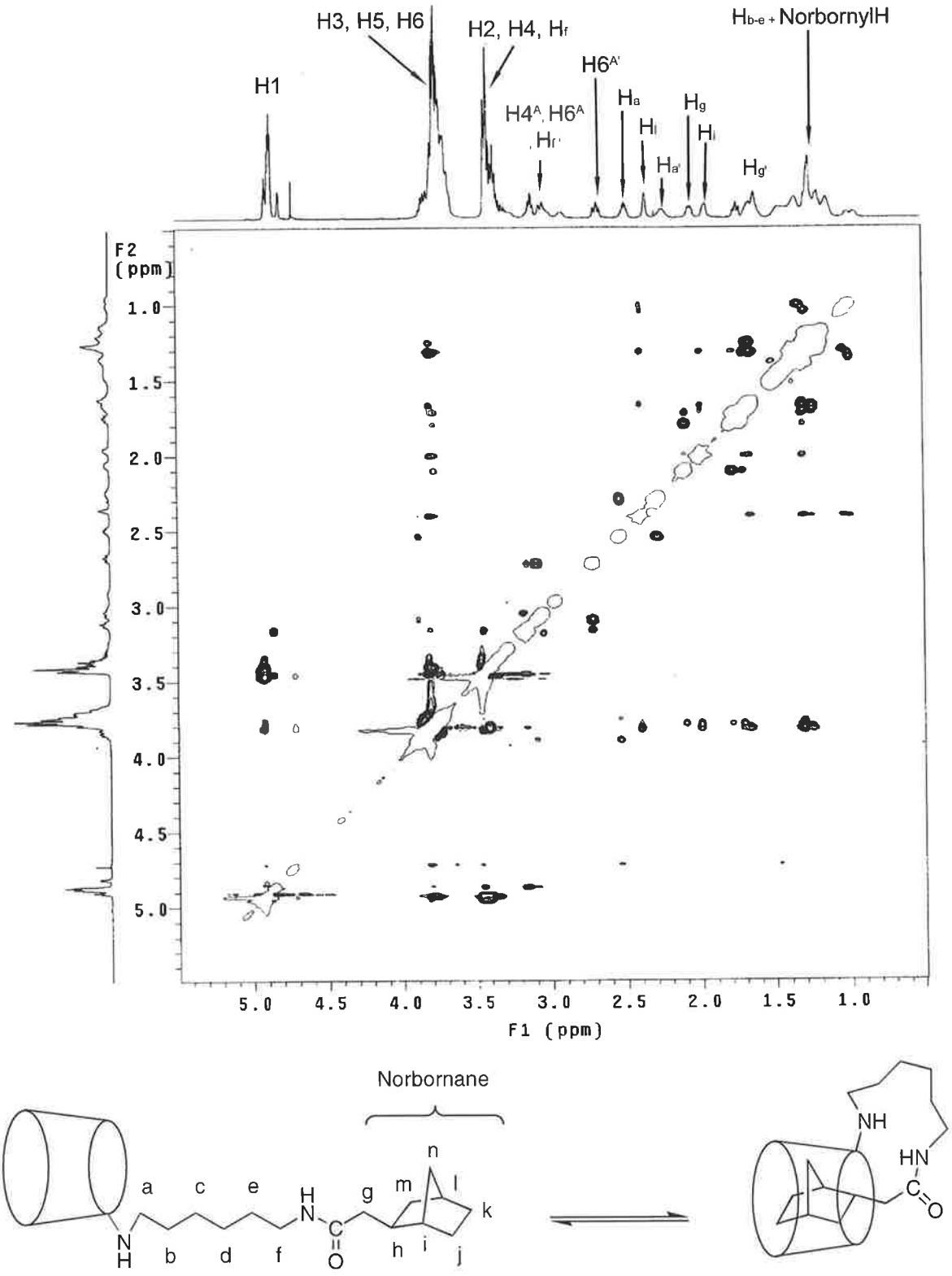


Figure 3.4.2.1: Contour plot of the ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz, 0.3 secs mixing time) performed on a sample containing 0.06 mol dm^{-3} in **1**. The protons are labelled as shown above.

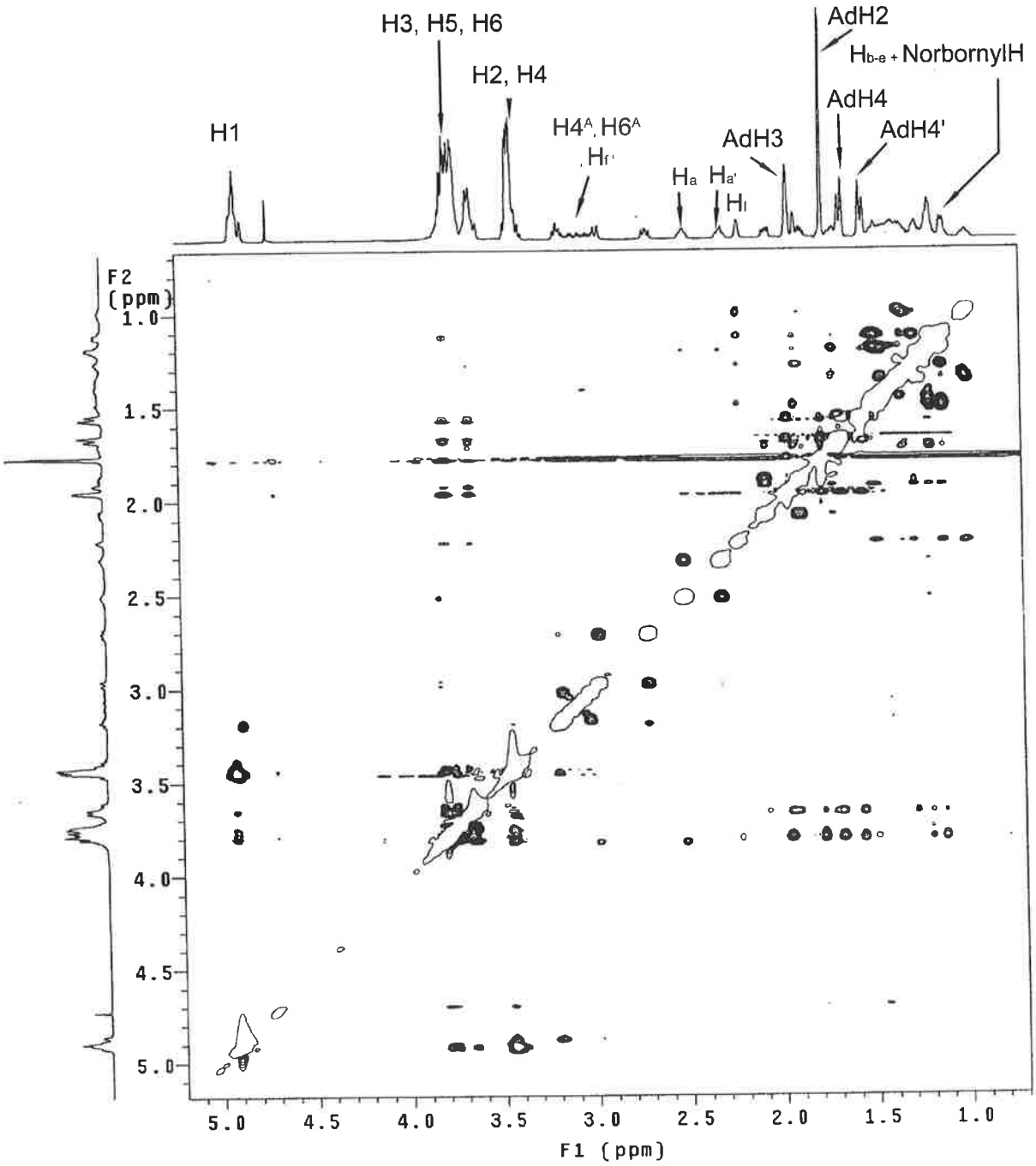
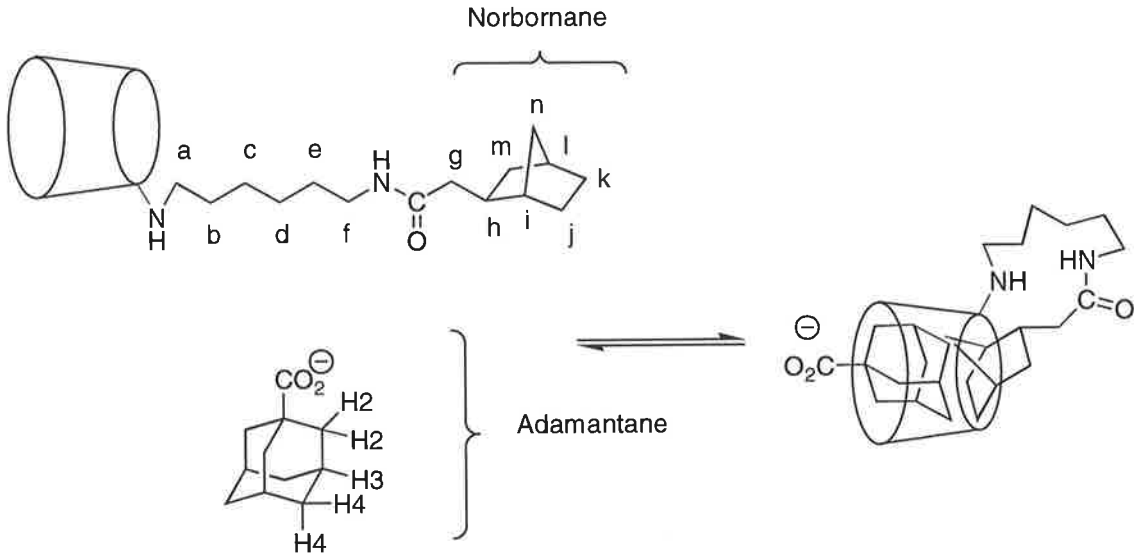


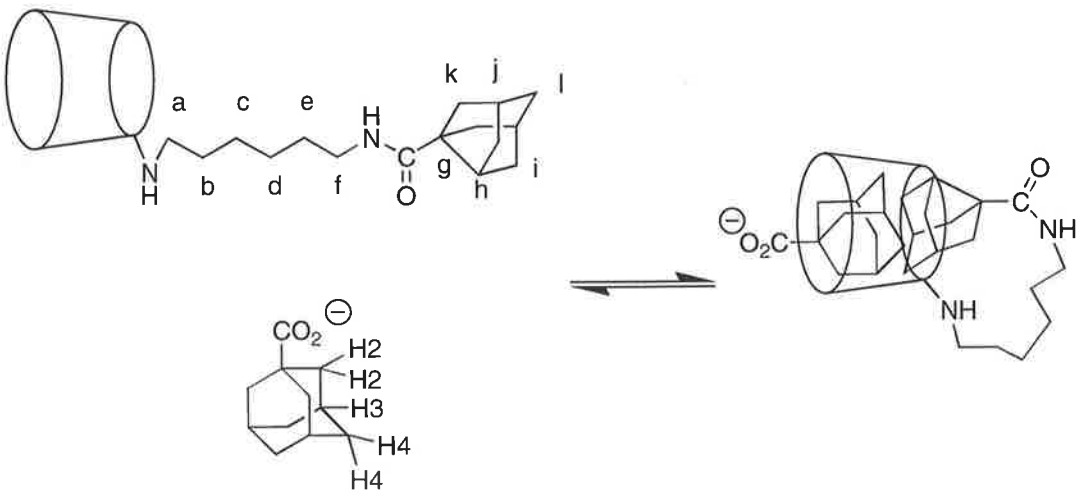
Figure 3.4.2.2: Contour plot of the ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz and 0.3 secs mixing time) performed on a sample contained 0.06 mol dm^{-3} each in **1** and adamantane-1-carboxylate. This spectrum shows residual NOE interactions between the norbornyl substituent and the annular H3 and H5 protons of the cyclodextrin. The protons are labelled as shown in Scheme 3.4.2.1.



Scheme 3.4.2.1: Schematic representation of the complexation of adamantane-1-carboxylate in the modified β-cyclodextrin **1** in aqueous solution at pD ≥ 12. Cross-peaks arising from NOE interactions are visible between the annular H3 and H5 cyclodextrin protons and both the adamantyl and the norbornyl groups, consistent with the norbornyl substituent not being completely expelled from the annulus and being held close to the primary face of the β-cyclodextrin entity.

The 600 MHz ^{1}H 2D ROESY NMR spectrum of **4** showed cross-peaks arising from the NOE interactions between the protons of the noradamantyl group (δ1.7 ppm - δ2.0 ppm) and the annular protons H3 and H5 of the β-cyclodextrin entity (Figure 3.4.2.3 and Table 3.4). This was consistent with complexation of the noradamantyl group into the annulus of the β-cyclodextrin entity. There were no visible interactions between the substituent chain and the annular protons H3 and H5 of the cyclodextrin indicating that it was not complexed into the annulus of the β-cyclodextrin entity. The addition of one mole of adamantane-1-carboxylate to the solution of **4/4'** saw the ^{1}H NMR resonances from the protons of adamantane-1-carboxylate and those from the noradamantyl substituent overlap between δ1.7 – δ2.2 ppm. Therefore, distinguishing the extent of adamantyl complexation was difficult. The adamantyl protons AdH2, AdH3 and AdH4 all show strong NOE interactions with the annular H3 and H5 protons of the β-cyclodextrin entity which are consistent with adamantane-1-carboxylate being complexed within the β-cyclodextrin annulus (Figure 3.3.2.4 and Table 3.3). The ROESY spectrum also shows cross peaks associated with the NOE

interactions between the H_h and H_j protons of the noradamantyl substituent and the H3 and H5 annular protons. These results are consistent with the complexation of the noradamantyl substituent in the same way as was seen with the norbornyl system **1/1'** and the dimethyl cubyl system **3/3'**. The adamantane-1-carboxylate was unable to fully expel the noradamantyl substituent from the annulus due to its high hydrophobicity. The hydrophobicity of the noradamantyl substituent of **4/4'** was assumed to be slightly lower than that of adamantane-1-carboxylate due to adamantane-1-carboxylate having an extra methylene group and a more symmetric and rigid shape. The difference in hydrophobicity allows competitive binding within the annulus between the two groups. The noradamantyl substituent may be partially complexed near the primary end of the β-cyclodextrin annulus as shown in Scheme 3.4.2.2.



Scheme 3.4.2.2: Schematic representation of the complexation of adamantane-1-carboxylate in the modified β-cyclodextrin **4** in aqueous solution at pD ≥ 12. There are cross-peaks arising from NOE interactions visible between the annular H3 and H5 protons of the modified β-cyclodextrin entity and both the adamantyl and the noradamantyl groups, indicating that the noradamantyl substituent is not completely expelled from the annulus and competes for complexation in a dynamic equilibrium.

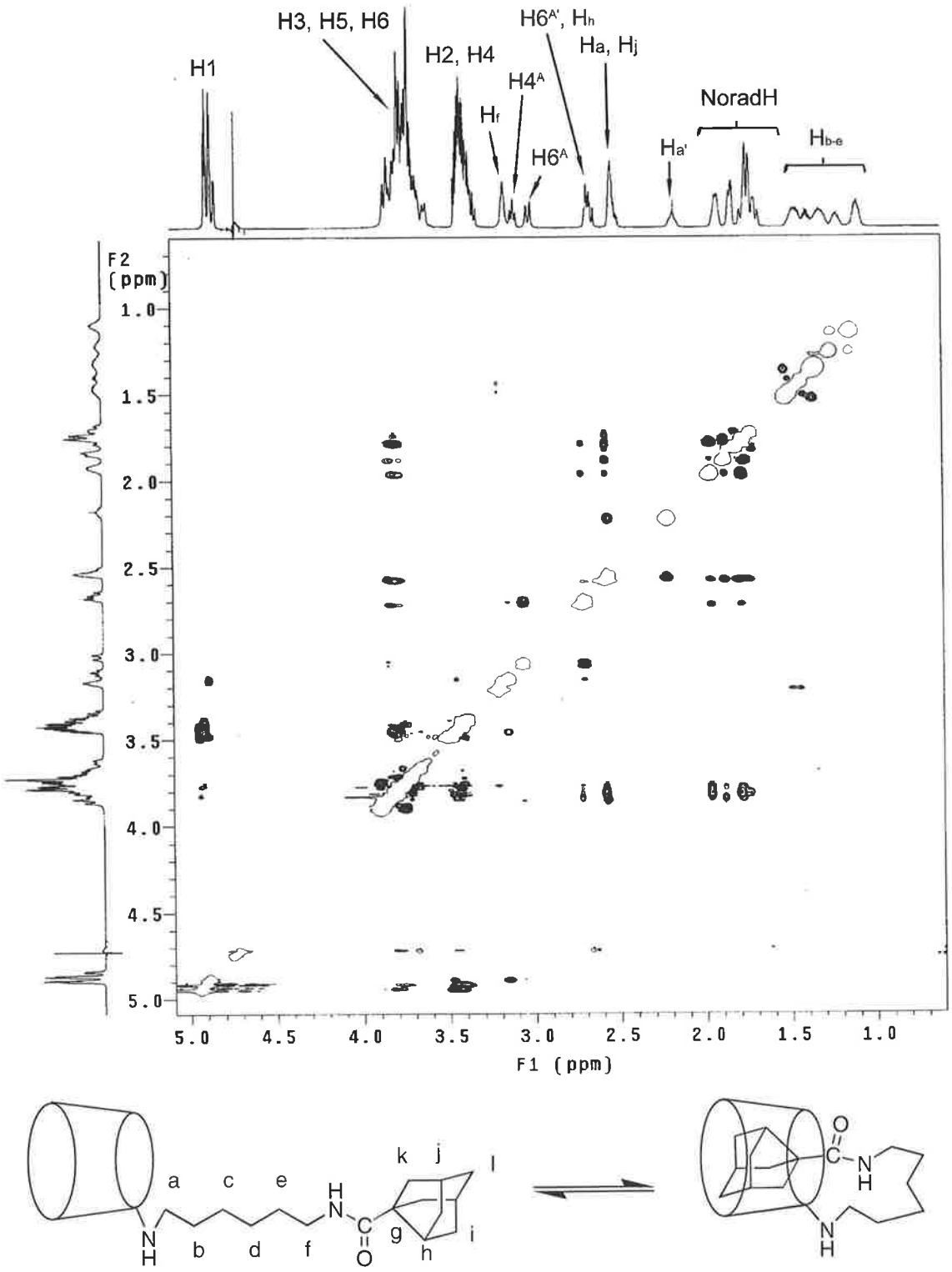


Figure 3.4.2.3: Contour plot of the ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz. 0.3 sec mixing time) performed on a sample containing 0.06 mol dm^{-3} of **4**. The protons are labelled as shown above.

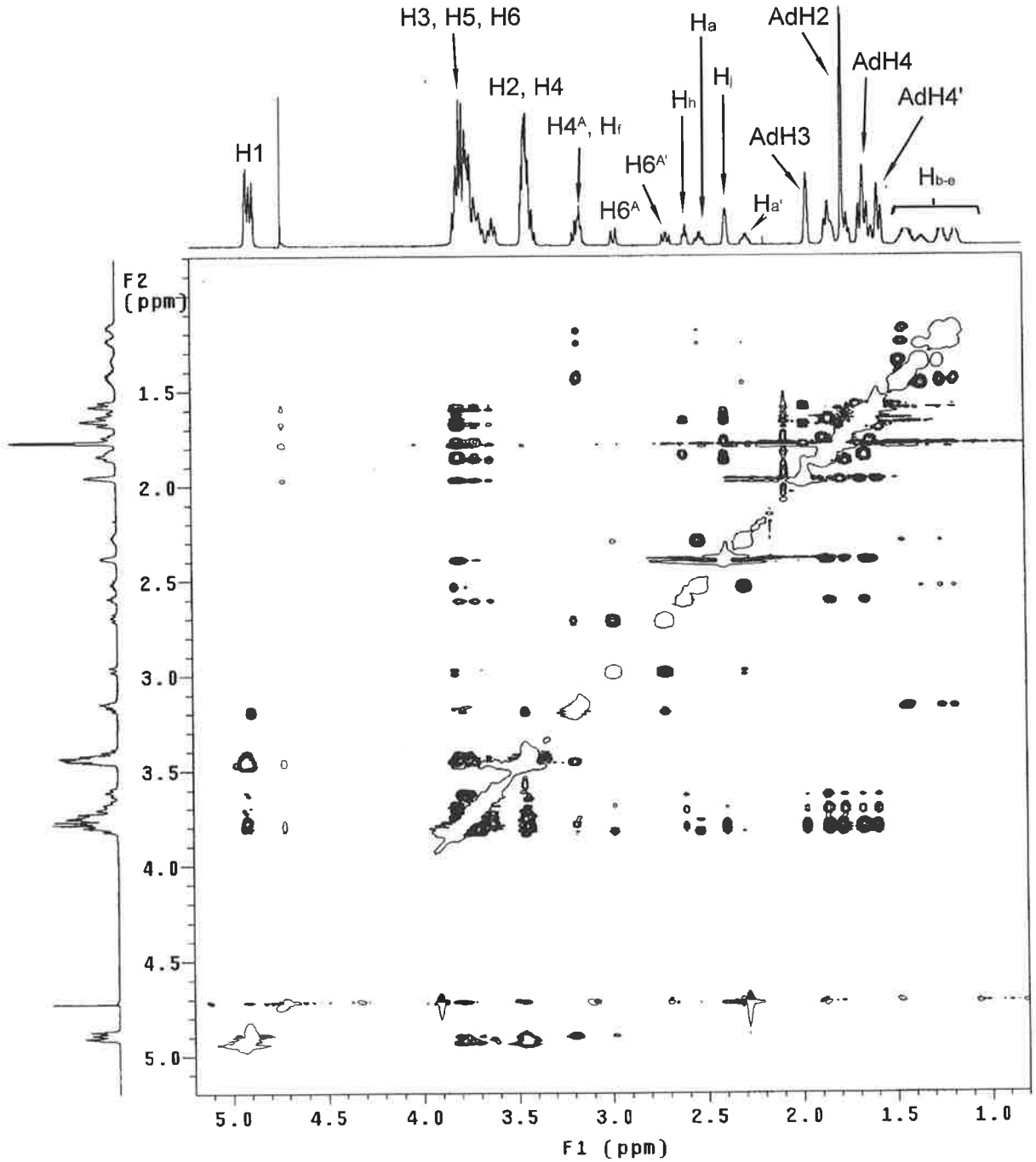


Figure 3.4.2.4: Contour Plot of ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz, 0.3 secs mixing time) performed on a sample contained 0.06 mol dm^{-3} each of **4** and adamantane-1-carboxylate. The protons are labelled as shown in Scheme 3.4.2.2.

Similar intramolecular complexation as that discussed above for the modified β-cyclodextrin monomers **1** and **4** to form **1'** and **4'** was reported of the cubyl and adamantyl moieties of **2** and **5** to form **2'** and **5'**, respectively.[23] While adamantane-1-carboxylate was seen to displace the cubyl moiety from the annulus of **2'**, it does not displace the adamantyl moiety from the annulus of **5'**. The intramolecularly complexed adamantyl substituent of **5'** may have an entropic advantage in competing with adamantane-1-carboxylate for occupancy of the β–cyclodextrin annulus. Alternatively, it may be that substitution of **8'** through the secondary face of the β–cyclodextrin annulus produces **5'** and the adamantyl moiety is too large to pass through the primary face. In the latter case, **5'** is a mechanically constrained molecular knot.

Despite uncertainty as to whether the entropic or the mechanical constraint rationale was correct, the ability of the norbornyl and noradamantyl moieties of **1'** and **4'** to compete with adamantane-1-carboxylate for occupancy of the β–cyclodextrin annulus, whereas the dimethyl cubyl and cubyl moieties of **2'** and **3'** appear to be less effective,[23] is consistent with a decreasing strength of intramolecular retention in the β–cyclodextrin annulus in the sequence: adamantyl > noradamantyl ≈ norbornyl > dimethylcubyl ≈ cubyl. This suggests that a combination of closeness of fit and degree of hydrophobicity of the substituent determine the relative stabilities of the modified β–cyclodextrin intramolecular complexes. It is also consistent with the closer fit of the adamantyl moiety of adamantane-1-carboxylate, in combination with its hydrophobicity, stabilising its intermolecular complex with **2** by comparison with the intramolecular cubyl complex **2'**.

This interpretation is in accord with a recent study[19] which showed adamantan-1-ol, adamantan-2-ol and adamantane-1-carboxylic acid compete more effectively with the dansyl moiety of N^{α}-dansyl-L-lysine-β-cyclodextrin for occupancy of the β–cyclodextrin annulus than do the smaller sized *l*-borneol, *d*- and *l*-camphor and *d*- and *l*-fenchone. Intramolecular complexation of aromatic substituents of modified β–cyclodextrins has been well established, particularly in the case of those incorporating a dansyl moiety.[9, 18-20]

3.4.3 – Dimethylcubane Substituted β-Cyclodextrin Dimer

The previously reported β-cyclodextrin dimer **6/6'** exists as an asymmetric species in solution through complexation of the cubyl group within one of the β-cyclodextrin entities (Figure 3.4.1.1). The cubyl substituent of **6/6'** does not compete for binding within the annulus in the presence of adamantane-1-carboxylate. The cubyl substituent is expelled from the annulus in preference to the complexation of adamantane-1-carboxylate.

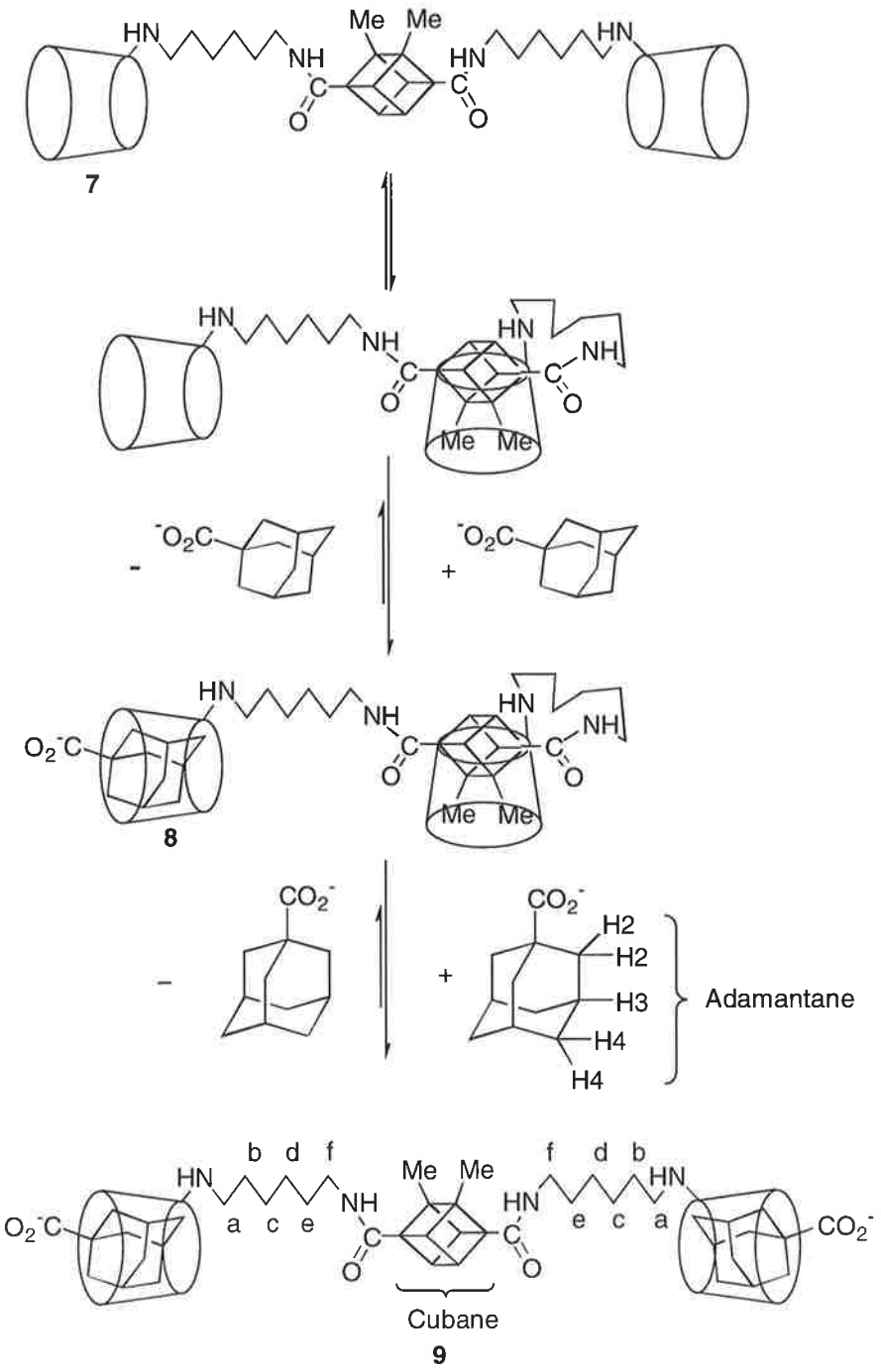
The dimethyl cubane substituent of **3/3'** competes for complexation within the modified β-cyclodextrin annulus upon addition of one mole of adamantane-1-carboxylate.[1] This substituent is investigated as a linker group component for the cyclodextrin dimer **7/7'**.

The 1D ^{1}H NMR spectrum of **7/7'** shows three multiplet resonances for the dimethylcubyl methine protons and a single resonance for the methyl protons. These results are consistent with **7/7'** existing in solution as an asymmetric species. The 600 MHz ^{1}H 2D ROESY spectrum of **7** in D_2O at pD ≥ 12 shows cross-peaks associated with NOE interactions between the dimethylcubyl methine and methyl protons (δ3.8 – δ4.3 ppm) and β-cyclodextrins annular H3 and H5 protons (Figure 3.4.3.1 and Table 3.4). This is consistent with the intramolecular complexation of the dimethyl cubyl moiety in one of the annuli of **7'** (Scheme 3.4.3.2). Cross-peaks also arose from the interaction of the hexyl H_b-H_e protons (δ1.1 ppm – δ1.4 ppm) with the annular H3 and H5 protons of β-cyclodextrins annulus, consistent with complexation of a hexyl moiety in a second β–cyclodextrin annulus.

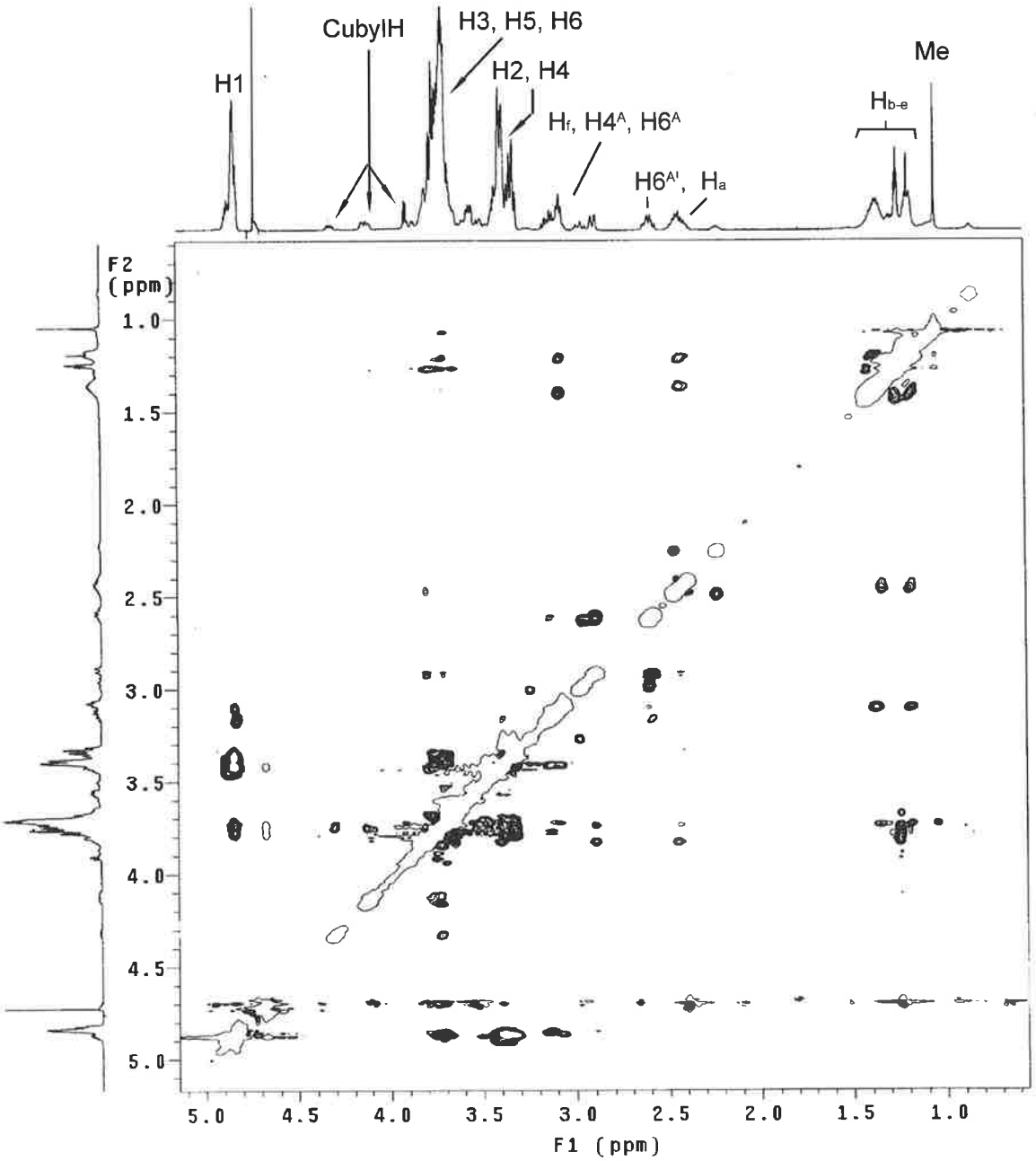
This was similar to the self-complexation observed for the β-cyclodextrin dimer **6**, where the cubanyl group was partially complexed at the primary hydroxyl face of the β-cyclodextrin (Scheme 3.4.1.1). This type of complexation allows only limited mobility of the cubyl group before steric interactions prevents its progress any further into the annulus.

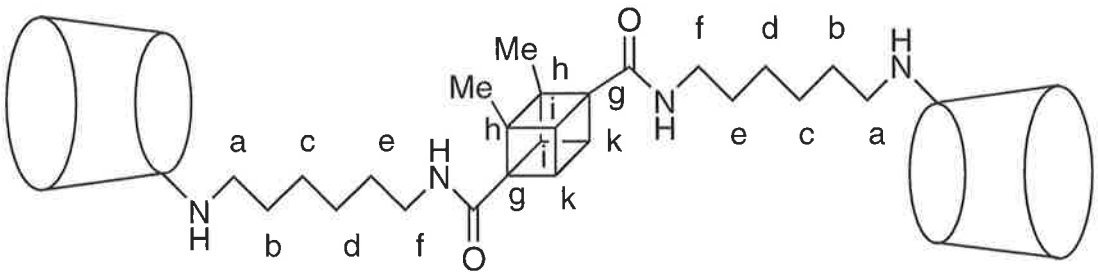
The addition of two moles of adamantane-1-carboxylate to a solution of **7/7'** causes chemical shift changes to occur consistent with intermolecular complexation of adamantane-1-carboxylate. New cross-peaks arise from the NOE interactions of the adamantyl protons AdH1-AdH4 with the annular H3 and H5 protons of the

intermolecular complex **9** (Scheme 3.4.3.1 and Table 3.4). The cross-peaks due to hexyl H_b-H_e protons disappear upon addition of two moles of adamantane-1-carboxylate, consistent with adamantane-1-carboxylate displacing the hexyl moiety from the β–cyclodextrin annulus. Changes in chemical shift render any change in the cross-peaks that arise from the dimethyl cubyl methine protons difficult to detect, due to other overlapping cross-peaks of the protons of adamantane-1-carboxylate. A cross-peak arising from the methyl protons of the cubyl moiety of **7/7'** interacting with the annular H3 or H5 protons remains. This is consistent with the dimethyl cubyl moiety competing with adamantane-1-carboxylate for occupancy of the β–cyclodextrin annuli through the equilibria between **7'**, **8** and **9** shown in Scheme 3.4.3.1. In the 1D ^{1}H NMR spectrum, only a single resonance was observed for the cubyl protons consistent with both β–cyclodextrin moieties becoming equivalent and complexed by adamantane-1-carboxylate in **9**, the dominant species in solution. (Similar equilibria have previously been reported for **6** and **6'**).[23]



Scheme 3.4.3.1: Schematic representation of the series of equilibria leading to the complexation of adamantane-1-carboxylate into the asymmetric β-cyclodextrin dimer **7'** to give **8** and the symmetric dimer **9**.





7

Figure 3.4.3.1: Contour plot of the ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz. 0.3 sec mixing time) performed on a sample 0.06 mol dm^{-3} in **7**. Protons are labelled as shown above.

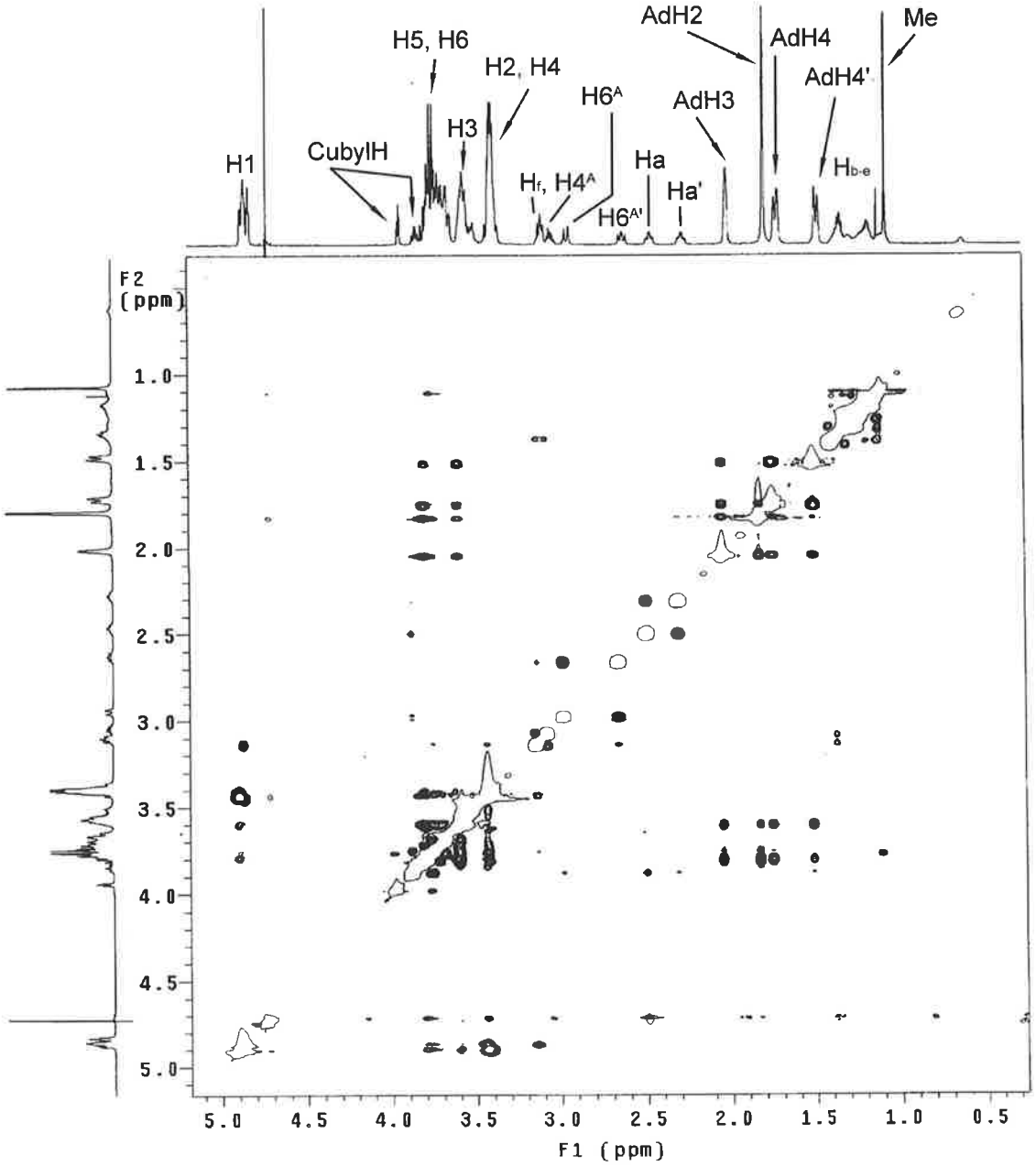


Figure 3.4.3.2: Contour plot of the ROESY experiment (D_2O, pD ≥ 12, 298 K, 600 MHz, 0.3 secs mixing time) performed on a sample 0.06 mol dm^{-3} in **7** and 0.12 mol dm^{-3} in adamantane-1-carboxylate. The protons are labelled as shown in Scheme 3.3.3.2.

Table 3.4: 600 MHz ^{1}H NMR ROESY cross-peaks observed in D_2O solution at pD ≥ 10 and identified as arising from intra- and intermolecular complexation.

	System **1/1'**				
Annular protons	Norbornyl protons				
	nor H_i	nor H_l	nor H_g	nor H_g'	nor H*
H3	+	+	+	+	+
H5	+	+	+	+	+

	System **1/1'** + Adamantane-1-carboxylate								
Annular protons	Norbornyl and adamantyl protons								
	nor H_i	nor H_l	nor H_g	nor H_g'	nor H*	AdH2	AdH3	AdH4	AdH4'
H3	+	+	+	+	+	+	+	+	+
H5	+	+	+	+	+	+	+	+	+

	System **4/4'**					
Annular protons	Noradamantyl protons					
	norad H_j	norad H_h	norad H*			
H3	+	+	+			
H5	+	+	+			

	System **4/4'** + Adamantane-1-carboxylate					
Annular protons	Noradamantyl and adamantyl protons					
	norad H_j	norad H_h	norad H*	AdH2	AdH3	AdH4
H3	+	+	+	+	+	+
H5	+	+	+	+	+	+

	System **7/7'**		
Annular protons	Hexyl and dimethylcubyl protons		
	hexyl H_a-H_e	cubyl H*	cubyl Me
H3	+	+	+
H5	+	+	+

	System **7/7'** + Adamantane-1-carboxylate				
Annular protons	Dimethylcubyl and adamantyl protons				
	cubyl Me	AdH2	AdH3	AdH4	AdH4'
H3	+	+	+	+	+
H5	+	+	+	+	+

* Identification of individual resonances uncertain. Crosses indicate the presence of a cross peak.

These interpretations are supported by ^{13}C NMR studies. In D_2O solution, three carbonyl and five methyl ^{13}C resonances are observed which is consistent with both **7** and **7'** existing in solution. On addition of two moles of adamantane-1-carboxylate, only single carbonyl and methyl ^{13}C resonances are observed consistent with the dominant formation of **9** (Scheme 3.4.3.2). In **7'**, the diastereomeric dimethylcubyl moieties are distinguished by two distinct pairs of methyl ^{13}C resonances arising from differing interactions of the methyl groups with the homochiral β–cyclodextrin annulus. It appears that the combination of deep penetration of the methyl groups into the β–cyclodextrin annulus of **7'** and the large chemical shift scale for ^{13}C is responsible for this detection of chiral discrimination however, no other evidence for it was provided by either the ^{13}C or ^{1}H spectra. The 2,3-dimethylcubane diester used to prepare the dimer **7** was racemic and therefore **7** should exist as two diastereomers, as should **7'**, **8**and **9**.

3.5 - Conclusions

The results described above indicate that the hydrophobicity of the blocking group was a major factor in determining the extent of self-complexation of the modified cyclodextrins **1-7**. Adamantane-1-carboxylate was able to completely exclude the smaller cubyl terminal group of **2** and linker group of **6** from the annulus of the β-cyclodextrin entity. The norbornyl and noradamantyl groups of **1**, **4** and **7** show an increased affinity to compete for complexation within the β-cyclodextrin annulus. The associated ^{1}H 2D-ROESY spectra reported that some self-complexation of these terminal groups occur in the presence of adamantane-1-carboxylate. However, the ability of adamantane-1-carboxylate to push these groups from the β-cyclodextrin annulus and compete for complexation in a dynamic equilibrium suggest that of the systems **1-7**, only **5** may exist as a molecular knot.

3.6 - Potentiometric Titration Studies

The pK_as of the new modified β–cyclodextrins, **1**, **4** and **7**, and also those of their earlier reported analogues, **2**, **5** and **6**[1, 23], were determined by pH titration (Table 3.6.1 and Equations 3.1 – 3.2) in this study. The increased acidity of the protonated secondary amine of the cyclodextrins (**1-7**) by comparison with that expected for a secondary amine (pK_a1 ~ 10), is similar to that observed in other amino-substituted β-cyclodextrins (Table 3.6.2)[24]. This increase may partially arise from either the electronic and steric effects of the attached β-cyclodextrin moiety, or by a difference in solvation experienced by protonated nitrogens adjacent to the hydrophobic β-cyclodextrin annulus, or a combination of both. The pK_a of the protonated amine decreases as the size and hydrophobicity of the **X** moiety increased. This is consistent with an increasingly hydrophobic environment destabilising the protonated amine.

The results suggest that the pK_as of **6** are either extremely similar in magnitude or identical. The theoretical model was successfully fit for a single pK_a system. This model was consistent with the experimental data implying the cyclodextrin dimer **6** had a single pK_a. The theoretical fit of the pH titration curve of **7** was like-wise fitted to a single pK_a system. The modified β–cyclodextrin dimers **6** and **7** are each characterised by identical pK_as for the two protonated amines, consistent with each amine being sufficiently insulated from a change in the protonation state of its twin to behave independently. This may also be reflected through the identical pK_as of **2** and **6**.

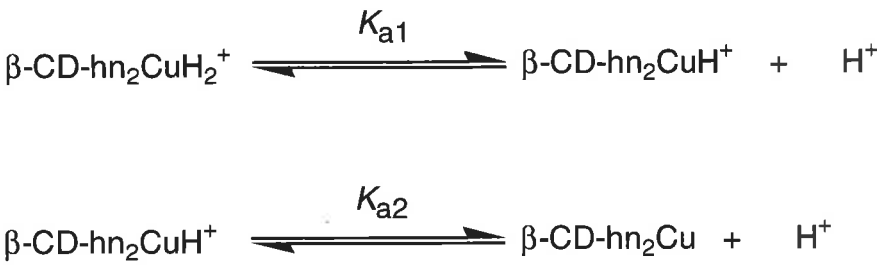
The protonated amine groups of the modified β–cyclodextrins experience different environments in their uncomplexed and intramolecularly complexed forms. However, two distinct pK_as were not observed. This was consistent with either the intramolecular complex being greatly dominant, or the exchange between it and its uncomplexed analogue being sufficiently rapid to yield an averaged pK_a, or a combination of these effects. (Above pH = 10, a time dependent pH change occurs for **2** that is thought to arise from hydrolysis of the methyl ester).

Table 3.6.1: pK_as[a] for the protonated β-cyclodextrins in aqueous solution (I = 0.10 mol dm^{-3}, $NaClO_4$) at 298.2 K. [a] Errors represent one standard deviation

Species	pK_a
β-CDNorb6 (**1**)	8.91 ± 0.02
β-CDCube6 (**2**)	8.87 ± 0.02
β-CDNorad6 (**4**)	8.47 ± 0.02
β-CDAdam6 (**5**)	8.15 ± 0.02
β-CDhn$_2$Cu (**6**)	8.87 ± 0.02
β-CDhn$_2$CuMe$_2$ (**7**)	8.80 ± 0.02

Equation (3.1)

X = Norb6 (**1**), Cube6 (**2**), Norad6 (**4**), Adam6 (**5**)



Equation (3.2)

Table 3.6.2: pK_a's of some ω-aminoalkylamino substituted cyclodextrins.

Cyclodextrin	pK_{a1}	pK_{a2}
β-CDen[7]	9.42 ± 0.01	5.70 ± 0.02
β-CDpn[25]	7.39 ± 0.04	9.9 ± 0.1
β-CDbn[7]	10.26 ± 0.02	8.06 ± 0.01
β-CDhn[1]	10.27 ± 0.03	8.72 ± 0.03

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Chapter 4: Attempted Synthesis of β-Cyclodextrins Mono-substituted With Multi-dentate Metal Ion Binding Substituents

4.1 – Introduction

Most metallocyclodextrin studies concern the coordination of a metal ion by a modified cyclodextrin to produce a binary metallocyclodextrin. Subsequently, a guest may be complexed in the cyclodextrin annulus and may also be coordinated by the metal centre to give a ternary metallocyclodextrin (see Chapter 1.4.2). Under these circumstances the opportunity to study the effects of the metal centre and cyclodextrin interactions on metallocyclodextrin stability and guest complexation presents itself. Many examples exist in the literature of such studies incorporating a wide variety of multi-dentate metal ion binding substituents.[1] These substituents include linear and cyclic polyamines,[2-4] porphyrins[5] and specifically tailored organic compounds[1].

The modified β-cyclodextrins 6^A-(aminopropylamino)-6^A-deoxy-β-cyclodextrin and 6^A-(2-(bis(2-aminoethyl)amino)ethylamino)-6^A-deoxy-β-cyclodextrin ((a) and (b) in Figure 4.1.1) coordinate metal ions such as Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} to form binary metallocyclodextrins.[2-4] These binary metallocyclodextrins complex (*R*)- and (*S*)-tryptophan anions to form ternary metallocyclodextrins. The stabilities of the binary metallocyclodextrin complexes formed are greater for $[M(\beta\text{-CDtren})]^{2+}$ by comparison with those for the corresponding $[M(\beta\text{-CDpn})]^{2+}$ system (Table 4.1.1).

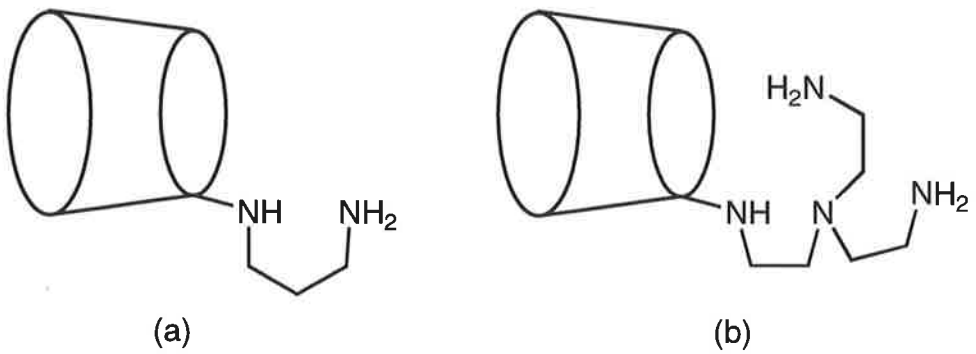


Figure 4.1.1: (a) 6^A-(aminopropylamino)-6^A-deoxy-β-cyclodextrin (β-CDpn) and (b) 6^A-(2-(bis(2-aminoethyl)amino)ethylamino)-6^A-deoxy-β-cyclodextrin (β-CDtren).

Table 4.1.1: Stability constants (*K*) for metallocyclodextrins of β-CDpn and β-CDtren in aqueous solution at 298.2K and $I = 0.1$ ($NaClO_4$).[1]

Equilibrium	M^{2+} and $\log(K/dm^3\ mol^{-1})$			
	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
$M^{2+} + \beta\text{-CDpn} \leftrightharpoons [M(\beta\text{-CDpn})]^{2+}$	4.22	5.2	7.35	4.96
$M^{2+} + \beta\text{-CDtren} \leftrightharpoons [M(\beta\text{-CDtren})]^{2+}$	-	11.65	17.29	12.25
$M^{2+} + \beta\text{-CDpnH}^{+} \leftrightharpoons [M(\beta\text{-CDpnH})]^{3+}$	2.5	3.1	3.09	3.0
$M^{2+} + \beta\text{-CDtrenH}^{+} \leftrightharpoons [M(\beta\text{-CDtrenH})]^{3+}$	-	8.46	11.56	7.92

The greater stabilities of $[M(\beta\text{-CDtren})]^{2+}$ result from the tetradentate nature of β-CDtren. The increase in metallocyclodextrin stability as the number of donor atoms of similar type increases in the chelating group(s) substituted on cyclodextrins is consistent with expectations arising from coordination chemistry. Other factors involved in the variation of stability arise through a combination of M^{2+} size and ligand-field variations.

The attachment of pendant "arms" which possess additional donor atoms onto a nitrogen containing ligand via substitution reactions is a standard technique for increasing the chelating potential of the ligand. Generally, the pendant arms posses a nitrogen, an oxygen, a sulfur or a phosphorus atom (or a combination of these groups) which help coordinate metal ions. The polyamine group of β-CDtren is an example of the most basic synthetic modification to β-CDen to achieve better coordination of metal ions and higher stability of the binary metallocyclodextrin formed.

A common synthetic modification of cyclic polyamine supra-molecules such as 1,4,7-triazacyclononane, 1,5,9-triazacyclododecane and 1,4,7,10-tetraazacyclododecane (Figure 4.1.2) is the attachment of acetic acid "arms" onto the nitrogens to afford a "binding

pocket" or "basket" in which a metal ion can be coordinated (DOTA, Figure 4.1.2). 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) forms complexes with the lanthanides which are very stable and have been used as NMR probes for biomedical applications.[6-8]

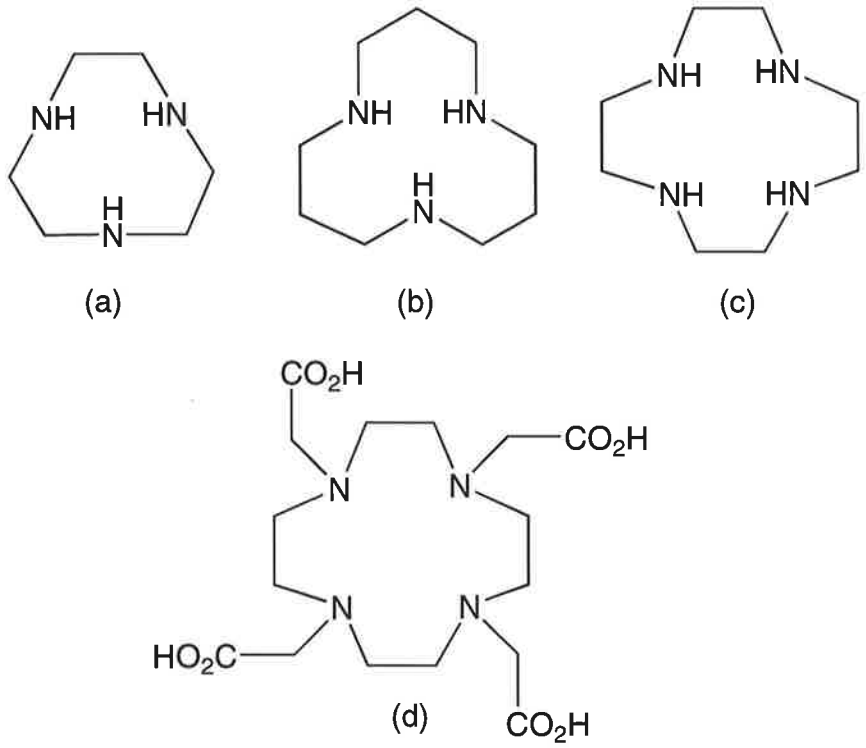


Figure 4.1.2: (a) 1,4,7-triazacyclononane (b) 1,5,9-triazacyclododecane (c) 1,4,7,10-tetraazacyclododecane and (d) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)

The cyclic polyaza systems (a), (b) and (c) shown in Figure 4.1.2 have also been successfully substituted onto β-cyclodextrin. All three modified β-cyclodextrins form binary metallocyclodextrins with Zn(II) which in turn form ternary metallocyclodextrins in the presence of *p*-nitrophenyl acetate.[9]

The aim of the work presented here was to synthetically modify β-CDen and β-CDpn with acetic acid arms (Figure 4.1.3) and study the stabilities and catalytic nature of the reactions of metallocyclodextrins formed by these modified cyclodextrins.

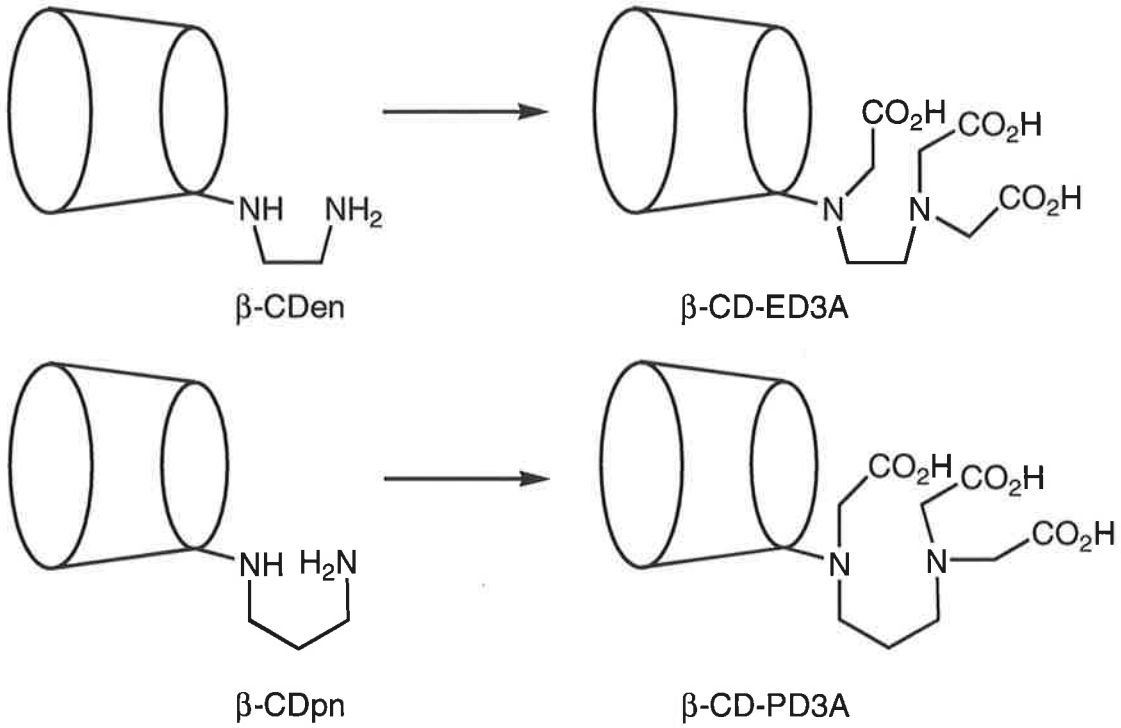
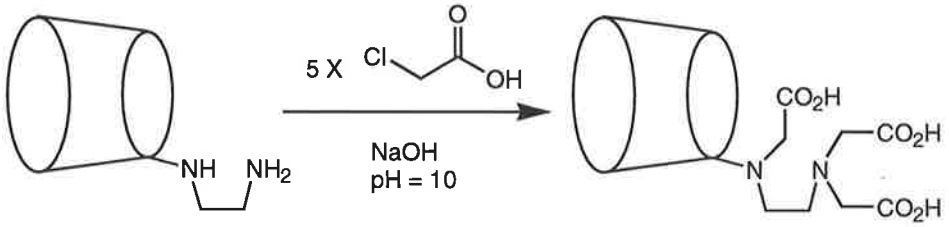


Figure 4.1.3: β-CDen and β-CDpn and their proposed acetic acid "armed" multi-dentate analogues, 6^A-(aminoethylamino-*N,N',N'*-triacetic acid)-6^A-deoxy-β-cyclodextrin (β-CD-ED3A) and 6^A-(aminopropylamino-*N,N',N'*-triacetic acid)-6^A-deoxy-β-cyclodextrin (β-CD-PD3A).

4.2 – Attempted Synthetic Preparation of β-CD-ED3A

The conversion of β–CDen to 6^A-(1,2- diaminoethane-*N, N', N'*-triacetic acid)-6^A-deoxy-β-cyclodextrin (β–CD-ED3A) was attempted using the method of Desreux (Scheme 4.2.1).[7] A 5:1 ratio of α-chloroacetic acid to cyclodextrin was used to ensure maximum substitutions occurred.



Scheme 4.2.1: Schematic representation of the reaction between β-CDen and α-chloro acetic acid to give the amino-acid functionalised derivative β-CD-ED3A.

This procedure required careful manipulation of pH to ensure that multiple substitutions occurred at each nitrogen of β-CDen due to the formation of hydrochloric acid as a by-product. Hydrochloric acid was neutralised as it was formed by constant addition of a concentrated solution of a stoichiometric amount of sodium hydroxide using a calibrated syringe pump to maintain the pH at the desired level. The pK_as of β–CDen have been determined previously as 9.42 and 5.7.[10] β–CDen exists in aqueous solution with partial protonation of both the primary and secondary nitrogens and thus a pH of 10~12 was maintained for the duration of the reaction to ensure the nitrogens where largely unprotonated. Extremes of pH (ie pH = 14) saw substantial hydrolysis of β-CDen, especially when heated. Sodium hydroxide solution was added to the reaction mixture at 1 cm^3/hour over 10 hours with regular pH measurements and TLC analysis. Reaction initiation was achieved only at elevated temperatures of 80 - 90°C.

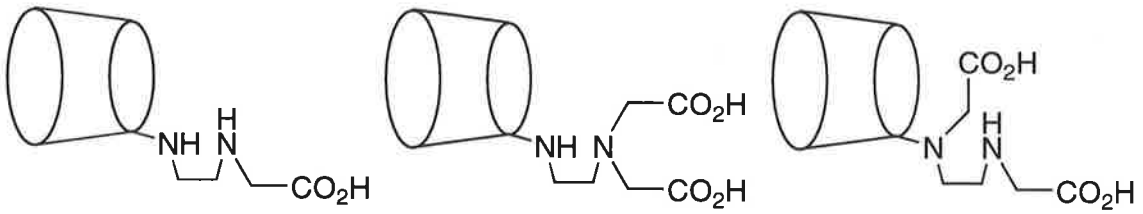
Test reactions of the acid arm attachment reaction on 1,2-diaminothane indicated that the substituted amino-acid compounds appeared as streaks on the TLC plates under standard conditions due to the dynamic protonic equilibria participated in by each attached pendant arm. These streaks give little information about the degree of substitution that had occurred. By varying the TLC solvent system to 7:7:5:4 ethyl acetate / propan-2-ol / 0.1M sodium hydroxide solution / water, the acid groups were deprotonated and the major species in solution was the fully deprotonated modified β-cyclodextrin product. This solvent system removed the streaking to leave a single spot at R_f = 0.38. This spot did not correspond to β–cyclodextrin (R_f = 0.48) or to β–CDen (R_f = 0.05). This result did not

indicate complete pendant arm substitution, but does indicate the extent of substitution (i.e. one spot inferred that ALL cyclodextrin molecules had been substituted to the same degree, either once or twice or three times).

The crude amino acid armed cyclodextrin was isolated as an orange, crystalline solid after ion exchange chromatography (strong gel). The orange compound was recrystallised from a minimum amount of hot water ($\approx 80^{\circ}C$) to afford a fine white crystalline solid.

^{13}C NMR studies of the isolated amino-acid cyclodextrin showed five sp2 hybridised carbon resonances between 170-180 ppm in the ^{13}C spectrum. These resonances correspond to the carbonyl groups of the carboxylic acids. Five distinct carbonyl resonances are consistent with a mixture of products and hence full characterisation studies where therefore undertaken to identify the amino-acid compound.

FAB-MS of the isolated cyclodextrin adduct showed a base peak of m/z= 1275 ([M+H]) amu which is not consistent with β–CD-ED3A which has a molecular weight of 1351 amu (the peak corresponding to m/z = 1275 is discussed later in this section) . There were no peaks corresponding to molecular ions above 1275 amu indicating that the substitution had not gone to completion. The possible products from the acid arm attachment reaction are the mono and di substituted cyclodextrins shown in Figure 4.2.2.



(a) m/z = 1234 (b) m/z = 1292 amu (c) m/z = 1292 amu

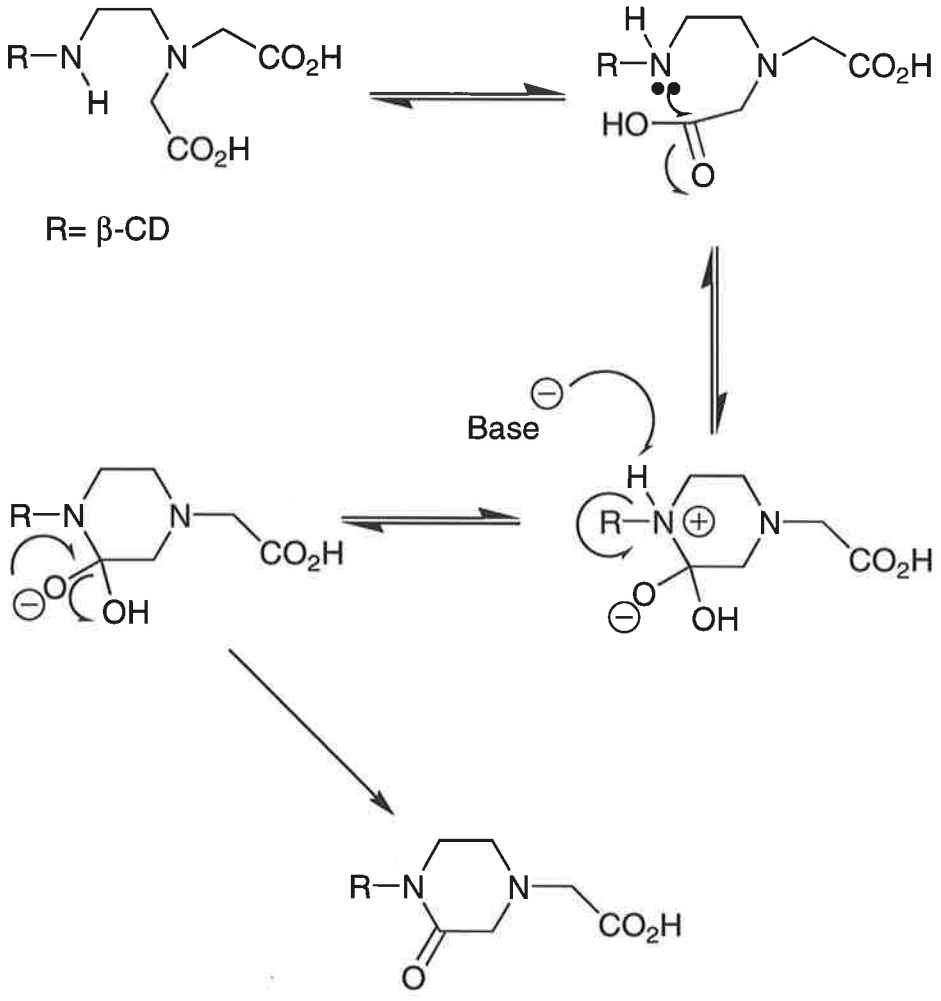
Figure 4.2.2: Possible products from the reaction of β-CDen with α-chloro acetic acid. These products are the possible products of the reaction not going to completion.

The mono-substituted product (Figure 4.2.2(a)) has a molecular mass of m/z = 1234 amu which was not observed by mass spectrometry, so is not a considered as a reaction product. The di-substituted products (Figure 4.2.2(b) and Figure 4.2.2(c)) both weigh 1292 amu which is 18 amu more than the calculated molecular weight of 1274. Microanalyses of the isolated cyclodextrin adduct reported a two-fold abundance of nitrogen over that expected from theoretical calculations. This is consistent with carboxylic acid groups of the cyclodextrin being isolated as the ammonium salts, furthermore it also suggests that only two substitutions of the acid arms have been achieved. The ammonium salts of amino-acid compounds are unsuitable for potentiometric titrations due to the ammonium ion having a titratable proton. When titrated, each carboxylic acid group will show a unique pK_a that will identify the extent of substitution. The ammonium salt was converted to the sodium analogue that would allow these pK_as to be determined by potentiometric titrations. This conversion was accomplished by adding two equivalents of sodium hydroxide to an aqueous solution of the cyclodextrin adduct and passing a steady stream of nitrogen gas through the solution to remove the ammonia formed. The resultant sodium salt was precipitated using excess ethanol to afford a white crystalline solid. The pK_as of this modified β-cyclodextrin were not determined as the mass spectrum data (discussed below) indicates that the isolated compound was not the desired product

The FAB-MS spectrum for this sodium salt showed a new peak corresponding to 1292 amu that was not seen in the mass spectrum of the ammonium analogue. This is consistent with base catalysed hydrolysis, as addition of 18 amu has been achieved in the presence of alkaline solution. Registered patents by Parker *et al.*[11, 12] outlining the synthesis of ethylene diamine tri-acetic acid (ED3A) reported similar chemistry to that observed in these reactions. In their synthesis, the authors reported that under mildly basic conditions, ED3A ring-closed to form 2-oxo-1,4-piperazine diacetic acid (Scheme 4.2.2). This piperazine intermediate consists of a very stable six-membered ring with one of the acid groups in the amide form.

This ring-closure probably occurs in the β-CDen system and is consistent with the experimental data. It was shown that this cyclization could occur in acidic or basic conditions provided that the nitrogens involved possess a proton (ie be primary or

secondary). It was determined that during the conversion of the crude cyclodextrin product from the ammonium salt to the sodium salt the amide ring is hydrolysed to an extent and regenerates a small amount of the di-acid. The cyclized system was still present as indicated by the peak at m/z = 1275 in the mass spectrum of the sodium salt. The molecular weight of the cyclized cyclodextrin adduct was determined to be 1274 amu, this explains why the derivatives shown in Figure 4.2.2. (b) and (c) have molecular weights exactly 18 amu greater than reported by mass spectrometry. If the primary amine of β-CDen is substituted twice before cyclization occurs, then the amide carbonyl group will be



Scheme 4.2.2: Schematic representation of the ring-closure to the piperazine intermediate. As shown, when R = β-cyclodextrin the ring closure disallows the addition of a third acetic acid pendant arm and thus the preparation of β-CDED3A.

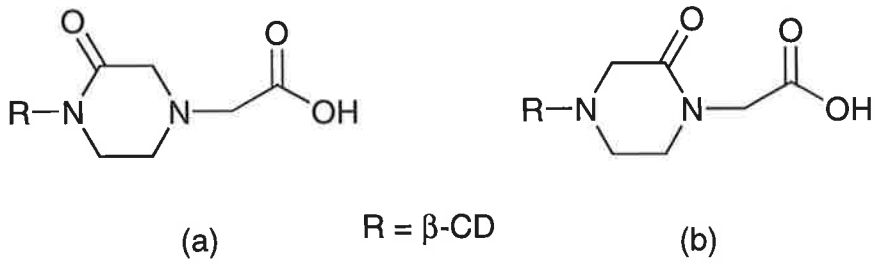


Figure 4.2.3: Possible ring-closed piperazine-cyclodextrin system produced during the attachment of α-chloro acetic acid arms to β-CDen.

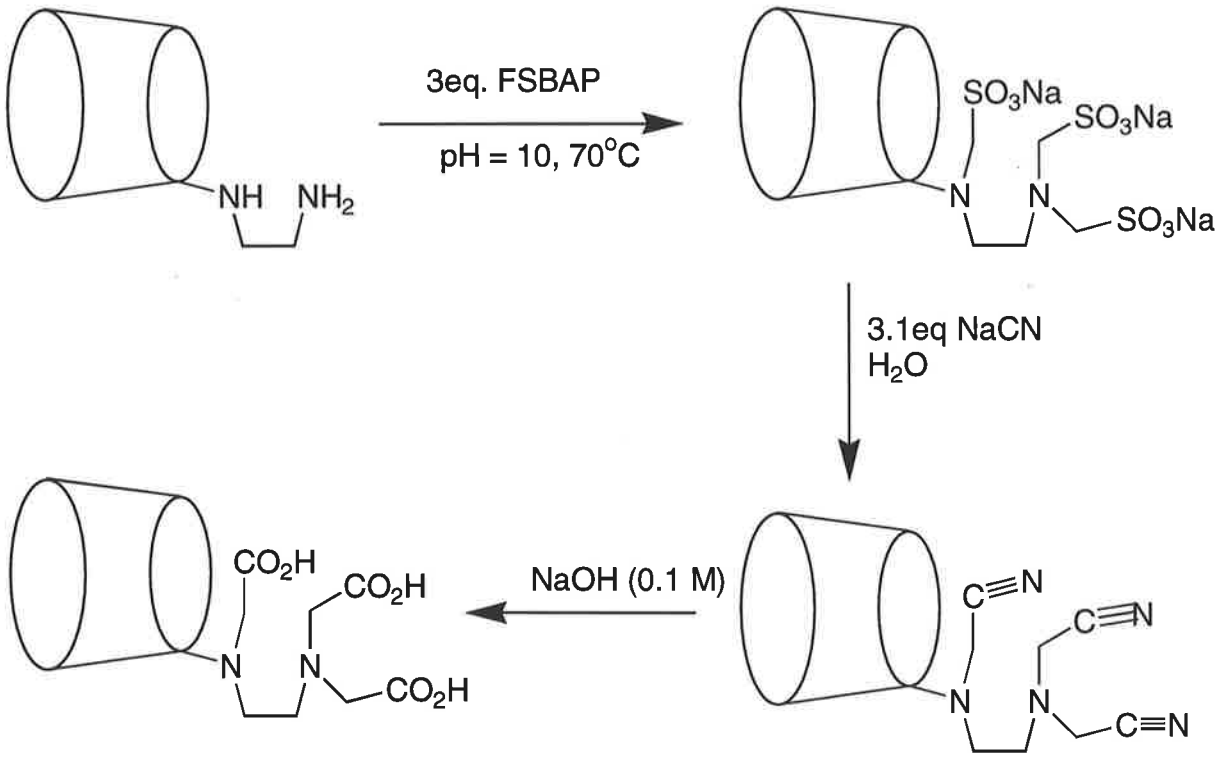
positioned next to the amine of the ethylene diamine arm attached to the $C6^A$ carbon of the β-cyclodextrin (Figure 4.2.3 (a)). If each amine is substituted once, then the amide carboxyl group can form at the primary amine end of the ethylene diamine arm (Figure 4.2.3 (b)), although the existence of this isomer was not supported by the experimental data.

This cyclization presented a significant synthetic hurdle and there is no evidence to suggest that a third acid arm was substituted onto the $C6^A$-attached nitrogen. Mass spectroscopy does not show a compound corresponding to β-CD-ED3A. The acid arm attachment reaction was attempted numerous times, always with the same result.

The formation of the amide does not allow the attachment of a third acetic acid arm. To combat this sulphomethyl pendant arms (which would not react with the amines and form cyclization products) where investigated as possible precursors. Following the method of Westrenen *et al.*[13] (Scheme 4.2.3), β-CDen was reacted with three equivalents of formaldehyde sodium bisulphite addition product (FSBAP). This procedure introduces the carboxylic acid groups at the last synthetic step, when both the nitrogens of the ethylene diamine backbone are tertiary. This ensures that the piperazine cyclization cannot occur, as a requirement of the cyclization is that the nitrogen involved in the amide formation possesses at least one proton. By manipulating the pH of the reaction mixture it is theoretically possible to substitute at either the secondary or primary amine of β-CDen. However, as mentioned previously, the protonated forms of β-CDen did not react with

p-nitrophenyl acetate at all, indicating that the mono-protonated β-CDenH$^+$ is not nucleophillic and hence will not attack electrophiles.[10]

This reaction was seen to initiate only at pH ≥ 10 and at elevated temperatures. Once initiated, all the starting materials were consumed leaving no unreacted β-CDen. The intermediate sulphonate modified β-cyclodextrin was isolated as a pale orange crystalline solid via ion exchange chromatography and shows a broad absorption at 1155 cm^{-1} in its infrared spectrum corresponding to the sulphonyl group stretch. This indicates that substitution of



Scheme 4.2.3: Schematic representation of the sulphomethylation of β-CDen. The acid groups are not formed until after the attachment of all the pendant arms circumventing the cyclization problem.

the sulphomethylene group onto the nitrogens has occurred. The conversion of the sulphonyl groups to cyano groups was achieved using the method of Neelakantan[14] by reacting the sulphomethyl intermediate β-cyclodextrin with three equivalents of aqueous sodium cyanide at 80°C. The addition of the sodium cyanide solution is an exothermic

reaction and causes the instant appearance of a white precipitate. Upon raising the reaction temperature to 110°C, this precipitate redissolves and a bright yellow precipitate forms upon cooling the solution to room temperature. The I.R. spectrum of this intermediate indicated a strong absorption at 2225 cm^{-1} corresponding to the nitrile groups. The absorption due to the sulphonyl groups was no longer present in the spectrum indicating that the conversion was quantitative.

The nitriles were converted to the corresponding carboxylic acids by stirring in 0.1M sodium hydroxide solution and the amino acid cyclodextrin product is isolated and purified by ion exchange chromatography. The I.R. spectrum of this compound was identical to that of the cyclized piperazine β-cyclodextrin produced by the α-chloro acetic acid substitution reaction attempted earlier. The I.R. spectrum indicated that the nitriles were successfully converted into carboxylic acids by the introduction of two strong carbonyl absorbances at 1710 cm^{-1} and 1720 cm^{-1}.

The mass spectrum of this compound had the distinctive 2-oxo-piperazine cyclization product peak at m/z = 1274 seen previously in the α-chloro acetic acid synthesis. Again there was no evidence of a third substitution onto the secondary amine attached to the cyclodextrin during the sulphomethylation step of the synthesis. This suggests that the secondary amine was not reacting in either of the substitution reactions and that the primary amine was substituted twice followed by the ring-closure to give the unwanted piperazine cyclodextrin adduct.

As the secondary amine is attached directly to the cyclodextrin its free electron pair may be involved in hydrogen bonding with the other primary hydroxyl groups on the primary face of the cyclodextrin and be unavailable for covalent bonding, though this would also effect the cyclization reaction. It may simply be that the substitution of the primary amine renders the secondary amine too hindered to react with electrophiles. Therefore the synthesis of β-CD-ED3A was not pursued further.

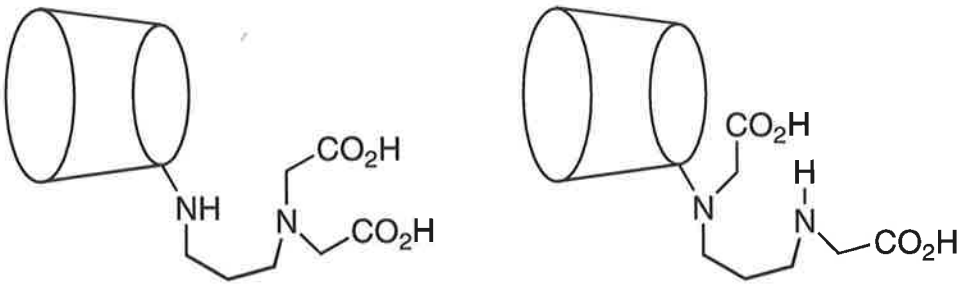
4.3 – Attempted Synthetic Preparation of β-CD-PD3A

The cyclization problem experienced in the attempted synthesis of β-CD-ED3A was not anticipated to occur with the β-CDpn system. Should the ring-closure reaction form a cyclic amide, the result would be a seven-membered ring that is both sterically and kinetically unfavourable. For this reason, the attachment of acetic acid pendant arms was again attempted using the method of Desreux.[7]

The pK_as of β-CDpn have been determined previously as 9.90 and 7.39 (± 0.1 and ± 0.04 respectively)[15] and the reaction of β-CDpn with α-chloroacetic acid was carried out at pH = 10 ~ 12 so as unprotonated β-CDpn was the major species in solution. As for β-CDen, the substitution reaction was only seen to initiate at elevated temperatures of 80 – 90°C and progress was followed by TLC analysis.

The ^{13}C NMR spectra of the isolated modified β-cyclodextrin product was consistent with the substitution of acetic acid arms onto the nitrogens of β-CDpn. Resonances associated with the carbonyl carbons of the acid groups are observed between δ160-165 ppm. The extent of nitrogen substitution is not discernible from the ^{13}C NMR spectra.

The FAB-MS spectrum of the isolated modified β-cyclodextrin shows a base peak of m/z = 1306 amu. This peak corresponds to the di-acid substituted analogue of β-CDpn (Figure 4.3.1 (a) and (b)). Although Figure 4.3.1(b) is a possible structure for the ion with m/z = 1306 amu, it seems unlikely that this compound would be the final product. The excess of α-chloroacetic acid in solution would facilitate the full substitution of both nitrogens of β-CDpn, making Figure 4.3.1(b) an intermediate compound. It would be expected that the two substitutions take place at the primary nitrogen of β-CDpn, leaving the nitrogen attached to the $C6^A$ carbon of the β-cyclodextrin entity unsubstituted in a similar way to that seen previously with the β-CDen system which also exhibited this chemistry.



m/z = 1305 m/z = 1305

(a) (b)

Figure 4.3.1: Possible structures of the reaction products of the reaction between α-chloroacetic acid and β-CDpn with a molecular weight of 1305 amu as seen by FAB-MS.

The non-reactivity of the $C6^A$-attached nitrogens of β-CDen and β-CDpn may be the result of hydrogen bonding with the hydroxyl groups of the primary face of β-cyclodextrin. Such interactions may render these nitrogens non-nucleophillic and inhibit their effectiveness to attack the α-chloroacetic acid. It may also that the substitution onto the primary nitrogen of β-CDen and β-CDpn results in the $C6^A$- attached nitrogen becoming too sterically hindered to allow the close positioning of an α-chloroacetic acid molecule needed for reaction to occur, or a combination of both factors.

While this synthesis was not successful in producing β-CD-PD3A the isolated product 6^A-(3-aminopropylamino-*N, N*-di-acetic acid)-6^A-deoxy-β-cyclodextrin (β-CD-PD2A) is itself a multi-dentate system and may be able to complex metal ions. Potentiometric titrations of this compound with Barium, Cadmium and Zinc were undertaken and no binding was observed with Ba^{2+} or Cd^{2+}. Propylene diamine tetra-acetic acid (PDTA) has low critical stability constants for both Ba^{2+} and Cd^{2+} (log K = 3.95 and 7.26 dm^3 mol^{-1} respectively)[16] and the critical stability constant would be expected to be lower for the β-CD-PD2A system due to it having fewer donor atoms to participate in complexation. Complexation of Zn^{2+} was observed, however the complex was very slow to form and the titration curves were not reproducible due to the slow complexation of the Zn^{2+} ions causing considerable electrode drift. Increasing the maximum equilibrium time delay on the auto-titrimeter from 300 to 1000 seconds (hence allowing the

metallocyclodextrin more time to form and stabilise) made no difference to the titration curve.

Due to the considerable difficulties encountered in the synthesis of the modified β-cyclodextrins β-CD-ED3A and β-CD-PD3A no further study of these compounds was undertaken.

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Chapter 5: Experimental Procedures and Protocols

5.1 – General Physical Methods

Water refers to de-ionised water that has undergone the Milli-Q ultra filtration process. All aqueous solutions are prepared using Milli-Q water unless otherwise stated.
Melting points were determined using a Kofler hot-stage apparatus under a Reichert microscope and are uncorrected. As cyclodextrin derivatives generally decompose without melting above 180 °C melting points were not determined for these compounds.

Elemental analyses were carried out by the Microanalytical Service of the Chemistry Department, University of Otago, Dunedin, New Zealand. Cyclodextrin derivatives were characterised as the hydrates by adding whole molecules of water to the molecular formula to give the best fit to the microanalytical data.

Infrared spectra were recorded on an ATI Mattson Genesis FT-IR. The abbreviations strong (s), medium (m), weak (w) and broad (b) are used in reporting the infrared data.

^{1}H and ^{13}C 1D NMR were recorded on a Varian Gemini 200 or 300 spectrometer. ^{1}H 2D ROESY NMR spectra of cyclodextrins were recorded on a Varian Inova 600 operating at 599.975 MHz using a standard sequence with a mixing time of 0.3 seconds. The cyclodextrins (and added guest when present) were dissolved in 0.1 mol dm^{-3} NaOH in D_2O to give final concentrations of 0.06 mol dm^{-3} of each component and a final pD=11. The resultant solutions were filtered (0.22 μm) and degassed by freeze-thawing before the spectra were recorded and the signals were referenced to aqueous trimethylsilylpropiosulfonic acid as an external standard.

Electrospray mass spectroscopy (Electrospray-ms) was carried out at the Research School of Chemistry at the Australian National University, Canberra, ACT. Samples were dissolved in 10% acetonitrile for injection and the cone voltage was set to 120 V.

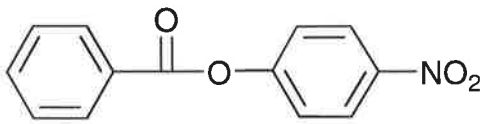
Thin layer chromatography (TLC) was carried out on Kieselgel 60 F_{254} (Merck) on aluminium backed plates. Unless otherwise stated, plates were developed with 7:7:5:4

v/v ethyl acetate/propan-2-ol/ammonium hydroxide/water for the analysis of all cyclodextrin samples (referred to as 'Standard TLC conditions' in this thesis). Compounds bearing amino groups were visualised by drying the plate then dipping it into a solution of 0.5% ninhydrin in ethanol and heating it with a heat-gun. Cyclodextrin compounds were further visualised by dipping the plate into a solution of 1% sulfuric acid in ethanol and heating it with a heat-gun. Iodine vapour was also used to visualise cyclodextrins. The value R_C represents the R_f of a cyclodextrin derivative relative to the R_f of β-cyclodextrin. Squat column chromatography was carried out using Merck Kieselgel 60 PF_{254} thin layer chromatography silica.[1, 2]

Column cation exchange chromatography was performed using Bio-Rex 70 50-100 mesh cation exchange resin (Bio-rad Chemicals) for amino compounds and Dowex 50 x 2 cation exchange resin for amino-acid compounds. For simplicity, Dowex gel will be referred to as 'strong gel' in this thesis. Columns where eluted with water and 1.4% ammonia solution unless otherwise stated.

5.2 – Experimental Protocols and Results

p-Nitrophenyl benzoate.

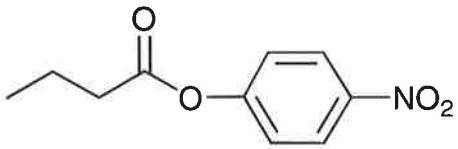


Powdered potassium hydroxide (2.68g, 48 mmols) was added to gently stirring dimethylsulphoxide (20 cm^3). To the DMSO / KOH solution is added *p*-nitrophenol (1.71g, 12 mmols) and stirred for 5 minutes. The solution turns a deep orange/yellow colour on addition of the phenol. Benzoyl chloride (2.9 cm^3, 24 mmols) is then added neat to the *p*-nitrophenol solution, upon addition solution turns bright yellow. The reaction mixture is then stirred at room temperature for 30 minutes after which time 100 cm^3 of water is added to the reaction mixture. The reaction mixture is then extracted with dichloromethane (3 x 50 cm^3) and the organic extracts combined, dried over

$MgSO_4$ and evaporated to afford the title compound as a white crystalline solid (2.2g, 75% Yield).

^{1}H NMR (D_2O, 300 MHz): δ 8.26 (d, *J* = 9.2 Hz, 2H, ArH), 8.13 (d, *J* = 9.2 Hz 2H, ArH), 7.43 (dd, 2H), 7.42 (dd, 2H)

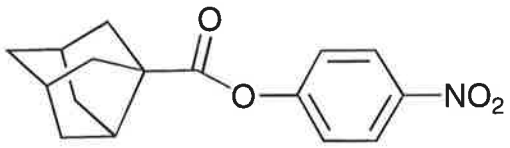
p-Nitrophenyl propanoate.



Butyric acid (10 cm^3, 0.1 mols) was added neat to thionyl chloride (37 cm^3, 0.5 mols) and refluxed for 16 hours under an N_2 atmosphere. The resulting brown mixture had the excess thionyl chloride distilled off (b.p. 79°C) and the residue dissolved in 50 cm^3 dichloromethane under an N_2 atmosphere. *p*-Nitrophenol (18 g, 0.15 mols) was added in one portion and triethylamine (TEA) dripped into the reaction flask over 10 minutes (Heat and HCl gas are expelled during the addition of TEA and care must be taken to avoid boiling the reaction mixture). The reaction mixture was then cooled using cold water bath and stirred for 3 hours at room temperature. The Solvent was removed and the resulting dark brown slurry dissolved in dichloromethane (100 cm^3) and purified by flash chromatography (20% hexane: 80% Dichloromethane). The fractions containing the ester product where combined and the solvent removed to give a brown oil. This brown oil was distilled (125°C @ 0.7 TORR) to afford of the title compound as a golden oil (12.52 g, 60%).

^{1}H NMR (D_2O, 300 MHz): δ_H 8.26 (d, *J* = 9.2 Hz 2H, ArH), 7.28 (d, *J* = 9.2 Hz, 2H, ArH), δ 2.59 (t, 2H, $-CH_2$), δ1.8 (sextet, 2H, $-CH_2$), δ1.05 (t, 3H, $-CH_3$).

p-Nitrophenyl noradamantane-3-carboxylate.



A solution of noradamantane-3-carboxylic acid (0.505 g, 3.04 x 10^{-3} mol), *p*-nitrophenol (0.430 g, 3.09 x 10^{-3} mol) and dicyclohexylcarbodiimide (0.624 g, 3.03 x 10^{-3} mol) was stirred at room temperature for 3 h. The reaction mixture was filtered and the filtrate was washed with 5% sodium bicarbonate solution (3 x 20 cm^3) and brine (20 cm^3) and dried over sodium sulfate. The filtered solution was evaporated under reduced pressure and the oily residue was suspended in 1:1 dichloromethane/hexane and loaded onto a squat column (4.5 cm i.d., 30 g silica gel). Elution of the column was commenced with 1:1 dichloromethane/hexane and the proportion of dichloromethane was progressively increased. Fractions containing the product were combined and evaporated under reduced pressure to give the product as a colourless oil which solidified on standing (0.812 g, 93%).

M.p.: 82-83 °C.

Accurate mass data: calculated for $C_{15}H_{18}NO_4$ $(M+H)^+$ 276.1236. Found 276.1249.

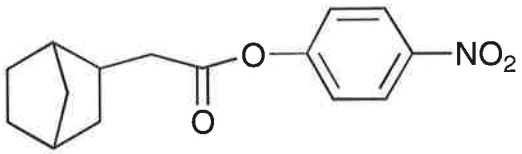
Electrospray-MS: *m/z* 287 (M^+H). (Found C 66.81;H 5.91;N 4.99. Calculated for ($C_{16}H_{17}NO_4$) C, 66.89; H, 5.96; N, 4.87%.)

^{1}H NMR (200 MHz, $CDCl_3$): δ_H 8.26 (d, *J* = 9.2 Hz, 2H, ArH); 7.28 (d, *J* = 9.2 Hz, 2H, ArH); 2.86 (t, *J* = 6.6 Hz, 1H, H7); 2.24 (m, 4H); 1.91 (m, 4H); 1.73 (m, 4H).

^{13}C NMR (50.4 MHz, $CDCl_3$): δ_C175.0 (C=O); 156.0, 145.1, 125.1, 122.4 (ArC); 54.1 (C3); 46.8 (C7); 44.5 (C9); 43.5 (C6); 37.4 (C1); 34.5 (C2).

I.R. (nujol): 1747 (s), 1614 (m), 1591 (m), 1521 (s), 1346 (s), 1305 (m), 1274 (m), 1209 (m), 1182 (s), 1097 (m), 1074 (m), 1020 (m), 997 (m), 873 (m), 862 (m), 742 (m) cm^{-1}.

p-Nitrophenyl norbornan-2-acetate.



A mixture of norbornan-2-acetic acid (0.571 g, 3.70 x 10^{-3} mol), *p*-nitrophenol (0.516 g, 3.71 x 10^{-3} mol) and dicyclohexylcarbodiimide (0.748 g, 3.63 x 10^{-3} mol) in dichloromethane (10 cm^3) was stirred at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite and the filtrate was evaporated under reduced pressure to give the crude ester as a yellow oil. This material was further purified by passage through a squat column eluted with dichloromethane. Fractions containing the ester were combined and evaporated to give the product as a colourless oil (0.830 g, 83%). An attempt to further purify the ester by bulb to bulb distillation (150 °C/0.05 Torr) resulted in some decomposition.

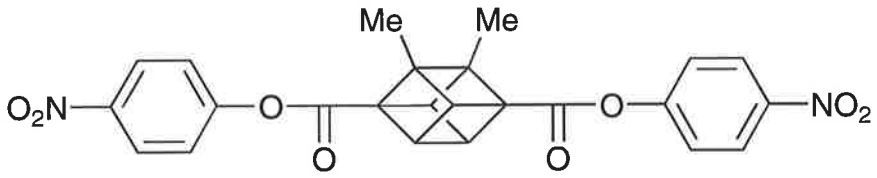
Accurate mass data: calculated for ($C_{15}H_{18}NO_4$) $(M+H)^+$ 276.1236. Found 276.1249.

^{1}H NMR (200 MHz, $CDCl_3$):δ_H 8.27 (d, J = 9.2 Hz, 2H, ArH); 7.28 (d, J = 9.2 Hz, 2H, ArH); 2-2.5 (m, 5H); 1-1.6 (m, 8H).

^{13}C NMR (50.4 MHz, $CDCl_3$):δ_C 170.6 (C=O), 155.5, 145.1, 125.0, 122.4 (ArC); 41.1, 38.3, 38.1, 37.6, 36.7, 35.2, 29.6, 28.4.

I.R. (film): 3115 (w), 3088 (w), 2951 (s), 2871 (s), 1768 (s), 1706 (m), 1615 (m), 1593 (m), 1525 (s), 1490 (m), 1455 (w), 1347 (s), 1208 (s), 1162 (s), 1106 (s), 915 (m), 864 (m), 749 (w), 716 (w).

1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane.



A mixture of 2,3-dimethylcubane-1,4-dicarboxylic acid (0.320 g, 1.45 x 10^{-3} mol), 4-nitrophenol (0.409 g, 2.94 x 10^{-3} mol) and dicyclohexylcarbodiimide (0.613 g, 2.98 x 10^{-3} mol) in dichloromethane (8 cm^3) was left to stir at room temperature for 18 hours. The reaction mixture was filtered and the collected solid was washed with dichloromethane (3 x 10 cm^3). The combined filtrate was washed with 5% sodium bicarbonate solution (3 x 20 cm^3) and dried over sodium sulfate. The solution was concentrated to approx. 20 cm^3 and loaded onto a squat column (30 g silica gel, 4.5 cm i.d) and the column was eluted successively with dichloromethane (3 x 25 cm^3) and chloroform (3 x 25 cm^3). Fractions containing the product were combined and evaporated to give the diester as a white powder (0.451 g, 67%). A portion of this material was recrystallised from dichloromethane/hexane.

M.p.: 202-204 °C.

Micro Analysis Data: (Found C, 62.32; H, 3.90; N, 6.08. Calculated for $C_{24}H_{18}N_2O_8$ C, 62.34; H, 3.92; N, 6.06%.)

^{1}H NMR (200 MHz, $CDCl_3$): δ_H 8.31 (d, J = 8.4 Hz, 4H, ArH); 7.29 ((d, J = 8.4 Hz, 4H, ArH); 4.38 (t, J = 4.0 Hz, 2H, cubaneH); 4.12 (t, J = 4.0 Hz, 2H, cubaneH); 1.46 (s, 6H, Me).

^{13}C NMR (54 MHz, $CDCl_3$): δ_C 167.9 (CO); 155.3, 145.4, 125.2, 122.4 (ArC); 57.8, 55.3, 48.3, 45.0 (cubylC); 12.4 (Me).

I.R. (nujol): 1731 (s), 1614 (m), 1591 (m), 1519 (s), 1319 (s), 1203 (s), 1187 (s), 1155 (s), 1108 (s), 1095 (s), 995 (s), 873 (m), 860 (m), 740 (m).

6^A-(2-Aminoethylamino)-6^A-deoxy-β-Cyclodextrin (β-CDen).

To a stirring solution of β-CD-OTs (2 g, 1.5 mmols) in dry N-methyl pyrolidin-2-one (5 cm^3) was added potassium iodide (25 mg, 0.15 mmols) and 1,2-diamino ethane (300 mg, 5 mmols). The reaction mixture was heated to 70°C and stirred for 7 hrs and then cooled to room temperature and stirred for 16 hrs. Ethanol (100 cm^3) was added drop wise over 2 hrs to afford an off-white precipitate. This precipitate was collected by vacuum filtration and washed successively with ethanol (100 cm^3) and ether (50 cm^3), dried under vacuum over P_2O_5 to give the crude product. This crude material was dissolved in water (10 cm^3) and purified by ion exchange chromatography. The fractions containing the amino moiety where combined and evaporated to dryness to give the crude product. This crude product was then redissolved in water (10 cm^3) and freeze-dried to afford the title compound as a dull yellow/white solid (860 mg, 49%).

^{13}C NMR. (pD = 10, D_2O 200 MHz): δ_C 104.6, 104.3, 86.3, 83.8, 83.7, 75.8, 75.7, 73.1, 74.8, 74.5, 73.2, 63.0, 52.4, 52.0, 42.2.

Electrospray-MS: m/z [M+H] = 1178.

6^A-(3-Aminopropylamino)-6^A-deoxy-β-Cyclodextrin (β-CDpn).

To a stirring solution of β-CD-OTs (2.052 g, 1.59 mmols) in dry N-methyl pyrolidin-2-one (5 cm^3) was added potassium iodide (25 mg, 0.15 mmols) and 1,3-diamino propane (420 mg, 5.6 mmols). The reaction mixture was heated to 70°C and stirred for 7 hrs and then cooled to room temperature and stirred for 16 hrs. Ethanol (100 cm^3) was added drop wise over 2 hrs to afford an off-white precipitate. This precipitate was collected by vacuum filtration and washed successively with ethanol (100 cm^3) and ether (50 cm^3), dried under vacuum over P_2O_5 to give the crude product. This crude material was dissolved in water (10 cm^3) and purified by ion exchange chromatography. The fractions containing the amino moiety where combined and evaporated to dryness to give the crude product. This crude product was then redissolved in water (10 cm^3) and freeze-dried to afford the title compound as a dull yellow/white solid (792 mg, 42%).

R_f = 0.15 (**R_c** = 0.5).

Electrospray-ms: *m/z* 1191 [M+]

^{13}C NMR. (pD ~ 9, D_2O 300 MHz): δ_C 104.6, 104.3, 86.4, 83.9, 83.6, 75.8, 75.75, 74.8, 74.5, 73.12, 63.0, 62.9, 52.2, 49.2, 41.15, 32.9.

6^A-(6-Aminohexylamino)-6^A-deoxy-β-Cyclodextrin (β-CDhn).

To a stirring solution of β-CD-OTs (2 g, 1.5 mmols) in dry N-methyl pyrolidin-2-one (5 cm^3) was added potassium iodide (25 mg, 0.15 mmols) and 1,6-diamino hexane (300 mg, 5 mmols). The reaction mixture was heated to 70°C and stirred for 7 hours. The reaction mixture was then cooled to room temperature and stirred for 16 hours. Ethanol (100 cm^3) was then added drop wise over 2 hours to afford an off-white precipitate. The precipitate was collected by vacuum filtration and washed successively with ethanol (100 cm^3) and ether (50 cm^3) then dried under vacuum over P_2O_5 to give the crude product. The crude material was dissolved in water (10 cm^3) and purified by ion exchange chromatography. The fractions containing the amino moiety where combined and evaporated to dryness to give the crude product. The crude product was then redissolved in water (10 cm^3) and freeze dried. The pure title compound was afforded as a white solid (960 mg, 52%).

R_f = 0.37 (**R_c** = 0.75)

Electrospray-ms: *m/z* 1234 [M+H]

^{13}C NMR. (pD = 9, D_2O 300 MHz): δ_C 104.3, 103.7, 85.4, 83.7, 83.6, 82.95, 75.6, 75.4, 75.35, 75.3, 75.25, 74.5, 74.33, 71.66, 62.76, 51.3, 50.75, 41.9, 29.45, 29.3, 28.2, 27.9.

6^A-(2-Aminoethylamino-N, N', N'-triacetic acid)–6^A–deoxy-β–cyclodextrin (β-CD-ED3A).

α-Chloro acetic acid (682mg, 7.2 mmols) dissolved in water (10 cm^3) and cooled to 0°C. This solution was neutralised by slow addition of a NaOH solution (288 mg, 7.2 mmols dissolved in 10 cm^3 of water) and the resulting solution cooled to 0°C. Concurrently, β-CDen (1.7g, 1.4 mmols) was dissolved in water (5 cm^3) and cooled to 0°C in a separate round bottom flask.

The two solutions where then added together at 0°C and then transferred to an oil bath and heated to 80°C for 10 hours. NaOH solution (288 mg in 10 cm^3 water) was dripped into the reaction vessel at 1 cm^3 per hour for the duration of the reaction. Once complete the reaction mixture was acidified to pH 2.5 with 10M HCl solution and purified by ion exchange chromatography (strong gel). Fractions containing the amino cyclodextrin product where combined and evaporated to afford the crude product as an orange/yellow crystalline ammonium salt. The ammonium salt was converted to the sodium analogue by dissolving in 50 cm^3 of water and adding two equivalents of NaOH (112mg, 2.8 mmols) and bubbling nitrogen gas through the solution for 3 hours. The cyclodextrin was isolated via precipitation with excess ethanol (approx. 30 cm^3) to afford a white crystalline solid. This compound was determined not to be the title compound.

R_C = 0.85

^{13}C NMR (pD = 10, D_2O 300 MHz): δC 179.1, 173.4, 171.7, 103.9, 83.2, 75.2, 74.1, 73.8, 62.2, 61.9, 59.5, 18.7.

FAB-MS: 1294, 1275 [M+H], 1215.

IR: 1632 (C=O), 1590 (C=O), 3000-3600 (-OH)

Method 2: Methyl Sulphonation of the nitrogens of β-CDen.

β-CD-en, **1**, (1gm, 0.8 mmols) was dissolved in water (5 cm^3) and the pH adjusted to 10 using 0.1 M NaOH solution. To this was added an aqueous solution of formaldehyde sodium bisulphite addition compound (FSBAP) (340mg, 2.5 mmols) and the reaction

vessel was heated to 80°C for 18 hours. To the resulting yellow solution was added 50 cm^3 of dry ethanol and the resulting precipitate collected by vacuum filtration. The pale orange crystals were washed with ethanol and ether (50 cm^3) and dried under vacuum over P_2O_5 to give the sulphonated intermediate compound (4) as a pale orange crystalline solid.

IR: 3325 (SO_3H), 1657, 1158, 1030.

The sulphonated intermediate was dissolved in water (5 cm^3) and to this was added sodium cyanide (NaCN) (25mg, 0.51 mmols) and reaction heated to reflux for 16 hours. 20 cm^3 of 0.1M NaOH solution is then added to the reaction vessel and stirred for 60 hours. The reaction mixture was then acidified to pH=5 (from pH=10) and purified by ion exchange chromatography (strong gel). The relevant fractions where combined and the ammonia removed by stirring with 3eq NaOH and bubbling N_2 gas through the solution. The solutions pH was raised to 10 and the solvent volume was reduced to ≈2ml by rotary evaporation. Ethanol (20 cm^3) was added drop wise over one hour to afford a white precipitate, which was collected by vacuum filtration and dried, under vacuum, over P_2O_5. This isolated compound was determined not to be the title compound (see chapter 4.1.2).

^{13}C NMR (pD = 10, D_2O 300 MHz): δ_C 179.1, 173.4, 171.7, 103.9, 83.2, 75.2, 74.1, 73.8, 62.2, 61.9, 59.5, 18.7

FAB-MS: 1294, 1275 [M+H], 1257, 1215.

IR: 1631 (C=O), 1590 (C=O), 3000-3600 (-OH)

6^A-(2-Aminopropylamino-N, N', N'-triacetic acid)-6^A-deoxy-β-cyclodextrin (β-CD-PD3A).

α-Chloro acetic acid (800 mg, 8 mmols) dissolved in water (10 cm^3) and cooled to 0°C. This solution was neutralised by slow addition of a NaOH solution (320 mg, 8 mmols dissolved in 10 cm^3 of water) and the resulting solution cooled to 0°C.

Concurrently, β-CDpn (1.07g, 0.8 mmols) was dissolved in water (5 cm^3) and cooled to 0°C in a separate round bottom flask.

The two solutions where then added together at 0°C and then transferred to an oil bath and heated to 80°C for 10 hours. NaOH solution (288 mg in 10 cm^3 water) was dripped into the reaction vessel at 1 cm^3 per hour for the duration of the reaction. Once complete the reaction mixture was acidified to pH = 2.5 with 10M HCl solution and purified by ion exchange chromatography (strong gel). Fractions containing the amino cyclodextrin product where combined and evaporated to afford the crude product as an orange/yellow crystalline ammonium salt. The ammonium salt was converted to the sodium analogue by dissolving in 50 cm^3 of water and adding three equivalents of NaOH (112mg, 2.8 mmols) and bubbling nitrogen gas through the solution for 3 hours. The cyclodextrin moiety was isolated via precipitation with excess ethanol (approx. 30 cm^3) to afford a white crystalline solid. This compound was determined not to be the title compound.

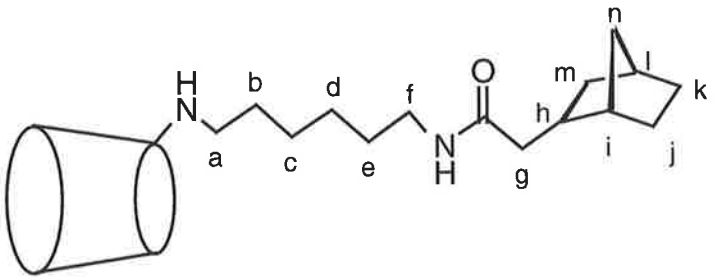
R_f = (**R_C** = 0.85)

^{13}C NMR (pD = 10, D_2O 300 MHz): δC 179.1, 173.4, 171.7, 103.9, 83.2, 75.2, 74.1, 73.8, 62.2, 61.9, 59.5, 18.7.

FAB-MS: 1366 [M+H], 1329, **1306** (base peak), 1248, 1220.

IR: 1601 (CO_2^-), 1500 (CO_2^-), 3000-3600 (-OH)

6^{A}-(6-N-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^{A}-deoxy-β-cyclodextrin (β–CDNorb6)



A mixture of β–CDhn (0.565 g, 4.58 x 10^{-4} mol) and 4-nitrophenyl 2-bicyclo[2.2.1]hept-2-ylacetate (0.131 g, 4.76 x 10^{-4} mol) in DMF (5 cm^3) was stirred at room temperature for 3 hours and then diluted with ether (100 cm^3). The resultant yellow precipitate was collected by vacuum filtration and washed with ether (100 cm^3). The solid was dissolved in water (20 cm^3) and the solution was acidified by addition of 3 mol dm^{-3} HCl (1 cm^3). The solution was washed with dichloromethane (3 x 20 cm^3) and then treated with AG 4-X4 anion exchange resin (free base form, 10 g). The filtered solution was evaporated and the residue was dissolved in water (10 cm^3) and loaded on to a column of BioRex 70 cation exchange resin (NH_4^+ form, 4.5 x 4.5 cm). The column was eluted with water (100 cm^3) and fractions containing the product were combined and evaporated under reduced pressure to give β–CDNorb6 as a white powder (0.320 g, 51%).

R_C = 1.4.

Electrospray-ms: *m/z* 1370 $(M+H)^+$.

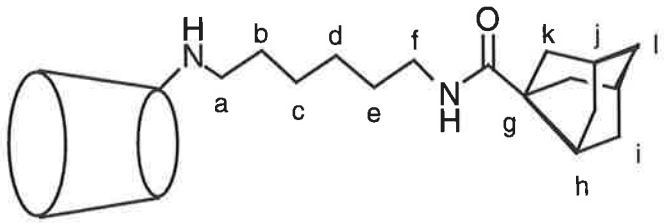
Calculated for βCDNorb6.5H$_2$O (C$_{57}$H$_{106}$N$_2$O$_{40}$): C, 46.91; H, 7.32; N, 1.92%.)(**Found** C, 46.65; H, 7.22; N, 1.83.

^{1}H NMR (600 MHz, D$_2$O, pH = 11): δ_H 4.89 (bs, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.2-3.5 (m, 14H, H2, H4, H$_f$); 2.9-3.2 (m, 3H, H4^A, H6^A, H$_{f'}$); 2.67 (t, *J* = 12.0 Hz, 1H,

$H6^{A'}$); 2.51 (m, 1H, H_a); 2.35 (bs, 1H, H_l); 2.24 (m, 1H, $H_{a'}$); 2.06 (m, 1H, H_g); 1.95 (bs, 1H, H_i); 1.74 (m, 1H, $H_{g'}$); 0.9-1.7 (m, 17H, H_{b-e} + norbornylH).

^{13}C NMR (75.4 MHz, D_2O, pH = 11): δ_C 176.7 (C=O); 105.9, 105.6 (C1); δ87.45 ($C4^A$); 85.1, 84.4 (C4); 76.8, 76.6, 76.2, 75.9, 75.3, 74.8 (C2, C3, C5); 70.3 ($C5^A$); 63.0 (C6); 52.2 ($C6^A$); 49.4 (C_a); 46.4 (C_g); 44.3, 42.2, 40.4, 39.3, 38.3, 38.2, 32.7, 31.4, 31.1, 30.7, 28.8, 27.3.

6^A-deoxy-6^A-(6-N-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)aminohexyl)amino-β-cyclodextrin (βCDNorad6)



A mixture of βCDhn (0.504 g, 4.09 x 10^{-4} mol) and 4-nitrophenyl noradamantane-3-carboxylate (0.125 g, 4.36 x 10^{-4} mol) in DMF (7 cm^3) was stirred at room temperature for 3 hours and then diluted with ether (100 cm^3). The resultant yellow precipitate was collected by vacuum filtration and washed with ether (100 cm^3). The solid was dissolved in water (20 cm^3) and the solution was acidified by addition of 3 mol dm^{-3} HCl (1 cm^3). The solution was washed with dichloromethane (3 x 20 cm^3) and then treated with AG 4-X4 anion exchange resin (free base form, 10 g). The filtered solution was evaporated and the residue was dissolved in water (10 cm^3) and loaded on to a column of Bio-Rex 70 cation exchange resin (NH_4^+ form, 4.5 x 4.5 cm). The column was eluted with water (100 cm^3) and fractions containing the product were combined and evaporated under reduced pressure to give βCDNorad6 as a white powder (0.307 g, 54%).

R_C = 1.4.

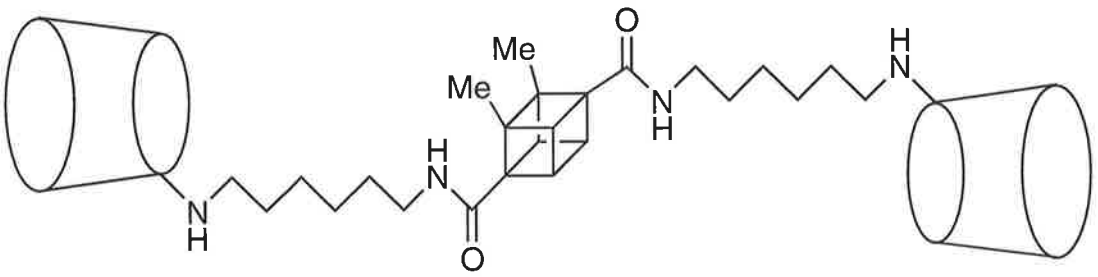
Electrospray-ms: *m/z* 1382 $(M+H)^+$.

(Found C, 46.46; H, 7.30; N, 1.83. Calculated for βCDNorad6•7H_2O ($C_{58}H_{110}N_2O_{42}$) C, 46.21; H, 7.35; N, 1.86%.)

^{1}H NMR (600 MHz, D_2O, pH = 11): δ_H 4.8 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.16 (bs, 2H, H_f); 3.10 (t, *J* = 9.0 Hz, 1H, $H4^A$); 3.01 (d, *J* = 13.0 Hz, 1H, $H6^A$); 2.67 (m, 2H, $H6^{A'}$, H_h); 2.53 (m, 3H, H_a, H_j); 2.17 (m, 1H, $H_{a'}$); 1.7-2.0 (m, 10H, NoradH); 0.9-1.5 (m, 8H, H_{b-e}).

^{13}C NMR (75.4 MHz, D_2O, pH = 11): δ_C 181.6 (C=O); 106.1, 106.0, 105.9 (C1); 88.0 ($C4^A$); 85.1, 85.0, 84.9, 84.8, 84.7 (C4); 76.7, 76.5, 76.4, 76.1, 75.9, 75.1, 75.0, 74.9, 74.8, 74.7 (C2, C3, C5); 70.5 ($C5^A$); 63.4, 63.3, 63.0, 62.9, 62.8 (C6); 7.9 (C_g); 52.4 ($C6^A$); 51.4, 50.8; 49.6 (C_a); 46.7, 46.5; 45.4 (C_h); 40.1, 39.9 (C_f, C_j); 36.7; 31.0, 30.8, 28.2, 26.1 (C_{b-e}).

1,4-bis((6-N-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocabonyl)-2,3-dimethylcubane (15) (βCDhn$_2$CuMe$_2$).



A mixture of βCDhn (0.550 g, 4.46 x 10^{-4} mol) and 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethyl cubane (0.098 g, 2.21 x 10^{-4} mol) in dry DMF (5 cm^3) was stirred at room temperature for 18 hours. The yellow reaction mixture was diluted with ether (100 cm^3) and the resultant precipitate was collected by vacuum filtration and washed with ether (100 cm^3 in portions). The crude product was dissolved

in water (20 cm^3) and passed through a column of AG 4-4X anion exchanger (free-base form, 4.5 x 4.5 cm) which was further eluted with water (150 cm^3). The eluant was concentrated under reduced pressure to ~15 cm^3 and this solution was loaded onto a column of BioRex 70 cation exchanger (NH_4^+ form, 4.5 x 4.5 cm i.d.) which was then eluted sequentially with water (150 cm^3) and 0.05 mol dm^{-3} ammonium hydrogen carbonate (200 cm^3). Fractions containing the product were combined and evaporated under reduced pressure to give $\beta CDhn_2CuMe_2$ as a white powder (0.340 g, 60%).

$\mathbf{R_C}$ = 0.76.

Electrospray-ms: *m/z* 2651 ($M+H^+$). (Found C, 45.83; H, 6.96; N, 2.06. Calculated for $\beta CDhn_2CuMe_2 \bullet 10H_2O$ ($C_{198}H_{196}N_4O_{80}$) C, 45.83; H, 6.98; N, 1.98%.)

^{1}H NMR (600 MHz, D_2O, pH = 11): δH 4.88 (bs, 14H, H1); 4.30 (m, 1H, cubylH); 4.10 (m, 2H, cubylH); 3.80 (m, 1H, cubylH); 3.5-3.8 (m, 52H, H3, H5, H6); 3.3-3.5 (m, 26H, H2, H4); 2.8-3.2 (m, 8H, H_f, $H4^A$, $H6^A$); 2.2-2.7 (m, 6H, $H6^{A'}$, H_a); 1.0-1.5 (m, 22H, H_{b-e}, Me).

^{13}C NMR (75.4 MHz, D_2O, pH = 11): δ_C 176.1, 175.7, 174.4 (C=O); 106.0, 105.7, 105.5, 105.2 (C1); 87.3 ($C4^A$); 84.8, 84.7, 84.4 (C4); 76.6, 75.8, 74.9, 74.8, 74.6 (C2, C3, C5); 72.8, 72.5, 72.3, 69.9, 63.1 (C6); 59.6, 59.5, 58.8, 58.7, 58.5, 52.1, 51.3, 51.0, 49.5, 48.6, 46.3, 46.1, 41.9, 41.7, 40.3, 35.4, 33.5, 32.1, 31.5, 31.2, 31.1, 30.2, 30.1, 28.9, 28.8, 28.6, 28.1, 27.0 ($C6^A$, C_{a-f}, cubylC); 16.0, 15.8, 14.7, 14.4, 13.7 (Me).

5.3 - Self-complexation Studies of Cyclodextrins

Self-complexation of the norbornyl group of 6^A-(6-N-(2-bicyclo[2.2.1]hept-2-ylacetyl) aminohexyl)amino-6^A-deoxy-β-cyclodextrin (βCDNorb6).

1D proton spectrum data: δ_H δ4.89 (bs, 7H, H1); δ3.6-3.9 (m, 26H, H3, H5, H6); 3.2-3.5 (m, 14H, H2, H4, H_f); 2.9-3.2 (m, 3H, $H4^A$, $H6^A$, $H_{f'}$); 2.67 (t, *J* = 12.0 Hz, 1H, $H6^{A'}$); 2.51 (m, 1H, H_a); 2.35 (bs, 1H, H_l); 2.24 (m, 1H, $H_{a'}$); 2.06 (m, 1H, H_g); 1.95 (bs, 1H, H_i); 1.74 (m, 1H, $H_{g'}$); 0.9-1.7 (m, 17H, H_{b-e} + norbornylH).

2D-ROESY spectrum data: δ_H 0.9-1.7 (H_{b-e} + norbornylH) shows cross-peaks with 1.74 ($H_{g'}$), 1.95 (H_i), 3.6-3.9 (H3, H5, H6); 1.74 ($H_{g'}$) shows cross-peaks with 0.9-1.7 (H_{b-e} + norbornylH), 1.95 (H_i), 2.06 (H_g), 2.35 (H_l), 3.6-3.9 (H3, H5, H6); 1.95 (H_i) shows cross-peaks with 0.9-1.7 (H_{b-e} + norbornylH), 1.74 ($H_{g'}$), 3.6-3.9 (H3, H5, H6); 2.06 (H_g) shows cross-peaks with 1.74 ($H_{g'}$), 3.6-3.9 (H3, H5, H6); 2.35 (H_l) shows cross-peaks with 1.74 ($H_{g'}$), 0.9-1.7 (H_{b-e} + norbornylH); 3.6-3.9 (H3, H5, H6) shows cross-peaks with 2.35 (H_l), 2.06 (H_g), 1.95 (H_i), 1.74 ($H_{g'}$), 0.9-1.7 (H_{b-e} + norbornylH).

Complexation of adamantane-1-carboxylate in 6^A-(6-N-(2-bicyclo[2.2.1]hept-2-ylacetyl) aminohexyl)amino-6^A-deoxy-β-cyclodextrin.

1D proton spectrum data: δH 4.9 (bs, 7H, H1); 3.7-3.95 (m, 26H, H3, H5, H6); 3.4-3.6 (m, 14H, H2, H4, H_f); 2.9-3.2 (m, 3H, $H4^A$, $H6^A$, $H_{f'}$); 2.7 (t, *J* = 12.0 Hz, 1H, $H6^{A'}$); 2.51 (m, 1H, H_a); 2.35 (bs, 1H, H_l); 2.24 (m, 1H, $H_{a'}$); 2.06 (m, 1H, H_g); 1.97 (bs, 3H, AdH3); 1.95 (bs, 1H, H_i); 1.8 (s, 3H, AdH2); 1.74 (m, 1H, $H_{g'}$); 1.69 (d, *J*=12 Hz, 3H, AdH4); 1.59 (d, *J*=12 Hz, 3H, AdH4'); 0.9-1.7 (m, 17H, H_{b-e} + norbornylH).

2D-ROESY spectrum data: δH 0.9-1.7 (H_{b-e} + norbornylH) shows cross-peaks with, 1.97 (AdH3); 1.59 (AdH4') shows cross-peaks with 1.69 (AdH4), 1.97 (AdH3), 3.7-3.95

(H3, H5, H6); 1.69 (AdH4) shows cross-peaks with 1.59 (AdH4'), 1.97 (AdH3), 3.7-3.95 (H3, H5, H6), 0.9-1.7 (H_{b-e} + norbornylH); 1.8 (AdH2) shows cross-peaks with 1.97 (AdH3), 3.7-3.95 (H3, H5, H6); 1.97 (AdH3) shows cross-peaks with 1.59 (AdH4'), 1.69 (AdH4), 1.8 (AdH2), 3.7-3.95 (H3, H5, H6); 3.7-3.95 (H3, H5, H6) shows cross-peaks with 1.59 (AdH4'), 1.69 (AdH4), 1.8 (AdH2), 1.97 (AdH3), 0.9-1.7 (H_{b-e} + norbornylH).

Self-complexation of 6^A-deoxy-6^A-(6-N-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)aminohexyl) amino-β-cyclodextrin (βCDNorad6).

1D proton spectrum data: δH 4.8 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.16 (bs, 2H, H_f); 3.10 (t, *J* = 9.0 Hz, 1H, $H4^A$); 3.01 (d, *J* = 13.0 Hz, 1H, $H6^A$); 2.67 (m, 2H, $H6^{A'}$, H_h); 2.53 (m, 3H, H_a, H_j); 2.17 (m, 1H, $H_{a'}$); 1.7-2.0 (m, 10H, NoradH); 0.9-1.5 (m, 8H, H_{b-e}).

2D-ROESY spectrum data: δH 1.7-2.0 (NoradH) shows cross-peaks with 2.53 (H_a, H_j), 2.67 ($H6^{A'}$, H_h), 3.6-3.9 (m, 26H, H3, H5, H6); 2.53 (H_a, H_j) shows cross-peaks with 1.7-2.0 (NoradH), 3.6-3.9 (m, 26H, H3, H5, H6); 2.67 ($H6^{A'}$, H_h) shows cross-peaks with 1.7-2.0 (NoradH), 3.6-3.9 (m, 26H, H3, H5, H6); 3.6-3.9 (m, 26H, H3, H5, H6) shows cross-peaks with 1.7-2.0 (NoradH), 2.53 (H_a, H_j), 2.67 ($H6^{A'}$, H_h).

complexation of adamantane-1-carboxylate in 6^A-deoxy-6^A-(6-N-(tricyclo[3.3.1.$0^{3,7}$] nonan-3-oyl)aminohexyl) amino-β-cyclodextrin.

1D proton spectrum data: δH 4.9 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.4-3.5 (m, 13H, H2, H4); 3.19 (bs, 3H, H4A, Hf); 3.01 (d, *J* = 13.0 Hz, 1H, $H6^A$); 2.67 (m, 2H, $H6^{A'}$); 2.61 (t, J = 8 Hz, 1H, H_h); 2.53 (m, 3H, H_a); 2.43 (bs, 1H, H_j); 2.27 (m, 1H, $H_{a'}$); 1.99 (bs, 3H, AdH3); 1.8 (s, 3H, AdH2); 1.71 (d, *J*=12 Hz, 3H, AdH4); 1.61 (d, *J*=12 Hz, 3H, AdH4'); 1.65-2.0 (m, 10H, NoradH); 1.1-1.5 (m, 8H, H_{b-e}).

2D-ROESY Spectrum data: δH 1.1-1.5 (H_{b-e}) shows cross-peaks with 3.19 (H4A, Hf); 1.61 (AdH4') shows cross-peaks with 1.65-2.0 (NoradH), 1.71 (AdH4), 1.99 (AdH3), 2.43 (H_j), 3.6-3.9 (H3, H5, H6); 1.71 (AdH4) shows cross-peaks with 1.65-2.0 (NoradH), 1.61 (AdH4'), 1.99 (AdH3), 2.61 (H_h), 2.43 (H_j); 3.6-3.9 (H3, H5, H6); 1.8 (AdH2) shows cross-peaks with 1.65-2.0 (NoradH), 1.99 (AdH3), 2.43 (H_j), 3.6-3.9 (H3, H5, H6); 1.99 (AdH3) shows cross-peaks with 1.65-2.0 (NoradH), 1.61 (AdH4'), 1.71 (AdH4), 1.8 (AdH2), 3.6-3.9 (H3, H5, H6); 1.65-2.0 (NoradH) signals overlap with the signals of adamantane-1-carboxylate and hence it is very difficult to assign specific interactions. Extensive NOE interactions with all adamantyl protons (AdH2, AdH3, AdH4 and AdH4') and those of the cyclodextrins interior (H3 and H5) are visible; 2.43 (H_j) shows cross-peaks with 1.61 (AdH4'), 1.71 (AdH4), 1.65-2.0 (NoradH), 1.99 (AdH3), 3.6-3.9 (H3, H5, H6); 2.61 (H_h) shows cross-peaks with 1.65-2.0 (NoradH), 1.71 (AdH4), 3.6-3.9 (H3, H5, H6); 3.19 (H4A, Hf) shows cross-peaks with 1.1-1.5 (H_{b-e}); 3.6-3.9 (H3, H5, H6) shows cross-peaks with 1.61 (AdH4'), 1.71 (AdH4), 1.65-2.0 (NoradH), 1.8 (AdH2), 1.99 (AdH3), 2.43 (H_j), 2.61 (H_h).

Self-complexation of 1,4-bis((6-N-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl) aminocabonyl)-2,3-dimethylcubane (βCDhn$_2$CuMe$_2$).

1D proton spectrum data: δH 4.88 (bs, 14H, H1); 4.44 (m, 1H, cubylH); 4.25 (m, 2H, cubylH); 4.14 (m, 1H, cubylH); 3.6-3.85 (m, 52H, H3, H5, H6); 3.2-3.45 (m, 26H, H2, H4); 2.9-3.15 (m, 8H, H_f, $H4^A$, $H6^A$); 2.7-2.8 (m, 6H, $H6^{A'}$), 2.48 (d, 1H, H_a); 1.3-1.5 (m, 16H, H_{b-e}), 1.1 (s, 6H, Me).

2D-ROESY spectrum data: δH 1.1 (cubyl Me) shows cross-peaks with 3.6-3.85 (H3, H5, H6); 1.3-1.5 (H_{b-e}) shows cross-peaks with 2.48 (H_a), 2.9-3.15 (H_f, $H4^A$, $H6^A$), 3.6-3.85 (H3, H5); 2.48 (H_a) shows cross-peaks with 1.3-1.5 (H_{b-e}), 3.6-3.85 (H3, H5); 2.9-3.05 ($H6^{A'}$) shows cross-peaks with 3.6-3.85 (H3, H5); 2.9-3.15 (H_f, $H4^A$, $H6^A$) shows cross-peaks with 1.3-1.5 (H_{b-e}); 3.6-3.85 (H3, H5) shows cross-peaks with 1.1 (cubyl

Me), 1.3-1.5 (H_{b-e}), 2.48 (H_a), 2.9-3.05 ($H6^{A'}$), 4.14-4.44 (cubylH); 4.14-4.44 (cubylH) shows cross-peaks with 3.6-3.85 (H3, H5).

Complexation of adamantane-1-carboxylate in 1,4-bis((6-N-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl) aminocarbonyl)-2,3-dimethylcubane.

1D proton spectrum data: δH 4.86 (m, 14H, H1); 3.86, (m, 2H, cubylH); 3.84 (t, J = 10.0 Hz, 2H, cubylH); 3.5-3.8 (m, 52H, H3, H5, H6); 3.4 (m, 26H, H2, H4); 3.1 (m, 6H, H_f, $H4^A$); 2.94 (d, J = 13.2 Hz, 2H, $H6^A$); 2.63 (m, 2H, $H6^{A'}$); 2.46 (m, 2H, H_a); 2.27 (m, 2H, $H_{a'}$); 2.01 (bs, 6H, AdH3); 1.79 (bs, 12H, AdH2), 1.72 (d, J = 11.4 Hz, 6H, AdH4); 1.47 (d, J = 11.4 Hz, 6H, AdH4'); 1.0-1.4 (m, 22H, H_{b-e}, Me).

2D-ROESY spectrum data: δH 1.47 (AdH4') shows cross-peaks with 1.72 (AdH4), 2.01 (AdH3), 3.5-3.8 (H3, H5); 1.72 (AdH4) shows cross-peaks with 1.47 (AdH4'), 2.01 (AdH3), 3.5-3.8 (H3, H5); 1.79 (AdH2) shows cross-peaks with 2.01 (AdH3), 3.5-3.8 (H3, H5); 2.01 (AdH3) shows cross-peaks with 1.47 (AdH4'), 1.72 (AdH4), 1.79 (AdH2), 3.5-3.8 (H3, H5); 3.5-3.8 (H3, H5) shows cross-peaks with 1.47 (AdH4'), 1.72 (AdH4), 2.01 (AdH3), 1.79 (AdH2).

5.4 - Kinetic measurements of the de-esterification of *p*-nitrophenylbenzoate and *p*-nitrophenylpropanoate

The de-esterification rate for *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate where determined using pseudo-first order conditions. The spectra where obtained by monitoring the appearance over time of the 4-nitro phenolate anion as mentioned previously. The reaction was monitored over at least seven half-lives and is seen to show pseudo first order kinetics, as determined from the variation in absorbency over time. The rate constants k_{obs} were determined from the exponential increase in absorbance with time (Figure 5.4.1).

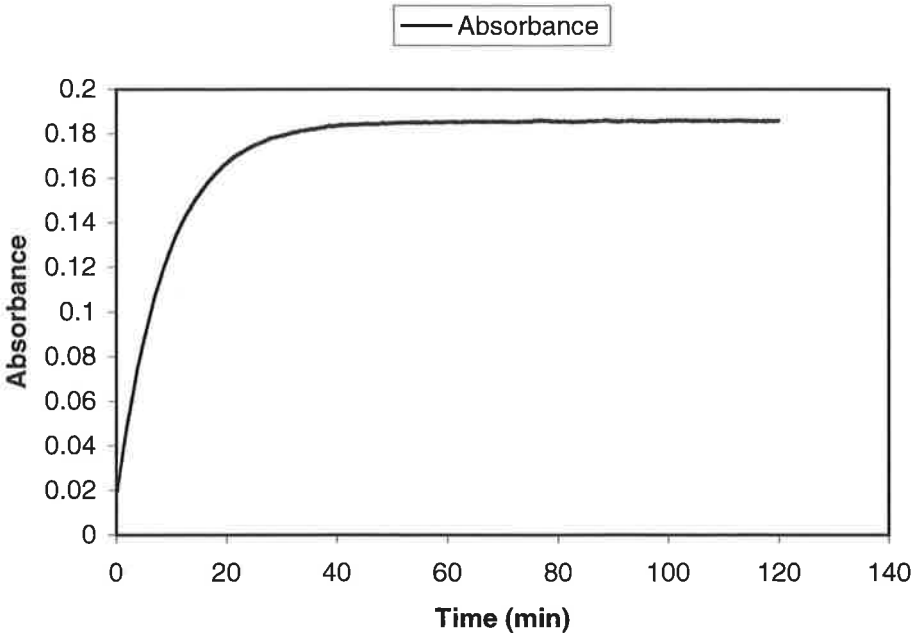


Figure 5.4.1: A plot of absorbance versus time for the deacylation of p-nitrophenyl benzoate by β–CDen (4×10^{-3} mol dm^{-3}).

The pseudo-first order method requires one of the reactants is in ten-fold excess or greater. Thus, the concentration of the excessive reactant does not change appreciably over the course of the reaction and the kinetics follow a first order rate law (Equation 5.4.1), where *k* is the pseudo-first order rate constant. A plot of $\ln(Abs_t/Abs_\infty)$

$$A_t = A_0 e^{-kt} \qquad (5.4.1)$$

versus time gives a straight line with a slope equal to *the* rate constant *k* (Abs_t and Abs_∞ are the absorbency at time *t* and infinite reaction time respectively). Plotting the logarithm of the change in the absorbency at 400nm as a function of time gave values for the first order rate constant. Repeating the experiments gave k_0 values that varied by less than 5%.

The concentration of β-CDen was varied between 10^{-3} and 10^{-5} mol dm^{-3} and rate constants for the reaction in the presence of cyclodextrin (k_{obs}) were determined for at least 7 cyclodextrin concentrations. The rate constant in the absence of the modified cyclodextrin (k_0) was subtracted from the k_{obs} values and the result (k_{obs}-k_0) is plotted

against the concentration of cyclodextrin. This followed Michaelis-Menton kinetics and the associated plot has a slope of k_C/K_{diss}. This procedure was repeated for *p*-nitrophenyl benzoate and *p*-nitrophenyl propanoate.

The reaction medium, 2 cm^3 of 0.05 mol dm^{-3} borate buffer at pH = 9.0, was placed in the reference cell holder of a Cary 2000 spectrometer and thermostated to 298.2 K. A solution of β-CDen in 0.05 mol dm^{-3} borate buffer at pH = 9.0 was placed into the 'reaction' cell holder and the reaction initiated by the addition of a 50 μm^3 aliquot of 2 $x10^{-4}$ mol dm^{-3} solution of a p-nitrophenyl ester in acetonitrile. The final ester concentration in the reaction mixture was 2.5 x 10^{-8} mol dm^{-3}. The reactions were followed by monitoring the appearance of the 4-nitrophenolate anion at 400 nm.

5.4.1- Preparation of solutions for catalysis studies.

These stock solutions were used to prepare all the other solutions required for the UV/vis spectroscopy study.

- Water stock.

Water refers to de-ionised water that has undergone the Milli-Q ultra filtration process. Water is boiled prior to use and stored under "Carbasorb " at all times.

- 70:30 H_2O : CH_3CN stock.

300 cm^3 of HPLC grade acetonitrile, was combined with 700 cm^3 of boiled Milli-Q water to make 1 dm^3 stock solution.

- *p*-nitrophenyl ester solutions.

The required *p*-Nitrophenyl ester was dissolved in acetonitrile to make 10 cm^3 of stock solution [$4x10^{-3}$ mol dm^{-3}]. 1 cm^3 of stock solution was diluted to 10 cm^3 with acetonitrile, and then 1 cm^3 of this solution is diluted to 10 cm^3 for use in the reaction cell.

- 0.05 Mol L^{-1} Borate Buffer (I=0.1, 293K)

For the 100% water solvent system: Boric acid (LR, 6.184g) was dissolved in 1 mol cm^3 $NaClO_4$ solution (100 cm^3) and made up to 1 dm^3 with stock solution. The stock solution of 0.1 mol cm^3 $H_3BO_3/NaClO_4$ (50 cm^3) and 0.1 M NaOH (see Table 5.4.1.1

below) are combined then made up to 100 cm^3 using 0.1 mol cm^3 (70:30 Stock) $NaClO_4$ solution. The pH was manipulated with 0.01 mol cm^3 $HClO_4$ solution to give the required pH.

For the 70/30 water/acetonitrile solvent system: As above using the 70/30 H_2O/MeCN stock solvents (Table 5.4.1.2).

Table 5.4.1.1: 100% water buffer solutions made for various pH's. "Final pH" denotes the pH attained after manipulation with 0.01 M $HClO_4$ solution.

pH needed	NaOH (cm^3 added)	Actual pH	Final pH
10	20	9.84	10
9.8	10	9.53	9.8
9.4	10	9.53	9.4
9.1	9	9.36	9.1
8.8	9	9.36	8.8
8.4	9	9.36	8.4
8.0	9	9.36	8.09

Table 5.4.1.2: 70/30 Water/Acetonitrile buffer solutions made for various pH's. "Final pH" denotes the pH attained after manipulation with 0.01 M $HClO_4$ solution.

pH needed	NaOH (cm^3 added)	Actual pH	Final pH
10	47.3	11.23	10
9.8	40.6	10.77	9.8
9.4	32.1	10.53	9.38
9.1	23.6	10.23	9.1
9.0	20.8	10.12	9.0
8.4	8.6	9.52	8.33

Cyclodextrin Solutions.

The required amount of β-CDen was dissolved in borate buffer to give the desired concentration as indicated in Tables 5.4.1.3 – 5.4.1.5, and reaction initiated by the addition of stock acetonitrile ester solution.

Table 5.4.1.3: Rate data for the de-esterification of 4-nitrophenyl propanoate in the presence of varying concentrations of β-CDen in aqueous solution.

[β-CDen] ($x10^{-3}$ mol dm^{-3})	k_{obs}-k_{un} ($x10^{-3}$ s^{-1})	mean rate ($x10^{-3}$ s^{-1})	STDEV
1.00	1.77, 1.84,1.74	1.78	4.86×10^{-5}
1.50	2.34, 2.47, 2.56	2.46	1.10×10^{-4}
2.00	3.24, 3.30	3.27	4.31×10^{-5}
2.50	3.96, 3.90, 3.79	3.89	8.56×10^{-5}
3.00	4.63, 4.49, 4.25	4.46	1.92×10^{-4}
3.50	5.16, 5.26, 5.36	5.26	1.00×10^{-4}
4.00	5.97, 6.05, 6.07	6.03	5.29×10^{-5}

Table 5.4.1.4: Rate data for the de-esterification of *p*-nitrophenyl benzoate **3** in the presence of varying concentrations of β-CDen **8** in 70:30 H_2O:MeCN solution.

[β-CDen] ($x10^{-3}$ mol dm^{-3})	k_{obs}-k_{un} ($x10^{-5}$ s^{-1})	mean rate ($x10^{-4}$ s^{-1})	STDEV
0.0536	0.100, 0.093	0.96	4.81×10^{-6}
0.120	0.217, 0.231	2.24	1.00×10^{-6}
0.140	0.240, 0.240	2.40	7.07×10^{-9}
0.208	0.354, 0.355	3.54	4.95×10^{-8}
0.386	0.636, 0.639, 0.642	6.39	3.00×10^{-7}
0.529	0.930, 0.939	9.35	6.36×10^{-7}
0.639	1.10, 1.09	11.04	2.83×10^{-7}
1.0	1.50, 1.60, 1.51	15.4	5.74×10^{-6}
2.0	3.0, 3.08	30.4	5.66×10^{-6}
3.12	4.55, 4.604	45.8	3.54×10^{-6}
4.3	6.69, 6.68	66.85	6.36×10^{-7}

Table 5.4.1.5: Rate data for the de-esterification of 4-nitrophenyl propanoate in the presence of varying concentrations of β-CDen in 70:30 H_2O:MeCN solution.

[β-CDen] ($x10^{-3}$ mol dm^{-3})	k_{obs}-k_0 ($x10^{-4}$ s^{-1})	mean rate ($x10^{-4}$ s^{-1})	STDEV
9.00	3.80, 3.76	4.53	$3.54 x10^{-6}$
1.40	5.36, 5.29	6.06	$4.81 x10^{-6}$
1.90	7.02, 7.03, 7.03	7.80	$1.14 x10^{-6}$
2.50	10.6, 10.4, 10.4	10.8	$4.88 x10^{-5}$
3.00	13.0, 12.2	12.6	$1.34 x10^{-5}$
3.70	15.1, 15.3	15.8	$3.46 x10^{-5}$
4.30	17.0, 16.9	17.7	$1.84 x10^{-5}$

The rate constants where then plotted in the form of a Michealis-Menton plots to give linear correspondence (see chapter 2).

5.5 – pH Titration Studies.

Potentiometric titrations were carried out using a Metrohm Dosimat E665 titrimator, an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode that was filled with aqueous 0.10 mol dm^{-3} $NaClO_4$ solution. All titration solutions were saturated with nitrogen by passing a fine stream of bubbles (previously passed through aqueous 0.10 mol dm^{-3} NaOH followed by 0.10 mol dm^{-3} $NaClO_4$) through them for at least 15 minutes before the commencement of the titration. For the mixed solvent system (70:30 water/acetonitrile) the electrode was filled with 0.10 mol dm^{-3} $NaClO_4$ prepared using a stock 70:30 H_2O/Acetonitrile solution. During the titrations a similar stream of nitrogen bubbles was passed through the titration solution which was magnetically stirred and held at 298.2 ± 0.1 K in a water-jacketed 20 cm^3 titration vessel that was closed to the atmosphere except for a small exit for nitrogen. In all titrations, standardised 0.1 mol dm^{-3} NaOH was titrated against solutions that were 1 x 10^{-3} mol dm^{-3} in the species of interest, 5 x 10^{-3} mol dm^{-3} in $HClO_4$ and 9.5 x 10^{-2} mol dm^{-3} in $NaClO_4$ (I = 0.1). Values of E_o and pK_W were determined by titration of a solution that was 1 x 10^{-4} mol dm^{-3} in $HClO_4$ and 9 x 10^{-4} mol dm^{-3} in $NaClO_4$ against 0.1 mol dm^{-3} NaOH and are listed for each titration in Section 4.4.

Values of pK_a's were determined using the computer program SUPERQUAD.[3] At least three runs were performed for each system and at least two of these runs were averaged; the criterion for selection for this averaging being that $\chi^2 < 12.6$ for each run at the 95% confidence level (see Figures 5.5.1 – 5.5.6 below).

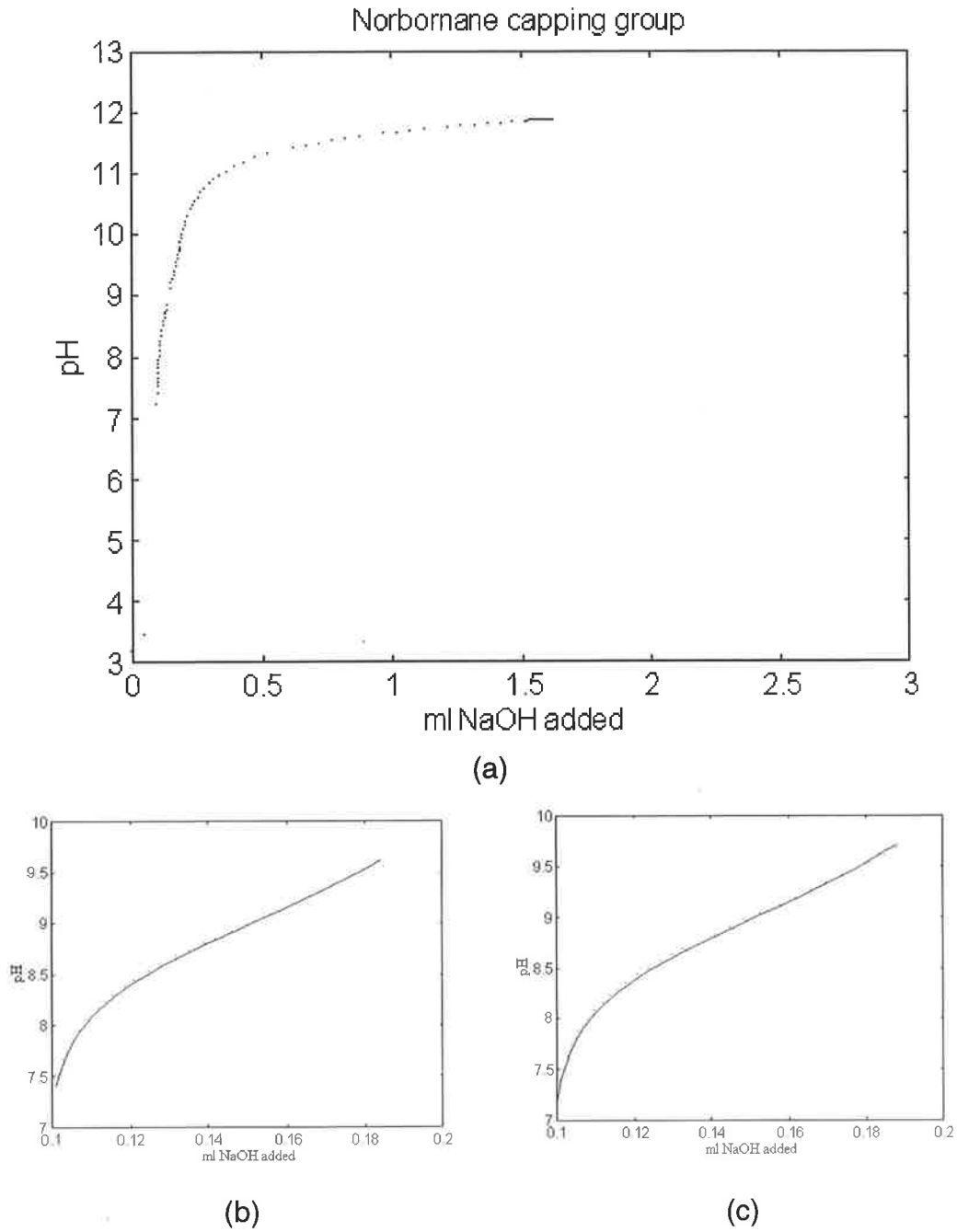


Figure 5.5.1: (a) pH titration curve of β-CDNorb6 with two equivalents of added acid ($HClO_4$). The pK_a of the secondary amine is visible between pH 8 –10. (b)-(c) The theoretical fit of the pK_a determined to be 8.91 ± 0.02. ε_o = 440.899, pK_w = 13.731.

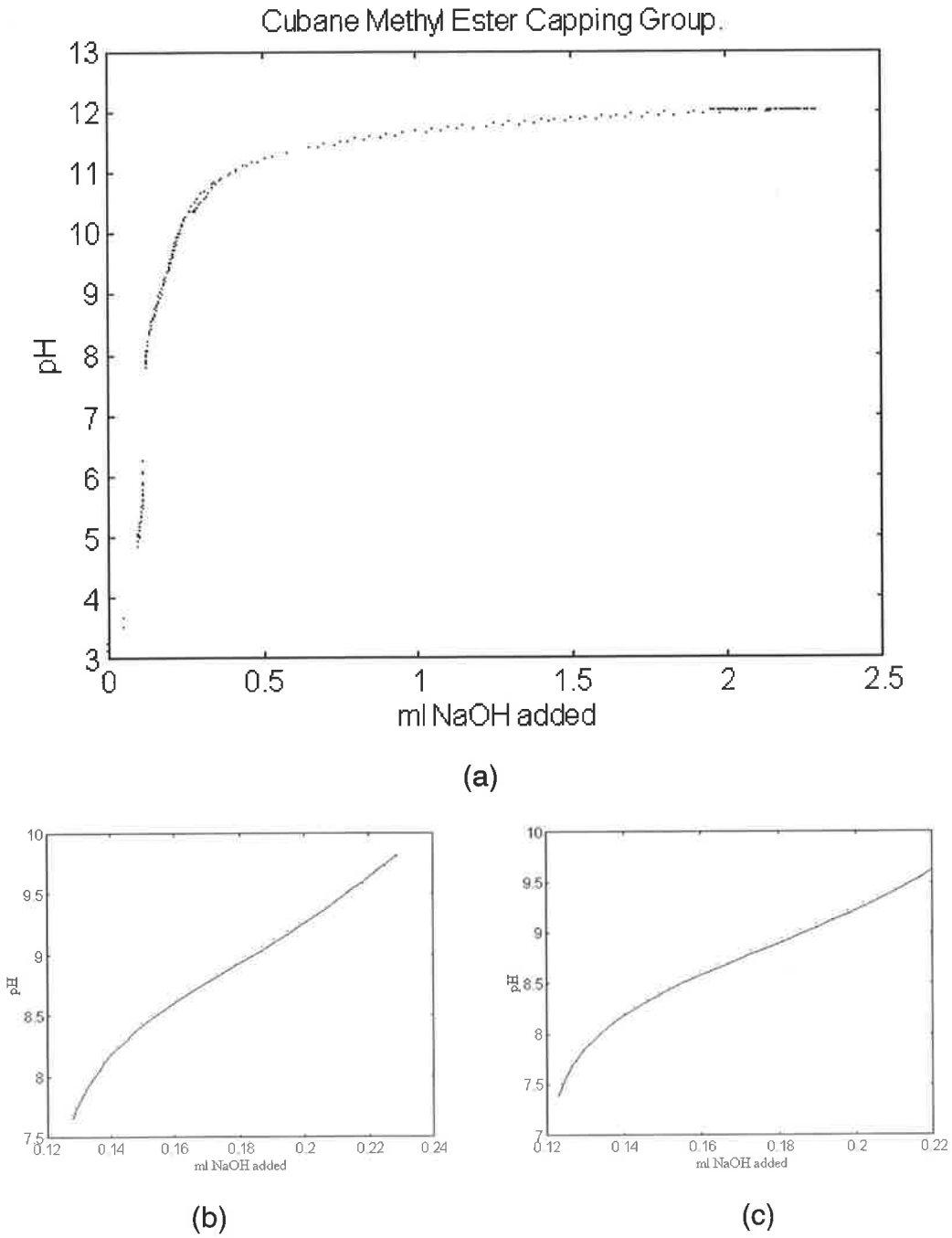


Figure 5.5.2: (a) pH titration curve of β-CDCube6 with two equivalents of added acid ($HClO_4$) the methyl ester is seen to hydrolyse at around pH=10.5. The pK_a of the secondary amine is visible between pH 8 – 10. (b)-(c) The theoretical fit of the pK_a determined to be 8.87 ± 0.02. ε_o = 443.874, pK_w = 13.745.

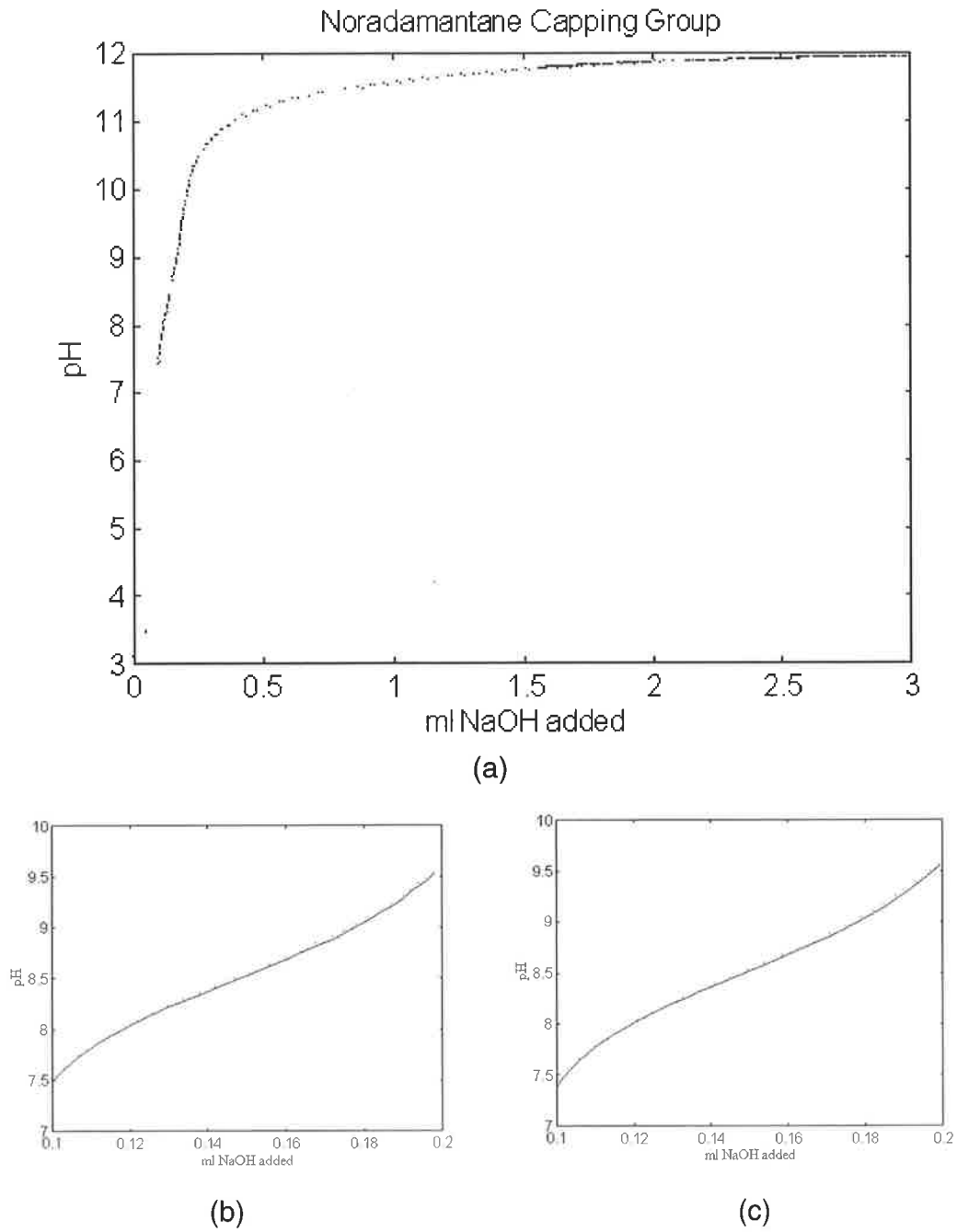


Figure 5.5.3: (a) pH titration curve of β-CDNorad6 with two equivalents of added acid ($HClO_4$). The pK_a of the secondary amine is visible between pH 8 –10. (b)-(c) The theoretical fit of the pK_a determined to be 8.47 ± 0.02. ε_0 = 438.345, pK_w = 13.822

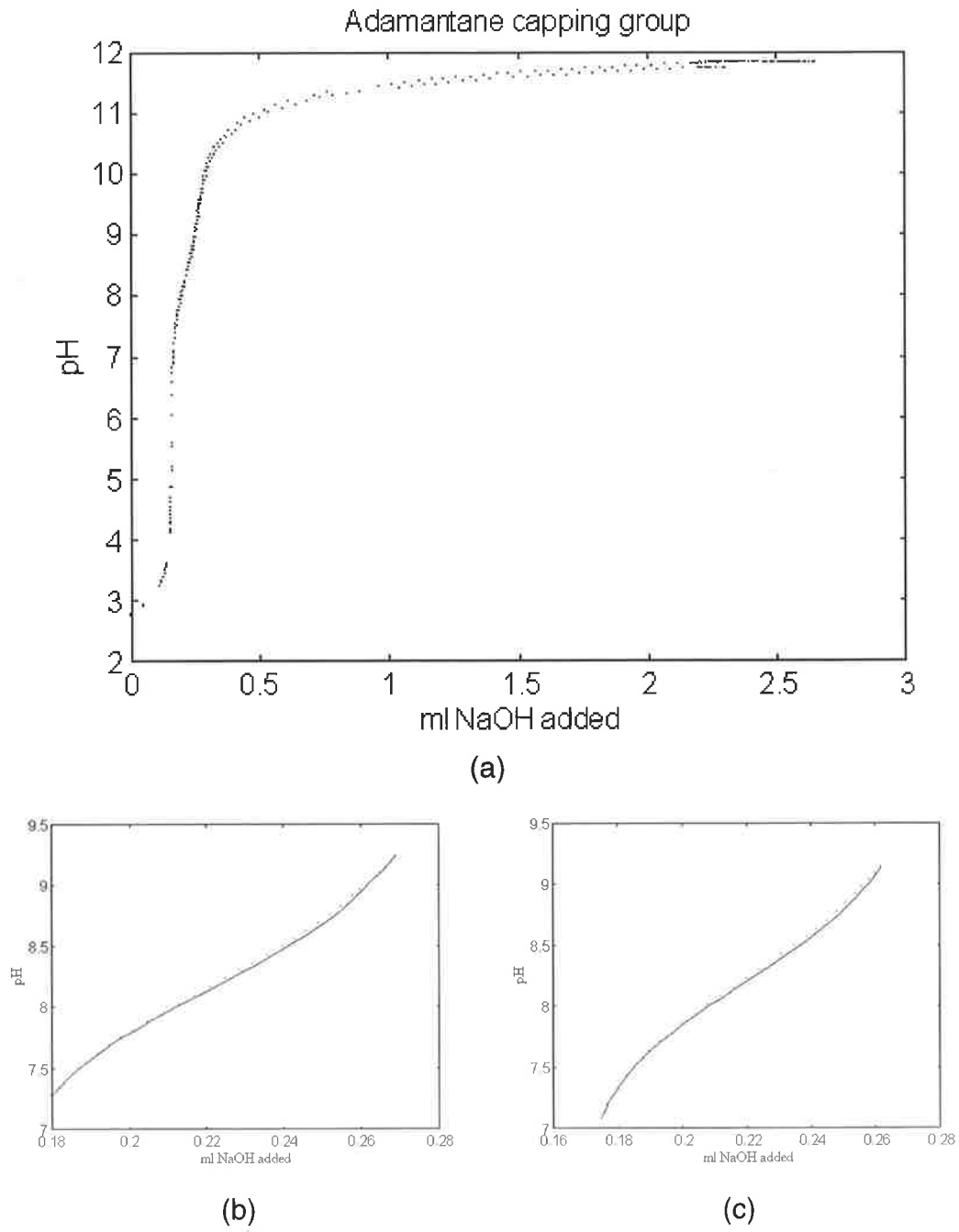


Figure 5.5.4: (a) pH titration curve of β-CDAdam6 with two equivalents of added acid ($HClO_4$). The pK_a of the secondary amine is visible between pH 8 –10. (b)-(c) The theoretical fit of the pK_a determined to be 8.15 ± 0.02. ε_0 = 419.236, pK_w = 13.619.

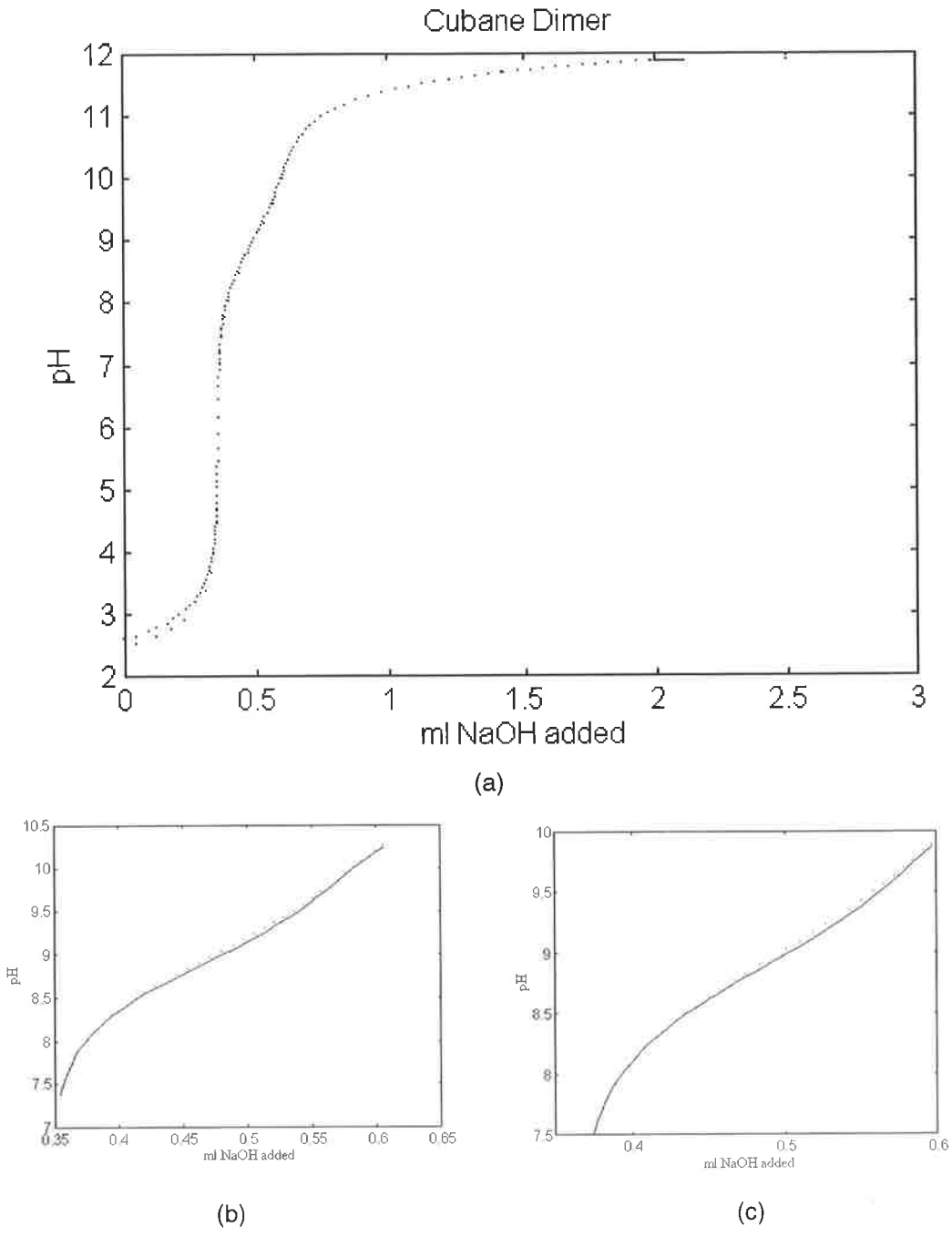


Figure 5.5.5: (a) pH titration curve of β-CDHn$_2$Cu$_2$ with four equivalents of added acid ($HClO_4$). The pK_a of the secondary amine is visible between pH 8 –10. (b)-(c) The theoretical fit of the pK_a determined to be 8.87 ± 0.02. ε_o = 432.512, pK_w = 13.69.

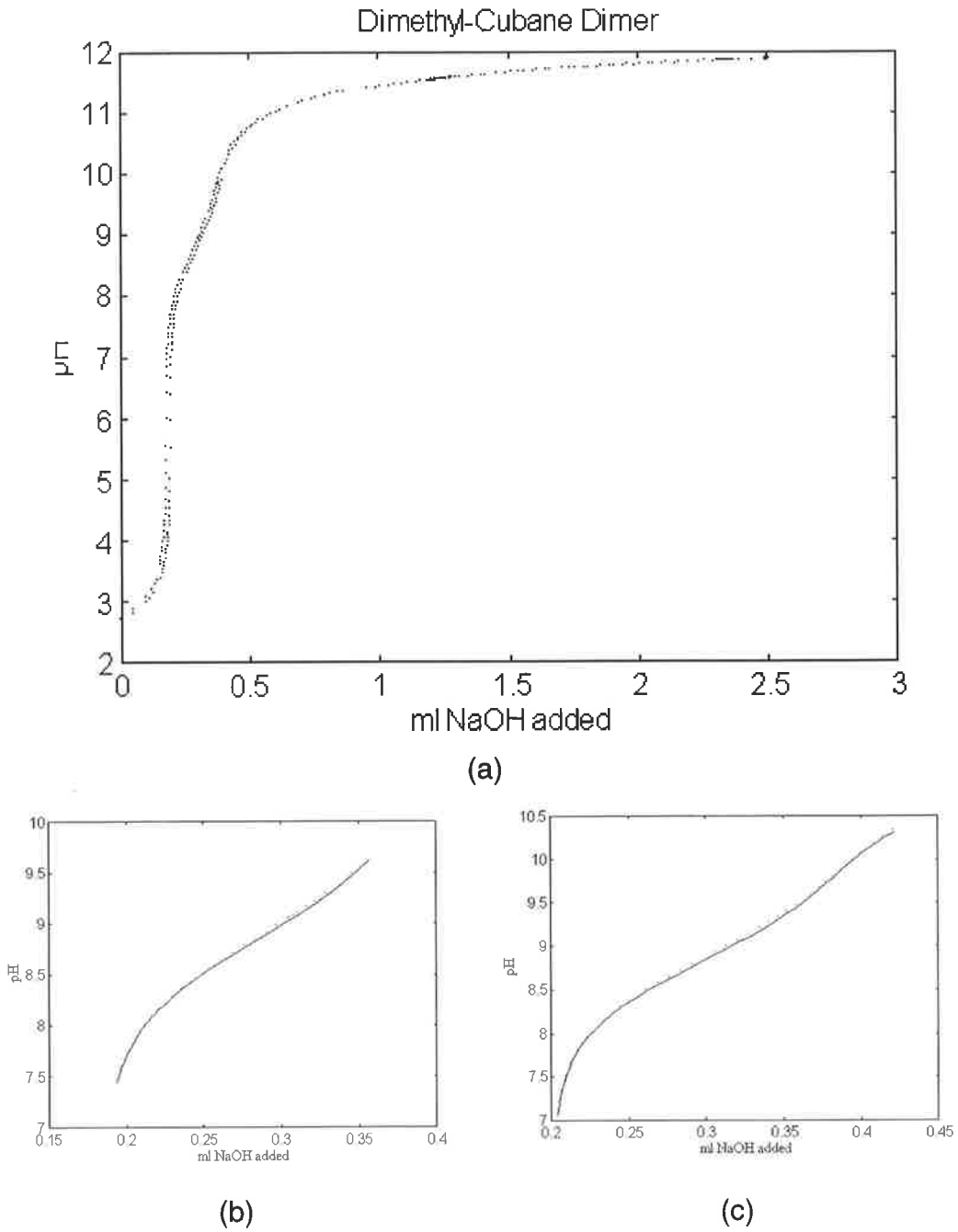


Figure 5.5.6: (a) pH titration curve of β-CDHn$_2$CuMe$_2$ with four equivalents of added acid ($HClO_4$). The pK_a of the secondary amine is visible between pH 8 –10. (b)-(c) The theoretical fit of the pK_a determined to be 8.80 ± 0.02. ε_o = 425.512, pK_w = 13.69.

PUBLICATIONS.

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Intramolecular Complexation in Modified β-Cyclodextrins†: A Preparative, Nuclear Magnetic Resonance and pH Titration Study

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†β-cyclodextrin = cycloheptamaltose

The reactions of 4-nitrophenyl norbornan-2-acetate and 4-nitrophenyl noradamantane-3-carboxylate with 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin, **1**, produce 6^A-(6-*N*-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin (**2**, pK_a = 8.98) and 6^A-deoxy-6^A-(6-*N*-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)amino-β-cyclodextrin (**4**, pK_a = 8.47), respectively, in good yield together with 4-nitrophenolate. The reaction of 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethylcubane, with two moles of **1** produces dimeric 1,4-bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocarbonyl)2,3-dimethylcubane (**7**, pK_a = 8.80) in good yield together with two moles of 4-nitrophenolate. The pK_as in brackets are those of the single protonated amine functions of **2** and **4**, and of both protonated amine functions of **7** which have identical pK_as (in each case at 298.2 K and I = 0.10 mol dm^{-3} ($NaClO_4$)). ^{1}H NMR ROESY studies are consistent with the norbornyl, noradamantyl and dimethylcubyl entities of **2**, **4** and **7** complexing inside the βCD annuli in D_2O at pD ≥ 11. Under the same conditions, adamantane-1-carboxylate forms intermolecular complexes with **2**, **4** and **7** and displaces their norbornyl, noradamantyl and the dimethylcubyl entities from the β-cyclodextrin annulus to varying degrees dependent on the relative size, shape and hydrophobicity of these groups. These data are compared with those for analogous modified β-cyclodextrins.

Introduction

The intramolecular complexation of the 6-aminohexylamino substituent in the β-cyclodextrin (βCD) annulus of 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin (**1**, Fig. 1)[1] provides the possibility of generating a range of intramolecularly complexing modified βCDs through substitution of the primary nitrogen of **1** with a variety of entities, X.[2,3] When the precursor of the X substituent has either one or two groups participating in this substitution, a modified βCD monomer and a linked βCD dimer are obtained, respectively (Schemes 1 and 2, where the truncated cones represent the βCD annuli where the secondary faces are delineated by 14 secondary hydroxy groups and the primary faces are delineated by 6 primary hydroxy groups and a secondary amine group). We have previously prepared such monomers where X is either a cubyl, a dimethylcubyl or an adamantly entity and a dimer where X incorporates a cubyl entity (**3**, **5** and **6** in Fig. 1).[2] We now report preparations where X incorporates norbornyl and noradamantyl entities into the modified βCD monomers (**2** and **4** in Fig. 1 and Scheme 1), and the dimethylcubyl entity into the βCD dimer (**7** in Fig. 1 and Scheme 2). The X entities of **2**, **4** and **7** were selected on the basis that they are hydrophobic and likely to enter the largely hydrophobic βCD annulus to form intramolecular complexes stabilised by a combination of secondary forces in aqueous solution.[4-10] The modified βCDs incorporating these X entities, together with analogous species reported earlier, facilitate an experimental assessment of the extent to which the size of X places a mechanical constraint on its occupancy of the annuli of the modified βCD monomers and dimers. Also shown in Fig. 1 is adamantane-1-carboxylate, **8**, which competes with the substituents for intramolecular complexation in the βCD annulus as is discussed below. The ability of the guest **8** to displace the X entity from the annulus is dependent on the relative size, shape and hydrophobicity of the polycyclic entity X.

Fig. 1 and Schemes 1 and 2 here

Results and Discussion

The reaction of 4-nitrophenyl norbornan-2-acetate, **9**, and 4-nitrophenyl noradamantane-3-carboxylate, **10**, with **1** produce 6^A-(6-*N*-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin, **2**, and 6^A-deoxy-6^A-(6-*N*-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)amino-β-cyclodextrin, **4** (Scheme 1), respectively, in good yield together with 4-nitrophenolate, **11**. The reaction of 1,4-bis(4-nitrophenoxycarbonyl)-2,3-dimethylcubane, **14**, with two moles of **1** produces 1,4-bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocarbonyl)2,3-dimethylcubane, **7**, in good yield together with two moles of **11** (Scheme 2). These new modified βCDs may either exist as **2**, **4** and **7** with their substituents outside the βCD annulus or as **2'**, **4'** and **7'** where the substituents are intramolecularly complexed inside the βCD annulus.

The experimental evidence for the equilibria shown in Schemes 1 and 2 depends on solution ^{1}H and ^{13}C NMR spectroscopy, of which the major aspects are presented below. Detailed resonance assignments based on the COSY and HSQC spectra of the new derivatives appear at the conclusion of each of the preparative sections for **2**, **4** and **7** in Experimental

6^A-(6-*N*-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin (2) and 6^A-deoxy-6^A-(6-*N*-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)amino-β-cyclodextrin (4)

The ^{1}H ROESY NMR spectrum of **2/2'** shows significant cross-peaks arising from NOE interactions between the norbornyl protons and the annular H3 and H5 protons which line the interior of the βCD annulus (Table 1). The analogous spectrum of **4/4'** shows-cross peaks between the noradamantyl protons and the annular H3 and H5 protons (Fig. 2 and Table 1). This is consistent with intramolecular complexation of the norbornyl and noradamantyl substituent in the annulus of **2'** and **4'** as shown in Scheme 1. Usually, the annular H3 and H5 resonances occur at δ ~ 3.8 and ~ 3.5 ppm, respectively, but in Fig. 2 they can neither be separately distinguished from each other nor from the resonances of H6 which lies on the outside of the annulus. Hence, in the assignment of cross-peaks the annular H3 and H5 resonances are not distinguished between. However, as the variations in the ROESY spectra discussed herein are consistent with intra- and intermolecular complexation in the βCD annulus,

it seems unlikely that any of the cross-peaks arise from NOE interactions of the protons of complexed entities with H6 on the outside of the βCD annulus. (Cross-peaks also arise from interactions between protons which are at small through-bond separations from each other, but these provide little information on complexation and are not further considered.)

On addition of **8**, new cross-peaks (Table 1) arising from the NOE interactions of the adamantyl H1- H4 protons with the annular H3 and H5 protons appear consistent with **8** competing with the intramolecularly complexed norbornyl and noradamantyl entities to form the intermolecular complexes **12** and **13** in Scheme 1. (The orientation of the carboxylate group of **8** towards the secondary face of the βCD annuli of **12** and **13** shown in Scheme 1 is consistent with modelling studies of intermolecular complexes formed by **8** with βCD and **1**.[2,11,12]) However, the cross-peaks arising from the protons of the norbornyl and noradamantyl entities remain, at a lower intensity, consistent with the intramolecular complexes **2'** an **4'** coexisting in solution with the intermolecular complexes **12** and **13**, respectively. The complexation of the adamantyl group within the annuli of **2** and **4** in competition with the norbornyl and noradamantyl groups, respectively, is consistent with the measured values of the formation constants for complexation of adamantane-1-carboxylate, noradamantane-3-carboxylate and norbornane-2-acetate within β-cyclodextrin ($K = 4.2 \times 10^4$, 8.2×10^3 and 4.3×10^3 dm^3 mol^{-1} at pH 7.2, respectively).[12] The stability of the intramolecular complexes **2'** and **4'** is probably enhanced by the favourable entropy contribution arising from the tethering of the norbornyl and noradamantyl groups to the βCD entity.

Fig. 2 and Table 1 here

Similar intramolecular complexation of the cubyl and adamantyl entities of **3** and **5** to form **3'** and **5'**, respectively, has been reported.[3] However, while **8** displaces the cubyl entity from the annulus of **3'**, it does not displace the adamantyl entity from the annulus of **5'** possibly because the intramolecularly complexed adamantyl substituent of **5'** has an entropic advantage in competing with **8** for occupancy of the βCD annulus. Alternatively, it may be that substitution of **1'** through the secondary face of the βCD annulus produces **5'** and the adamantyl entity is too

large to pass through the primary face. In the latter case, **5'** is a mechanically constrained molecular slip-knot. Despite uncertainty as to whether the entropic or the mechanical constraint rationale is correct, the ability of the norbornyl and noradamantyl entities of **2'** and **4'** to compete with **8** for occupancy of the βCD annulus, whereas the dimethyl and cubyl entities of **3'** and **5'** appear to be less effective,[3] is consistent with a decreasing strength of intramolecular retention in the βCD annulus in the sequence: adamantyl > noradamantyl ≈ norbornyl > dimethylcubyl ≈ cubyl. This suggests that a combination of closeness of fit and degree of hydrophobicity of the substituent determines the relative stabilities of the modified βCD intramolecular complexes. It is also consistent with the closer fit of the adamantyl entity of **8**, in combination with its hydrophobicity, stabilising its intermolecular complex with **3** by comparison with the intramolecular cubyl complex **3'**. This interpretation is in accord with adamantan-1-ol, adamantan-2-ol and adamantane-1-carboxylic acid competing more effectively with the dansyl entity for occupancy of the βCD annulus of N^{α}-dansyl-L-lysine-β-cyclodextrin than do *l*-borneol, *d*- and *l*-camphor and *d* and *l*-fenchone which are smaller.[5] Intramolecular complexation of aromatic substituents of modified βCDs is well established, particularly in the case of those incorporating the dansyl entity.[4-8]

1,4-Bis((6-*N*-(6^{A}-deoxy-β-cyclodextrin-6^{A}-yl)aminohexyl)aminocarbonyl)2,3-dimethylcubane (7)

The substituted βCD, **7**, prepared from the racemic diester **14** and homochiral **1**, is a 1:1 mixture of diastereomers. The 1D ^{1}H NMR spectrum of **7/7'** shows three multiplet resonances for the dimethylcubyl methine protons and a single resonance for the methyl protons. The ^{1}H ROESY NMR spectrum shows cross-peaks arising from NOE interactions between the dimethylcubyl methine and methyl protons and the annular H3 and H5 protons (Fig. 3 and Table 1). Cross-peaks also arise from the interaction of the hexyl H2-H5 protons with the annular H3 and H5 protons which is consistent with complexation of a hexyl entity in the second βCD annulus. These observations are consistent with exchange of the dimethylcubyl entity between the two equivalent annular intramolecular complexation sites of **7'** (Scheme 2) occurring slowly on the

^{1}H NMR timescale and resulting in an inequivalence of the βCD entities. There is no detectable spectral differentiation of the diastereomers of **7/7'** in the ^{1}H NMR spectra.

On addition of two moles of **8**, ^{1}H NMR spectral changes occur consistent with step-wise intermolecular complexation of **8** to give a symmetric 2:1 guest: host complex, **16**, through the 1:1 intermediate complex **15**. New cross-peaks arise from the NOE interaction of the adamantyl protons H1-H4 with the annular H3 and H5 protons of the intermolecular complex **16** (Scheme 2 and Table 1). The cross-peaks from the hexyl H2-H5 protons disappear consistent with **8** displacing the hexyl entity from a βCD annulus. While the observed changes in chemical shift render any change in the cross-peaks arising from the dimethylcubyl methine protons difficult to detect because of other overlapping cross-peaks, a cross-peak arising from the methyl protons interacting with the annular H3 or H5 protons remains. This is consistent with the dimethylcubyl entity competing with **8** for occupancy of a βCD annulus through the equilibria between **7'**, **15** and **16** shown in Scheme 2. In the 1D ^{1}H NMR spectrum, only a single resonance is observed for the cubyl protons consistent with both βCD entities becoming equivalent and complexed by **8** in **16**, the dominant species in solution. (Similar equilibria have previously been reported for **6** and **6'**.[3])

Fig. 3 here

These interpretations are supported by ^{13}C NMR studies. In D_2O solution, three carbonyl and five methyl ^{13}C resonances are observed, arising from both the asymmetry of **7'** and some spectral differentiation of the diastereomers of **7/7'**. On addition of two moles of **8**, only single carbonyl and methyl ^{13}C resonances are observed consistent with the dominant formation of the symmetric 2:1 guest:host complex **16** (Scheme 2). In **7'**, the distereomeric dimethylcubyl entities are distinguished by two distinct pairs of methyl ^{13}C resonances arising from differing interactions of the methyl groups with the homochiral βCD annulus. It appears that a combination of the deep penetration of the methyl groups into the βCD annulus of **7'** and the

large chemical shift scale for ^{13}C is responsible for this detection of chiral discrimination but no other evidence for it is provided by either the ^{13}C or ^{1}H spectra.

pK_a Studies

The pK_as of the new modified βCDs, **2**, **4** and **7**, and those of their earlier reported analogues, **3**, **5** and **6**, were determined by pH titration (Fig. 1). The increased acidity of the protonated amine group of the modified βCDs, by comparison with that expected for simple aliphatic diamines, is similar to that observed in other amino-substituted βCDs.[13] This may partially arise from either the electronic and steric effects of the βCD entity, or a change in solvation experienced by a protonated amine adjacent to the βCD annulus, or combination of these factors. The pK_a of the protonated amine decreases as the size and hydrophobicity of the X entity increases consistent with an increasingly hydrophobic environment destabilising the protonated amine. The βCD dimers **6** and **7** are each characterised by identical pK_as for the two protonated amines, consistent with each amine being sufficiently insulated from a change in the protonation state of its twin to behave independently. This may also be reflected through the identical pK_as of **3** and **7**. The protonated amine groups of the modified βCDs experience different environments in their uncomplexed and intramolecularly complexed forms. However, two distinct pK_as are not observed consistent with either the intramolecular complex being greatly dominant, or for exchange between it and its uncomplexed analogue being sufficiently rapid to yield an averaged pK_a, or a combination of these effects. (Above pH 10 a time dependent pH change occurs for **3** which is thought to arise from hydrolysis of the methyl ester.)

Experimental

Physical methods

Elemental analyses were carried out by the Microanalytical Service of the University of Otago. Modified βCDs were characterised as the hydrates by adding whole molecules of water to the molecular formula to give the best fit to the microanalytical data. Melting points were determined using a Kofler hot-stage apparatus under a Reichert microscope and are uncorrected. As βCD derivatives generally decompose without melting above 180 °C melting points were not

determined for these compounds. Thin layer chromatography (TLC) was carried out on Kieselgel 60 F_{254} (Merck) on aluminium backed plates. Unless otherwise stated, plates were developed with 7:7:5:4 v/v ethyl acetate/propan-2-ol/ammonium hydroxide/water for the analysis of all cyclodextrin samples. Compounds bearing amino groups were visualised by drying the plate then dipping it into a solution of 0.5% ninhydrin in ethanol and heating it with a heat-gun. Modified βCDs were further visualised by dipping the plate into a solution of 1% sulfuric acid in ethanol and heating it with a heat-gun. Iodine vapour was also used to visualise cyclodextrins. The value R_c represents the R_f of a modified βCD relative to the R_f of βCD. Squat column chromatography was carried out using Kieselgel 60 F_{254} thin layer chromatography silica.[14]

Infrared spectra were recorded on an ATI Mattson Genesis FT-IR. The abbreviations strong (s), medium (m), weak (w) and broad (b) are used in reporting the infrared data. Electrospray mass spectroscopy (electrospray-ms) was carried out at the Australian National University. Samples were dissolved in 10% acetonitrile for injection and the cone voltage was set to 120 V.

All ^1H and ^{13}C NMR spectra were run on solutions 0.10 mol dm^{-3} in the cyclodextrin of interest using Varian Gemini 200 and 300 spectrometers except for the ROESY ^1H NMR spectra which were run on a Varian Inova 600 using a standard sequence with a mixing time of 0.3 s.[15] The modified βCDs **2**, **4** and **7** (and **8** when present) were dissolved in D_2O to give final concentrations of 0.06 mol dm^{-3} of each component and a final pH ≥ 11 adjusted with NaOD. The resultant solutions were filtered (0.22 μm) and degassed by freeze-thawing before the spectra were recorded. Resonances were assigned on the basis of COSY and HSQC spectra. The spectral assignments presented below with the preparative details of each modified βCD are listed according to the atom labelling in Fig. 1. All chemical shifts are referenced to aqueous trimethylsilylpropiosulfonic acid as an external standard.

pH Titrations were carried out using a Metrohm Dosimat E665 titrimator, an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode filled with 0.10 mol dm^{-3} $NaClO_4$. Titration solutions were saturated with nitrogen by passing a fine stream of bubbles (previously passed through aqueous 0.10 mol dm^{-3} NaOH followed by 0.10 mol dm^{-3} $NaClO_4$) through them for at least 15 minutes before the commencement of the titration. During

the titrations a similar stream of nitrogen bubbles was passed through the titration solution which was magnetically stirred and held at 298.2 ± 0.1 K in a water-jacketed 20 cm^3 titration vessel which was closed to the atmosphere except for a small exit for nitrogen. In all titrations, standardised 0.100 mol dm^{-3} NaOH was titrated against solutions which were 1 x 10^{-3} mol dm^{-3} in the species of interest, 5×10^{-3} mol dm^{-3} in $HClO_4$ and 9.5×10^{-2} mol dm^{-3} in $NaClO_4$ (I = 0.1). Values of E_o and pK_w were determined by titration of a solution which was 1×10^{-4} mol dm^{-3} in $HClO_4$ and 9×10^{-4} mol dm^{-3} in $NaClO_4$ against 0.100 mol dm^{-3} NaOH. Values of pK_a were determined using the program SUPERQUAD.[16] At least three runs were performed for each system and at least two of these runs were averaged; the criterion for selection for this averaging being that χ^2 for each run was < 12.6 at the 95% confidence level.

Preparations of modified β-cyclodextrins and their precursors

Literature methods were used to prepare 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin[13] and dimethyl-2,3-dimethylcubane-1,4-dicarboxyate.[17] Either standard procedures were employed in the preparation of the other compounds used in the preparations described below, or they were of good commercial grade. **CAUTION**. While no instability was noted in the cubyl-substituted βCDs prepared in this study, it should be noted that cubanes are high energy materials and should be handled accordingly.

4-Nitrophenyl norbornan-2-acetate (9)

A mixture of norbornan-2-acetic acid (0.571 g, 3.70×10^{-3} mol), 4-nitrophenol (0.516 g, 3.71×10^{-3} mol) and dicyclohexylcarbodiimide (0.748 g, 3.63×10^{-3} mol) in dichloromethane (10 cm^3) was stirred at room temperature for 18 h. The reaction mixture was filtered through a pad of Celite and the filtrate was evaporated under reduced pressure to give the crude ester as a yellow oil. This material was further purified by passage through a squat column eluted with dichloromethane. Fractions containing the ester were combined and evaporated to give the product as a colourless oil (0.830 g, 83%). An attempt to further purify the ester by bulb to bulb distillation (150 °C/0.05 Torr) resulted in some decomposition. Accurate mass data: calculated for **9** ($C_{15}H_{18}NO_4$) $(M+H)^+$ 276.1236. Found 276.1249. δ_H (200 MHz, $CDCl_3$) 8.27 (d, J =

9.2 Hz, 2H, ArH); 7.28 (d, J = 9.2 Hz, 2H, ArH); 2-2.5 (m, 5H); 1-1.6 (m, 8H). δ_C (50.4 MHz, $CDCl_3$) 170.6 (C=O), 155.5, 145.1, 125.0, 122.4 (ArC); 41.1, 38.3, 38.1, 37.6, 36.7, 35.2, 29.6, 28.4. I.R. (film) 3115 (w), 3088 (w), 2951 (s), 2871 (s), 1768 (s), 1706 (m), 1615 (m), 1593 (m), 1525 (s), 1490 (m), 1455 (w), 1347 (s), 1208 (s), 1162 (s), 1106 (s), 915 (m), 864 (m), 749 (w), 716 (w).

6^A-(6-*N*-(2-bicyclo[2.2.1]hept-2-ylacetyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin (2)

A mixture of 1 (0.565 g, 4.58×10^{-4} mol) and **9** (0.131 g, 4.76×10^{-4} mol) in DMF (5 cm^3) was stirred at room temperature for 3 h and then diluted with ether (100 cm^3). The resultant yellow precipitate was collected by vacuum filtration and washed with ether (100 cm^3). The solid was dissolved in water (20 cm^3) and the solution was acidified by addition of 3 mol dm^{-3} HCl (1 cm^3). The solution was washed with dichloromethane (3×20 cm^3) and then treated with AG 4-X4 anion exchange resin (free base form, 10 g). The filtered solution was evaporated and the residue was dissolved in water (10 cm^3) and loaded on to a BioRex 70 cation exchange column (NH_4^+ form, 4.5×4.5 cm). The column was eluted with water (100 cm^3) and fractions containing the product were combined and evaporated under reduced pressure to give 2 as a white powder (0.320 g, 51%). R_c = 1.4. Electrospray-ms *m/z* 1370 $(M+H)^+$. (Found C, 46.65; H, 7.22; N, 1.83. Calculated for $2.5H_2O$ ($C_{57}H_{106}N_2O_{40}$) C, 46.91; H, 7.32; N,-1.92%.) δ_H (**2/2'**, 600 MHz, D_2O, pH ≥ 11) 4.89 (bs, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.2-3.5 (m, 14H, H2, H4, hexyl H6); 2.9-3.2 (m, 3H, $H4^A$, $H6^A$, hexyl H6'); 2.67 (t, J = 12.0 Hz, 1H, $H6^{A'}$); 2.51 (m, 1H, hexyl H1); 2.35 (bs, 1H, H_I); 2.24 (m, 1H, hexyl H1'); 2.06 (m, 1H, norbornyl H8); 1.95 (bs, 1H, norbornyl H2); 1.74 (m, 1H, norbornyl H8'); 0.9-1.7 (m, 17H, hexyl H2-H5 + norbornyl H). δ_C (**2/2'**,75.4 MHz, D_2O, pH ≥ 11) 176.7 (C=O); 105.9, 105.6 (C1); 87.45 ($C4^A$); 85.1, 84.4 (C4); 76.8, 76.6, 76.2, 75.9, 75.3, 74.8 (C2, C3, C5); 70.3 ($C5^A$); 63.0 (C6); 52.2 ($C6^A$); 49.4 (hexyl C1); 46.4 (norbornyl C8); 44.3, 42.2, 40.4, 39.3, 38.3, 38.2, 32.7, 31.4, 31.1, 30.7, 28.8, 27.3. δ_H (**2/2'** + 1 mol **8**, 600 MHz, D_2O, pH ≥ 1) 4.89 (bs, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.2 (bs, 13H, H2, H4); 2.9-3.2 (m, 4H, $H4^A$, $H6^A$, hexyl H6); 2.70 (m,

1H, $H6^{A'}$); 2.50 (m, 1H, hexyl H1); 2.30 (m, 1H, hexyl H1'); 2.20 (bs, 1H, norbornyl H5); 1.8-2.1 (m, 6H, norbornyl H2, norbornyl H8, adamantyl H3); 1.76 (s, 6H, adamantyl H2); 0.8-1.7 (m, 23 H, adamantyl H4, norbornyl H, hexyl H2-H5).

4-Nitrophenyl noradamantane-3-carboxylate (10)

A solution of noradamantane-3-carboxylic acid (0.505 g, 3.04×10^{-3} mol), 4-nitrophenol (0.430 g, 3.09×10^{-3} mol) and dicyclohexylcarbodiimide (0.624 g, 3.03×10^{-3} mol) was stirred at room temperature for 3 h. The reaction mixture was filtered and the filtrate was washed with 5% sodium bicarbonate solution (3×20 cm^3) and brine (20 cm^3) and dried over sodium sulfate. The filtered solution was evaporated under reduced pressure and the oily residue was suspended in 1:1 dichloromethane/hexane and loaded onto a squat column (4.5 cm i.d., 30 g silica gel). Elution of the column was commenced with 1:1 dichloromethane/hexane and the proportion of dichloromethane was progressively increased. Fractions containing the product were combined and evaporated under reduced pressure to give the product as a colourless oil which solidified on standing (0.812 g, 93%). Mp 82-83 °C. EI-MS *m/z* 287 ($M^{+\cdot}$). (Found C 66.81;H 5.91;N 4.99. Calculated for **10** ($C_{16}H_{17}NO_4$) C, 66.89; H, 5.96; N, 4.87%.) δ_H (200 MHz, $CDCl_3$) 8.26 (d, J = 9.2 Hz, 2H, ArH); 7.28 (d, J = 9.2 Hz, 2H, ArH); 2.86 (t, J = 6.6 Hz, 1H, H7); 2.24 (m, 4H); 1.91 (m, 4H); 1.73 (m, 4H). δ_C (50.4 MHz, $CDCl_3$)) 175.0 (C=O); 156.0, 145.1, 125.1, 122.4 (ArC); 54.1 (C3); 46.8 (C7); 44.5 (C9); 43.5 (C6); 37.4 (C1); 34.5 (C2). I.R. (nujol) 1747 (s), 1614 (m), 1591 (m), 1521 (s), 1346 (s), 1305 (m), 1274 (m), 1209 (m), 1182 (s), 1097 (m), 1074 (m), 1020 (m), 997 (m), 873 (m), 862 (m), 742 (m) cm^{-1}.

6^A-Deoxy-6^A-(6-*N*-(tricyclo[3.3.1.$0^{3,7}$]nonan-3-oyl)aminohexyl)amino-β-cyclodextrin (4)

A mixture of 1 (0.504 g, 4.09×10^{-4} mol) and 4-nitrophenyl noradamantane-3-carboxylate (0.125 g, 4.36×10^{-4} mol) in DMF (7 cm^3) was stirred at room temperature for 3 h and then diluted with ether (100 cm^3). The resultant yellow precipitate was collected by vacuum filtration and washed with ether (100 cm^3). The solid was dissolved in water (20 cm^3) and the solution

was acidified by addition of 3 mol dm^{-3} HCl (1 cm^3). The solution was washed with dichloromethane (3 × 20 cm^3) and then treated with AG 4-X4 anion exchange resin (free base form, 10 g). The filtered solution was evaporated and the residue was dissolved in water (10 cm^3) and loaded on to a BioRex 70 cation exchange column (NH_4^+ form, 4.5 × 4.5 cm). The column was eluted with water (100 cm^3) and fractions containing the product were combined and evaporated under reduced pressure to give 4 as a white powder (0.307 g, 54%). R_c = 1.4. Electrospray-ms *m/z* 1382 $(M+H)^+$. (Found C, 46.46; H, 7.30; N, 1.83. Calculated for $4.7H_2O$ ($C_{58}H_{110}N_2O_{42}$) C, 46.21; H, 7.35; N, 1.86%.) δ_H (**4/4'**, 600 MHz, D_2O, pH ≥ 11) 4.8 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.16 (bs, 2H, hexyl H_6); 3.10 (t, J = 9.0 Hz, 1H, $H4^A$); 3.01 (d, J = 13.0 Hz, 1H, $H6^A$); 2.67 (m, 2H, $H6^{A'}$, noradamantyl H6); 2.53 (m, 3H, hexyl H1, noradamantyl H3); 2.17 (m, 1H, hexyl H1'); 1.7-2.0 (m, 10H, noradamantyl H); 0.9-1.5 (m, 8H, hexyl H2-H5)). δ_C (**4/4'**, 75.4 MHz, D_2O, pH ≥ 11) 181.6 (C=O); 106.1, 106.0, 105.9 (C1); 88.0 ($C4^A$); 85.1, 85.0, 84.9, 84.8, 84.7 (C4); 76.7, 76.5, 76.4, 76.1, 75.9, 75.1, 75.0, 74.9, 74.8, 74.7 (C2, C3, C5); 70.5 ($C5^A$); 63.4, 63.3, 63.0, 62.9, 62.8 (C6); 57.9 (noradamantyl C1); 52.4 ($C6^A$); 51.4, 50.8 ; 49.6 (hexyl C1); 46.7, 46.5; 45.4 (noradamantyl C6); 40.1, 39.9 (hexyl C6, noradamantyl C5); 36.7; 31.0, 30.8, 28.2, 26.1 (hexyl C2-C5). δ_H (**4/4'** + 1 mol **8**, 600 MHz, D_2O, pH ≥ 11) 4.8 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.15 (m, 3H, $H4^A$, hexyl H6); 2.96 (d, J = 13.0 Hz, 1H, $H6^A$); 2.70 (dd, J = 9.0, 13.0 Hz, 1H, $H6^{A'}$); 2.60 (m, 1H, noradamantyl H6); 2.50 (m, 1H, hexyl H1); 2.28 (bs, 2H, noradamantyl H5); 2.26 (m, 1h, hexyl H1'); 1.95 (s, 3H, adamantyl H3); 1.5-1.9 (m, 22H, adamantyl H, noradamantyl H); 1-1.5 (m, 8H, hexyl H2-H5).

1,4-Bis(4-nitrophenoxycarbonyl)-2,3-dimethylcubane (14)

A mixture of 2,3-dimethylcubane-1,4-dicarboxylic acid (0.320 g, 1.45 × 10^{-3} mol), 4-nitrophenol (0.409 g, 2.94 × 10^{-3} mol) and dicyclohexylcarbodiimide (0.613 g, 2.98 × 10^{-3} mol) in dichloromethane (8 cm^3) was left to stir at room temperature for 18 hours. The reaction mixture was filtered and the collected solid was washed with dichloromethane (3 × 10 cm^3). The combined filtrate was washed with 5% sodium bicarbonate solution (3 × 20 cm^3) and dried over

sodium sulfate. The solution was concentrated to approx. 20 cm^3 and loaded onto a squat column (30 g silica gel, 4.5 cm i.d.) and the column was eluted successively with dichloromethane (3 × 25 cm^3) and chloroform (3 × 25 cm^3). Fractions containing the product were combined and evaporated to give the diester as a white powder (0.451 g, 67%). A portion of this material was recrystallised from dichloromethane/hexane. Mp 202-204 °C. (Found C, 62.32; H, 3.90; N, 6.08. Calculated for **14** ($C_{24}H_{18}N_2O_8$) C, 62.34; H, 3.92; N, 6.06%.) δ_H (200 MHz, $CDCl_3$) 8.31 (d, J = 8.4 Hz, 4H, ArH); 7.29 (d, J = 8.4 Hz, 4H, ArH); 4.38 (t, J = 4.0 Hz, 2H, cubyl H); 4.12 (t, J = 4.0 Hz, 2H, cubyl H); 1.46 (s, 6H, Me). δ_C (54 MHz, $CDCl_3$) 167.9 (CO); 155.3, 145.4, 125.2, 122.4 (ArC); 57.8, 55.3, 48.3, 45.0 (cubyl C); 12.4 (Me). I.R. (nujol) 1731 (s), 1614 (m), 1591 (m), 1519 (s), 1319 (s),1203 (s), 1187 (s), 1155 (s), 1108 (s), 1095 (s), 995 (s), 873 (m), 860 (m), 740 (m).

1,4-Bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocabonyl)-2,3-dimethylcubane (7)

A mixture of 1 (0.550 g, 4.46×10^{-4} mol) and **13** (0.098 g, 2.21×10^{-4} mol) in dry DMF (5 cm^3) was stirred at room temperature for 18 h. The yellow reaction mixture was diluted with ether (100 cm^3) and the resultant precipitate was collected by vacuum filtration and washed with ether (100 cm^3 in portions). The crude product was dissolved in water (20 cm^3) and passed through an AG 4-4X anion exchange column (free-base form, 4.5 × 4.5 cm) and was further eluted with water (150 cm^3). The eluant was concentrated under reduced pressure to ~15 cm^3 and this solution was loaded onto a BioRex 70 cation exchange column (NH_4^+ form, 4.5 × 4.5 cm) which was then eluted sequentially with water (150 cm^3) and 0.05 mol dm^{-3} ammonium hydrogen carbonate (200 cm^3). Fractions containing the product were combined and evaporated under reduced pressure to give **7** as a white powder (0.340 g, 60%). R_c = 0.76. Electrospray-ms *m/z* 2651 ($M+H^+$). (Found C, 45.83; H, 6.96; N, 2.06. Calculated for $7.10H_2O$ ($C_{198}H_{196}N_4O_{80}$) C, 45.83; H, 6.98; N, 1.98%.) δ_H (**7**/**7'**,600 MHz, D_2O, pH ≥ 11) 4.88 (bs, 14H, H1); 4.30 (m, 1H, cubyl H); 4.10 (m, 2H, cubyl H); 3.80 (m, 1H, cubyl H); 3.5-3.8 (m, 52H, H3, H5, H6); 3.3-3.5 (m, 26H, H2, H4); 2.8-3.2 (m, 8H, H_f, $H4^A$, $H6^A$); 2.2-2.7 (m, 6H,

$H6^{A'}$, H_a); 1.0-1.5 (m, 22H, hexyl H2-H5, Me). δ_C (**7/7'**,75.4 MHz, D_2O, pH ≥ 11) 176.1, 175.7, 174.4 (C=O); 106.0, 105.7, 105.5, 105.2 (C1); 87.3 ($C4^A$); 84.8, 84.7, 84.4 (C4); 76.6, 75.8, 74.9, 74.8, 74.6 (C2, C3, C5); 72.8, 72.5, 72.3, 69.9 ($C5^A$?); 63.1 (C6); 59.6, 59.5, 58.8, 58.7, 58.5, 52.1, 51.3, 51.0, 49.5, 48.6, 46.3, 46.1, 41.9, 41.7, 40.3, 35.4, 33.5, 32.1, 31.5, 31.2, 31.1, 30.2, 30.1, 28.9, 28.8, 28.6, 28.1, 27.0 ($C6^A$, hexyl C1-C6, cubyl C); 16.0, 15.8, 14.7, 14.4, 13.7 (Me). δ_H (**7/7'** + 2 mol **8**, 600 MHz, D_2O, pH ≥ 11 + 2 eq. **8**) 4.86 (m, 14H, H1); 3.86, (m, 2H, cubyl H); 3.84 (t, *J* = 10.0 Hz, 2H, $H5^A$); 3.5-3.8 (m , 52H, H3, H5, H6, cubyl H); 3.4 (m, 26H, H2, H4); 3.1 (m, 6H, H_f, $H4^A$); 2.94 (d, *J* = 13.2 Hz, 2H, $H6^A$); 2.63 (m, 2H, $H6^{A'}$); 2.46 (m, 2H, H_a); 2.27 (m, 2H, $H_{a'}$); 2.01bs, 6H, adamantyl H3); 1.79 (bs, 12H, adamantyl H2), 1.72 (d, *J* = 11.4 Hz, 6H, adamantyl H4); 1.47 (d, *J* = 11.4 Hz, 6H, adamantyl H4'); 1.0-1.4 (m, 22H, hexyl H2-H5, Me). δ_C (75.4 MHz, D_2O, pH ≥ 11 + 2 eq. **8**) 189.9 (CO_2^-); 176.0 (CONHR); 106.3, 106.0, 105.9, 105.3 (C1); 87.7 ($C4^A$); 84.6, 84.5, 84.4, 84.1 (C4); 76.8, 76.5, 76.4, 76.0, 75.0, 74.9, 74.7 (C2, C3, C5); 72.3, 69.8 ($C5^A$?); 62.7 (C6); 59.6, 58.8, 50.7, 49.5, 49.2, 46.1, 44.8, 42.4, 41.3, 39.8, 34.6, 31.3, 31.0, 30.6, 29.1, 28.5 ($C6^A$, hexyl C1-C6, adamantyl C, cubyl C); 13.7 (Me).

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Table 1 600 MHz ^{1}H NMR ROESY cross-peaks observed in D_2O solution at pD ≥ 10 and identified as arising from intra- and intermolecular complexation

	System **2/2'**								
Annular protons	Norbornyl protons								
	nor H2	nor H5	nor H8	nor H8'	nor H*				
H3	+	+	+	+	+				
H5	+	+	+	+	+				
	System **2/2' + 8**								
Annular protons	Norbornyl and adamantyl protons								
	nor H2	nor H5	nor H8	nor H8'	nor H*	adam 2	adam 3	adam 4	adam 4'
H3	+	+	+	+	+	+	+	+	+
H5	+	+	+	+	+	+	+	+	+
	System **4/4'**								
Annular protons	Noradamantyl protons								
	norad H3	norad H6	norad H*						
H3	+	+	+						
H5	+	+	+						
	System **4/4' + 8**								
Annular protons	Noradamantyl and adamantyl protons								
	norad H3	norad H6	norad H*	adam 2	adam 3	adam 4			
H3	+	+	+	+	+	+			
H5	+	+	+	+	+	+			
	System **7/7'**								
Annular protons	Hexyl and dimethylcubyl protons								
	hexyl H2-H5	cubyl H*	cubyl Me						
H3	+	+	+						
H5	+	+	+						

	System **7/7' + 8**				
Annular protons	Dimethylcubyl and adamantyl protons				
	cubyl Me	adam 2	adam 3	adam 4	adam 4'
H3	+	+	+	+	+
H5	+	+	+	+	+

* Identification of individual resonances uncertain. Crosses indicate the presence of a cross-peak

Figure and Scheme captions

Fig. 1. Structures of C(6) substituted βCDs, **1/1' - 7/7'** where the substituent is intramolecularly complexed in the primed species, adamantane-1-carboxylate (**9**) and 4-nitro norborane acetate (10) showing the atom labelling schemes. The prefixes annular, hexyl, norbornyl, cubyl, noradamantyl and adamantyl are used as appropriate in referring to ^{1}H and ^{13}C resonances in the NMR spectra. The pK_as (± 0.02) in brackets are those of the protonated secondary amine function in each case at 298.2 K and I = 0.10 mol dm^{-3} ($NaClO_4$). In each case the amine groups of 6 and 7 have identical pK_as.

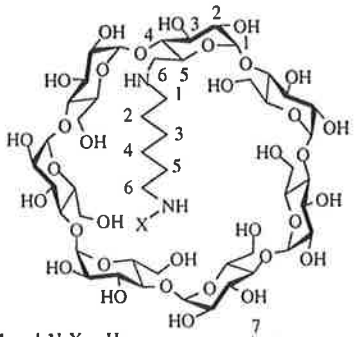
Fig. 2. 600 MHz ^{1}H NMR ROESY spectrum of **4/4'** in D_2O at pH ≥ 11.

Fig. 3. 600 MHz ^{1}H NMR ROESY spectrum of **7/7'** in D_2O at pH ≥ 11.

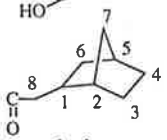
Scheme 1

Scheme 2. A form of **7'** where the hexyl entity is complexed in the second βCD annulus probably also exists but this is not shown in the Scheme.

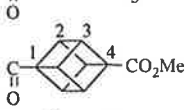
Fig. 1.



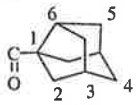
1 and **1'** X = H



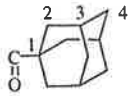
2 and **2'** X = pK_a = 8.98



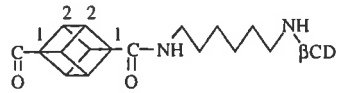
3 and **3'** X = pK_a = 8.87



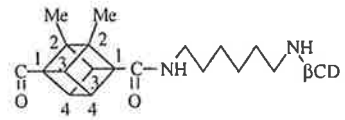
4 and **4'** X = pK_a = 8.47



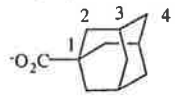
5 and **5'** X = pK_a = 8.15



6 and **6'** X = pK_a = 8.87



7 and **7'** X = pK_a = 8.80



8

Fig. 2.

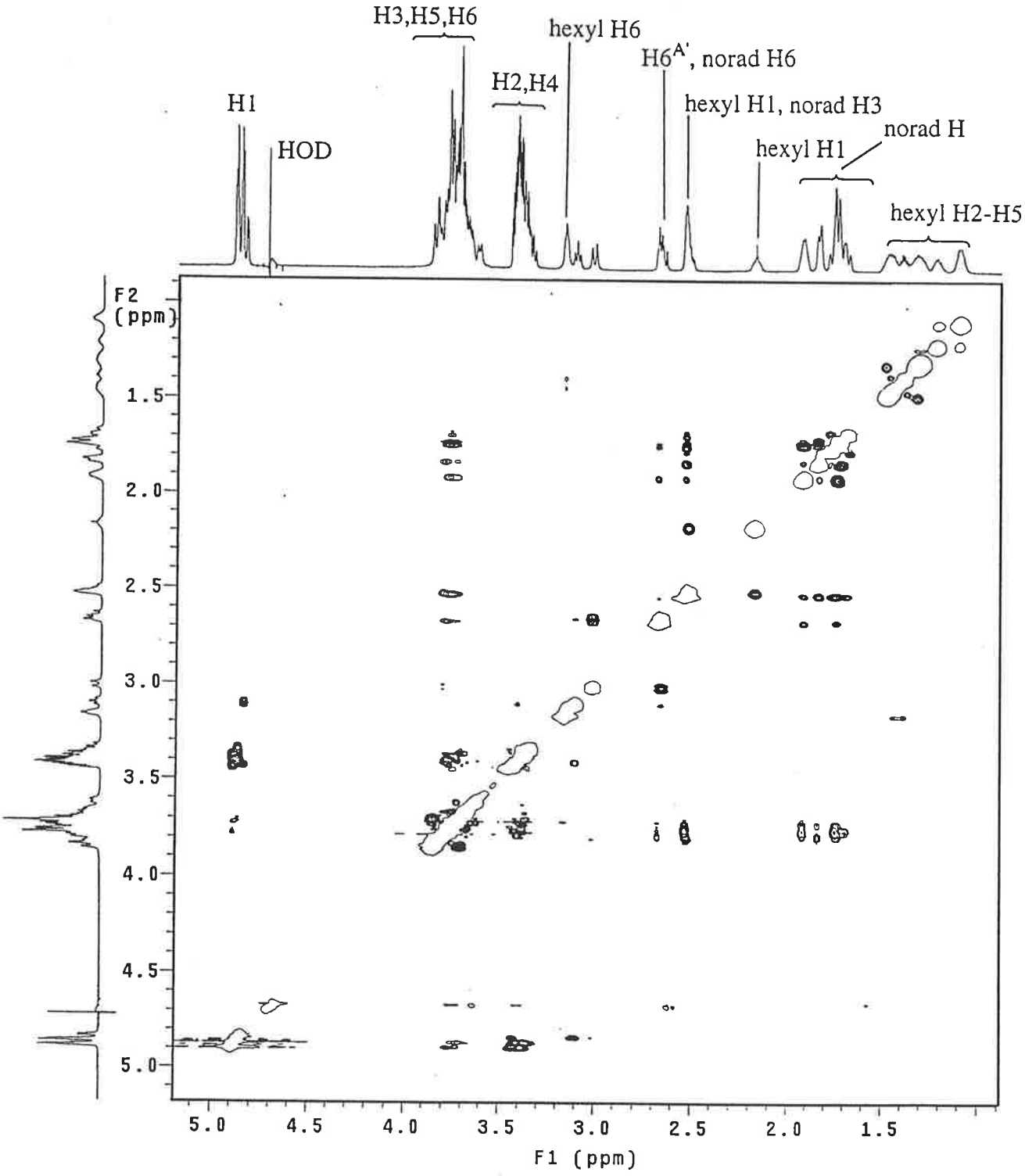
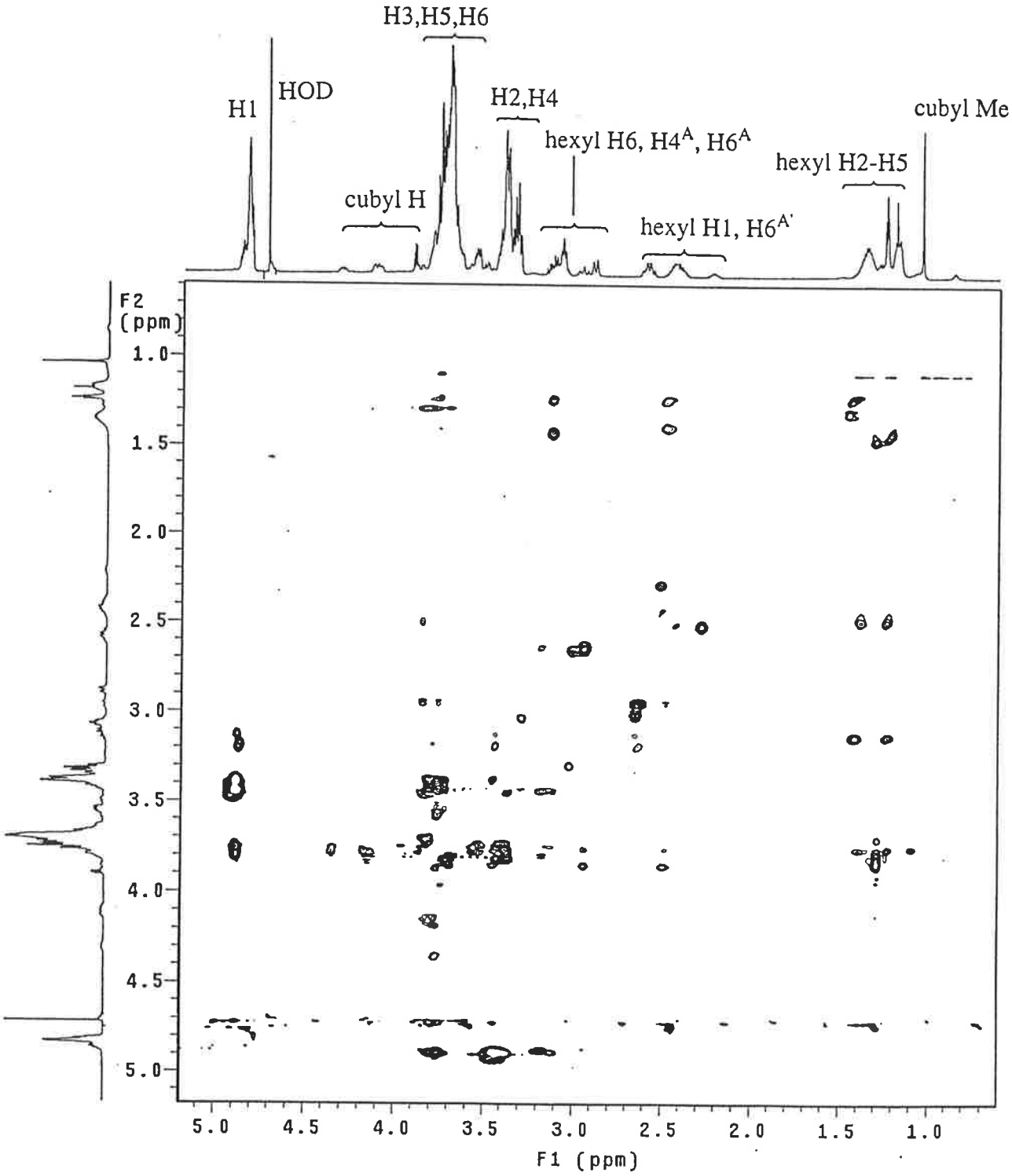
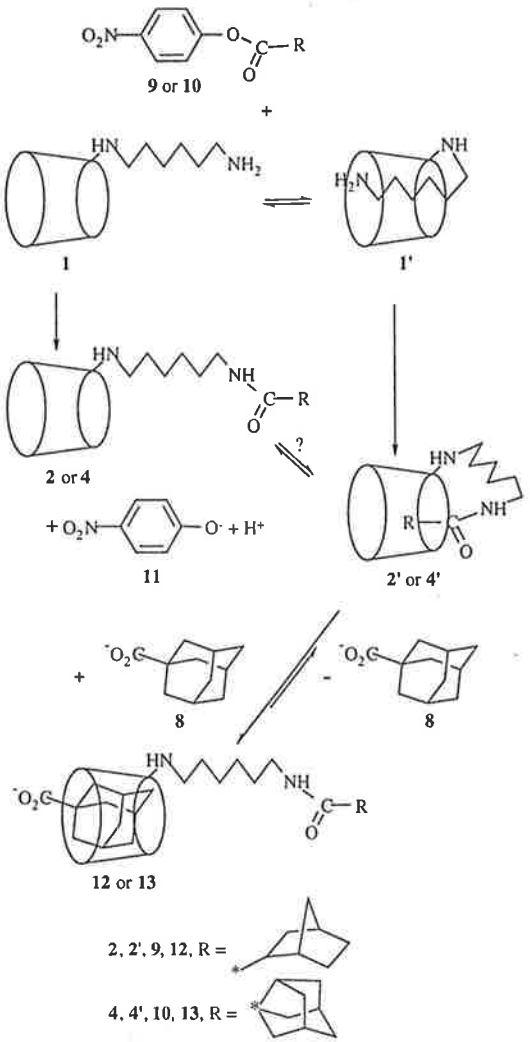

H3,H5,H6
hexyl H6
H6A', norad H6
H2,H4
H1
hexyl H1, norad H3
norad H
HOD
hexyl H1
hexyl H2-H5
F2 (ppm)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
F1 (ppm)

Fig. 3.


H3,H5,H6
H1
HOD
H2,H4
cubyl Me
hexyl H6, H4A, H6A
hexyl H2-H5
cubyl H
hexyl H1, H6A'
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F1 (ppm)

Scheme 1.



where * indicates the bonding carbon

Scheme 2.