6^{A}-Amino-Cyclodextrins: Their Preparation, Reactions and Host-guest Chemistry

Bruce L. May

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Contents

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Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abstract

This thesis describes the preparation and characterisation of a series of 6^A-amino-substituted β-cyclodextrins. The reactions of 6^A-*O*-(4-methylbenzenesulfonyl)-β-cyclodextrin **32** with a range of primary and secondary amines in 1-methyl-pyrrolidin-2-one at 70 °C produce thirteen amino-substituted β-cyclodextrins in yields of 30-50%. The product cyclodextrins have been fully characterised by NMR, electrospray-ms and elemental analysis.

Potentiometric titration was used to determine the pK_a values of the protonated amines and the formation constants of the complexes formed by them with benzoate, 4-methylbenzoate and (*R*)- and (*S*)-2-phenylpropionate. The formation constants of these complexes were much greater than those found for the complexes formed with β-cyclodextrin. In particular, the complexes formed with cyclodextrins bearing a cyclic polyamine substituent were extremely stable, with formation constants in the range 9650-44000 $dm^3\ mol^{-1}$ for the complexes formed between the cyclodextrins bearing a 1,5,9-triazacyclododecanyl or a 1,4,7,10-tetraaza-cyclododecanyl substituent and these carboxylates. NMR studies suggest that the high stability of these complexes may be due to the capping of the primary face of the cyclodextrin by the cyclic substituent.

The solution structures of the complexes formed between the carboxylates and some of the modified cyclodextrins were examined by 2D-ROESY NMR spectroscopy. Hydrophobic linear substituents are included within the annulus at high pH, when the substituent is non-protonated. The spectra of the complexes formed between 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin and the carboxylates indicate that the 6-aminohexyl substituent is included simultaneously with the carboxylate.

The Zn(II) complexes of 6^A-(1,5,9-triazacyclododecan-1-yl)- and 6^A-(1,4,7,10-tetraazacyclododecan-1-yl)-6^A-deoxy-β-cyclodextrin were examined as mimics of esterases. The reactions of 6^A-(1,5,9-triazacyclododecan-1-yl)-6^A-deoxy-β-cyclodextrin with 4-

nitrophenyl acetate are inhibited by the presence of Zn(II). The reactions of 6^A-(1,4,7,10-tetra-azacyclododecan-1-yl)-6^A-deoxy-β-cyclodextrin with 4-nitophenyl acetate were marginally enhanced by the presence of Zn(II) at pH ≤ 7.5 but were inhibited by the presence of Zn(II) at higher pH.

The reactions of ω-aminoalkylamino-substituted β-cyclodextrins with 4-nitrophenyl acetate involve the nucleophilic attack of the primary nitrogen on the carbonyl of the ester to give the acetamides. The major reactive species is the non-protonated species as shown by the pH dependence of the reaction of 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin with 4-nitrophenyl acetate. The reaction this ester with the ω-aminoalkylamino-substituted β-cyclodextrins involves the inclusion of the ester within the annulus as shown by the inhibition of the reaction in the presence of adamantane-1-carboxylate. The inhibition is not quantitative, some of the reaction between the ester and the cyclodextrins occurs by a normal S_N2 pathway. The solution structure of the complex formed between adamantane-1-carboxylate and 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin was examined by 2D-ROESY NMR spectroscopy. The adamantyl group is deeply included within the annulus while the 6-aminohexylamino substituent forms a rigid structure within the primary face of the cyclodextrin.

The effect of the hydrophobicity of the substituent on the inclusion chemistry of modified cyclodextrins was examined by 2D-ROESY NMR spectroscopy. A 12-aminododecyl substituent is much more strongly included within the annulus than is 6-aminohexyl substituent, preventing the inclusion of 4-methylbenzoate within the annulus of 6^A-(12-amino-dodecyl)amino-6^A-deoxy-β-cyclodextrin. Adamantane-1-carboxylate is able to displace most of the alkyl chain of the 12-aminododecyl substituent from the annulus but is itself only partially included within the annulus.

The reactions of 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin with the 4-nitrophenyl esters of 1-methoxycarbonyl-cubane-4-carboxylic acid, 2,3-dimethyl-1-methoxycarbonyl-cubane-4-carboxylic acid and adamantane-1-carboxylic acid lead to the formation of the corresponding 6-amidohexylamino-substituted cyclodextrins. The substituents of each of these derivatives is included within the annulus. Addition of adamantane-1-carboxylate to solutions of these modified cyclodextrins causes the cubanyl substituents to be

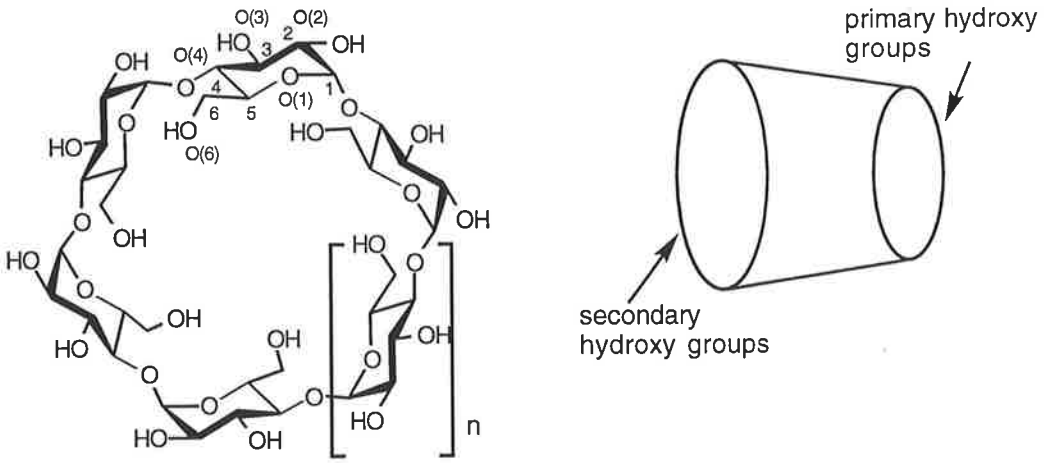
excluded from the annulus as the adamantane-1-carboxylate is included. The adamantyl substituent of 6^A-(6-*N*-(adamantan-1-oyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin is not excluded from the annulus by adamantane-1-carboxylate under these conditions and no inclusion of the added adamantane-1-carboxylate occurs. 6^A-(6-*N*-(adamantan-1-oyl)aminohexyl)amino-6^A-deoxy-β-cyclodextrin may be a molecular knot.

The reaction of 1,4-bis(4-nitrophenoxycarbonyl)-cubane with 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin gives a cyclodextrin dimer. The cubanyl group is included within the annulus of one of the cyclodextrin moieties leading to a product which is asymmetric on the NMR time-scale. 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 moiety has included a molecule of adamantane-1-carboxylate.

Chapter 1: The Cyclodextrins

1.1. The natural cyclodextrins

The cyclodextrins are a class of natural, cyclic oligosaccharides first isolated by Villiers in 1891.[1] They were determined to be cyclic oligosaccharides by Schardinger in 1904.[2] For this reason they are often referred to in the early literature as Schardinger-dextrins. More recently they have been referred to as cycloamyloses following the full structure determination by Freudenberg and Cramer in 1948.[3] However, most current reports use the general name cyclodextrins and this terminology is used throughout this thesis.



1 n = 1 α-cyclodextrin
2 n = 2 β-cyclodextrin
3 n = 3 γ-cyclodextrin

Figure 1.1. Schematic representations of the cyclodextrins **1-3**. In this thesis a truncated cone is used to represent a natural or modified cyclodextrin. When a substituent is drawn at the narrow end of the cone, it indicates that it has replaced a C6 hydroxyl group, whereas a substituent drawn at the wider end of the cone indicates that it replaces either a C2 or a C3 hydroxyl group.

The cyclodextrins are a series of cyclic oligimers composed of α-(1→4)-linked D-glucopyranose units. The most common cyclodextrins are composed of 6, 7, or 8 glucose units corresponding to α-, β-, and γ-cyclodextrin, respectively, (Figure 1.1) although cyclodextrins with up to 21 glucopyranose units have been described.[4]

Cyclodextrins are prepared commercially by the enzymatic degradation of starch with cyclodextrin-glucanosyltransferase (EC 2.4.1.19) from *Bacillus macerans* and other *Bacillus* species. Starch hydrosylates prepared from such bacterial fermentations contain mixtures of the various cyclodextrins and it is necessary to separate the products by selective precipitation in order to isolate the individual cyclodextrins. Considerable effort has been put into developing commercial processes to provide high yields of the pure cyclodextrins, particularly β-cyclodextrin **2**, which is the most commonly used of the cyclodextrins. The current world-wide production of β-cyclodextrin **2** is around 10 tonnes per annum.[5] One recent report describes the use of a debranching enzyme, pullulinase (EC 3.2.1.41), and cyclodecanone (which preferentially complexes with and precipitates β-cyclodextrin **2**) to obtain a 92% yield of β-cyclodextrin **2** from amylopectin. When cyclodecanone is replaced with decan-1-ol, α-cyclodextrin **1** is obtained in 84% yield from the same source, while γ-cyclodextrin **3** is obtained in 72% yield in the presence of cyclotridecanone.[6]

Crystallographic x-ray studies of cyclodextrin hydrates show that each glucopyranose unit is held in a rigid $^4C_1$ chair conformation such that each molecule exists in an annular structure resembling a shallow truncated cone where the narrow end is delineated by the primary hydroxyl groups on C6 and the wider end by the secondary hydroxyl groups on C2 and C3.[7] This structure is stabilised by intramolecular hydrogen bonding. The interior of a cyclodextrin molecule is lined with two layers of methine groups (C3 and C5, hydrogens inside the annulus) sandwiching a layer of glycosidic oxygens, making it a hydrophobic surface, while the exterior of the molecule is hydrophilic due to the presence of the hydroxyl groups. It is the hydrophobic nature of the cavity that brings about the formation of non-covalent host-guest complexes between cyclodextrins and many organic molecules in aqueous solution, and it is the ability to form such complexes which has directed the large amount of interest in the

chemistry of the cyclodextrins.

The crystal structures of cyclodextrin hydrates show some distortion of the cone and an inherent asymmetry in the smaller cyclodextrins.[7] This apparent rigidity disappears in solution where, on the NMR time-scale, the cyclodextrins appear to be perfectly symmetrical. Further experimental evidence that cyclodextrins are more flexible than is commonly portrayed comes from a recent study on the conformational flexibility of cyclodextrins using vibrational Raman optical activity.[8] Theoretical studies, using molecular dynamics, indicate that cyclodextrins are able to modify their shape in order to accommodate different guest molecules.[9-14]

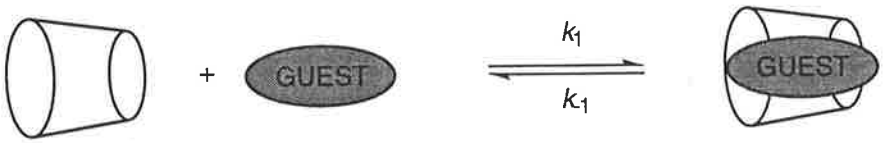
Table 1.1. Some physicochemical properties of the natural cyclodextrins

	Cyclodextrin			
Property	**1**	**2**	**3**	Reference
no. glucose units	6	7	8	
anhydrous molecular weight	972.85	1134.99	1297.14	
cavity length (Å)	8	8	8	15
cavity diameter (Å)	~5.2	~6.6	~8.4	15
solubility in water, 25 °C (mol dm^{-3})	0.121	0.0163	0.168	16
pK_a (25 °C)	12.33	12.20	12.08	17, 18

Some physical data for the cyclodextrins **1**, **2** and **3** are given in Table 1.1. The depth of the cavity is the same for all cyclodextrins, being determined by the width of a glucose molecule (~ 8 Å), but the width varies with the number of glucopyranose units in the cyclodextrin. It is the difference in the widths of the annuli which brings about the selectivity in complex formation based on the size of the guest. Benzenes form tight complexes with α-cyclodextrin **1** while naphthalenes form tight complexes with β-cyclodextrin **2**. This size selectivity forms the basis for the methods for the purification of cyclodextrins from hydrosylates as discussed above.

The cyclodextrins are all much less soluble in water than their linear analogues, with β-cyclodextrin **2** being the least soluble. The lower solubility of β-cyclodextrin **2** has been ascribed to self-aggregation[19] and to the presence of intramolecular hydrogen bonding around the rim of β-cyclodextrin **2**.[20] Alkylation of cyclodextrin hydroxyl groups, which would be expected to increase the hydrophobicity of the cyclodextrin, actually increases the solubility of the product relative to that of the native cyclodextrin. This suggests that hydrogen bonding is a factor in the relatively low solubility of β-cyclodextrin **2**. There are no satisfactory explanations for this anomalous solubility of β-cyclodextrin **2** given that hydrogen bonding effects should be similar for all of the cyclodextrins.

1.2. Host-guest complexation



$$K = \frac{k_1}{k_{-1}} = \frac{[\text{CD.GUEST}]}{[\text{CD}][\text{GUEST}]}$$

Scheme 1.1. Schematic representation of the formation of a host-guest (inclusion) complex between a guest molecule and a cyclodextrin host.

Most of the current interest in cyclodextrins arises from their ability to partially or fully include a wide range of guest species within their annuli to form host-guest (inclusion) complexes (Scheme 1.1).[20-30] Their ability to form host-guest complexes has lead to the use of cyclodextrins in the formulation of pharmaceuticals,[31, 32] as stationary phases in gas liquid chromatography[33] and high pressure liquid chromatography,[34, 35] as a mobile phase modifier in capillary electrophoresis,[26] and as catalysts for chemical reactions.[36-39]

Several hypotheses have been proposed to account for the formation of host-guest

complexes with cyclodextrins: (1) the release of "high energy" water from the cavity;[40] (2) the relief of conformational strain energy of the uncomplexed cyclodextrin (particularly for α-cyclodextrin **1**);[41] (3) the hydrophobic interaction;[42] and (4) the electrostatic interactions: dipole-induced dipole, dipole-dipole, London dispersion force and hydrogen bonding.[43] A recent review of this area discusses each of these effects and concludes that the main driving force for inclusion of an organic molecule within the cyclodextrin annulus is the hydrophobic effect.[28] A model has been developed to predict binding constants of guests with α-cyclodextrin **1**, based on differences in the solvent interactions of the free guest, the free cyclodextrin **1** and the complex that is formed between them.[44] More recently, molecular modelling studies have shown that there is a linear relationship between the logarithm of the binding 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.[45] Structures of the host-guest complexes predicted by this model system are in accord with the reported crystal structures of these complexes.

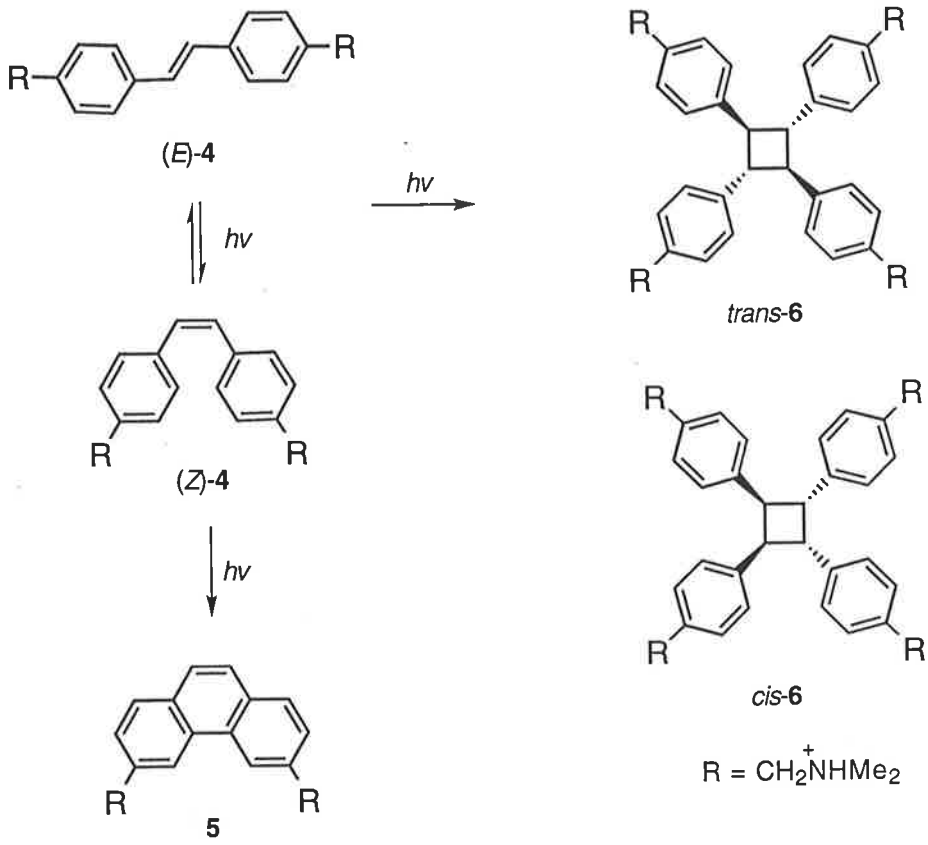
The variation in the size of the annuli of the cyclodextrins **1-3** allows for discrimination in the inclusion process based on size and shape of the guest. The cyclodextrins are homo-chiral molecules. The chirality of the cyclodextrins leads to the formation of diastereomeric complexes with racemic guests. The diastereomeric complexes which are formed will have different stabilities leading to chiral discrimination by cyclodextrins. However, in practice this discrimination is only small due to the inherent symmetry of the cyclodextrin hosts.[30]

1.3. Catalysis by natural cyclodextrins

Cyclodextrins can affect the course of chemical reactions in two distinct ways. Firstly, inclusion of a guest molecule within the annulus changes the micro-environment of that molecule. It may experience an effective solvent polarity or dielectric constant which is different from that of the bulk solution, thereby altering levels or positions of ionisation within the guest. One particular conformation of a guest may be more favoured by inclusion, giving

rise to a product of a reaction carried out in the presence of a cyclodextrin, which has a different stereo- or regiochemistry from that obtained by the same reaction carried out in the absence of the cyclodextrin. Inclusion of a guest may favour attack by a reagent at a specific position on the guest through the protection of other reactive sites on the guest by the steric bulk of the cyclodextrin.[46] The cyclodextrin can act as a "reverse phase-transfer" catalyst, carrying organic molecules into the aqueous phase for reaction with a water soluble reagent.[47] In reactions where a cyclodextrin is only involved through the inclusion of reactants within the annulus, the cyclodextrin acts as a "non-covalent catalyst".

Non-covalent catalysis may also involve the pre-organisation of two or more reactants to favour the formation of a particular product. In such reactions the size of the cyclodextrin annulus becomes an important factor in the control of the reaction pathway. The presence of cyclodextrins can greatly affect the photochemical reactions of the stilbene derivative (*E*)-**4** (Scheme 1.2).[48] Under acidic conditions, in the absence of cyclodextrins, irradiation of the trans isomer (*E*)-**4** yields mainly the *cis*- isomer (*Z*)-**4** and the secondary photo-product **5**. In the presence of the cyclodextrins **1** and **2** dimerization is totally suppressed as the inclusion of the stilbene moieties, to give 1:1 complexes with the cyclodextrins, shields them from each other and only *cis-trans* isomerization is possible. In the presence of the larger γ-cyclodextrin **3**, however, 2:1 guest:host complex formation is most favoured. This generates a high local concentration of the stilbene (*E*)-**4** and consequently dimerization to give the cyclobutanes **6** proceeds readily. This chemistry has been extended to the formation of a [3]-rotaxane made up of the cyclodextrins **2** and **3** threaded onto a poly-(*trans*-stilbene).[49]

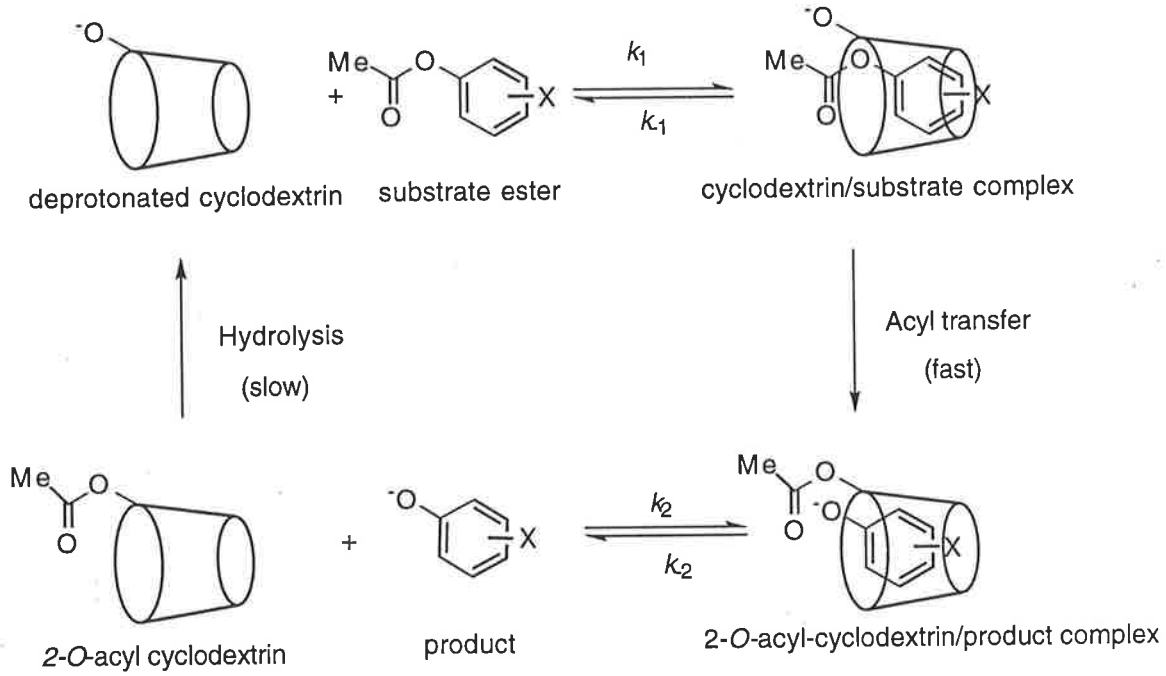


		% Composition of reaction mixture				
Cyclodextrin	t/h	(*E*)-**4**	(Z)-**4**	**5**	*trans*-**6**	*cis*-**6**
none	24	10	62	19	7	2
1	24	20	60	20	0	0
2	24	16	83	1	0	0
3	72	0	0	2	79	19

Scheme 1.2. Effects of the cyclodextrins **1-3** on the photochemical reactions of the stilbene **4**. From ref. 48.

Cyclodextrins can also act as "covalent catalysts". Cyclodextrins catalyse the base hydrolysis of phenyl esters through the formation of a transient *O*-acyl cyclodextrin (Scheme 1.3). Pioneering work by Bender's group in the 1960's established the mechanism for this catalysis.[50 -54] A deprotonated C2 hydroxyl group (pK_a ~ 12[17, 18]) makes a nucleophilic attack on the substrate, previously included within the annulus of the cyclodextrin, to give an *O*-acyl cyclodextrin and a phenoxide. Under the conditions of the reaction (usually pH ≥ 10.5)

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 chymotrypsin, an enzyme which has a hydrophobic binding pocket, adjacent to the active site, capable of binding aryl groups (eg. tyrosine, phenylalanine) and a hydroxyl group of a serine residue which is activated as a nucleophile through a charge relay network.[55]



Scheme 1.3. Schematic representation of the hydrolysis of phenyl esters catalysed by cyclodextrins. A C2 hydroxyl group is deprotonated ($pK_a \sim 12$) to generate the active catalyst.

Generally, the greatest rate enhancement of the hydrolysis occurs when α-cyclodextrin **1** is the catalyst and the phenyl ester substrate is *meta*-substituted.[56 -58] With this catalyst, *meta*-substituted esters are bound within the annulus of the cyclodextrin such that the carbonyl group is held in close proximity to the ionised secondary hydroxyl group, while the carbonyl group of an included *para*-substituted ester is positioned further away from the active hydroxyl group. This leads to a greater enhancement of the rate of reaction of *meta*-substituted esters relative to that of the *para*-substituted esters in the presence of the cyclodextrin **1**. When the cyclodextrin **2** is the catalyst, both types of substrate are held more loosely in the larger annulus and the resultant greater number of degrees of freedom of movement of the substrate leads to a

lowering of both the overall rate of catalysis, and the difference between the reactivities of the *meta*- and *para*-substituted esters. With the much larger cyclodextrin **3** there is little difference in the in the reactivities of *meta*- and *para*-substituted esters. The differences in the reactivity of the different cyclodextrins and their substrates has been correlated to differences in transition state binding.[36] The hydrolysis of phenyl esters by the cyclodextrin **2** has been modelled using a molecular dynamics methodology and the results, which are in good agreement with experimental data, support this observation.[59, 60]

1.4. Modification of natural cyclodextrins

While the natural cyclodextrins are themselves of interest as molecular hosts, they are limited in their applications through their inherent symmetry and lack of specific binding groups. For example, enantioselective binding requires a minimum of three specific interactions between a host and a guest. Addition of new functionality to the cyclodextrin structure allows for the possibility of specific interactions between these groups and sites on a guest molecule, which may lead to much greater host-guest specificity.[30]

1.4.1. Nomenclature

The following nomenclature system has become generally accepted for the naming of modified cyclodextrins and is used throughout this thesis. The cyclodextrin is named α-, β-, γ-, etc., depending on the number of glucose residues that make up the annulus, as for the natural cyclodextrins. Each atom of a single glucose residue is numbered as for glucose itself. For a cyclodextrin with a modification to a single glucose residue, that residue is labelled as A and the remaining residues are labelled B, C, D..., etc., going from the C1 of the modified residue to the C4 of the next residue (clock-wise around the cyclodextrin if it is viewed from the primary face, Figure 1.2). Thus, a cyclodextrin of seven glucose residues, where the C6 hydroxyl group of one of the residues has been converted to a bromide, is named 6^A-bromo-6^A-deoxy-β-cyclodextrin.

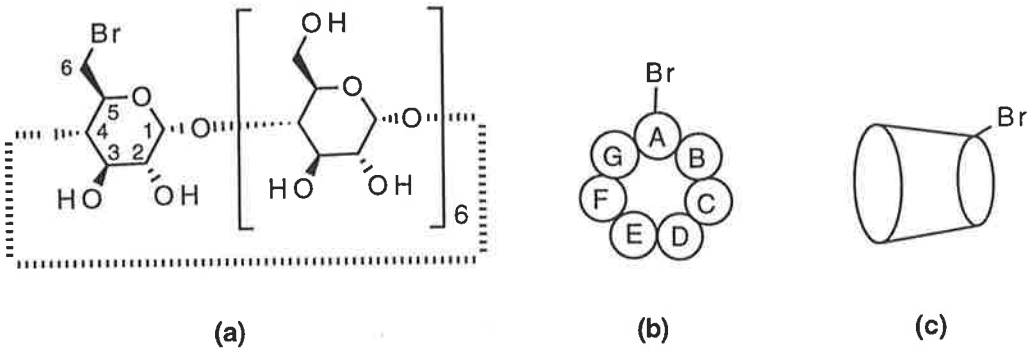


Figure 1.2. Schematic representations of the structure of 6^{A}-bromo-6^{A}-deoxy-β-cyclodextrin showing (a) the atom labelling of an individual glucose residue, (b) a view of the modified cyclodextrin from above the primary face showing the labelling of the glucose residues, and (c) the truncated cone representation of the modified cyclodextrin that is used throughout this thesis.

1.4.2. Modification of all hydroxyl groups

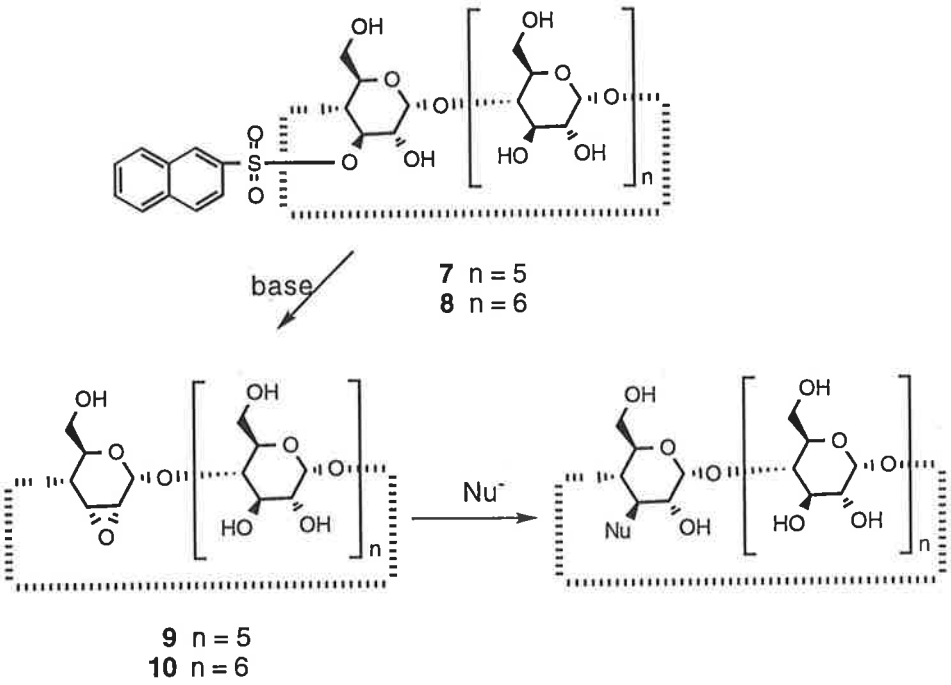
Most modifications of the cyclodextrin structure involve a reaction at one or more of the hydroxyl groups. The simplest modifications involve the alkylation or acylation of all of the hydroxyl groups. When a cyclodextrin is treated with an excess of base and an alkylating agent (eg. alkyl halide or epoxide) or an acylating agent (eg.. acid anhydride) the per(*O*-alkyl)- or per(*O*-acyl)-cyclodextrin is obtained. The product of such a reaction is usually non-homogenous and contains a mixture of cyclodextrins with one or more unmodified hydroxyl groups.[33] The cyclodextrins modified in this way have improved solubility properties over the natural cyclodextrins. The crystalline per-*O*-methyl derivatives are much more soluble in water than the parent cyclodextrins. As the length of the attached alkyl chain is increased, the modified cyclodextrins become increasingly more hydrophobic and are generally isolated as viscous oils. Such compounds have been found to be of great utility as liquid phases for enantioselective gas-liquid chromatography.[33]

1.4.3. Modification of specific hydroxyl groups

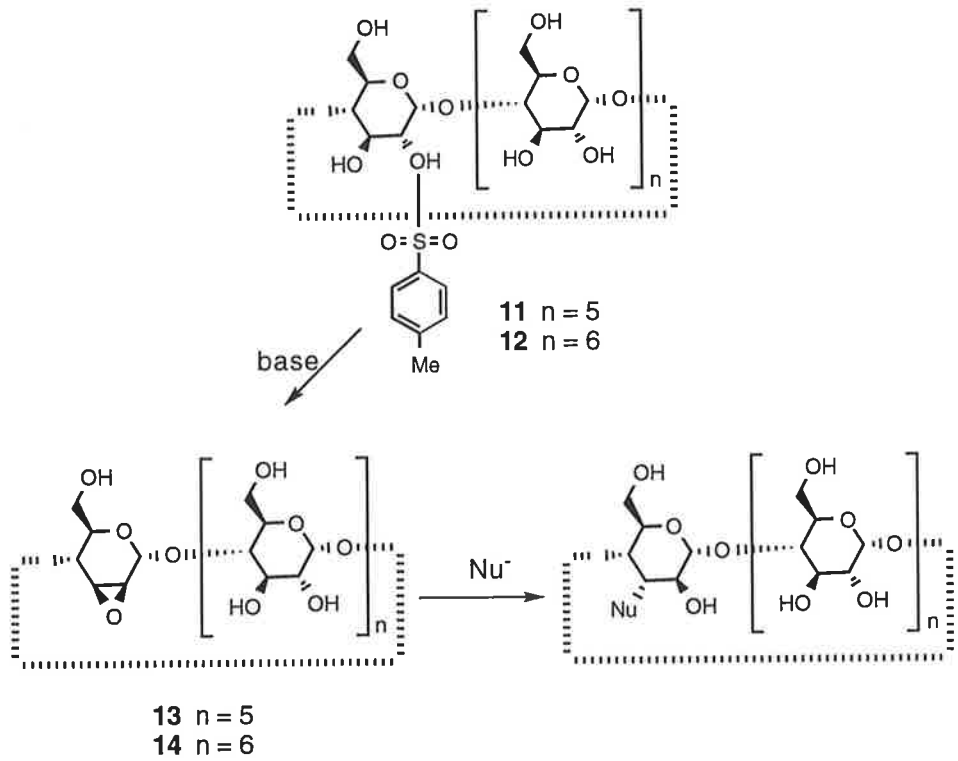
More specific modifications of the natural cyclodextrins rely on the different reactivities of the C2, C3 and C6 hydroxyl groups. The C2 hydroxyl groups are the most acidic and can be selectively alkylated without the need for protection of the C3 and C6 hydroxyl groups. Treatment of a solution of β-cyclodextrin **2** in dimethylsulfoxide (DMSO) with one equivalent of sodium hydride, followed by one equivalent of *N*-methyl-4-chloromethyl-2-nitro aniline gave the 2^{A}-*O*-substituted cyclodextrin in 35% yield.[61] Similarly, per(2-*O*-methyl)-β-cyclodextrin was obtained in 83% yield by treatment of β-cyclodextrin **2** with seven equivalents each of sodium hydride and methyl iodide. This chemistry has been extended to the formation of a γ-cyclodextrin dimer[62] and some calixarene-appended β-cyclodextrins.[63, 64] Nucleophilic substitution at C2 is difficult to achieve. The mono- and per-sulfonates of the C2 hydroxyl groups are readily available through the use of a number of reagents.[65, 66] However, attempts at substitution of these sulfonates by nucleophiles lead to the formation of *manno*-2,3-epoxides as the major products of the reactions, only small amounts of the C2 substituted product are formed in these reactions.[67]

The C3 hydroxyl groups are the least reactive of the hydroxyl groups on a cyclodextrin. The selective modification of these groups usually requires the prior protection of the C2 and C6 hydroxyl groups or an indirect approach, through transfer of functionality from adjacent centres. β-Naphthalenesulfonyl chloride reacts with the cyclodextrins **1** and **2** to give the corresponding 3^{A}-*O*-sulfonates **7** and **8** which when treated with mild bases are converted to *allo*-2^{A},3^{A}-epoxycyclodextrins **9** and **10**.[68 -71] The epoxides **9** and **10** react with nucleophiles to give mainly the corresponding $C3^{A}$-substituted cyclodextrins with retention of stereochemistry at $C2^{A}$ and $C3^{A}$ (Scheme 1.4).[67]

The treatment of the $C2^{A}$-*O*-sulfonates **11** and **12** with a mild base generates the *manno*-2^{A},3^{A}-epoxycyclodextrins **13** and **14** which react with nucleophiles to give mainly the corresponding $C3^{A}$-substituted cyclodextrins with inversion of the stereochemistry at $C2^{A}$ and $C3^{A}$ (Scheme 1.5).[68, 72 -74]

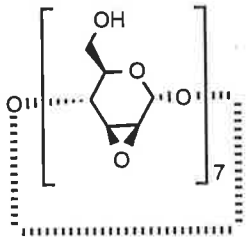


Scheme 1.4. Preparation of cyclodextrins substituted at $C3^A$ through the formation of an *allo*-2,3-epoxide. The stereochemistry at $C2^A$ and $C3^A$ is retained in this process.



Scheme 1.5. Preparation of cyclodextrins substituted at $C3^A$ through the formation of a *manno*-2,3-epoxide. The stereochemistry at $C2^A$ and $C3^A$ is inverted in this process.

The chemistry of the *manno*-epoxy-cyclodextrins has been developed further for the preparation of a novel β-cyclodextrin analogue, β-cycloaltrin **16**.[75] The per(*manno*-epoxy)cyclodextrin **15** (prepared from per(2-*O*-4-methylbenzenesulfonate)-β-cyclodextrin) was heated in water at reflux for five days to give β-cycloaltrin **16** in 73% yield.



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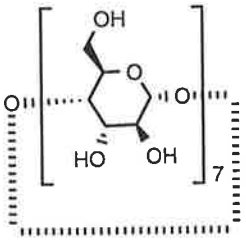


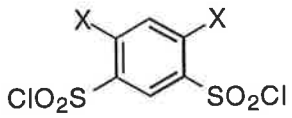
16

The C6 hydroxyl groups are the most nucleophilic and can be selectively modified by reaction with electrophilic species. The conversion of all of the C6 hydroxyls to halides can be achieved by reaction with Vilsmeier-Haack complexes ($[(CH_3)_2NCHX]^+.X^-$), prepared either *in situ* [76-78] or isolated as the crystalline salts.[79, 80] The product per(6-halo)-β-cyclodextrins are readily converted to the per(6-amino)-[80, 81] or per(6-thio)-β-cyclodextrins[82] which can be elaborated further to give new cyclodextrins with a range of complex functionalities. The per(6-bromo)- and per(6-*O*-4-methylbenzenesulfonate)-β-cyclodextrins have been converted to per(3,6-anhydro)-β-cyclodextrin by treatment with a mild base.[76, 83]

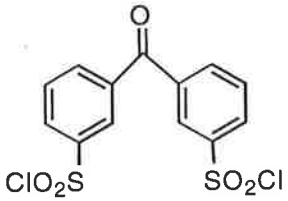
The selective substitution of a single C6 hydroxyl group is usually managed through the formation of the 6^A-*O*-4-methylbenzenesulfonate derivative and subsequent displacement of the sulfonate by a suitable nucleophile. Seminal work in this area was carried out by Melton and Slessor, who prepared pure 6^A-*O*-4-methylbenzenesulfonyl-α-cyclodextrin and converted this to the corresponding halo-, azido-, amino- and deoxy-α-cyclodextrin derivatives.[84] Matsui and Okimoto have developed a similar strategy for the preparation of derivatives of β-cyclodextrin **2**.[85]

A series of disulfonyl chloride reagents has been developed for the selective di-

sulfonation, and subsequent substitution, of primary face hydroxyl groups.[86, 87] The sulfonyl chlorides **17** and **18** give AB di-sulfonation, while the sulfonyl chlorides **19** and **20** give AC and AD disulfonation, respectively.



17 X = H
18 X = OMe



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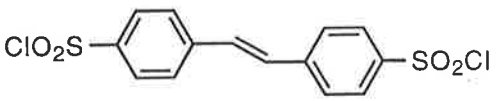


20

Once one or more functional groups, such as amino or thio, have been introduced into a cyclodextrin by the methods outlined above, they can then act as nucleophiles for further elaboration to a wide range of more extensively modified cyclodextrins.[30] Such modified cyclodextrins have been developed to improve the stability of host-guest complexes through ionic interactions between the guest and host, to form metal complexes which are able to mimic the reactions of enzymes and to act as molecular reactors to direct the stereo- or regiochemical ~~out come~~ outcome of a reaction.

The work discussed in the following chapters involves the preparation of a series of 6^A-amino-6^A-deoxy-β-cyclodextrins and an examination of their solution structures, and those of some of their complexes with small aromatic carboxylates, by 2D NMR spectroscopic techniques. The Zn(II) complexes of some of these derivatives are examined as mimics of metallo-enzymes through their reactions with 4-nitrophenyl acetate, and this ester is also used to probe the reactivity of the nitrogens of a series of 6^A-ω-aminoalkylamino-6^A-deoxy-β-cyclodextrins. The reactions of some 6^A-ω-aminoalkylamino-substituted cyclodextrins with the 4-~~nitophenyl~~ nitrophenyl esters of bulky carboxylic acids give rise to products with large hydrophobic substituents and the host-guest chemistry of these products is examined by NMR.

Chapter 2: Synthesis and Characterisation of 6^A-amino-6^A-deoxy-β-cyclodextrins

2.1. Introduction

The substitution of a hydroxyl group at C6 of β-cyclodextrin **2** by a series of polyamino species presents the opportunity to examine, in a systematic way, the factors affecting the formation of host-guest complexes.[27, 88] Metallo-cyclodextrins derived from such amino-substituted β-cyclodextrins, may show enantioselectivity in the formation of ternary complexes with chiral guests[89, 90] and can act as catalytic systems which mimic the actions of enzymes.[91] In order to carry out such a systematic survey it is necessary to be able to prepare a number of analogues in a simple and reproducible fashion. The compounds chosen for this study are shown in Figure 2.1 and represent several series of substituted β-cyclodextrins (βCDX).

The first series of substituents consists of linear α,ω-diaminoalkanes with a regular increase in the length of the alkyl chain separating the amino groups. Thus, there is an increase in the hydrophobicity of the substituent on going from 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin **21** to 6^A-(6-aminohexylamino)-6^A-deoxy-β-cyclodextrin **24**. An increase in the hydrophobicity of the host was expected to increase the stability of complexes with hydrophobic guests. Alternatively, as the length of the alkyl chain increases there may be an increasing tendency for the substituent to include (either completely or partially) in the annulus of the cyclodextrin and so compete with prospective guests, causing a relative decrease in the stability of host-guest complexes.[92]

The second series of substituents consists of linear polyamines containing three or four amino groups with variations in the distance between the amino groups. Increasing the number of amino groups on the substituent increases the number of available sites for additional ionic or

hydrogen bonding interactions between a guest and the cyclodextrin and so the formation constants of such host-guest complexes may be higher than for the diamino systems. In addition the higher polarity of these chains makes it less likely that they will include in the annulus of the cyclodextrin and so they will not compete with the guest for this site.

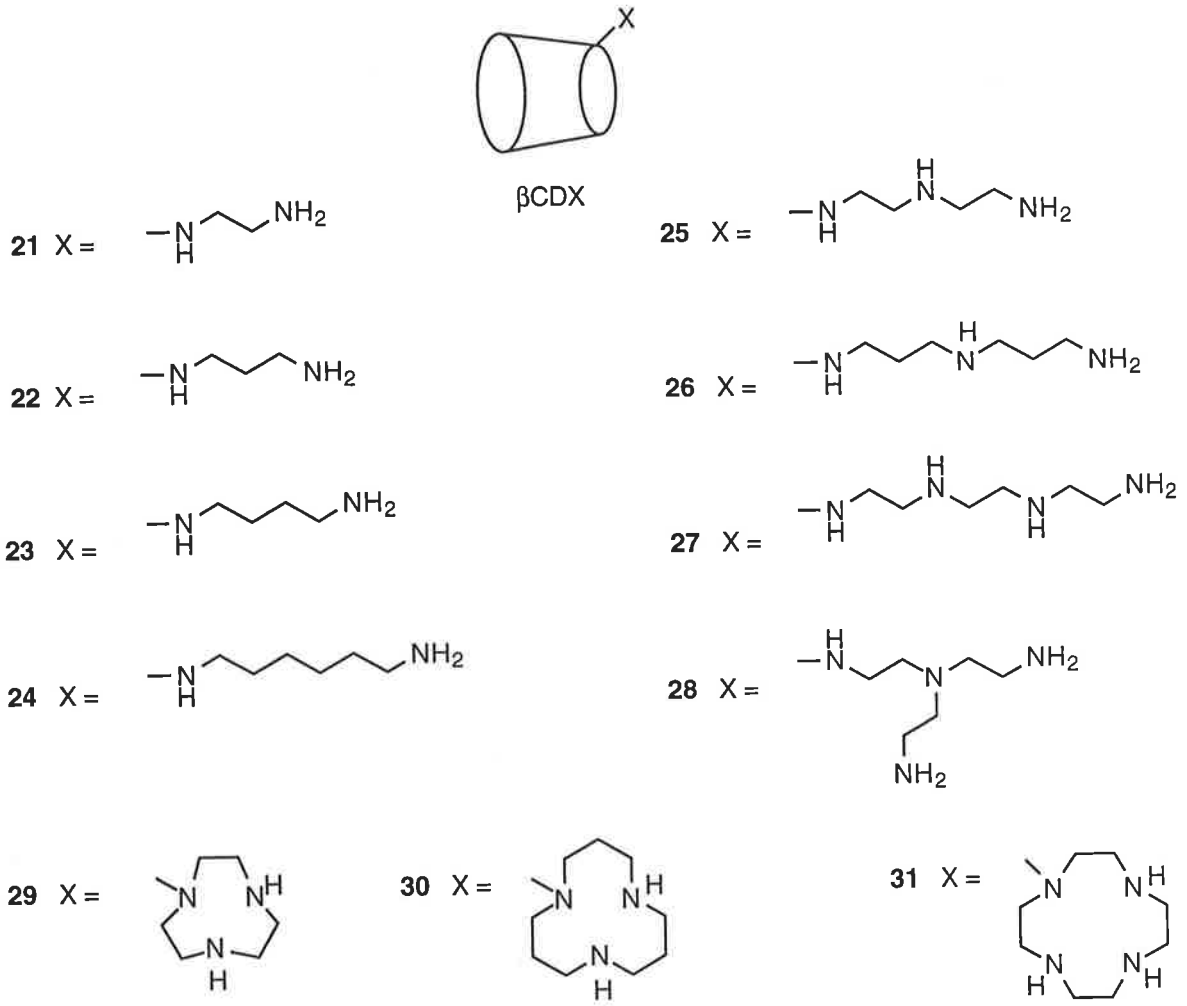
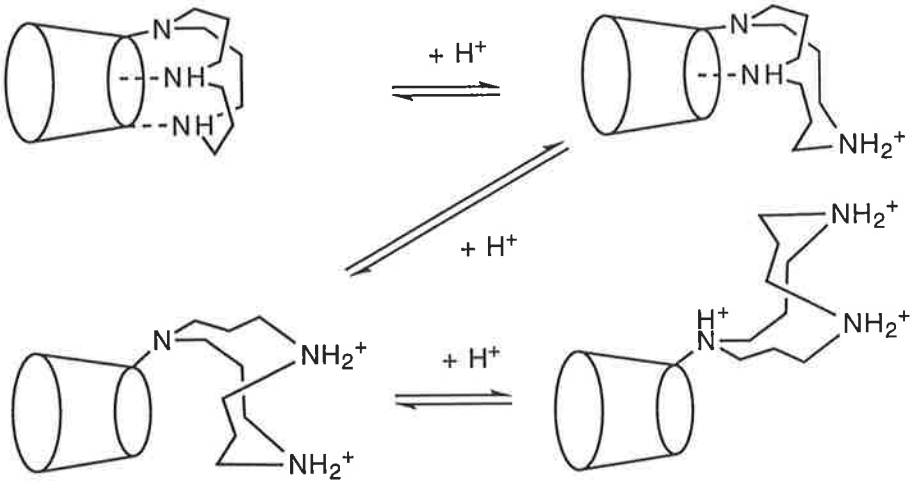


Figure 2.1 The series of polyamino-cyclodextrin derivatives discussed in this chapter

The third series of substituents consists of cyclic polyamines which may be considered to be the cyclic analogues of the linear polyamines described above. They contain three or four sites capable of ionic or hydrogen bonding interactions with suitable guests but, because of the cyclic nature of these groups, they are more constrained than their linear equivalents. This was expected to lead to more selective interactions with guest molecules and so to a greater degree of

molecular recognition by these compounds. The cyclic polyamines were not expected to include in the annulus of the cyclodextrin but rather to sit over the primary face of the cyclodextrin and possibly cap this end of the molecule. Strong hydrogen bonding interactions between the amino groups of the cyclic substituent and the primary hydroxyl groups of the cyclodextrin were expected to hold the substituent tightly against the primary face of the cyclodextrin. Capping of one end of a cyclodextrin has been shown to increase binding by guests.[93]



Scheme 2.1. Schematic representation of the cyclodextrin **30** behaving as a pH dependent "molecular peddle-bin". Dashed lines (---) represent hydrogen bonds between the nitrogen of the substituent and a hydroxyl group on the primary face of the cyclodextrin.

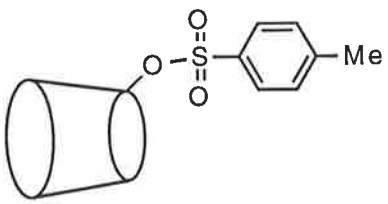
This hydrogen bonding will be pH dependent. At high pH (all amino groups deprotonated) it was expected that strong hydrogen bonding interactions between the substituent and the cyclodextrin would hold the substituent against the primary face of the cyclodextrin annulus. At low pH (all amino groups protonated) the high charge that will reside over the substituent was expected to cause it to move away from the hydrophobic cavity of the cyclodextrin . Effectively the modified cyclodextrin would be acting as a proton activated "molecular peddle-bin" (Scheme 2.1). This may have consequences for the host-guest chemistry of these compounds and raises the possibility of their use in drug delivery systems where the pH dependence of complexation can be an important factor in the delivery of drugs to

the appropriate site in the body .[94] Evidence is presented below that the cyclodextrins **30** and **31** most probably do behave in this fashion.

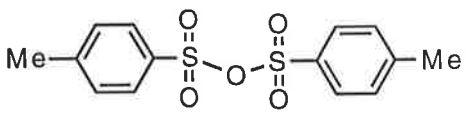
2.2. Synthesis

Although some of the required derivatives have been reported previously it was found that the material produced by the reported methods was not of sufficient purity to carry out further studies and so an alternative procedure was developed.

The synthesis of 6^A-modified cyclodextrins begins with the preparation of the monotosylate derivative **32**, which was prepared by the method of Matsui.[85, 95] Slow addition of one equivalent of 4-methylbenzenesulfonyl chloride to a solution of dry β-cyclodextrin **2** in pyridine at 0 °C gave a mixture of mono- and poly-tosylated cyclodextrins together with unchanged starting material. Pure mono-tosylated product **32** was obtained in 30% yield by repetitive recrystallization from water. At least two recrystallizations were required to remove all of the poly-tosylated cyclodextrin from the mixture and leave less than 5% of unsubstituted cyclodextrin. This material was of sufficient purity for subsequent steps as aminated cyclodextrins can be separated from β-cyclodextrin **2** by ion-exchange chromatography. It is important, however, to remove all of the poly-tosylated material because of the potential difficulties in removing contaminant polyaminated-cyclodextrins from the products of later reactions.



32



33

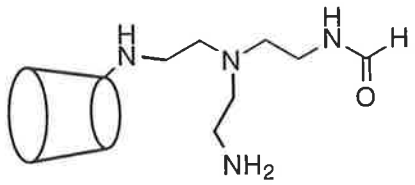
A new method for the preparation of the tosylate **32** from β-cyclodextrin **2** using 4-methylbenzenesulfonic anhydride **33** in aqueous solution has been reported.[96] Although the method reportedly gave good yields of pure tosylate **32**, a single attempt to prepare the anhydride reagent **33** was not successful in my hands. All of the tosylate **32** used in this present work was prepared by the method of Matsui.

Initially, the amination reactions were carried out by heating the tosylate **32** in *N,N*-dimethylformamide (DMF) containing a large excess of the required polyamine. Workup of these reactions involved repetitive precipitation of cyclodextrin containing material from aqueous solutions by dilution with ethanol or acetone to remove the excess amine reagent. In some cases ~~Sohxlet~~ Soxhlet extraction with ether or acetone was necessary in order to remove all of the excess amine. Chromatography using a cation exchange resin was then carried out to separate neutral cyclodextrins from those bearing amino groups. After this process the product cyclodextrins were often still contaminated by residual starting amine.

The preparation of 6^A-(2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrin **28** was used as a starting point for developing an improved method for the synthesis of amino-cyclodextrins.[90] Early preparations of this compound involved stirring a solution of the tosylate **32** with one equivalent of tris(2-aminoethyl)amine in DMF at 70 °C in a lightly stoppered flask. Only one equivalent of tris(2-aminoethyl)amine was used because it was found to be difficult to separate any excess amine from cyclodextrin products. Thin-layer chromatography (TLC) analysis of the dark coloured reaction mixture after 18 hours showed that most of the starting tosylate **32** had disappeared and that a new, low R_f spot was present together with a spot corresponding to β-cyclodextrin **2**. Some hydrolysis of the tosylate **32** to β-cyclodextrin **2** occurred in all of the reactions carried out due to the presence of bound water. This water remains associated with all cyclodextrin derivatives despite extensive drying over phosphorus pentoxide under vacuum. Cyclodextrin compounds were precipitated from the reaction mixture by addition of acetone-ether (3:1) and the collected precipitate was dissolved in water and reprecipitated by addition of ethanol. This step was repeated until there was no evidence of tris(2-aminoethyl)amine in the sample (TLC).

Neutral β-cyclodextrin **2** was separated from the amino-cyclodextrin **28** by passing an aqueous solution of the crude mixture down a column of a weak cation-exchange resin in its protonated form. Neutral compounds passed through the column in the void volume while the amino-cyclodextrin **28** was protonated and formed an ion pair with the carboxylate groups on the resin. The cyclodextrin **28** was eluted with a solution of 1.4 mole dm^{-3} ammonia solution and evaporation of the eluent gave the cyclodextrin **28** as a yellow powder.

BioRex 70 resin (Bio Rad Laboratories) was chosen for this separation because it was thought to be unlikely that cyclodextrins would interact with the poly-acrylate matrix of this resin. There have been reports of yield loss when cyclodextrins have been treated with poly-styrene based Amberlite and Dowex resins.[97] Sephadex (Pharmacia) based resins are also suitable for use with cyclodextrins but they can have additional binding interactions with cyclodextrins unless organic co-solvents are used.[98, 99] This effect has been used previously to separate mixtures of substituted cyclodextrins .[94]



34

The cyclodextrin **28** obtained from the above procedure was highly coloured. ^{1}H and ^{13}C NMR indicated that at least one of the amino groups had been formylated. The ^{1}H NMR spectrum showed a singlet at δ 8.1 corresponding to a formyl proton and the ^{13}C NMR showed a signal at δ 164.1 corresponding to a carbonyl carbon. There were no resonances which could be due to the methyl groups of any residual DMF present in either spectrum. This suggests that the extra signals do not arise from included DMF but are most likely to be due to a trans-acylation reaction between DMF and the cyclodextrin **28** to give the *N*-formyl derivative **34** as the most likely product.

The above sequence was repeated using pyridine as the solvent to avoid this side reaction but the cyclodextrin **28** isolated after the ion-exchange step appeared to be strongly associated with pyridine. Pure cyclodextrin **28** was obtained as a salt after acidification of an aqueous solution of the complex with methanesulfonic acid and precipitation of the cyclodextrin by dilution with acetone.[90] The tris(methanesulfonate) salt thus obtained was further characterised as the tetra-hydrochloride by addition of hydrochloric acid to a solution of the tris(methanesulfonate) followed by precipitation as described above.

While many amines are best stored as their hydrochloride salts, it was believed that the high acidity of the salt might cause some hydrolysis of the acid labile glycosidic linkages in the cyclodextrin over a period of time and this was not desirable. Therefore, as the use of pyridine as the solvent for the amination reaction appeared to require an acidic workup in order to obtain a pure product, as well as involving a number of additional precipitation steps with concurrent losses in yield, other solvents were considered for this reaction.

An ideal solvent for the amination reaction would be one in which the tosylate **32** is readily soluble and is itself highly water soluble, decreasing the tendency to form strong inclusion complexes with cyclodextrins. Polar aprotic solvents such as DMF will favour the S_N2-type reaction but DMF is not completely stable to the reaction conditions. Increasing the substitution on the formyl group might be expected to hinder attack at the carbonyl carbon by amines and so decrease the amount of acylation of the amino-cyclodextrin product. On this basis 1-methyl-pyrrolidin-2-one (NMP) was expected to be a good solvent for the reaction, as it is a highly water soluble dipolar aprotic solvent, that is readily available, and has been shown to be superior to DMF as a solvent in a number of applications.[100] In particular, Henbest and Jackson have shown that nucleophilic substitution of tosylates occurs more readily and in better yield when NMP is used as solvent rather than DMF.[101] In addition, NMP is more stable to acid and base than is DMF and it is also more stable to prolonged heating.[102]

When the tosylate **32** was heated with 3.3 equivalents of tris(2-aminoethyl)amine and 0.1 equivalents of potassium iodide in NMP at 70 °C for 4 hours pure cyclodextrin **28** was obtained in 60% yield following a single precipitation with ethanol and purification through ion-

exchange chromatography as described above. The addition of potassium iodide to the reaction mixture was to generate 6^A-iodo-6^A-deoxy-β-cyclodextrin **35** *in situ* as this had been shown earlier to react with tris(2-aminoethyl)amine more rapidly than did the tosylate 32 in both DMF and pyridine.[90] The cyclodextrin **28** obtained by this procedure was fully characterised by NMR, electrospray-mass spectroscopy and elemental analysis. The product was isolated as the trihydrate and, unlike our first reported synthesis[90], the product was obtained as a clean white powder and showed no evidence of inclusion or other association with solvents other than water.

Table 2.1. Reaction times and yields for the preparation of the 6^Aamino-6^A-deoxy-β-cyclodextrins **21-31**.[a]

βCDX	Time (hr)	Yield (%)	βCDX	Time (hr)	Yield (%)
21	6	55	**27**	7	40
22	4.5	52	**28**	4	57
23	4.5	52	**29**	5	33 (52[b])
24	5	51	**30**	7	34 (50[b])
25	4.5	54	**31**	14	35
26	6	50			

[a] Conditions: 3.3 equivalents of amine and catalytic KI in NMP at 70 °C. [b] Starting amine purified by distillation, no KI catalyst.

A series of substituted cyclodextrins was prepared under the conditions described above (Table 2.1). All of the preparations were reproducible in both yield and purity of the final product. Elemental analysis of each of the products showed that they were all of high purity and that all had been isolated as hydrates, most usually containing three molecules of water. Reactions of primary amines were complete within 4-6 hours and gave yields around 50%. The more hindered secondary amines required longer reaction times (5-14 hours) and gave lower yields (around 30%). In these reactions TLC showed that there was some unreacted tosylate **32** remaining after this time. Extending the reaction time to 18 hours gave no improvement in

yield and darkening of the reaction mixture suggested that some solvent decomposition may have occurred.

The crude product from the reaction of the tosylate **32** and 1,2-diaminoethane contained an unidentified minor compound together with the cyclodextrin **21**. This material did not separate from the cyclodextrin **21** on ion-exchange under the standard conditions. Pure cyclodextrin **21** was obtained only when the crude mixture was loaded onto the ion-exchange resin in its NH_4^+ form and eluted with 0.05 mol dm^{-3} ammonium bicarbonate. Repetitive evaporation of the combined fractions containing the cyclodextrin **21** gave the product as the free diamine as shown by NMR, elemental analysis and potentiometric titration.

Early preparations of the cyclodextrins **29** and **30** were carried out after an *in situ* neutralisation of the hydrochloride salts of the respective amines, 1,4,7-triazacyclononane[103] and 1,5,9-triazacyclododecane,[104] prepared by a modified Richman-Atkins procedure.[105, 106] A stirred suspension of the amine hydrochloride was treated with three equivalents of sodium hydroxide, the resultant solid (NaCl) was filtered off and the filtrate was evaporated to give the free amine, which was then used directly in the reaction with the tosylate **32** under the conditions described above. There is always the possibility that some sodium hydroxide may be introduced into the reaction mixture when the amine is prepared in this fashion and this will cause an increase in the amount of hydrolysis of the tosylate **32** so lowering the yield of the desired product. Therefore, in later preparations 1,4,7-triazacyclononane and 1,5,9-triaza-cyclododecane were purified by treatment of their salts with strong base followed by extraction of the free amine into dichloromethane and distillation at reduced pressure prior to reaction with the tosylate **32**.

When the tosylate **32** was allowed to react with 1,5,9-triazacyclododecane, prepared in this fashion, under the standard conditions the crude cyclodextrin **30**, obtained after precipitation of the reaction mixture by addition of ethanol, gave a bright pink solution when dissolved in water. This colour disappeared on dropwise addition of 10% sodium metabisulfite solution suggesting that the colouration was due to the formation of iodine in the reaction mixture. Repetitive precipitation of the cyclodextrin from aqueous solution by addition of

ethanol and purification by ion-exchange chromatography gave pure cyclodextrin **30** but only in low yield (16%). When this reaction was repeated under exactly the same conditions the work-up was again complicated by the formation of iodine in the reaction mixture. It is not clear how the iodide, added as catalyst, is being oxidised to iodine under these conditions.

The problems caused by the generation of iodine in the reaction between the tosylate **32** and 1,5,9-triazacyclododecane led to an examination of the need for the use of potassium iodide as a catalyst. The preparation of the cyclodextrin **21** was carried out under the standard conditions except that the potassium iodide was omitted from the reaction mixture. The product **21** was obtained in high purity and in the same yield as when potassium iodide was used. There appeared to be no decrease in the rate of reaction when the iodide was omitted. Several other substituted cyclodextrins were prepared under similar conditions, without the use of potassium iodide, and gave highly pure products in yields comparable to those obtained previously in reactions where the iodide had been added.

When the tosylate **32** was allowed to react with either 1,4,7-triazacyclononane or 1,5,9-triazacyclododecane, previously purified by distillation, under these modified conditions, the cyclodextrin **29** or **30** was obtained in a considerably higher yield (~50%) than had been obtained previously, indicating that in the earlier reactions some sodium hydroxide may have been carried through to the reaction mixture and so caused a loss in yield. Thereafter, all preparations of the substituted cyclodextrins, where the parent amine was best stored as the hydrochloride salt, were carried out using amine that was freshly isolated from the salt and distilled just prior to use. In all further preparations of the substituted cyclodextrins sodium iodide was omitted from the reaction mixture.

All of the modified cyclodextrins, with the exception of the cyclodextrins **24** and **30**, were considerably more soluble in water than β-cyclodextrin **2**, giving clear solutions around pH 9 at a concentration of approximately 0.06 mol dm^{-3}. (The solubility of β-cyclodextrin **2** in water is 0.016 mol dm^{-3}.[28]) For the cyclodextrins **24** and **30** solutions of 0.06 mol dm^3 were only obtained at low pH, when the amino groups were fully protonated, or at high pH when either the deprotonation of a secondary hydroxyl group or a salting in effect increased the

solubility of these compounds. Differences in the polarity of these compounds were also observed in their behaviour on TLC. Both of the cyclodextrins **24** and **30** had R_c (the value of R_f for the derivative relative to that of β-cyclodextrin **2**) values of 0.75. In contrast to this, the other linear diamines ran at R_c ~ 0.6 while the other polyamines ran at R_c ~ 0.3. This may suggest that a strong interaction exists between the substituent and the cyclodextrin moiety for the cyclodextrins **24** and **30** limiting the interaction of the amino groups with the polar sites on the silica surface.

2.3. NMR spectroscopy

On the NMR time scale all of the glucopyranose groups of β-cyclodextrin **2** are equivalent, consistent with complete conformational averaging and the ^{1}H and ^{13}C NMR spectra of β-cyclodextrin **2** are relatively simple. In particular, the ^{13}C NMR spectrum of β-cyclodextrin **2** shows only six signals each corresponding to a carbon of a glucose unit. Substitution of the C6 hydroxyl of a single glucopyranose of β-cyclodextrin **2** renders this ring (ring A) and the other glucopyranose groups (rings B-G) inequivalent, and as such they should each exhibit six unique ^{13}C resonances to give a total of forty-two individual signals. In practice, however, the difference in magnetic environment experienced by the carbons in the unsubstituted rings (B-G) is usually too small to resolve these separate signals and only the carbons of the substituted ring (A) can be differentiated. The ^{1}H spectra of these substituted cyclodextrins are usually too poorly resolved to give much information as to structure other than changes to the shift of the resonance due to $H6^{A}$.

The amino-cyclodextrins described above are poly-basic compounds so the ^{1}H and ^{13}C NMR spectra of these compounds are dependent on the pH at which the spectra are recorded. An NMR titration study of the cyclodextrin **25** has been reported.[107] This study was carried out by adding stoichiometric amounts of DCl to the cyclodextrin **25** and recording the 50 MHz ^{13}C NMR spectrum at 30 values of pH over the range 12-2. Changes in the positions of the carbon signals in the spectra recorded at different values of pH were then used to determine the

order of protonation of the amine groups.

The pH-dependence of the NMR spectra of the cyclodextrins **21-31** was examined at pH ≥ 12, pH ~9 and pH ≤ 2. Solutions of these cyclodextrins (0.06 mol dm^{-3}) in D_2O/H_2O have a pH ~ 9. After collecting the spectra of these solutions they were treated with NaOH to give solutions of pH ≥ 12, the spectra were recorded and the samples were then treated with HCl to give solutions of pH ≤ 2. Thus, spectra were recorded for the fully protonated and deprotonated compounds as well as for intermediate species with at least one protonated group.

The resolution of the 300 MHz ^1H NMR spectra of the cyclodextrins **21-31** does not allow much determination of structure. The resonances were assigned on the basis of literature assignments and some limited ^1H-^{13}C correlation spectroscopy. At pH ≥ 12 and pH 9, the two diastereotopic protons $H6^A$ (attached to the substituted carbon) often give separate resonances in the region δ 2.8-3.2, an apparent doublet (J ~ 12 Hz) due to geminal coupling, and a doublet of doublets (geminal coupling and coupling to $H5^A$) around 0.2 ppm further upfield which is not clearly resolved from the resonances of the methylene protons adjacent to the amino functionality of the substituent polyamine in most cases. An apparent triplet at around δ 3.1 (J ~ 9 Hz) due to the proton $H4^A$ is observed at pH ≥ 12 and this resonance was shifted to around δ 3.4 at pH 9. At pH ≤ 2 the resonances of the protons $H6^A$ and the aminomethylene protons on the substituent often merge together and the resonance of the proton $H4^A$ merges with those of the protons H2 and H4 of the unsubstituted rings while an apparent triplet (J ~ 9 Hz) due to the proton $H5^A$ appears at around δ 5.1. Interestingly, for the cyclodextrin **30** the resonance of the proton $H5^A$ is clearly resolved from the rest of the protons H5 at each pH. If the shift of the resonance of $H5^A$ at low pH for all of the substituted cyclodextrins is due to the build-up of positive charge on the nitrogens of the substituent, then this anomalous behaviour of the proton $H5^A$ of the cyclodextrin **30** may suggest that there is a strong hydrogen bonding interaction between the nitrogens of the substituent and the cyclodextrin moiety at high pH. This will result in an increase in the relative positive charge on the nitrogens and a resulting downfield shift of the resonance of the proton $H5^A$.

The 75.5 MHz ^{13}C NMR spectra of the substituted cyclodextrins **21-31** are more

informative about the structures of the modified cyclodextrins and the interactions between the substituents and the cyclodextrin than are the ^{1}H NMR spectra. For all of the substituted cyclodextrins, the resonances of the carbons $C1^{A}$, $C4^{A}$, $C5^{A}$ and $C6^{A}$ are often well separated from those of the carbons of the unsubstituted glucopyranose residues. In particular, the resonance of the carbon $C6^{A}$ is observed at around δ 50 ppm (about 10 ppm upfield from the resonances of the other carbons C6) as expected for a methylene attached to nitrogen of a secondary amine. The resonance of the carbon $C5^{A}$ is shifted about 2-5 ppm upfield from the rest of the carbon C5 resonances depending on the pH at which the spectrum was recorded. Protonation of an amine group causes upfield shifts of the resonances of the β-carbons.[107, 108] The resonance of the carbon $C4^{A}$ is shifted about 3 ppm downfield from those of the other carbons C4 while the resonance of the carbon $C1^{A}$ is shifted about 1 ppm upfield from those of the rest of the carbons C1.

The pH dependent changes in the ^{13}C NMR spectra of the cyclodextrins **21**-**28**, where the substituent is a linear polyamine, show no systematic variation. However, there is a general trend for the spectra to show more resolution of the resonances of the carbons of the unsubstituted glucose units at pH $\geq$ 12 and pH $\leq$ 2 than at pH 9 . This may indicate that the substituents are held in specific conformations with respect to the cyclodextrin moiety when either fully protonated or fully deprotonated and that the system is more flexible when in a partially protonated state.

In contrast, the ^{13}C NMR spectra of cyclodextrins bearing cyclic polyamino substituents show a great increase in the resolution of the resonances of the carbons of the unsubstituted glucose units as the pH is increased (Figures 2.2-2.3). This is consistent with the highly charged substituent moving away from the cyclodextrin annulus so that it has little or no interaction with the primary hydroxyl groups of the cyclodextrin moiety at low pH and thus causes little differentiation of the unsubstituted glucopyranose units of the cyclodextrin. Hydrogen bonding interactions between the primary hydroxyl groups and the substituent increase as the substituent becomes more deprotonated and less charged as the pH is increased. Thus, at high pH the substituent is firmly bound across the primary face of the cyclodextrin

annulus and so gives maximum differentiation of the unsubstituted glucopyranose units of the cyclodextrin. These results indicate that cyclodextrins bearing cyclic polyamine groups do act as "molecular peddle-bins" as shown in Scheme 2.1.

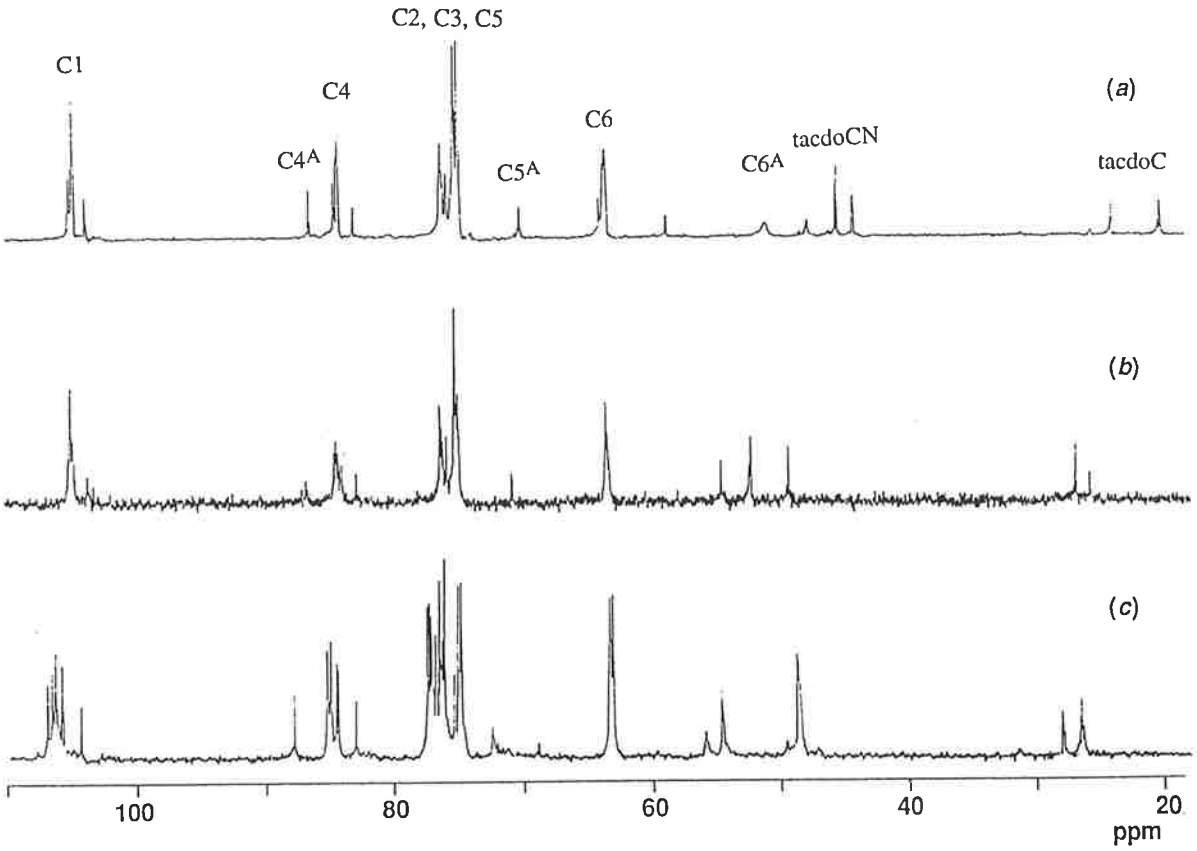


Figure 2.2. 75.5 MHz ^{13}C NMR spectra of the cyclodextrin **30** in D_2O at a concentration of 0.06 mol dm^{-3} at (a) pH ≤ 2, (b) pH ~ 9 and (c) pH ≥ 12.

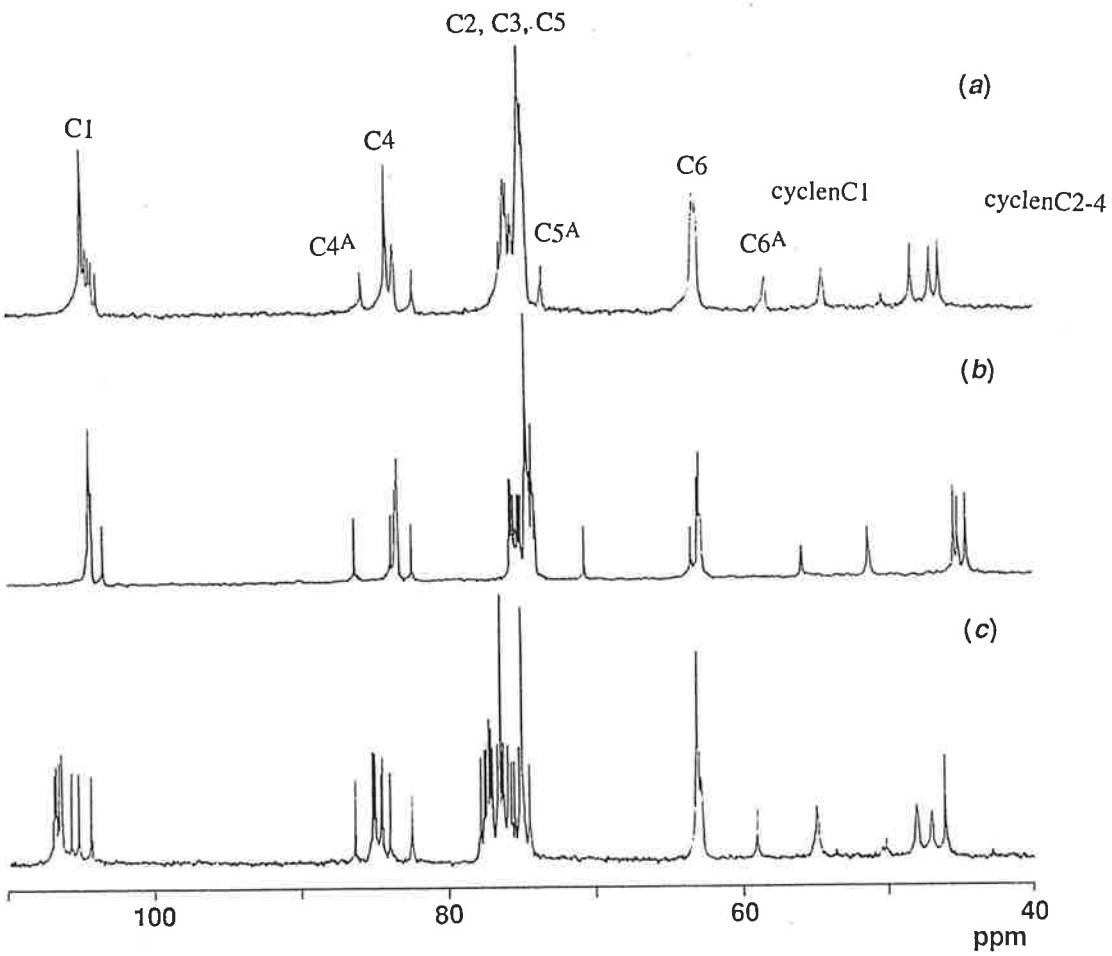


Figure 2.3. 75.5 MHz ^{13}C NMR spectra of the cyclodextrin **31** in D_2O at a concentration of 0.06 mol dm^{-3} at (a) pH ≤ 2, (b) pH ~ 9 and (c) pH ≥ 12.

2.4. pK_a Variation

The values for the pK_as of the protonated cyclodextrins **21-31**, determined by potentiometric titration, are given in Table 2.2 and the pK_as of the corresponding protonated free polyamines are included for comparison. In general, the amino nitrogens of the substituent of the cyclodextrin are less basic than those of the free amine.

Table 2.2. pK_as for some protonated 6^A-substituted-β-cyclodextrins and the corresponding protonated free polyamines in aqueous $NaClO_4$ (I = 0.10 mol dm^{-3}) at 298.2 K.

Cyclodextrin	pK_a	Free Amine	pK_a
21	9.42 5.70	1,2-diaminoethane	9.97 7.16
22	9.90 7.39	1,3-diaminopropane	10.56 8.97
23	10.26 8.06	1,4-diaminobutane	10.91 9.49
24	10.27 8.72	1,6-diaminohexane	11.01 10.04
25	9.52 7.63 3.88	2-(2-aminoethyl)aminoethylamine	9.78 8.99 4.32
26	10.06 8.44 6.72	3-(3-aminopropyl)aminopropylamine	10.56 9.44 7.54
27	9.33 8.22 5.61 3.1	2-(2-(2-aminoethyl)aminoethyl)aminoethylamine	9.83 8.93 5.40 3.0
28[a]	9.85 8.99 6.89 2.6	tris(2-aminoethyl)amine[b]	10.14 9.43 8.41
29	10.0 5.89 2.4	1,4,7-triazacyclononane	10.69 7.01
30	11.24 5.85 2.8	1,5,9-triazacyclododecane[c]	12.60 7.57 2.41
31	10.40 8.62	1,4,7,10-tetraazacyclododecane[d]	10.6 9.6

[a] Ref. 90. [b] Ref. 109. [c] Ref. 110. [d] Ref. 111.

Generally, *N*-alkylation of a polyamine system yields a product which is more basic than the starting polyamine. For example the pK_as of 2-(methylamino)ethylamine are 10.21

and 7.27 while for 1,2-diaminoethane (en) the pK_as are 9.97 and 7.16.[109] The observed decrease in basicity on *N*-alkylation of a polyamine by a cyclodextrin may be due to one or more of the following factors: a decrease in the ability of water to solvate the protonated species adjacent to the hydrophobic cavity of the cyclodextrin; electronic and steric effects due to attachment of a large oxygen-rich entity to the polyamine;and involvement of the nitrogen lone pair electrons in hydrogen bonding to the remaining hydroxyl groups on $C6^{B-G}$. Some evidence that such hydrogen bonding does exist has been presented above.

A calorimetric study of the protonation of the cyclodextrin **25** has shown that for each protonation of the cyclodextrin **25** there is a more favourable entropic contribution to $\Delta G°_{protonation}$ than for the corresponding protonation of 2-(2-aminoethyl)aminoethylamine while the reverse is true for the enthalpic contributions.[107] This suggests that protonation of the nitrogens allows a greater increase in the degrees of freedom of the chain in the cyclodextrin **25** than in 2-(2-aminoethyl)aminoethylamine and that this is due to the breaking of hydrogen bonds in the cyclodextrin **25**.

The variation of the pK_as across the series of cyclodextrins **21-31** parallels that of the parent polyamines. For example, for the ω-aminoalkylamino-substituted cyclodextrins **21-24** the two pK_as increase as the chain length increases while the difference between the two pK_as is decreased, and a similar tend is observed for the free diamine analogues.

The increase in the pK_a magnitude as the chain length of the substituent increases coincides with the increasing hydrophobicity of the substituents and a consequent lessening of the ability to lose a proton to the surrounding water as overall hydration is decreased. The effect of the number of methylenes in the chain decreases after a certain length.[112] While there is a large difference in the pK_as for the first protonation of the cyclodextrins **21** and **22** there is little difference between the first pK_as of the cyclodextrins **23** and **24**. The decrease in the difference between pK_{a1} and pK_{a2} as the chain-length increases is due to the increase in charge separation in the di-protonated species leading to a decreased electrostatic repulsion between the ammonium groups.

Each nitrogen of a linear polyamine substituent of a substituted cyclodextrin is unique while the corresponding free amines are symmetric and contain pairs of equivalent nitrogens. Thus, there is a question as to the order of protonation of a substituted cyclodextrin. It is generally accepted that secondary amines are more basic (pK_a ~11) than primary and tertiary amines (pK_a ~10-11)[113] however, there is evidence that in polyamine systems the first protonation involves all of the nitrogens and that the position of subsequent protonations is controlled mainly by charge separation effects.[114, 115] The involvement of all nitrogens in the first protonation of polyamines has been disputed on the basis of ^1H and ^{13}C NMR evidence that suggested that protonation of these systems involved only the terminal primary amino nitrogens.[116] More recently, a series of amine shift parameters has been developed for predicting ^{13}C chemical shift changes with the order of protonation, and this suggests that all nitrogens are involved in the first protonation of 2-(2-aminoethyl)aminoethylamine.[117]

A full study of the pH dependence of the ^{13}C NMR spectra of the cyclodextrin **25** concluded that the protonation order is terminal nitrogen followed by nitrogen bound to $C6^A$ of the cyclodextrin (with a partial overlap of the first and second protonations) and no involvement of the central nitrogen until the addition of the final proton.[107] However, such a conclusion does not fit all of the reported data. Protonation of an amine causes an upfield shift of the resonances of the β-carbons[108] and such a shift was observed for $C5^A$ after the first addition of acid to a solution of the cyclodextrin **25**. The resonance of the proton $H5^A$ is shifted downfield at the same time. These observations support the sharing of the first additional proton between the terminal nitrogens of the substituent and do not rule out involvement of the central nitrogen in the mono-protonated species. Further evidence in support of the sharing of the first protonation between the terminal nitrogens of the substituted cyclodextrins **21-24** is presented in the next chapter.

2.5. Inclusion phenomena

The presence of the substituent on the cyclodextrins **21-31** may affect the binding of a guest molecule within the annulus of the cyclodextrin moiety in a number of ways: (1) as the hydrophobicity of the substituent increases, the stability of complexes with hydrophobic guests may increase; (2) the protonation of one or more amino groups on the substituent may increase the stability of complexes with negatively charged guests through the formation of ion pair interactions; (3) interactions between the nitrogen groups on the substituent and the C6 hydroxyl groups may act to block the primary face of the annulus preventing exit of the guest and so increasing the stability of complexes (particularly for cyclic substituents); and (4) the substituent may be included within the annulus of the cyclodextrin moiety and limit the entry of guest molecules thus decreasing the stability of complexes (particularly for linear diamino substituents).

2.5.1. Self-inclusion of the substituent

There are a number of reports of substituted cyclodextrins where the substituent is included within annulus of the cyclodextrin moiety.[118 -126] Such "self-inclusion" has been utilised in molecular signalling devices. Most of these systems involve substituents bearing aromatic groups which include within the annulus, giving rise to a characteristic spectral phenomenon such as fluorescence. In the presence of an added guest molecule, able to bind within the annulus, the substituent is pushed out of the annulus with resultant quenching of the fluorescence by solvent, thus signalling the complexation of the added guest. Self-inclusion is often inferred by such changes but in one study self-inclusion of the aromatic substituent is confirmed through 2D-ROESY spectroscopy.[126]

For the substituted cyclodextrins **21-31** described above, it is most likely that the linear diamino substituents, and the 6-aminohexyl substituent in particular, would undergo self-inclusion. The reported crystal structure of the 6-aminohexylamino-cyclodextrin **24** shows that individual molecules come together to form polymer-like columns such that the 6-aminohexyl

chain enters the cavity of an adjacent cyclodextrin moiety in the column from the secondary side (Figure 2.4).[127] The likelihood of the self-inclusion of this substituent in the annulus in the solution phase led to the cyclodextrin **24** being chosen for a 2D-NMR study of the solution structures of these modified cyclodextrins and their inclusion complexes with several guests.

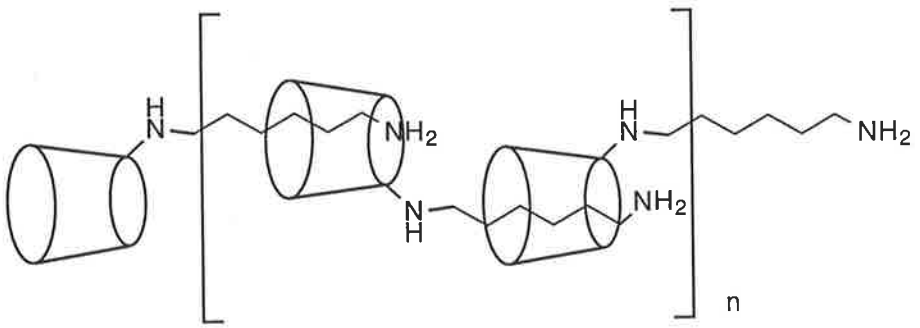
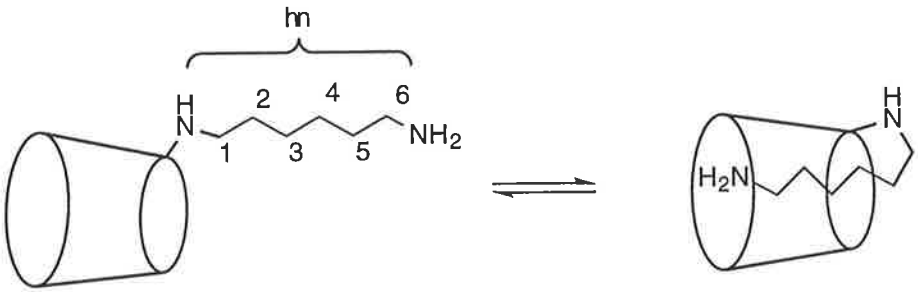


Figure 2.4. Schematic representation of the columnar structures formed in the crystals of the cyclodextrin **24**. From Ref. 127.

Recently, 2D-ROESY has been shown to be a powerful tool for examining the solution structure of host-guest complexes of cyclodextrins.[128 -132] When a guest (or a part of a guest) is included in the annulus of a cyclodextrin the attached protons may have through-space (nuclear Overhauser effect, NOE) interactions with the protons H3 and H5 of the cyclodextrin host which are located within the annulus. In the 2D-ROESY experiment these interactions produce cross-peaks between the resonances of the interacting protons. Some indication of the orientation and/or depth of inclusion of a guest within the annulus of a host cyclodextrin can be gained from the relative intensities of these cross-peaks.

The 600 MHz 2D ROESY spectrum of the cyclodextrin **24** (0.06 mol dm^{-3}) in D_2O at pH ≥ 12 was run using standard Varian software with a mixing time of 0.3 seconds (Figure 2.5). Strong cross-peaks were observed between the resonances of the methylene protons of the substituent hnH1-hnH6 and the annular protons H3 and H5 indicating that under these conditions the substituent is included within the annulus. The protons hnH3, hnH4 and hnH5 show equally strong NOE interactions with the annular protons H3 and H5 while the protons hnH2 show a weaker NOE interaction with the protons H3 than with the protons H5. The

protons hnH6 also show strong NOE interactions with the protons H3 and H5 but the protons hnH1 show an NOE interaction with the protons H5 only (and are alone in interacting with the proton $H5^A$). This spectrum represents a time averaged view of the solution structure of the cyclodextrin **24** at pH ≥ 12. The methylene groups of the 6-aminohexyl substituent are included in the annulus and become more mobile the further they are from the point of attachment to the cyclodextrin (Scheme 2.2).



24

Scheme 2.2. Schematic representation of the self-inclusion of the substituent of the cyclodextrin **24** in aqueous solution at pH ≥ 12.

In contrast to the picture at pH ≥ 12, the 2D-ROESY spectra of the cyclodextrin **24** recorded at pH 9 and pH ≤ 2 showed no evidence of inclusion of the 6-aminohexyl substituent within the annulus. Protonation of the amino nitrogens precludes their entry into the annulus of the cyclodextrin moiety.

The substituents of the cyclodextrins **25-28** were not expected to be included within the annulus of the cyclodextrin moiety as the extra nitrogens within the chain of these substituents should decrease the overall hydrophobicity of the substituent relative to that of the ω-aminoalkylamino substituents and so increase their solvation by water through additional hydrogen bonding interactions.

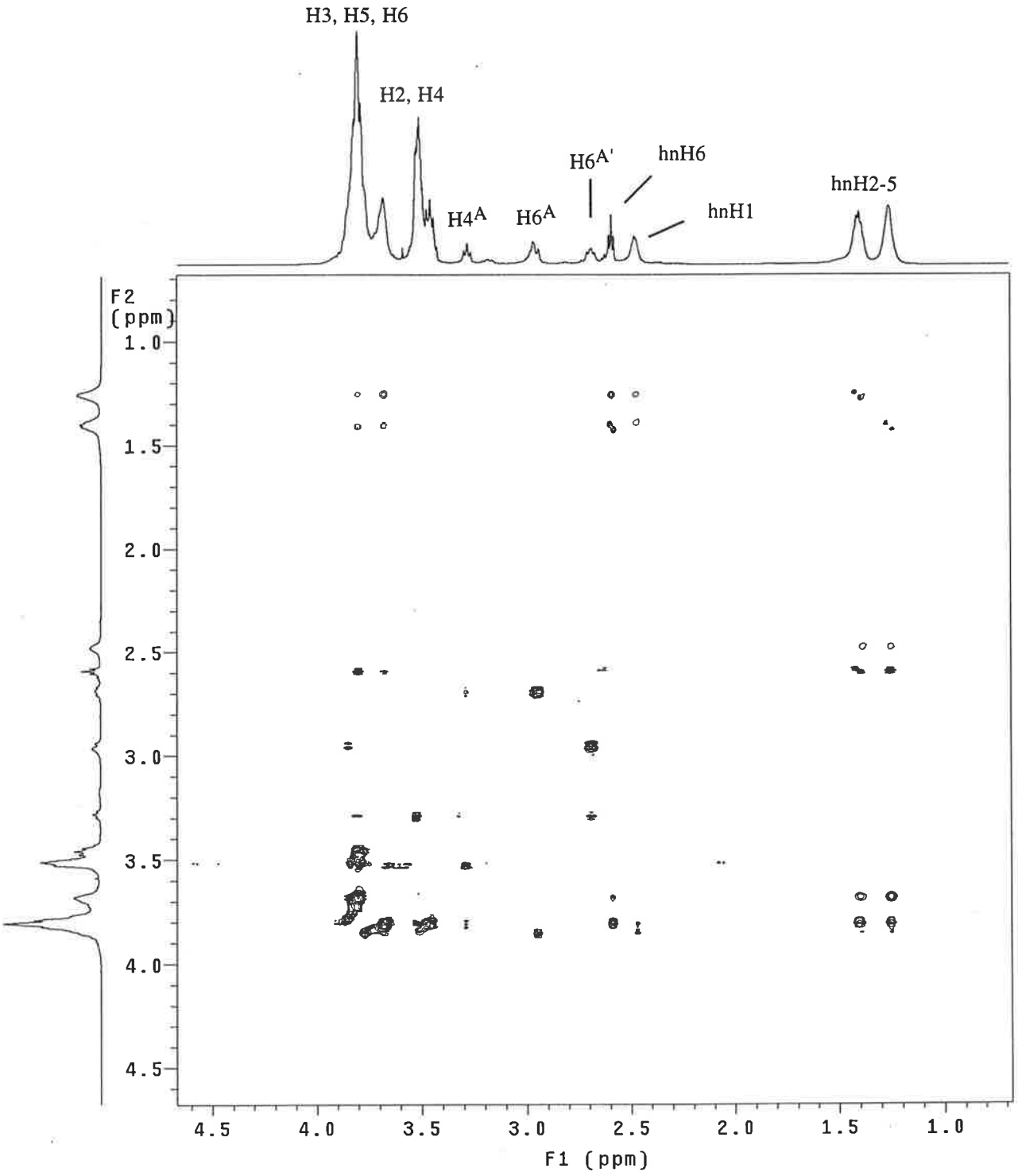


Figure 2.5. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} of the cyclodextrin **24**. The protons are labelled as shown in Scheme 2.2.

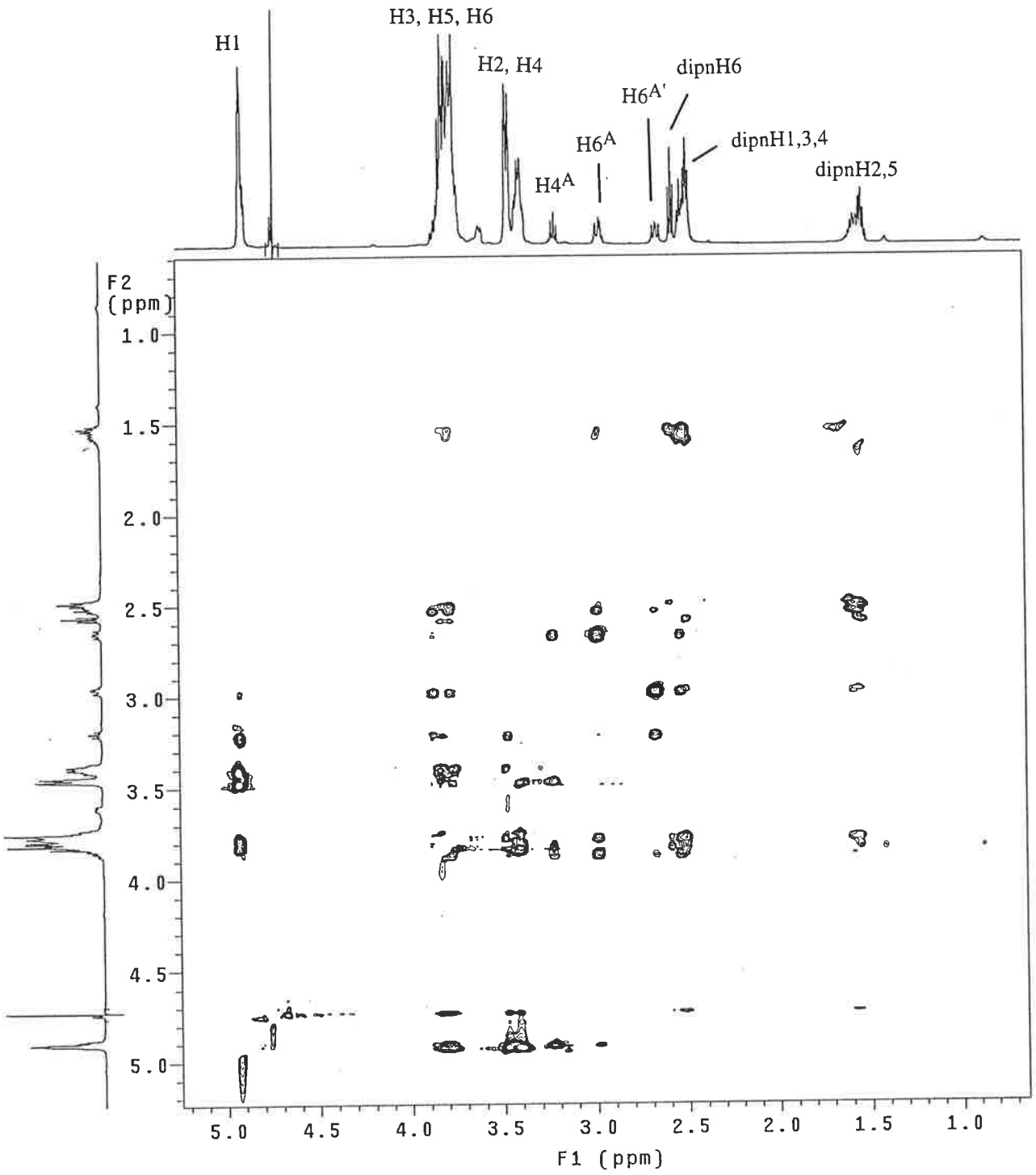
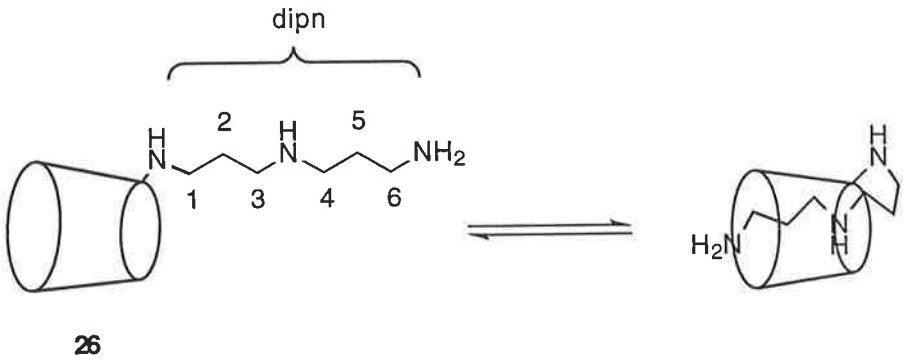


Figure 2.6. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~$\times 10^{-3}$~~ mol dm^{-3} of the cyclodextrin **26**. The protons are labelled as shown in Scheme 2.3.

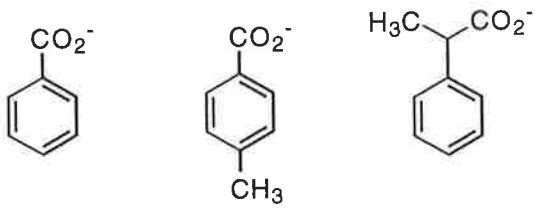
The 2D-ROESY spectrum of the cyclodextrin **25** at pH ≥ 12 shows no NOE interactions between the protons of the substituent and the annular protons H3 and H5, indicating that this substituent is not included within the annulus of the cyclodextrin. However, the 2D-ROESY spectrum of the cyclodextrin **26** at pH ≥ 12 does indicate self-inclusion of the substituent. Strong cross-peaks are observed between the resonances of the annular protons H3 and H5 and those of the protons of the substituent (Figure 2.6). The increased hydrophobicity of the substituent of the cyclodextrin **26**, over that of the cyclodextrin **25**, has decreased the ability of water to solvate this substituent relative to the increase in the hydrophobic interactions within the cyclodextrin annulus.



26

Scheme 2.3. Schematic representation of the self-inclusion of the substituent of the cyclodextrin **26** in aqueous solution at pH ≥ 12.

2.5.2. Host-guest complexes



36 **37** **38**

As part of a continuing systematic study of the properties of modified cyclodextrins, a series of potentiometric titrations was carried out to determine the formation constants of host-

guest complexes formed between the cyclodextrins **21-31** and benzoate **36**, 4-methylbenzoate **37** and (*R*)- and (*S*)-2-phenylpropionate **38**.[88, 133, 134] The formation constants obtained for the complexes formed between the unprotonated cyclodextrins **21-31** and the guests **36-38** are given in Table 2.3.

Table 2.3. Formation constants for host-guest complexes formed between the cyclodextrins **21-31** and the carboxylates **36-38** determined by potentiometric titration.[a]

	K dm^3 mol^{-1}			
Cyclodextrin	**36**	**37**	(*R*)-**38**	(*S*)-**38**
2	60[b]	110[b]	63[b]	52[b]
21	*c*	*c*	*c*	*c*
22	915	885	760	465
23	950	535	630	630
24	1000	750	1150	1760
25	*c*	*c*	*c*	*c*
26	300	1050	1900	3400
27	*c*	*c*	*c*	*c*
28	340	930	1740	1600
29	2500	1250	2700	1400
30	24000	12300	13500	9650
31	15000	6000	41000	44000

[a] Determined in aqueous solution at I = 0.10 mol dm^{-3} ($NaClO_4$) and 298.2 K. [b] Ref 88.
[c] No complex detected.

For the three cyclodextrins **21**, **25** and **27** no complexes were detected by this method. This does not mean that the complexes do not exist in solution but rather, that they are not reliably detected by the potentiometric method used in this study. Complexes that are present at ≤ 5% of the total concentration of the cyclodextrin host are eliminated from the fitting protocol and are listed as not detected.

In the cases where complexes between the host cyclodextrin and the carboxylates **36-38** were detected, several general trends can be observed. Substitution of a C6 hydroxyl group by an aminoalkyl chain substantially increases the stability of the complex formed compared to that of the complex formed between the same guest and β-cyclodextrin **2**. The complexes formed with cyclodextrins bearing a cyclic polyamine substituent are considerably more stable than those formed when the substituent is a linear polyamine. This may be attributed to capping of the primary face of the cyclodextrin by the cyclic substituent, or to competition between the guest and a linear substituent for binding within the annulus due to self-inclusion of the substituent, or a combination of both effects.

For the aminoalkylamino-substituted cyclodextrins **22**, **23** and **24** there are no great differences in the stabilities of the complexes formed with any of the guests **36-38**. The complexes formed with benzoate **36** are slightly more stable than those formed with the more bulky 4-methylbenzoate **37** but there is no real selectivity for any guest by any host. A slight selectivity can be seen in the stability of the diastereomeric complexes formed between these chiral hosts and the chiral phenylpropionate **38**. No selectivity is observed in complex formation with the cyclodextrin ~~2.3~~ **23** but with the 3-aminopropylamino-cyclodextrin **22** as host there is a small selectivity (1.6:1) for the (*R*)-isomer while the 6-aminohexylamino-cyclodextrin **24** shows a reversed selectivity (but of the same order).

The complexes formed with cyclodextrins bearing a cyclic substituent show much greater selectivity between the hosts and guests. For all the guests **36-38** the complexes formed with the cyclodextrin **29** are considerably less stable than those formed with the cyclodextrins **30** and **31**. However, the cyclodextrin **29** shows the greatest selectivity (2:1) in the complexes formed with the enantiomers of 2-phenylpropionate **38**.

The solution structures of several of these host-guest complexes were studied by 2D-ROESY NMR. Solutions were prepared at pH ≥ 12 with the host and guest each at a concentration of 0.06 mol dm^{-3}. Under these conditions all of the guests will be deprotonated and all of the amino groups on the hosts will be unprotonated. In addition, it is likely that one of the secondary hydroxyl groups of the cyclodextrin moiety will be deprotonated (pK_a ~ 12[17]).

These conditions are necessary to avoid complications in analysing the 2D spectra due to the presence of more than one type of species in solution.

The 2D-ROESY spectrum of a solution containing a 1:1 mixture of the cyclodextrin **24** and 4-methylbenzoate **37** (Figure 2.7) shows that both the aryl ring of the guest **37** and the 6-aminohexyl arm of the cyclodextrin host **24** are included in the annulus of the cyclodextrin moiety. The 1D spectrum of this solution shows that the resonances of the methylene protons hnH2-hnH5 are more clearly resolved and are shifted relative to the resonances of these protons for the cyclodextrin **24** alone. Cross-peaks between these resonances and those of the annular protons H3 and H5 indicate the inclusion of the 6-aminohexyl group within the annulus. Cross-peaks between the resonances of the protons H_o and H_m of the guest **37** and those of the annular protons H3 and H5 show that the aryl group of the guest **37** is included in the cyclodextrin annulus. The benzylic methyl protons also show some NOE interactions with the annular protons H3 and H5. There are no NOE interactions between the protons of the guest **37** and those of the 6-aminohexyl substituent of the host **24** indicating that these protons must be at least 4 Å apart from each other.

Changes to the NMR spectral resolution and chemical shift of the methylene protons hnH2-hnH5 on complexation of the guest **37** by the cyclodextrin **24** are consistent with the methylene chain lying inside the annulus parallel to the face of the aromatic ring of the included guest. The 2D spectrum does not give a clear indication of the orientation of the guest **37** within the annulus. That the protons H_o and H_m appear to interact equally with the annular protons H3 and H5 suggests that both head-first and tail-first inclusion may occur under these conditions as indicated in Scheme 2.4.

Gas-phase modelling of the complex formed between β-cyclodextrin **2** and 4-methylbenzoate **37** showed that "head-first" inclusion (carboxylate group positioned towards the primary face of the cyclodextrin) was favoured over "tail-first" inclusion (carboxylate group positioned towards the secondary face of the cyclodextrin). The most stable orientation corresponded to the formation of the maximum number of intramolecular hydrogen bonds and an anti-parallel alignment of the dipole moments of the host **2** and the guest **37**.[135]

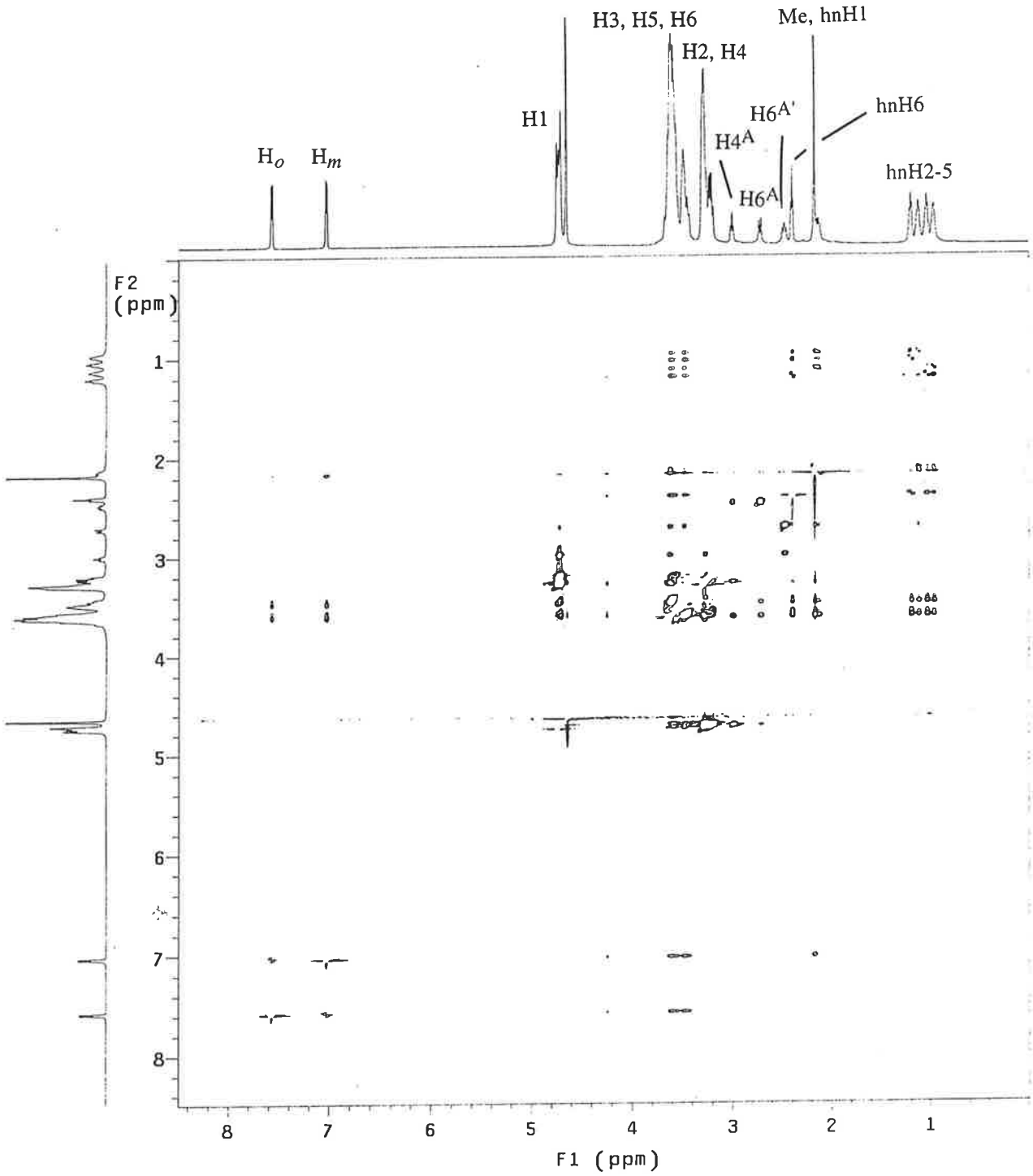
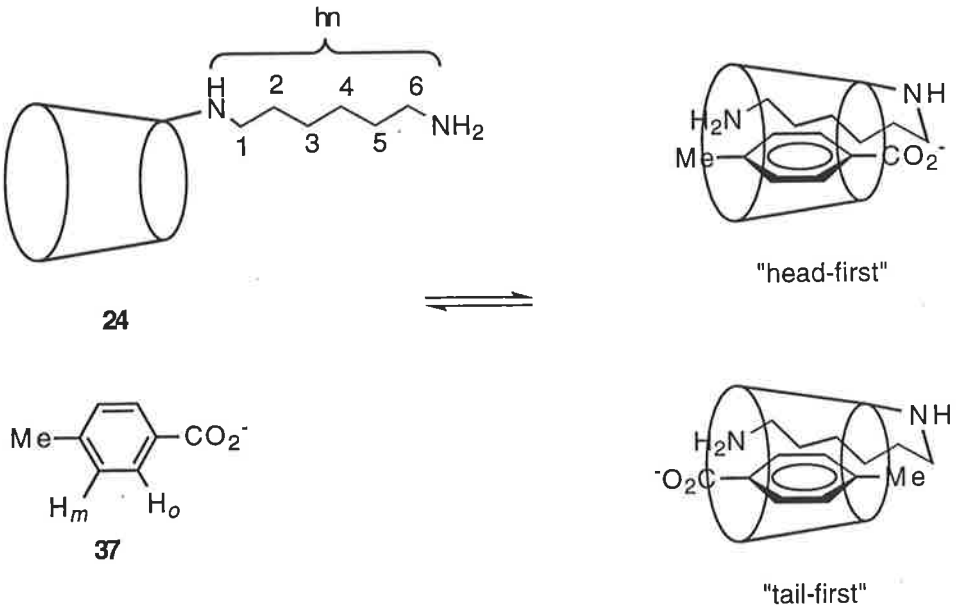
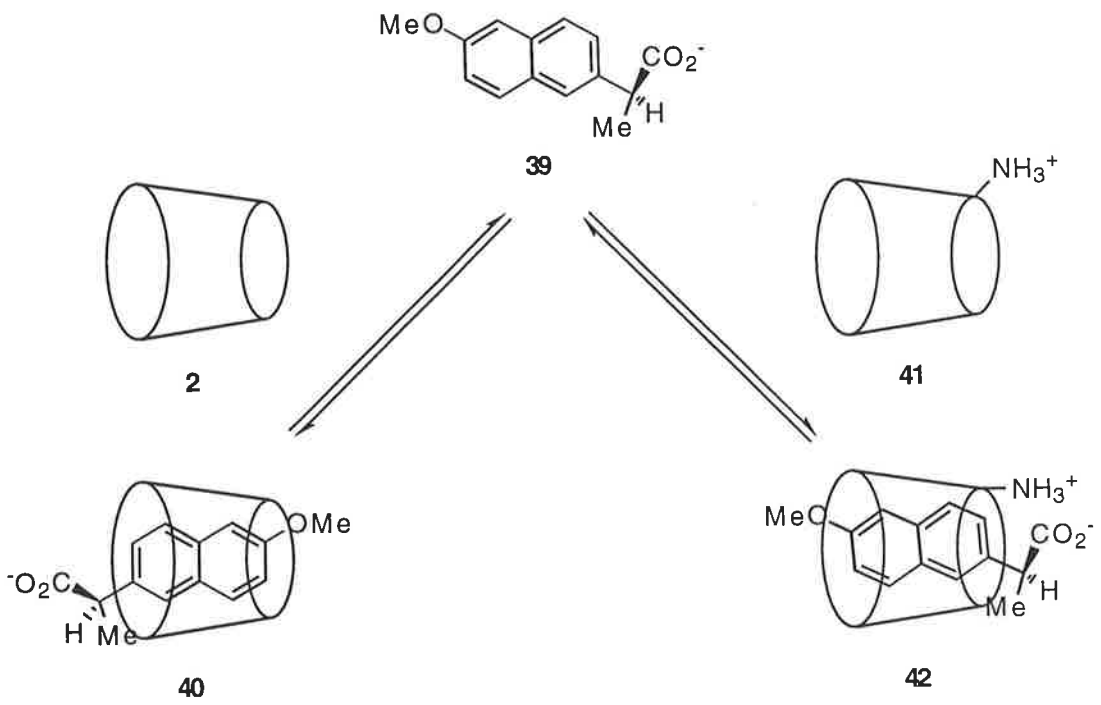


Figure 2.7. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} each of the cyclodextrin **24** and 4-methylbenzoate **37**. The protons are labelled as shown in Scheme 2.4.



Scheme 2.4. Schematic representation of the inclusion of both the 6-aminohexyl substituent and the guest **37** in the cyclodextrin **24** in aqueous solution at pH ≥ 12 indicating the two possible modes of inclusion of the guest **37.**

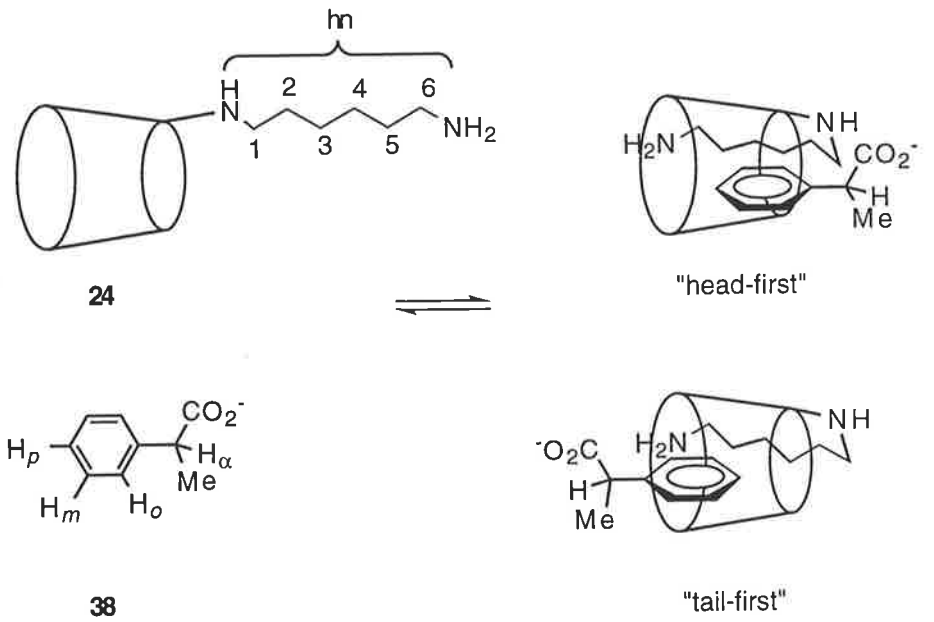


Scheme 2.5. Inclusion modes for Naproxen **39** in β-cyclodextrin **2** and 6^{A}-deoxy-6^{A}-amino-β-cyclodextrin **41** at pH 6.8 determined by 500 MHz 2D-ROESY NMR spectroscopy. From Ref. 132.

A 2D-ROESY spectroscopy study has shown that while Naproxen **39** forms a "tail-

first" complex **40** with β-cyclodextrin **2** at pH 6.8, the complex formed when Naproxen **39** is added to a solution of the amino-cyclodextrin **41** proceeds with "head-first" inclusion to give the complex **42** due to ion-pairing interactions between the ammonium group and the carboxylate group (Scheme 2.5).[132]

Under the conditions used above to study the complexes formed by the cyclodextrin **24**, no ion-pairing interactions are possible between the negatively charged guest **37** and the unprotonated, neutral host **24**. However, hydrogen bonding interactions between the carboxylate anion and either the terminal (primary) amino hydrogens or the secondary amino hydrogens may occur. The nature of the inclusion complexes formed under the conditions used to obtain the 2D-ROESY spectra will be dependent on a complex interplay between hydrogen bonding, solvation, hydrophobic and steric effects due to the self-inclusion of the aminohexyl substituent, and repulsive forces due to the deprotonation of a hydroxyl group on the secondary face of the cyclodextrin moiety.



Scheme 2.6 Schematic representation of the inclusion of the 6-aminohexyl substituent and the 2-phenylpropionate **38** in the cyclodextrin **24** in aqueous solution at pH ≥ 12 indicating the two possible modes of inclusion of the guest **38**.

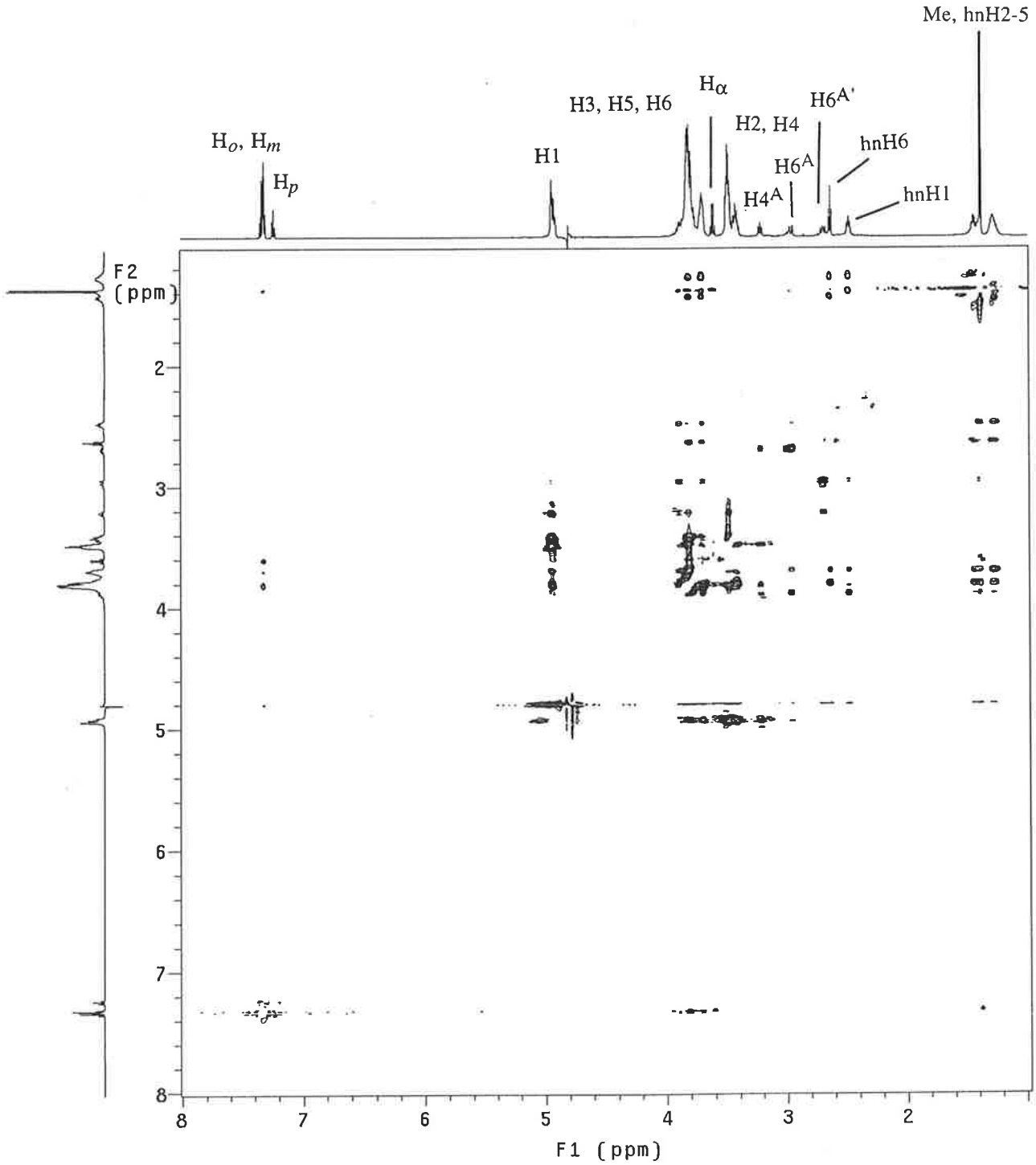


Figure 2.8. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} each of the cyclodextrin **24** and (*S*)-2-phenylpropionate **38**. The protons are labelled as shown in Scheme 2.6.

The 2D-ROESY spectrum of the complex formed between the cyclodextrin **24** and the guest (*S*)-**38** show cross-peaks indicating that both the 6-aminohexyl substituent and the guest (*S*)-**38** are simultaneously included within the annulus. Cross-peaks are observed between the resonances of the protons hnH2-5 of the 6-aminohexyl arm and the annular protons H3 and H5, and between the resonances of the protons H_o or H_m of the guest **38** and the annular protons H3. There are no NOE interactions between the methine proton H_α or the α-methyl protons and the annular protons H3 and H5, suggesting that the "tail-first" mode of inclusion is dominant under these conditions. The lack of NOE interactions between the protons of the guest (*S*)-**38** and the annular protons H5 suggests that there is only shallow inclusion of the aryl ring of the guest **38** into the cyclodextrin annulus. The 2D-ROESY spectrum obtained of a solution containing the cyclodextrin **24** and the guest (*R*)-**38** is identical to that obtained above. Shallow inclusion of the guest **38** into the annulus such that the chiral centre of the guest **38** is held away from the chiral environment of the cyclodextrin leads to a lack of spectroscopic (and thermodynamic) discrimination between the two diastereomeric complexes formed between the chiral host **24** and the enantiomers of the guest **38**.

While it was not possible to detect the formation of a complex between the cyclodextrin **25** and 4-methylbenzoate **37** under the conditions used for potentiometric titration, the 2D-ROESY spectrum of a solution containing both components at pH ≥ 12 shows that such a complex does exist under the conditions used for the NMR studies (Figure 2.9). There are strong NOE interactions between the aryl protons H_o and H_m and the annular protons H3 and H5. The methyl group also shows NOE interactions with the annular protons H3 and H5. The relative intensities of the cross-peaks due to these interactions suggest that "head-first" inclusion is the predominant inclusion mode in this system.

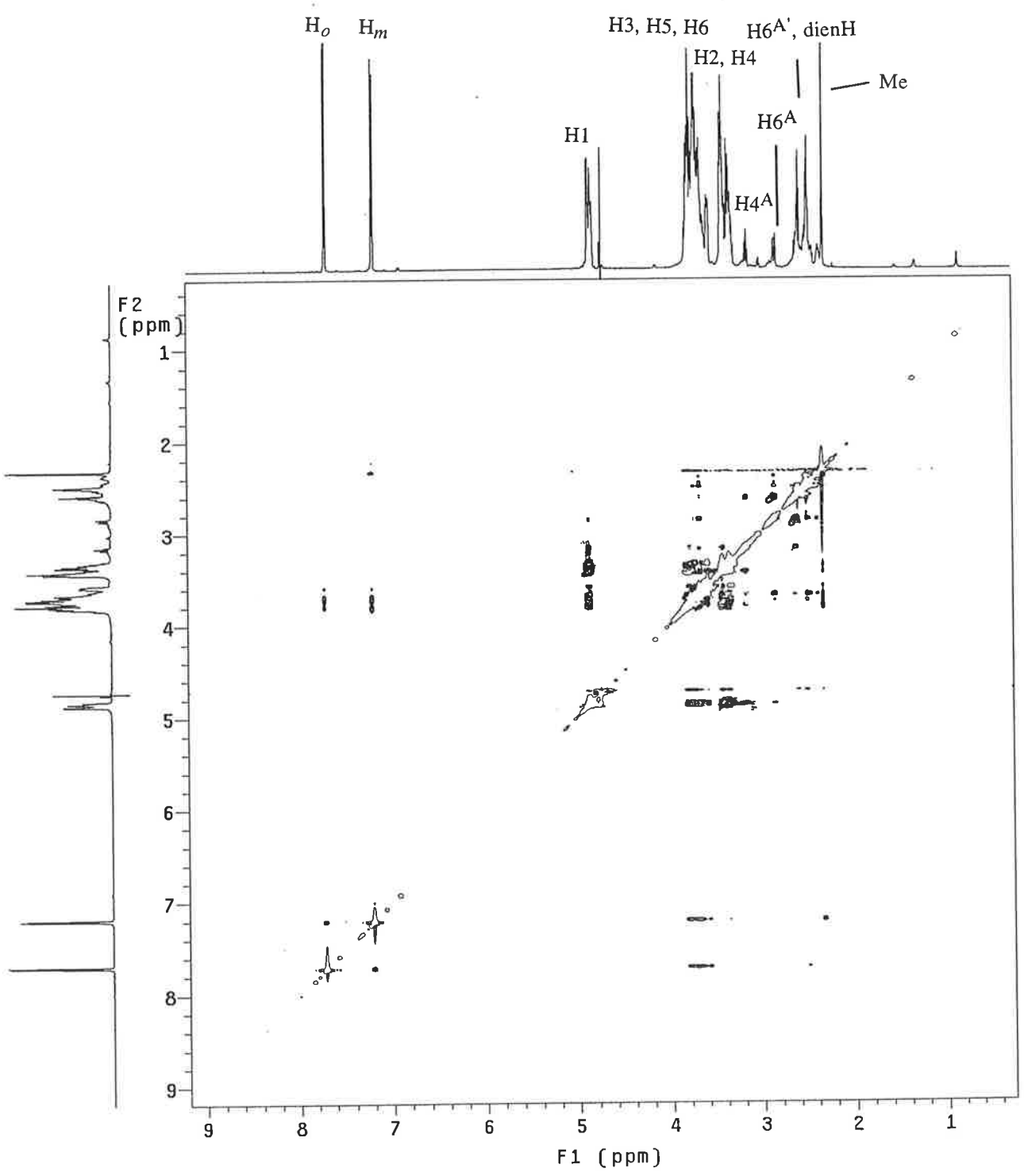
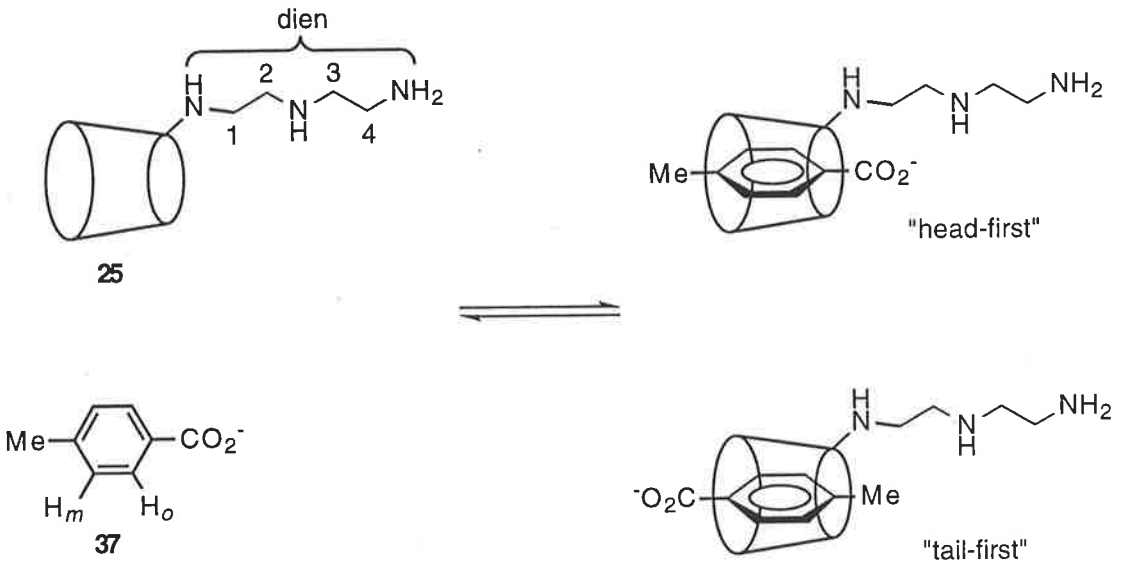


Figure 2.9. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} of the cyclodextrin **25** and 4-methylbenzoate **37**. The protons are labelled as shown in Scheme 2.7.



Scheme 2.7. Schematic representation of the inclusion of 4-methylbenzoate **37** in the cyclodextrin **25** in aqueous solution at pH ≥ 12 indicating the two possible modes of inclusion of the guest **37**.

The 1D ^{1}H NMR spectrum of a solution containing both the cyclodextrin **26** and 4-methylbenzoate **37** at pH ≥ 12 shows an increase in the resolution of the resonances of the aminomethylene protons relative to that observed for a solution of the cyclodextrin **26** alone, where only the signal due to the protons dipnH6 is well resolved. This suggests that the substituent is held in a fixed conformation such that each of the aminomethylene groups is in a different magnetic environment (compare Figure 2.6 with Figure 2.10).

The 2D-ROESY spectrum of this solution shows that 4-methylbenzoate **37** is included within the annulus of the cyclodextrin **26**. There are cross-peaks between the resonances of each of the protons of guest **37** and those of the annular protons H3 and H5, the relative intensities of which suggest that "head-first" inclusion is the predominant inclusion mode. There are no observable NOE interactions between the annular protons H3 and H5 and those of the substituent. Inclusion of the guest **37** pushes the substituent out of the annulus. The 2D-ROESY spectrum does show cross-peaks between the resonances of the protons dipnH4 and those of the protons dipnH3 and dipnH6, suggesting that the linear substituent is held in a conformation that brings these methylene protons near to each other.

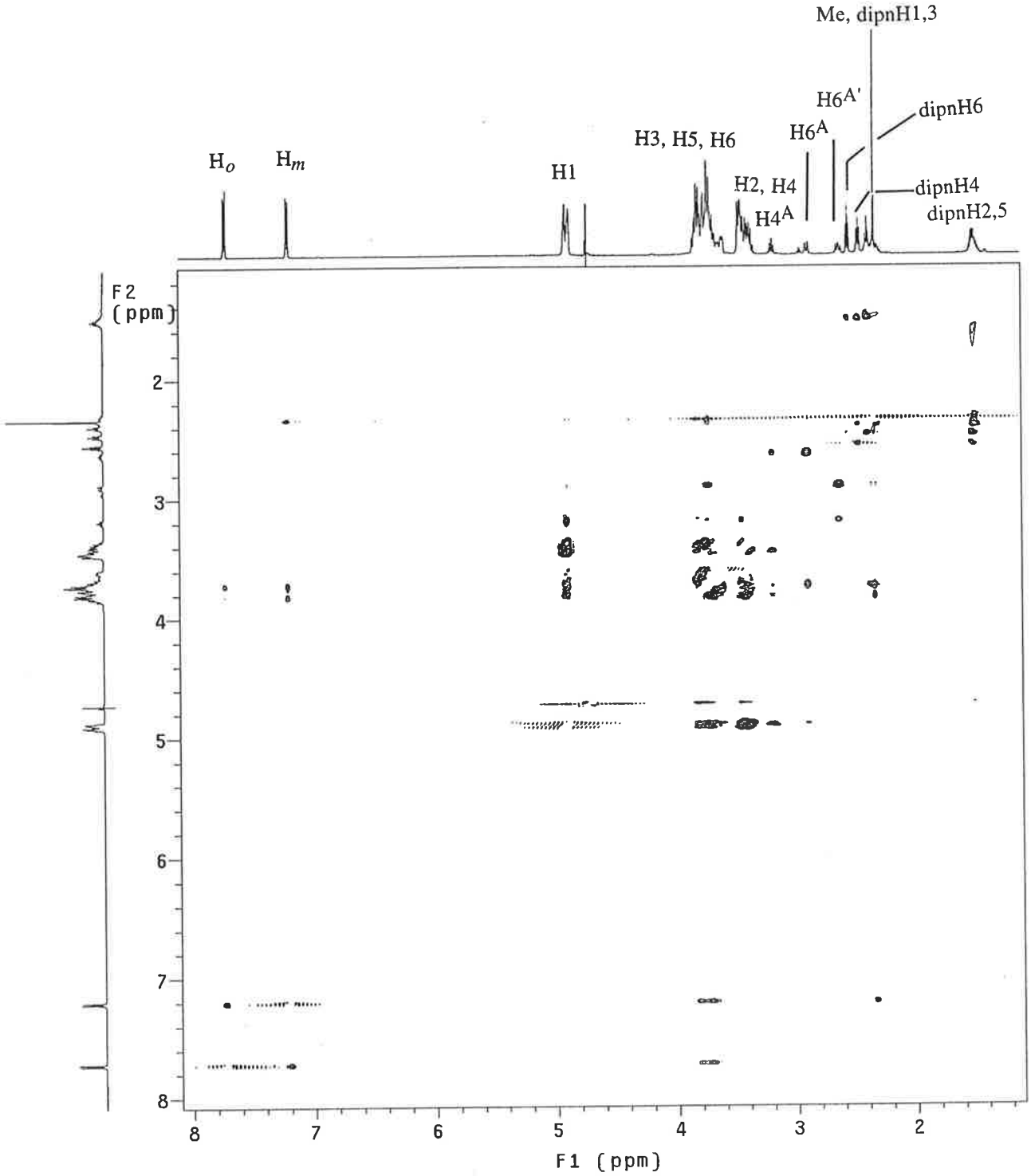
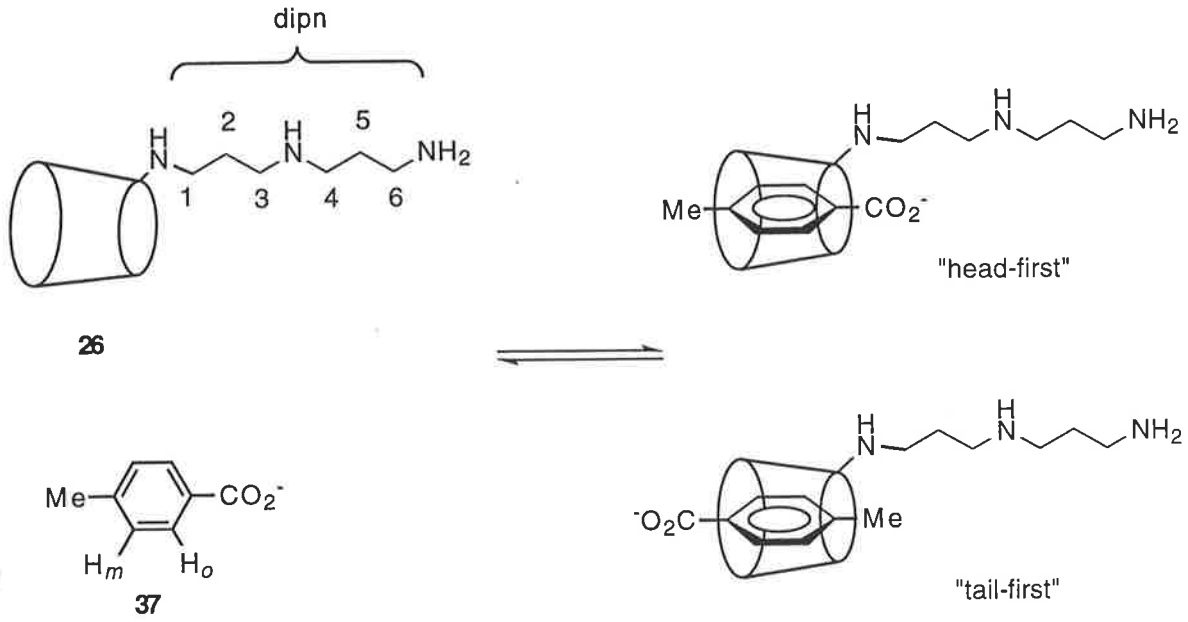


Figure 2.10 Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.~~06 × 10^{-3}~~ mol dm^{-3} each of the cyclodextrin **26** and 4-methylbenzoate **37**. The protons are labelled as shown in Scheme 2.8.



Scheme 2.8. Schematic representation of the inclusion of 4-methylbenzoate **37** in the cyclodextrin **26** in aqueous solution at pH ≥ 12 indicating the two possible modes of inclusion of the guest **37**.

Cyclic substituents were not expected to be included within the annulus of the attached cyclodextrin moiety and the 600 MHz 2D-ROESY spectrum of a solution of the cyclodextrin **30** at pH ≥ 12 shows no evidence for NOE interactions between the protons of the substituent and the annular protons H3 and H5. The 2D-ROESY spectrum of a solution of the cyclodextrin **30** containing one equivalent of 4-methylbenzoate **37** at pH ≥ 12 shows that the guest **37** is included within the annulus of the cyclodextrin **30** (Figure 2.11).

Cross-peaks indicate that the protons H_o and H_m are located near the annular protons H3 and H5, with the strongest NOE interactions being between protons H_m and the annular protons H5. There are cross-peaks indicating that the methyl group of the guest **37** is situated adjacent to the protons H5 and has NOE interactions with one or more of the aminomethylene groups of the substituent (Scheme 2.9). This suggests that "tail-first" inclusion, where deep penetration of the guest **37** allows the methyl group to push up into the "crown" of the substituent, is the major inclusion mode under these conditions.

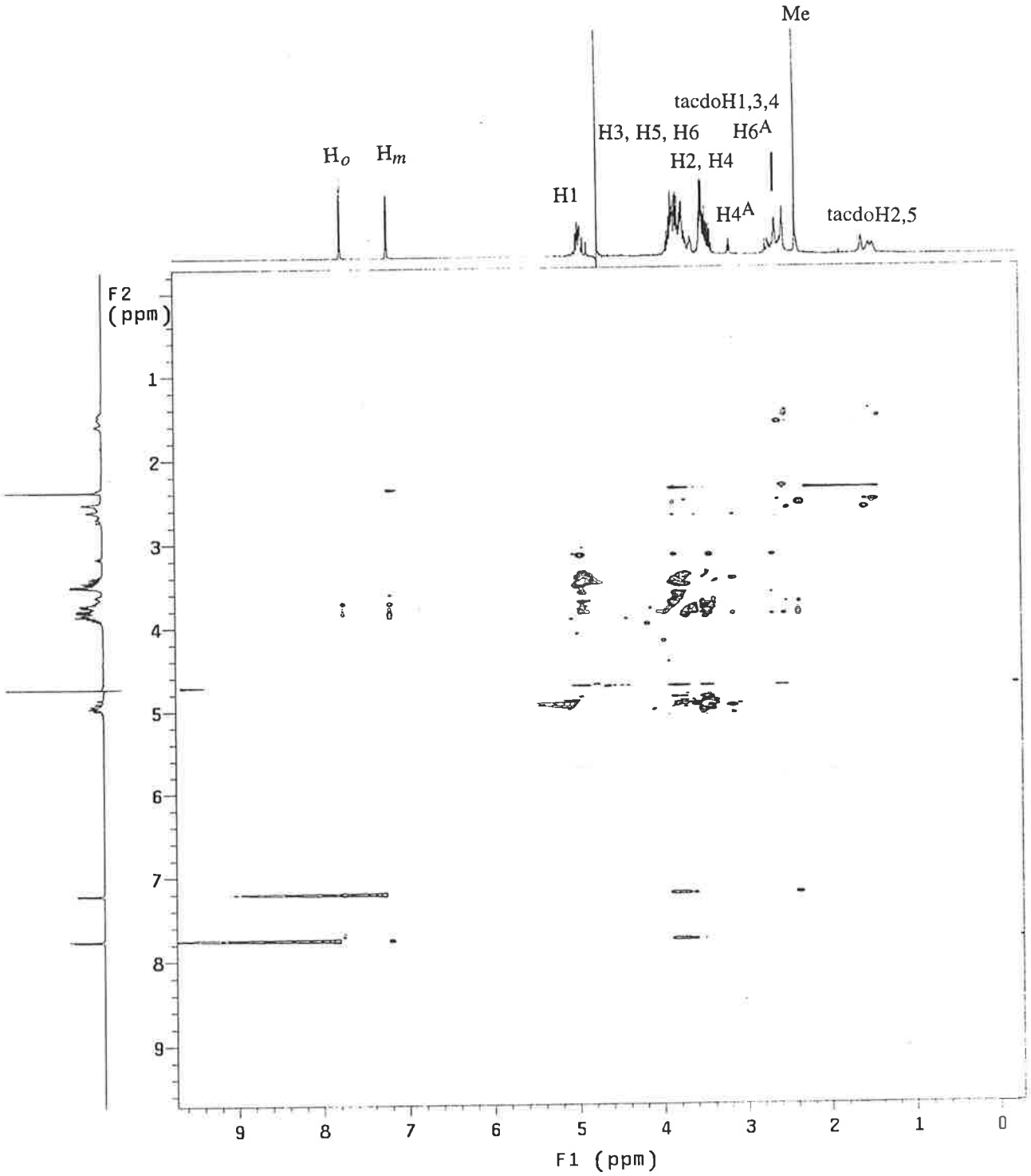
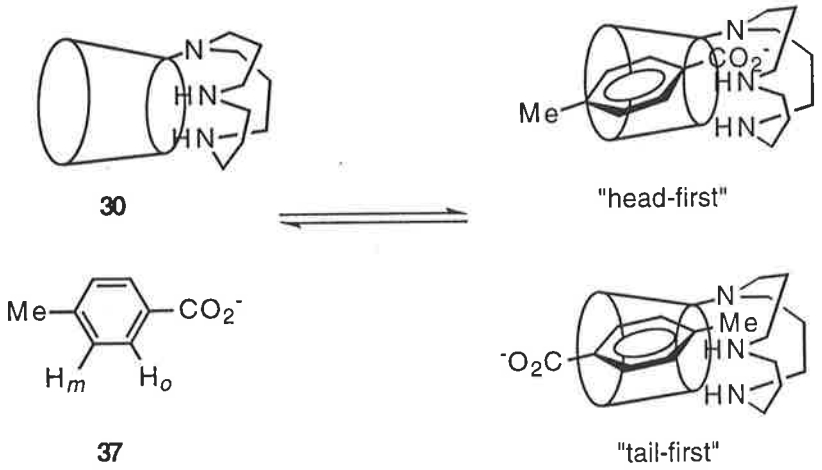


Figure 2.11. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10~~$^{-3}$ mol dm^{-3} each of the cyclodextrin **30** and 4-methylbenzoate **37**. The protons are labelled as shown in Scheme 2.9.



Scheme 2.9. Schematic representation of the inclusion of 4-methylbenzoate **37** in the cyclodextrin **30** in aqueous solution at pH ≥ 12 indicating the two possible modes of inclusion of the guest **37**. The substituent is held against the primary face of the cyclodextrin by hydrogen bonding.

Why do cyclodextrins bearing linear chains, which have been shown to include in the annulus at high pH, show stronger binding of aromatic carboxylates than does the parent β-cyclodextrin **2**? It has been shown that such self-inclusion can preclude binding of large guests.[92, 137] However, in the systems studied above the guests are small enough to fit inside the annulus alongside the included substituent, as shown above for the complexes formed by the cyclodextrin **24**. The hydrophobic nature of these alkyl chains must increase the hydrophobicity of the annulus, thus making inclusion of the guest within the annulus increasingly favourable over solvation of the guest by water. As the size of the guest is increased (benzoate **36** to 4-methylbenzoate **37**) steric factors cause some decrease in the stability of the complexes formed, this effect being smallest for the host with the shortest substituent (3-aminopropylamino-cyclodextrin **22**). The shallow inclusion of the aryl portion of 2-phenylpropionate **38** into the annulus of the host causes the steric effects to become less important than the hydrophobic effects and so the cyclodextrin **24** gives the more stable complex formed with this guest.

Cyclodextrins bearing cyclic polyamine substituents form the most stable complexes with the guests examined because the substituent acts to cap the primary face of the cyclodextrin

moiety at high pH, as shown by ^{13}C NMR. In particular, the 1,5,9-triazacyclododecyl substituent of the cyclodextrin **30**, acts to cap the primary face of this host and also causes an increase in the overall hydrophobicity of the system by increasing the apparent depth of the cavity to form the most stable complexes, of all the hosts examined, with the smaller guests.

2.6. Conclusion

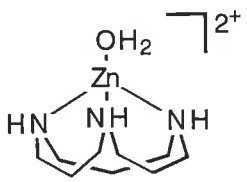
A clean, simple and reproducible synthesis of 6^A-amino substituted β-cyclodextrins has been developed. The key improvement over previous methods is the use of 1-methyl-pyrrolidin-2-one (NMP) as the solvent for the reaction. The use of this solvent allows the rapid substitution of a 4-methylbenzenesulfonate by a wide variety of primary and secondary amines at moderate temperature. This avoids the use of high pressure or sealed tube reactions and the use of large excesses of amine reagents, some of which may be expensive to obtain and difficult to separate from the desired product. The cyclodextrin products are obtained as pure materials after a simple and inexpensive ion-exchange step.

A series of amino-substituted β-cyclodextrins has been prepared by this procedure and systematic studies of their pH dependent solution structures and host-guest chemistry have been carried out using titrometric and 2D-NMR techniques. At high pH the hydrophobic, linear substituents are included within the annulus of the cyclodextrin moiety and remain included within the annulus when small aromatic guests are bound inside the cyclodextrin. Cyclic substituents form a tight cap over the primary face of the cyclodextrin at high pH resulting in the enhanced binding of aromatic guests. At lower pH both types of substituents move away from the annulus as the charged ammonium groups are better solvated by water, allowing easier dissociation of host-guest complexes.

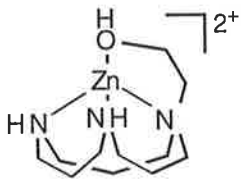
Chapter 3: Reactions of Amino-substituted Cyclodextrins and their Zn(II) Complexes with Activated Esters

3.1. Introduction

It has been reported that the Zn(II) complexes **43** and **44** are able to catalyse the hydrolysis of active esters and phosphates at physiological pH.[138 -142] These complexes also catalyse the hydration of carbon dioxide and are described as mimics of carbonic anhydrase, a metallo-enzyme which has in its active site a Zn(II) ion bound by three nitrogen donors. The physiological activity of this enzyme is ascribed to the ready deprotonation of a water molecule bound to Zn(II) (pK_a ~ 7.5). The pK_as of the water and hydroxyl bound to Zn(II) in the complexes ~~3.1~~ 43 and ~~3.2~~ 44 are 7.3 and 7.4 respectively.



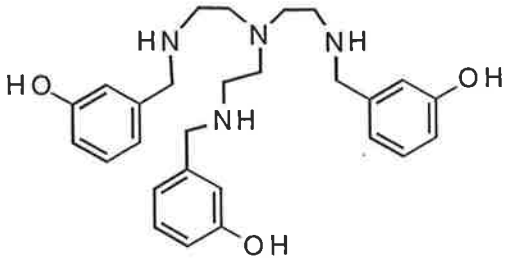
43



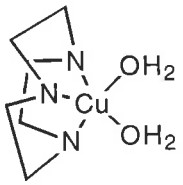
44

The Zn(II) complex of **45** has been shown to act as an esterase with Zn(II) acting both to bring together the aromatic groups to form an ill-defined cavity and to activate a phenolic hydroxyl to deprotonation (its pK_a is lowered about 1.3 units relative to the free phenol).[143,144]

The complex **46** has been shown to be a phosphatase and protease mimic, able to cleave the unactivated glycylglycine bond. It shows a high level of bond selectivity in the cleavage of bovine serum albumin.[145, 146]



45



46

The binding of a substrate within the active site of an enzyme (to form an enzyme-substrate complex) is an important step in any process catalysed by an enzyme. This binding places the substrate in an optimum position to react with the active groups within the active site. Modification of the conformation of the enzyme or the substrate or both species on binding can be a major driving force in the catalytic process.[147] Control of enzyme activity is dependent on the requirement for the substrate to be bound to the enzyme before any reaction can occur.[55] Feed-back inhibition, where a product of the reaction being catalysed can compete with the substrate for binding to the active site (competitive inhibition), is a process for preventing the over-production of metabolites by enzymes. Allosteric interactions, where binding of some species at a site remote from the catalytic centre causes changes to the three dimensional structure of the active site, are important in the control of enzyme activity. These changes can either activate the enzyme, by altering the active site to a form which can bind the substrate and then carry out the catalytic process, or they may distort the active site to prevent productive substrate binding (non-competitive inhibition) and so inhibit the catalytic process.

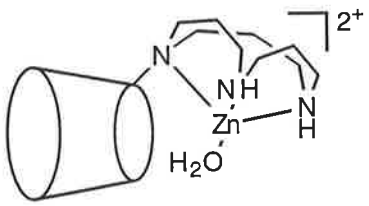
None of the complexes **43-46** is able to form such catalyst-substrate complexes prior to reaction with a substrate, so the complexes **43-46** are not truly enzyme mimics. It was anticipated that if these, or similar, catalytic groups could be attached to a cyclodextrin by the methods outlined in the previous chapter, better models of metallo-enzymes could be created. Unlike the complexes **43-46**, such catalysts were expected to show Michaelis-Menten kinetics in their reactions due to binding of the substrate within the cyclodextrin annulus prior to subsequent reaction.[50, 52, 54, 148, 149] The catalytic reactions of such modified cyclodextrins

were expected to be inhibited by the presence of molecules which can compete with the substrate for binding within the annulus of the cyclodextrin.

Cyclodextrins bearing substituents capable of binding metals have been shown to act as mimics of metallo-enzymes. It has been shown that the cyclodextrin **25** forms a complex with Zn(II) that acts as an efficient phosphatase mimic.[150] The esterase activity of the cyclen appended cyclodextrin **31** in the presence of a number of metal salts has been studied and it was shown that there was an enhanced reactivity of the metallo-cyclodextrins formed under these conditions over that observed for the corresponding 1,4,7,10-tetraazacyclododecanyl complexes.[91, 97, 151]

3.2. Reactions of Zn(II) Complexes

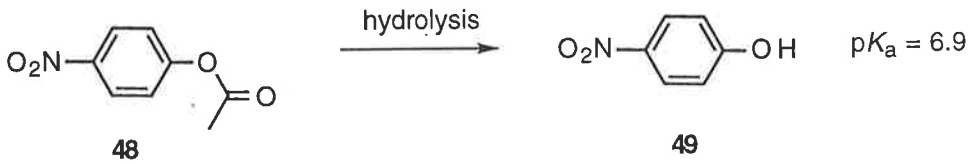
The high activity reported for complex **43** lead to the examination of the Zn(II) complex **47**, derived from the cyclodextrin **30**, as a potentially highly active mimic of carbonic anhydrase.



47

The reaction examined was the catalysed hydrolysis of 4-nitrophenyl acetate **48**. There have been some questions raised as to the validity of the use of the "active" ester **48** for studies of this type.[152 -154] However, this substrate is the most frequently utilised ester in studies of this kind, allowing comparisons of the results obtained here with those of previously reported studies. In addition, hydrolysis of the ester **48** generates 4-nitrophenol **49** which, on deprotonation, gives the yellow phenolate anion due to absorbance at 400 nm (Scheme 3.1). It

is by monitoring changes in absorbance at 400 nm that the reactions are followed.



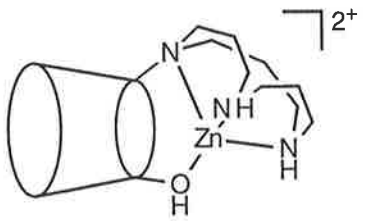
48 **49**

Scheme 3.1 Hydrolysis of 4-nitrophenyl acetate ~~**3.6**~~ **48** generates the phenol ~~**3.7**~~ **49**. The deprotonated phenol has an absorbance at 400 nm.

Studies on the reactivity of the complex **47** were carried out by the "initial rate method" used for the study of the reactivity of complex **43**.[138] This method is often employed for the study of slow reactions. In the initial rate method, reactions are monitored to less than 2% completion and the rate constant (mol dm^{-3} s^{-1}) for the formation of 4-nitrophenol **49** is determined from the slope of the straight line obtained by plotting the absorbance at 400 nm against time.

Deprotonation of bound water in the complex **43** is believed to be important in the hydrolyses catalysed by this complex and this was expected to be important in the reactions of complex **47**, the reactivity of both complexes therefore being pH dependent. A sigmoid curve was obtained for the pH dependence of the hydrolysis of ester **48** by complex **43**, with the inflection point at around pH 7.3, which is the pK_a of the bound hydroxyl as determined by potentiometric titration.[138] It was not known what effect the conjunction of a cyclodextrin moiety to the metal complex might be. It was anticipated that deprotonation of the complex **47**, which would decrease the charge adjacent to the hydrophobic annulus, may make this process more favoured than for the complex **43**, giving a lower pK_a of the bound water relative to that of the water bound to the complex **43**. This would parallel the decrease in basicity of amine groups attached to the annulus noted in the previous chapter. Alternatively, it was thought that a C6 hydroxyl group may be bound to the Zn(II) to form the complex **50**, related to the hydroxyethyl complex **44** which has a higher pK_a for the deprotonation of the hydroxyl group but is a much more efficient nucleophile than the deprotonated complex **43**.[140] Complexation

of a metal by a C6 hydroxyl group has been proposed for the cobalt (III) complex of the cyclodextrin **31**.[151]



~~3.8~~
50

Solutions of the complexes **43** and **47** were prepared by dissolving the appropriate weight of the free ligand in buffer (I = 0.1 mol dm^{-3}) over the range pH 6.6-9.1 and adding one equivalent of $Zn(ClO_4)_2$ from a stock solution to give a final concentration of 1.03×10^{-3} mol dm^{-3} of complex, assuming that complete complexation of Zn(II) occurs. Solutions of pH ≥ 8.5 containing complex **47** prepared in this manner became cloudy suggesting that either Zn(II) ion or the complex **47** was precipitating, possibly as a hydroxo species. To cover the full range of pH, both HEPES and borate buffers were used. The range of pH covered by each buffer was overlapped around pH 8 so that any differences in reactivity due to buffer effects could be taken into account. Buffer reagents may affect the formation of complexes with cyclodextrins.[155]

Reactions were carried out at 298.2 K by pipetting 2.0 cm^3 of the appropriate solution (buffer, buffer + **43** or buffer + **47**) into a quartz cell (1 cm pathlength) and placing this in the heated cell block of a Cary 2200 spectrophotometer. The solution was allowed to equilibrate and then 0.05 cm^3 of a stock solution of the ester **48** in acetonitrile (0.041 mol dm^{-3}) was added to give a final solution that was 2.5% acetonitrile and contained each reactant at a concentration of 1.0×10^{-3} mol dm^{-3}. The solution was mixed quickly and the increase in absorbance at 400 nm was recorded digitally for the first 2% of reaction. The absorbance was referenced against a solution of buffer placed in the reference beam. Each run was carried out in triplicate and the results were averaged. Variations in the determined rate constants between

runs were less than 5%. The calculated rate constants for the formation of the phenol **39** determined from the above experiments are given in Tables 3.1 and 3.2.

Table 3.1 Variation of molar absorbance and initial rate of formation of the phenol **49** for the reaction between the complex **43** and 4-nitrophenyl acetate **48** in aqueous buffered solutions (I = 0.1 mol dm^{-3}) at 298.2 K.

pH[a]	ε_{400} dm^3 mol^{-1}[b]	$10^8 k_o$/mol dm^{-3} s^{-1}[c]	$10^8 k_{obs} - k_o$/mol dm^{-3} s^{-1}[d]
6.9	10600	0.546	0.74
7.1	11300	0.689	1.18
7.3	12500	0.912	2.75
7.6	13500	1.39	3.10
7.8	14500	2.01	2.39
8.0	15300	2.78	4.34
8.2	15900	4.95	4.36

[a] 0.05 mol dm^{-3} HEPES buffer. [b] Determined from seven concentrations of 4-nitrophenol **49** at each pH. [c] Rate of formation of phenol **49** for buffer reaction calculated from rate of change of absorbance at 400 nm. [d] Observed rate of formation of phenol **49** in the presence of the complex **43** minus the buffer rate calculated from rate of change of absorbance at 400 nm. See Section E.3.2 for experimental data.

The molar extinction coefficient ε_{400} of 4-nitrophenol **49** (pK_a = 6.9[109]) varies with pH as it is the phenolate anion which absorbs at 400 nm. It was therefore necessary to determine the value of ε_{400} at each pH in order to convert the kinetic data, obtained as ΔAU s^{-1}, into the rate of formation of the phenol **49** (mol dm^{-3} s^{-1}). The absorbance of seven solutions of 4-nitrophenol **49** over the concentration range 0.337-8.41 $\times$ 10^{-5} mol dm^{-3} was determined at each pH studied and the value of ε_{400} at each pH was calculated by a linear least squares fit of this data (Table 3.1). It was found that the molar extinction coefficient of the phenol **49** was affected by the presence of the complex **47**, the value of ε_{400} being increased, most probably due to complexation of phenol **49** within the cyclodextrin annulus. Therefore, the value of ε_{400} for the phenol **49** in solutions containing cyclodextrins was determined by a single point method. Solutions of the complex **47** were placed in the spectrophotometer as described above

but 0.05 cm^3 of a solution of the phenol **49** in acetonitrile was added rather than the solution containing ester **48** (final concentration of phenol **49** = 3.65×10^{-5} mol dm^{-3}). The solution was allowed to equilibrate, the absorbance at 400 nm was recorded and a value for ε_{400} was calculated.

Table 3.2 Variation of molar absorbance and initial rate of formation of the phenol **49** for the reaction between complex **47** and the ester **48** in aqueous buffered solutions (I = 0.1 mol dm^{-3}) at 298.2 K.

pH	ε_{400} dm^3 mol^{-1} [d]	$10^8 k_o$/mol dm^{-3} s^{-1} [e]	$10^8 k_{obs} - k_o$/mol dm^{-3} s^{-1} [f]
6.6[a]	8900	0.218	0.489
7.2[a]	14700	0.417	1.81
7.8[a]	19300	1.08	7.47
8.1[a]	21300	2.47	13.5
8.1[b]	21300	2.47	20.5
8.4[a,c]	21300	3.27	21.6
9.1[b,c]	21300	18.9	111

[a] 0.05 mol dm^{-3} HEPES buffer. [b] 0.05 mol dm^{-3} borate buffer. [c] Some precipitate observed. [d] Determined from single measurement of a solution of 4-nitrophenol **49** (3.65×10^{-5} mol dm^{-3}) in buffer containing complex **47**. [e] Rate of formation of the phenol **49** for buffer reaction calculated from rate of change of absorbance at 400 nm. [f] Observed rate of formation of the phenol **49** in the presence of the complex **47** minus the buffer rate calculated from rate of change of absorbance at 400 nm. See Section E.3.2 for experimental data.

When the rate of reaction in buffer alone (k_o) is subtracted from the observed rate of reaction in the presence of either the complex **43** or **47** (k_{obs}) the value obtained is a measure of the reaction due to the complex. For the complex **43** a maximum catalytic effect is observed at around pH 7.3 where the catalytic rate is about three times that of the buffer reaction. At higher pH the increasing concentration of hydroxide begins to mask the effect of the complex. When the rate constant for the reaction of the complex **43** with ester **48** at pH 8.2 is converted to a second order rate constant (by division of the measured rate constant by the initial

concentrations of the reactants) a value of 4.36×10^{-2} dm^3 mol^{-1} s^{-1} is obtained which compares well with the reported value (4.1×10^{-2} dm^3 mol^{-1} s^{-1}).[138]

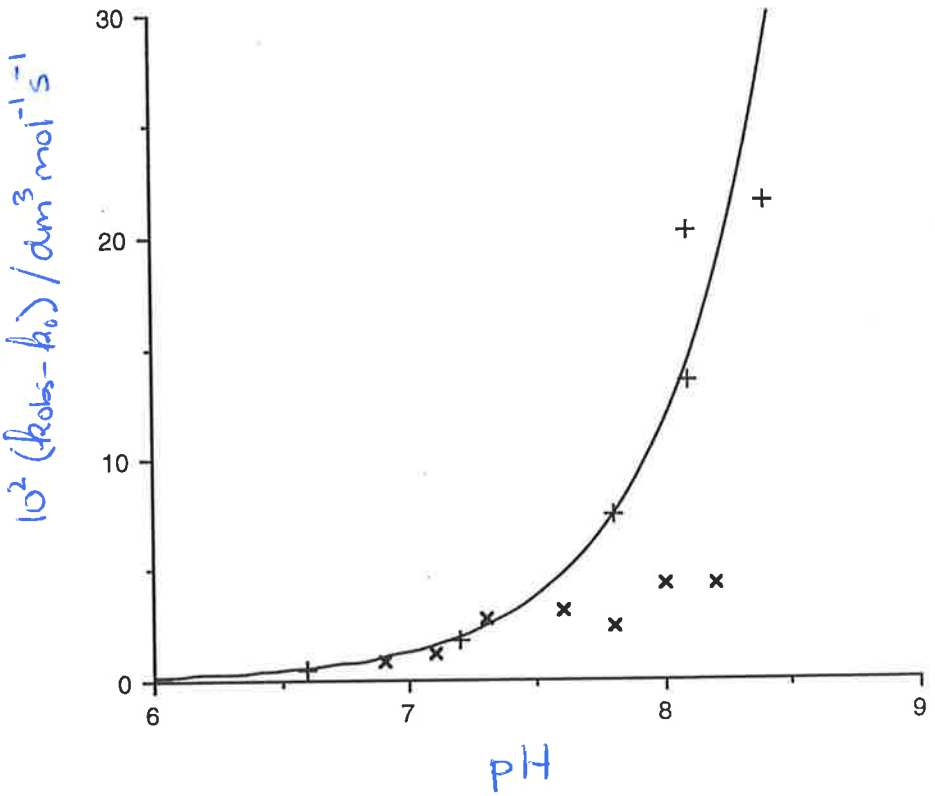


Figure 3.1. Plot of variation of rate (k_{obs}-k_o) with pH for the reaction of the ester **3.6** with the complexes **3.1** (×) and **3.5** (+). Rates determined by the initial rate method at 298.2 K.

Comparison between the reactivities of the two complexes **43** and **47** can only be made in a qualitative manner as each is presumed to follow a different kinetic pathway. At pH ≤ 7.3 the complexes **43** and **47** show similar rates of formation of the phenol **49**. However, above pH 7.3, where the reactivity of the complex **43** tends towards a plateau as the complex becomes fully deprotonated, there is a continuous increase in the reactivity of the complex **47** over the pH range studied (the observed catalytic rate is about seven times that of the rate of the buffer reaction for most of this range). This effect is enhanced by a change in buffer, an increase in rate being observed on changing from HEPES to borate buffer (compare observed rates at pH 8.1 with each buffer), suggesting that HEPES is able to compete with the ester **48** for inclusion

in the cyclodextrin annulus.

The continuing increase in the reactivity of the complex **47** as the pH of the solution is increased, and the observation that Zn(II) appears to be coming out of solution at pH ≥ 8.5, suggests that the reactive group is not a deprotonated hydroxy species activated by Zn(II) but one of the amino groups on the substituent of the cyclodextrin. The rate of formation of the phenol **49** from the reaction of the ester **48** with the cyclodextrin **30** in buffer in the absence of Zn(II) is 2.42×10^{-8} mol dm^{-3} s^{-1} and 1.70×10^{-6} mol dm^{-3} s^{-1} at pH 7.2 and pH 9.1, respectively. These results suggest that Zn(II) acts to inhibit the reactivity of the cyclodextrin **30**, most probably by binding to, and so decreasing the nucleophilicity of, a reactive nitrogen. The reaction of the cyclodextrin **30** with the ester **48** appears to involve attack of one of the nitrogens of the substituent on the carbonyl of the ester **48**.

These results were unexpected given the number of reports of metal induced activation of amino-substituted cyclodextrins towards reaction with esters and phosphate esters. In particular, metal ions have been reported to enhance the reactivity of the cyclodextrin **31** in the deacylation of the ester **48**.[91] The unexpected de-activation of the cyclodextrin **30** by Zn(II) in the reaction with the ester **48** lead to an examination of the previously reported reactions of the Zn(II) complex of the cyclodextrin **31**.

The reactivity of the cyclodextrin **31** with ester **48** has been reported to be enhanced in the presence of metal salts, including Zn(II).[91] The observed enhancement in the activity of the cyclodextrin-bound complex over the free complex was around a factor of two in most cases (cf. factor of two to three for complex **47** over complex **43** observed above). However, the presence of added metals had only a marginal effect on the reactivity of the cyclodextrin **31**, particularly at higher values of pH. The reported data show a trend similar to that observed above in the reactions of the complex **47**, that there is a continuous increase in the reactivity of the cyclodextrin complexes as the pH is increased.

These results had been obtained using a bis-tris-propane buffer and it is possible that this buffer, related to tris and in a large excess over the concentration of the cyclodextrin **31**,

will itself complex the added metals, so reducing the amount of the cyclodextrin complex in solution. This might then account for the observed low enhancement in reactivity of the cyclodextrin **31** on addition of the metals and so the rate analysis was repeated using HEPES, which does not bind metals, as the buffer agent.

The rates for reaction of the cyclodextrin **31** with the ester **48** were determined by the initial rate method as described above. (The reported rates had been determined under first order conditions.) The rates obtained in this experiment (see Section E.3.2 for the experimental data) were comparable to those reported earlier and confirmed that there was little effect of the metal on the reactions of the cyclodextrin **31** with the ester **48**. Although a small rate enhancement was observed at the lower pH (k_{obs}-k_o = 2.58 × 10^{-8} and 1.63 × 10^{-8} mol dm^{-3} s^{-1} for the reaction with and without added Zn(II), respectively, at pH 7.2), the presence of Zn(II) caused a decrease in the reaction rate at higher pH (k_{obs}-k_o = 5.56 × 10^{-7} and 8.31 × 10^{-7} mol dm^{-3} s^{-1} for the reaction with and without added Zn(II), respectively, at pH 9.1).

Thus, it appears that the Zn(II) complexes of the amino-substituted cyclodextrins **30** and **31** do not act through the activation of a bound water (or C6 hydroxyl) to deprotonation and subsequent reaction at the ester carbonyl but through reaction of a free amine nitrogen. The Zn(II) complexes of these cyclodextrin derivatives may involve co-ordination of the metal by one or more of the hydroxyl groups around the primary face, leaving one or more of the amine nitrogens free to react with suitable electrophiles. Zn(II) may play a role in the reactions with the ester **48** by polarising the carbonyl group and so enhancing the reactivity of the system, but this has only a minor effect on the reaction rate at near neutral pH, Zn(II) acting as an inhibitor at pH ≥ 8.5.

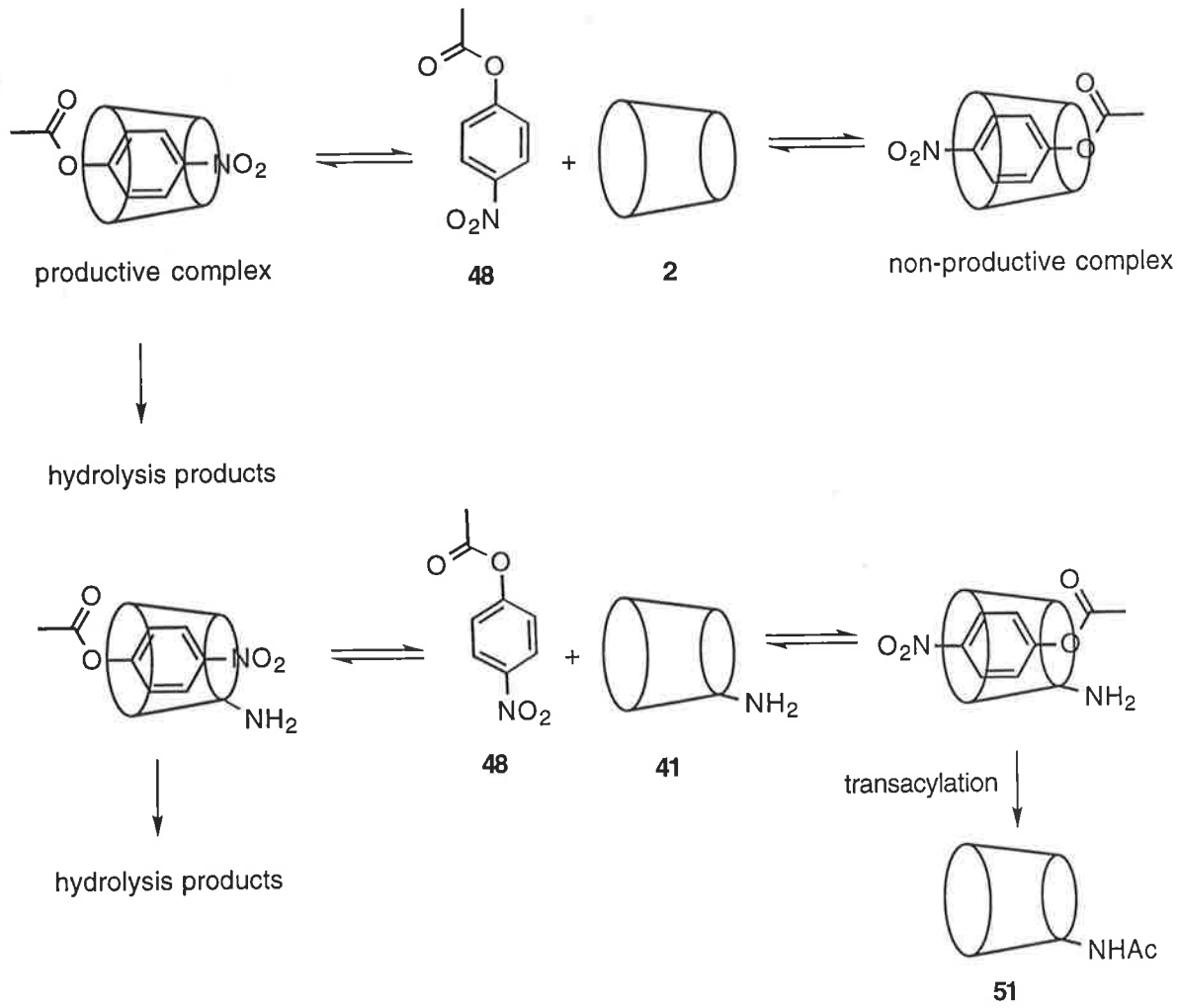
It has recently been shown, by a full kinetic analysis, that the complex **43** does not act through an activated hydroxyl bound to Zn(II) as was previously reported but that Zn(II) most probably acts as a Lewis acid to polarise the carbonyl bond of the ester and so facilitates the attack of hydroxide ion on the complexed ester.[156]

3.3. Reactions of Aminoalkylamino Cyclodextrins

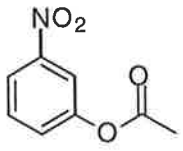
The reactivity of an amine group bound to a cyclodextrin moiety has been utilised to prepare derivatives selectively modified at nitrogen.[157] Reaction at nitrogen by esters leads to the formation of amide bonds and this type of reaction has been used to prepare a number of cross-linked cyclodextrins[158, 159] and several pro-drug compounds.[99] These reactions may show some diastereoselectivity when a chiral amino-cyclodextrin reacts with a racemic mixture of a reactive carbonyl compound. Such reactions may involve inclusion of the carbonyl compound within the cyclodextrin cavity prior to reaction at the amino function and so may be considered to model the reaction of an enzyme with a suicide substrate (a substrate which undergoes part of the catalysis sequence but becomes permanently covalently attached at the active site at some point).

In earlier work the mono-amino cyclodextrin **41** was used as a kinetic probe for examining the inclusion states of 4-nitrophenyl acetate **48** in cyclodextrins.[160] Reaction of the ester **48** with β-cyclodextrin **2** at pH > 10 forms a transient C2-*O*-acyl cyclodextrin which is slowly hydrolysed back to β-cyclodextrin **2**. The reaction occurs through the inclusion of the aryl portion of the ester **48** such that the acyl bond is adjacent to a deprotonated C2 hydroxyl group. A reversal of this inclusion mode places the acyl group adjacent to a C6 hydroxyl group, which is not reactive under these conditions, and so this is a non-productive binding mode (Scheme 3.2).

When the amino-cyclodextrin **41** is allowed to react with the ester **48** the previously non-productive binding mode places the acyl bond adjacent to a reactive amino nitrogen which reacts to form the stable acetamide **51**. This result showed, for the first time, that the ester **48** has two binding modes within the annulus of a cyclodextrin. The reaction between 3-nitrophenyl acetate **52** and the amino-cyclodextrin **41** gave none of the acetamide **51**, suggesting that for this substrate only one mode of inclusion (acyl group toward the secondary face) was possible.



Scheme 3.2. Schematic representation of the reactions of the ester **48** with β-cyclodextrin **2** and the amino-cyclodextrin **41** showing the formation of productive and non-productive complexes. The reaction of the ester **36** with the amino group of the cyclodextrin **41** leads to the formation of the stable acetamide **51**.



52

The reactions of the ester **48** with a series of ω-aminoalkylamino-cyclodextrins with alkyl chain lengths ranging from two to six carbons were examined in order to determine the effect of substituent length on the reactivity of the system. The ω-aminoalkylamino substituents were expected to modify the binding of the ester **48** within the annulus due to their inclusion within the cyclodextrin annulus. The increased flexibility of these aminoalkylamino chains was also expected to modify the reaction geometry relative to that of the reactions of the amino-cyclodextrin **41**.

3.3.1. Rate analysis

The reactions of the cyclodextrin derivatives **21**, **22** and **24** were compared with those of the corresponding "free" diaminoalkanes. All of the reactions were carried out under first order conditions ($[RNH_2]>>[\mathbf{48}]$).

Reactions were carried out at 298.2 K by pipetting 2.0 cm^3 of a stock solution (1.03×10^{-3} mol dm^{-3}) of the amino compound in 0.05 mol dm^{-3} borate buffer pH 9.1 into a quartz cell (1 cm pathlength) and placing this in the heated cell block of a Cary 2200 spectrophotometer. The solution was allowed to equilibrate and then 0.05 cm^3 of a stock solution of ester **48** in acetonitrile (4.1×10^{-4} mol dm^{-3}) was added to give a final solution that was 2.5% acetonitrile and contained the ester **48** at a concentration of 1.0×10^{-5} mol dm^{-3} and the amine at a concentration of 1.0×10^{-3} mol dm^{-3}. The solution was mixed quickly and the increase in absorbance at 400 nm was recorded digitally for at least eight reaction half-lives. The absorbance was referenced against a solution of buffer placed in the reference beam. Each run was carried out in triplicate and the results averaged. Variations in the determined rates between

runs were less than 5%.

The first order rate constant (k_{obs}-k_o) was determined by fitting the collected data to a first order rate equation by conventional methods (Table 3.3). Under these conditions the first order rate for the reaction of ester **48** in buffer alone (k_o) was found to be 2.00×10^{-4} s^{-1}.

Table 3.3 Pseudo-first order rates for the reaction of the ester **48** with ω-aminoalkylamines in 0.05 mol dm^{-3} borate buffer pH 9.1 at 298.2 K.[a]

βCDX	$10^3(k_{obs}-k_o)/s^{-1}$[b]	X	$10^3(k_{obs}-k_o)/s^{-1}$[b]
21	7.11	$NH_2(CH_2)_2NH_2$	0.69
22	3.61	$NH_2(CH_2)_3NH_2$	1.15
24	0.93	$NH_2(CH_2)_6NH_2$	1.08

[a] Initial concentration of the ester **48** and ω-aminoalkylamine 1.00×10^{-5} and 1.00×10^{-3} mol dm^3 respectively. [b] Difference in the observed first order rate, k_{obs}, and the rate observed with buffer alone, $k_o = 2.00 \times 10^{-4}$ s^{-1}. See Section E.3.3 for the experimental data.

Initial examination of the obtained data suggests that the presence of a cyclodextrin moiety enhances the reactivity of the attached amine groups over that of the "free" amine species and that this effect decreases with an increase in the length of the attached aminoalkyl chain. For cyclodextrins **21** and **22** the rate of reaction is increased over that of the "free" amine by a factor of 9 and 3.1, respectively, while for the cyclodextrin ~~34~~ 24 a decrease in rate (0.86) is observed. However, such an observation ignores the effect of the different pK_as of the amines examined. At pH 9.1 there will be different levels of protonation of each of the amines and the observed rate will be dependent on the reactivity of each of the protonated species in solution (Table 3.4). It was expected that only the mono-protonated and the non-protonated species would be able to react with the ester **48** as the di-protonated species have no free electrons to allow them to act as nucleophiles. The reactivity of the mono-protonated species was expected to be dependent on the degree of sharing of the additional proton between the nitrogens of these species.

Table 3.4 pK_as and speciation at pH 9.1 of the ω-aminoalkylamino-cyclodextrins **21**, **22** and **24** and the corresponding "free" diaminoalkanes.

		% Species at pH 9.1[b]	
Amine	pK_a	non-protonated	mono-protonated
$NH_2(CH_2)_2NH_2$	9.97, 7.16	11.8	87.2
$NH_2(CH_2)_3NH_2$	10.56, 8.97	2.0	56.3
$NH_2(CH_2)_6NH_2$	11.01, 10.04	0.13	10.3
21	9.42, 5.70	32.4	67.6
22	9.90, 7.39	13.7	86.3
24	10.27, 8.72	6.3	93.7

3.3.2. pH dependence

The pH dependence of the reaction between the cyclodextrin **24** and the ester **48** was examined under first order conditions, as described above, over the range pH 9.1 to pH 10.3. The plot of the reaction rate (k_{obs}-k_o) against the concentration of either the non- or mono-protonated species indicates that it is the non-protonated species which is the major reactive species (Figure 3.2).

There is a linear dependence between the first order rate of the reaction due to the cyclodextrin **24** and the concentration of the amount of non-protonated cyclodextrin **24**. In contrast, the plot shows that there is no linear correlation between the rate of the reaction due to the cyclodextrin **24** and the concentration of the mono-protonated species. The first order rate (k_{obs}-k_o) is decreased as the amount of mono-protonated species increases. The observed lack of reactivity of the mono-protonated species is most probably due to the sharing of the additional proton between both nitrogens of the 6-aminohexyl substituent of the cyclodextrin **24**.

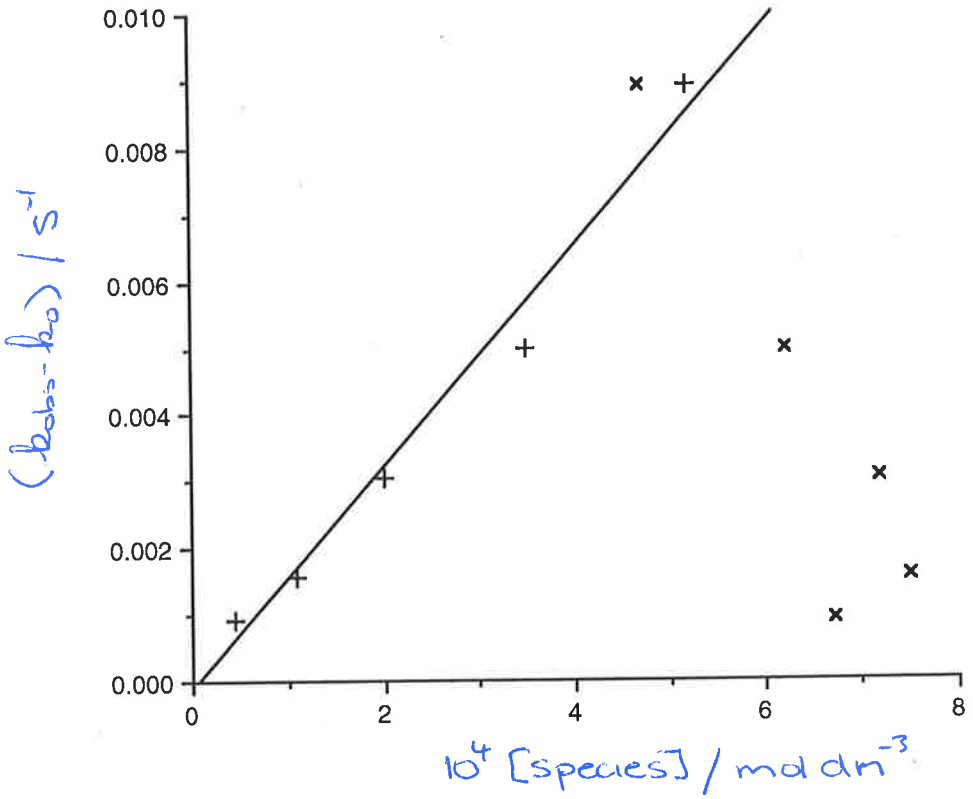


Figure 3.2. Plot of (k_{obs}-k_o) against the concentration of non-protonated (+) and mono-protonated (×) cyclodextrin **2.4** over the range pH 9.1-10.3.

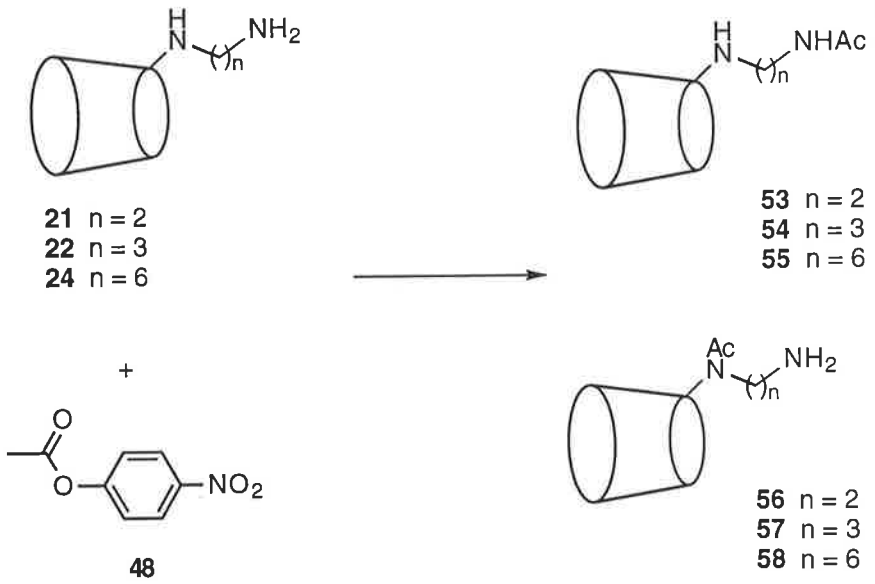
Dividing the observed rate of reaction at pH 9.1 by the concentration of the non-protonated species in solution at pH 9.1 gives a better measure of the relative reactivity of the cyclodextrin amines **21**, **22** and **24**, if the non-protonated species is the major reactive species . This gives first order rate constants of 0.022 s^{-1}, 0.026 s^{-1} and 0.014 s^{-1} for the cyclodextrins **21**, **22** and **24**, respectively. This suggests that the 3-aminopropylamino substituent of the cyclodextrin **22** provides the best balance between flexibility of the substituent to attain an optimum geometry for the reaction with the ester **48**, and self-inclusion of the substituent which may inhibit complexation of the ester **48** within the annulus. That inclusion of the ester **48** is involved in its reactions with amino-cyclodextrins is shown below.

In comparison, carrying out the same calculation for the "free" amines $NH_2(CH_2)_xNH_2$

gives first order rate constants of 0.006 s^{-1}, 0.057 s^{-1} and 0.83 s^{-1} for x = 2, 3 and 6, respectively. The apparent increase in reactivity, as the length of the alkyl chain is increased, is most likely due to the increase in the relative reactivity of the mono-protonated species of these amines. As the length of the alkyl chain is increased there will be less tendency for the sharing of the extra proton between the terminal nitrogens of the mono-protonated amine and so there will be an increase in the nucleophilicity of one of the nitrogens.

3.3.3. Product studies

The reaction of the ester **48** with the aminoalkylamino-cyclodextrins **21**, **22** and **24** generates stable acetamides as reaction products (Scheme 3.3). Acetylation may occur at either the primary or the secondary nitrogen. In order to determine the site of the acylation on the ω-aminoalkylamino-cyclodextrins, authentic samples were required to be synthesised, characterised and compared with the products observed in the reactions described above.



Scheme 3.3 Possible formation of isomeric acetylated aminoalkylamino-cyclodextrins from the reaction between the ester **48** and the cyclodextrins **21**, **22** and **24** at pH 9.1.

Each of the cyclodextrins **21**, **22** and **24** was treated with one equivalent of the ester **48** in *N,N*-dimethylformamide (DMF) at room temperature. When the reactions were complete, as determined by thin-layer chromatography (TLC), the yellow solutions were acidified to pH 1 with dilute acid and extracted with dichloromethane to remove most of the 4-nitrophenol **49** formed as a result of the trans-acylation reaction. The cyclodextrin products were precipitated by addition of ethanol and were further purified by passage of an aqueous solution of the crude product through a cation-exchange resin (BioRex 70) in its ammonium form and further elution with water. This process rendered the mono-acetylated products as the hydrochloride salts.

A single product was obtained from each reaction as indicated by TLC, which showed that each of the acetylated products that had been isolated was a single spot (R_c 1.1). Each of the products obtained in this manner gave a satisfactory elemental analysis and the molecular ion corresponding to the mono-acetylated product was the major ion observed in the electrospray mass spectrum. There was no evidence for the formation of any di-acetylated materials.

The 75.5 MHz ^{13}C NMR spectra confirmed the presence of the acetyl group in each of the products with signals at around δ 176 and δ 25 for the carbonyl and the methyl carbons of the acetyl group, respectively, but there was little change in the signals for the carbons of the rest of the molecule compared to those of the starting ω-aminoalkylamino-cyclodextrin. Therefore, it was not possible to determine which of the two nitrogens had been acetylated in these reactions from these spectra.

In contrast to the ^{13}C NMR spectra, the 300 MHz ^{1}H NMR spectra of these derivatives show that the primary nitrogens of the aminoalkylamino-cyclodextrins **21**, **22** and **24** are acetylated under the conditions described above to give the acyl derivatives **53-55**, respectively. In D_2O at pH 9 the triplet resonance for the methylene protons attached to the primary amino group of the starting diamine (identified by ^{1}H-^{13}C correlation spectroscopy) is shifted about 0.6 ppm downfield to around δ 3.2 in the acetylated products. The positions of the resonances for the protons on $C6^A$ and those of the methylene group attached to the secondary nitrogen are unchanged on acetylation. If the acetylation was to occur on the secondary nitrogen, attached to $C6^A$, then it will affect the chemical shift of the $C6^A$ protons as

well as those of the attached methylene group of the aminoalkyl chain. Thus, in solution in DMF, the reaction of the ester **48** with the ω-aminoalkylamino-cyclodextrins **21**, **22** and **24** gives the mono-acetylated products **53-55**, respectively, and not the isomeric acetamides **56-58**.

A temperature effect was noticed in the 300 MHz ^{1}H NMR spectra of the amide **54** in D_2O at pH 9. At room temperature, the spectrum showed only broad peaks and was poorly resolved. The signal due to the methyl protons of the acetyl group appeared as a doublet. The spectrum recorded at 50 °C, however, was well resolved and the methyl protons of the acetyl group gave rise to a sharp singlet. This may be due to a slow (on the NMR time-scale) exchange process, perhaps involving self-inclusion of the acetylated substituent within the cyclodextrin annulus. Why this is observed only with the 3-aminoalkyl derivative **54** and not the other two derivatives, prepared in the same manner, is not known.

The reaction in DMF may not involve the inclusion of the ester **48** which occurs under aqueous conditions (as discussed below) and this may affect the position of acetylation, so further analysis of the products of the reaction carried out under aqueous conditions was required in order to determine the position of reaction under aqueous conditions. It had been possible to examine the products of the reaction between the ester **48** and the mono-amino cyclodextrin **41** by comparison of the retention times of the reaction products with those of an independently prepared acetamide using high pressure liquid chromatography (HPLC).[160] It was not possible to follow the reactions of the amino-cyclodextrins **21**, **22** and **24** in this way as no solvent/column system could be found to give good separation between these compounds and the corresponding acetamides. However, the starting amines were able to be separated from the product acetamides by TLC so this technique was used to analyse the aqueous reactions between the ester **48** and the cyclodextrins **21**, **22** and **24**.

Small-scale reactions were carried out by adding one equivalent of the ester **48** to a solution of the amine **21**, **22** or **24** (2.2×10^{-6} mol dm^{-3}) in 0.05 mol dm^{-3} borate buffer pH 9.1 and stirring the resultant solution at room temperature until all of the ester had been consumed (TLC). Comparison of the cyclodextrin products of these reactions with the

previously prepared acetamides **53-55** by TLC indicated that the same acetamides are produced under aqueous conditions as when the reaction is carried out in DMF as solvent. It should be noted, however, that as there is no difference in the R_c values for the different acetamides **53-55**, and no authentic products of acetylation at the secondary nitrogen were available for comparison (these may have the same R_c values as well) this can only be a tentative assignment of the products of the aqueous reactions.

The products of the reaction between the ester **48** and the 6-aminohexylamino-cyclodextrin **24** were examined further by 300 MHz ^{1}H NMR spectroscopy. The crude reaction mixture from the reaction between the cyclodextrin **24** and the ester **36**, both at a concentration of 2.4×10^{-3} mol dm^{-3}, in 0.05 mol dm^{-3} borate buffer pH 9.1 was freeze-dried and the residue was dissolved in D_2O. This procedure was repeated for the reaction between the cyclodextrin **24** and 3-nitrophenyl acetate **52**.

Both solutions contained a similar mixture of products and unreacted cyclodextrin **24** as shown by TLC. The 300 MHz ^{1}H NMR spectra of these two solutions are too complex to clearly show which of the nitrogens had been acylated due to the mixture of cyclodextrins present in the solution. Two resonances due to acyl methyl groups can be seen at around δ 2.1 and δ 1.9 in a ratio of 1:2.7 and 3.3:1 for the products of the reaction with the esters **48** and **52**, respectively. Treatment of each solution with sodium hydroxide to give a pH $\geq$ 12 caused the resonance at δ 2.1 to disappear, suggesting that this signal is due to an *O*-acyl group, which is readily hydrolysed. After this treatment, the spectra became less complicated and it was possible to see the resonances of the protons of the acetamide **55** over the top of those due to the protons of the starting cyclodextrin **24**. Comparison of the integrations of the signals due to the acetyl groups with the integration of the signal due to the protons H1 allowed the determination of the relative amounts of the cyclodextrins in the crude products from these reactions (Table 3.5).

The reactions of the cyclodextrin **24** with the esters **48** and **52** in water at pH 9.1 produce mixtures of *O*- and *N*-acetylated products and unchanged starting material. The *O*-acylated products are most likely formed by reaction of a secondary hydroxyl group with the

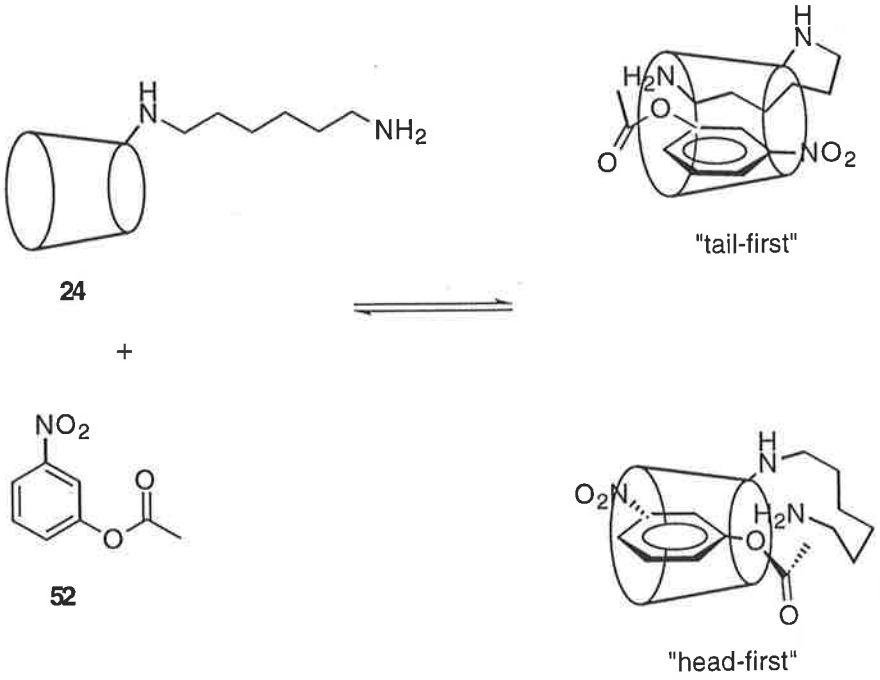
esters **48** and **52** in analogy with the reactions of these esters with β-cyclodextrin **2**.[50-54] It is surprising that this reaction is so competitive with that occurring at nitrogen, given that at pH 9.1 there is less than 0.1% of the total species with a deprotonated hydroxyl group (pK_a =12[17]) compared with 6.3% of total species with non-protonated amine groups.

Table 3.5. Percentages of the components of the crude mixtures from the reactions of the cyclodextrin **24** with the esters **48** and **52** in 0.05 mol dm^{-3} borate buffer at pH 9.1 (I = 0.1 mol dm^{-3}).[a, b]

ester	**24**	**55**	*O*-acetylated products
48	27	54	20
52	26	17	57

[a]Initial concentration of the reactants is 2.4×10^{-3} mol dm^{-3}. [b] Calculated from the relative integrations of the resonances at δ 1.94, 2.11 and 5.07.

The *N*-acetylated product **55** is formed by the reaction of the primary amino nitrogen of the cyclodextrin **24** with the esters **48** and **52**. The formation of the acetamide **55** in the reaction with the ester **52** is in contrast with the earlier report for the reactions of the ester **52** with the amino-cyclodextrin **41**, where no *N*-acetylated products were observed.[160] The lack of any *N*-acylated products in this reaction had ~~lead~~ iect to the conclusion that the ester **52** was only able to include within the annulus such that the acetyl group was located at the secondary face of the cyclodextrin annulus. The formation of the acetamide **55** in the reaction between the ester **52** and the cyclodextrin **24** may be due to the reaction of a self-included 6-aminohexylamino chain, with the ester **52** being included in a "tail-first" manner (Scheme 3.4). This complex would be similar to that found for the complex formed between 4-methylbenzoate **37** and the cyclodextrin **24** described in the previous chapter. Alternatively, the acetamide **55** may be formed in the reaction of a "head-first" included ester **52** with a non-included primary nitrogen group. If the latter case is the correct one, then the formation of an *N*-acetylated product in the reaction of the ester **52** with the cyclodextrin **24** but not with the cyclodextrin **41** is due to the increased flexibility of the 6-aminohexylamino substituent of the cyclodextrin **24**.

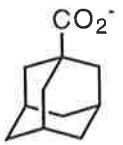


Scheme 3.4. Schematic representation of two possible modes of inclusion of the ester **52** in the cyclodextrin **24** in aqueous solution leading to formation of the acetamide **55.**

Under similar reaction conditions as described above, the first order rate (k_{obs}-k_o) for the reaction of the ester **48** with β-cyclodextrin **2** at pH 9.1 is 3.4×10^{-4} s^{-1}, which is 0.37 times the rate observed for the reaction with the cyclodextrin **24**. The amount of *O*-acetylated material formed in the reaction between the cyclodextrin **24** and the ester **48** is 0.37 times that of the *N*-acetylated product formed. This suggests that the product ratios determined above reflect a 1:1 ratio of "head-first" to "tail first" inclusion complexes of the ester **48** in the annulus of the cyclodextrin **24** as the rate of the reaction of the ester **48** with a secondary hydroxyl group of the cyclodextrin **24** is the same as that for the reaction with β-cyclodextrin **2**. Thus, the cyclodextrin **24** acts as a kinetic probe for the determination of the populations of the various reactive inclusion modes of the ester **48** within the annulus of this cyclodextrin.

3.3.4. Inhibition studies

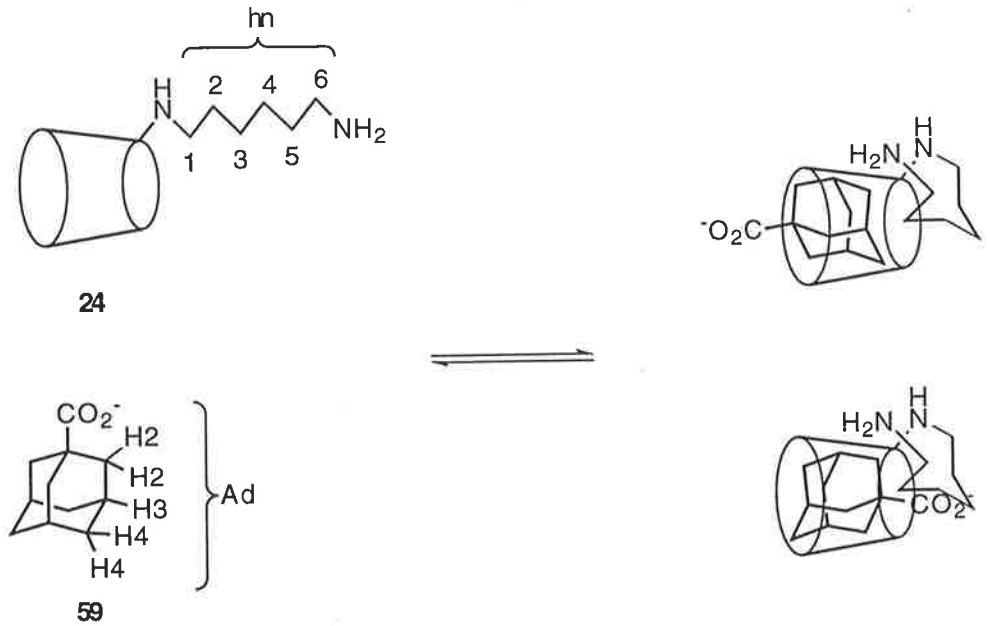
If inclusion of the esters **48** and **52** within the annulus of the cyclodextrin is important in the trans-acylation reaction with the ω-aminoalkylamino-cyclodextrins **21**, **22** and **24**, then this reaction will be inhibited in the presence of a non-reactive compound which is able to include in the cyclodextrin annulus. Depending on the relative binding constants of the ester **48** and the added "competitive inhibitor" the reaction would be slowed or, in the case of an inhibitor with an extremely high binding constant, stopped completely. A commonly used inhibitor of this type of reaction is adamantane-1-carboxylate **59** which forms an extremely stable complex with β-cyclodextrin **2** ($K = 1.8 \times 10^4$ dm^3 mol^{-1} at pH 8.5).[161] Adamantane-1-carboxylate **59** was found to be an effective inhibitor of the reactions of ester **48** with the cyclodextrins **2** and ~~52~~ 41.[160] The formation constants for the complexes formed between ester **48** and these cyclodextrins was determined to be around 100-200 dm^3 mol^{-1}.



59

When the reaction between the ester **48** and the cyclodextrins **21**, **22** and **24** were carried out as described above in the presence of 0.5 equivalents of the carboxylate **51** the calculated first order rate constants (k_{obs}-k_o) were ~~4.24~~ 4.44 × 10^{-3} s^{-1}, ~~1.98~~ 2.19 × 10^{-3} s^{-1} and ~~0.716~~ 0.916 × 10^{-3} s^{-1} for the cyclodextrins **21**, **22** and **24**, respectively. In the presence of two equivalents of the carboxylate **59**, the first order rate (k_{obs}-k_o) for the reaction between the ester **48** and the cyclodextrin **22** was ~~0.463~~ 0.663 × 10^{-3} s^{-1}. Thus, adamantane-1-carboxylate **59** acts as a competitive inhibitor of the trans-acylation reaction between ester **48** and the cyclodextrins **21**, **22** and **24**, and this reaction involves the formation of a substrate-host complex prior to the transfer of the acetyl group from the ester to the primary nitrogen of the ω-aminoalkylamino-substituent. That the reaction is not stopped quantitatively by the addition of the guest **59** suggests that some reaction is occurring outside of the annulus through a normal S_N2 process.

The complex formed between the cyclodextrin **24** and the carboxylate **59** was examined by NMR spectroscopy. The 600 MHz 2D-ROESY spectrum of a solution containing the cyclodextrin **24** and the carboxylate **59** both at 0.06 mol dm^{-3} in D_2O at pH ≥ 12 clearly shows that the adamantyl group is included within the annulus of the cyclodextrin (Figure 3.3). It was not possible to specifically assign the annular protons H3 and H5 to particular resonances in the region δ 3.4-3.7 but, generally, the resonances due to the protons H3 are found downfield of those due to the protons H5 (but not the proton $H5^A$). There are intense cross-peaks between the resonances due to the adamantyl protons and the annular protons H3 and H5. Only weak cross-peaks are seen for the NOE interactions between the protons of the 6-aminohexyl substituent and the annular protons (mainly) H5 suggesting that the substituent is not deeply included within the annulus of the cyclodextrin moiety. The strong NOE interactions between the protons hnH1 and hnH6 suggest that these methylene groups are held close together in the complex. There are no cross-peaks between any resonances due to the adamantyl protons or those of the protons of the 6-aminohexyl substituent. The substituent may be coiled around the primary face of the cyclodextrin **24**.



Scheme 3.5. Schematic representation of the inclusion of adamantane-1-carboxylate **59** in the cyclodextrin **24** in aqueous solution at pH ≥ 12.

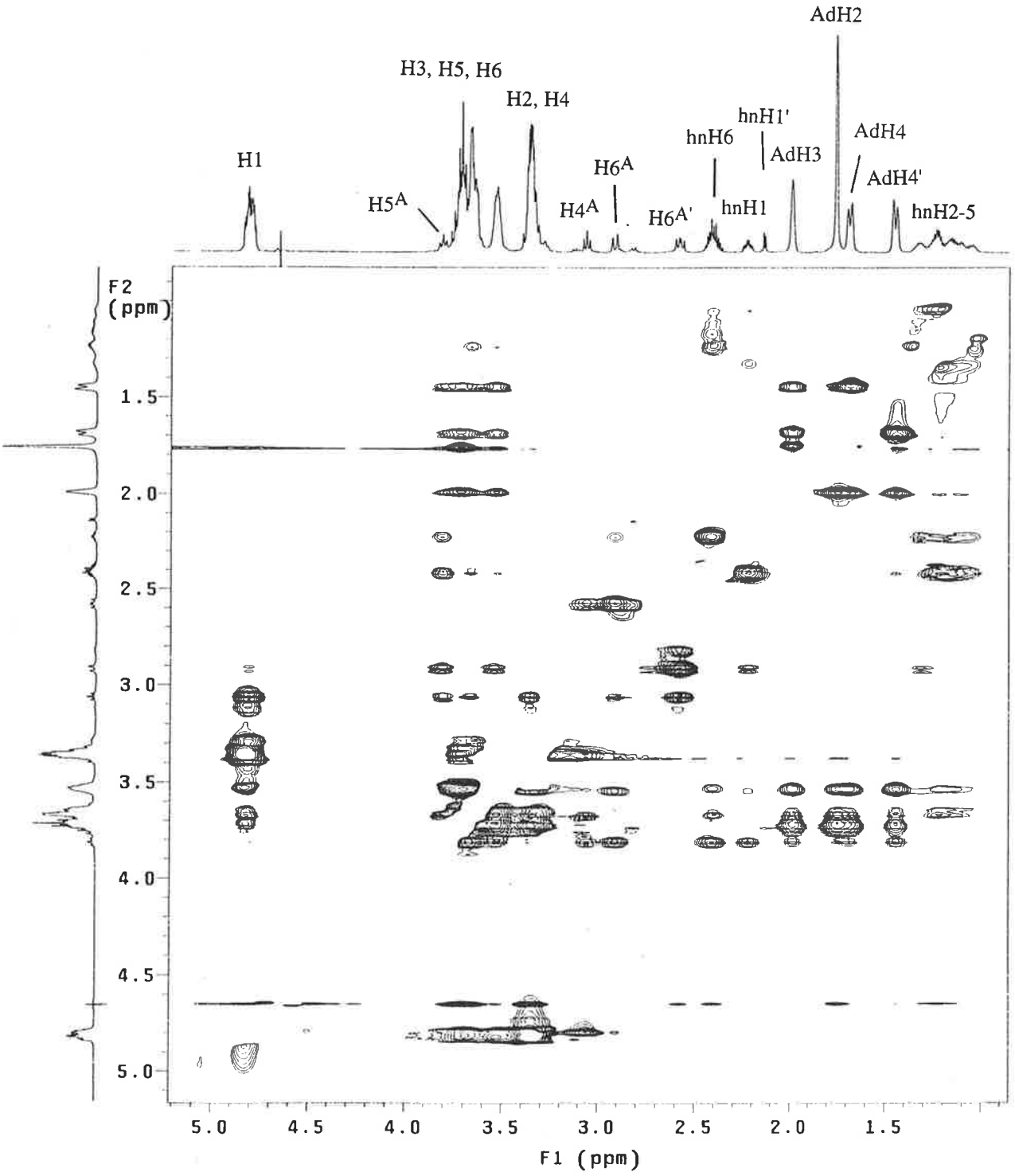


Figure ~~3.4~~ 3.3. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} each of cyclodextrin **24** and adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 3.5.

3.4. Conclusion

The reaction of the cyclodextrin **30** with 4-nitrophenyl acetate **48** is inhibited by the addition of Zn(II) ion to the reaction mixture. The reaction between the cyclodextrin **31** and the ester **48** is marginally enhanced in the presence of Zn(II) at neutral pH but is inhibited by Zn(II) at higher pH. The rate enhancement is most likely due to the increased polarisation of the carbonyl bond of the ester in the presence of Zn(II) and not due to the formation of a metallo-cyclodextrin hydroxy species.

The reactions of the ω-aminoalkylamino-cyclodextrins **21**, **22** and **24** with the esters **48** and **52** involve the nucleophilic attack of the primary nitrogen on the ester carbonyl to give *N*-acetylated derivatives. The site of reaction was confirmed by comparison of the reaction products with authentic samples of the amides **53-55** prepared by an independent synthesis. The non-protonated ω-aminoalkylamino-cyclodextrin species is the major reactive species in the trans-acylation reaction with the ester **48** as shown by pH dependence studies for the reaction of the cyclodextrin **24**.

The reactions of the cyclodextrin **24** with the esters **48** and **52** lead to both *N*- and *O*-acetylated products. These reactions involve the prior inclusion of the esters **48** and **52** within the cyclodextrin annulus and indicate that both "head-first" and "tail-first" inclusion may occur. The product ratios for the reaction between the cyclodextrin **24** and the ester **48** are in accord with the ratio of the rates for the reactions at a secondary hydroxyl and at the primary nitrogen of the substituent of the cyclodextrin **24**.

The addition of adamantane-1-carboxylate **59** to these reactions inhibits the trans-acylation by competitive inhibition. The inhibition is not quantitative as some trans-acylation is occurring outside of the annulus by an S_N2 attack of a primary nitrogen on a non-included ester. 2D-ROESY spectroscopy confirms that the adamantyl group is included within the cyclodextrin annulus of the cyclodextrin **24** at pH ≥ 12.

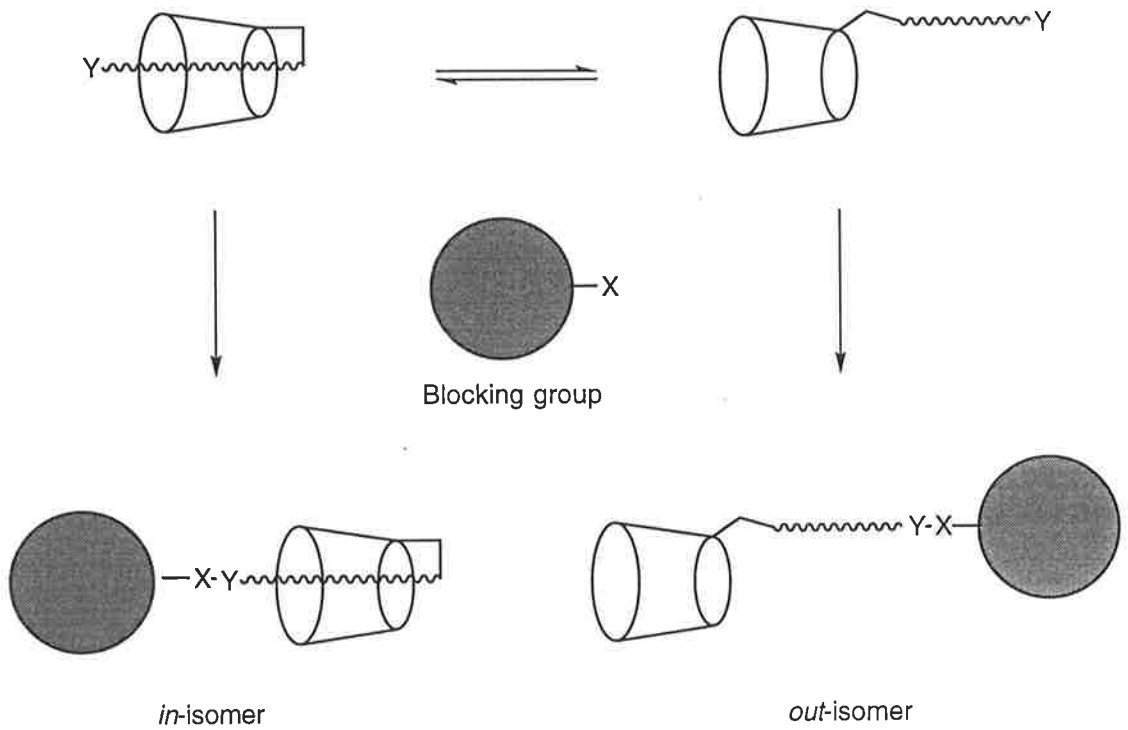
Chapter 4: Self-inclusion Complexes: An Approach to Molecular Knots

4.1. Introduction

The addition of a short hydrophobic substituent to a cyclodextrin increases the stability of the host-guest complexes formed with small aromatic guests over that found for the unsubstituted cyclodextrin (Chapter 2). However, the self-inclusion of hydrophobic substituents within the annulus of a cyclodextrin has been reported previously to limit the ability of larger guests to be included by the cyclodextrin and to result in the total exclusion of the guest in some cases.[92] A recent report described an attempt to limit the self-inclusion of ω-aminoalkylamino substituents attached to β-cyclodextrin **2** through *tert*-butyloxycarbonylation of the ω-nitrogen.[137] The resultant "Cup and Ball" molecules showed a decreased tendency for the inclusion of the substituent relative to that of the parent ω-aminoalkylamino-cyclodextrin but the self-inclusion was still evident. If a group that was larger than *tert*-butyl was attached to the end of the substituent, then the self-inclusion of the substituent may not be possible at all. Alternatively, the attachment of a large group to the end of a self-included hydrophobic chain covalently bound to a cyclodextrin raises the possibility of preparing novel derivatives of cyclodextrins which may be considered to be molecular knots.[162] If a sufficiently large group can be attached to the terminus of such a self-included chain, then it will prevent the dethreading of the chain. The chain will be knotted through the cyclodextrin, held in place by mechanical (steric) forces (Figure 4.1).

If an equilibrium exists between self-inclusion and non-inclusion of a substituent within the annulus of the cyclodextrin moiety then two isomeric products may be formed. The *in*-isomer (a molecular knot) will be produced when the substituent chain is included within the annulus of the cyclodextrin moiety while undergoing the reaction with the blocking group. The

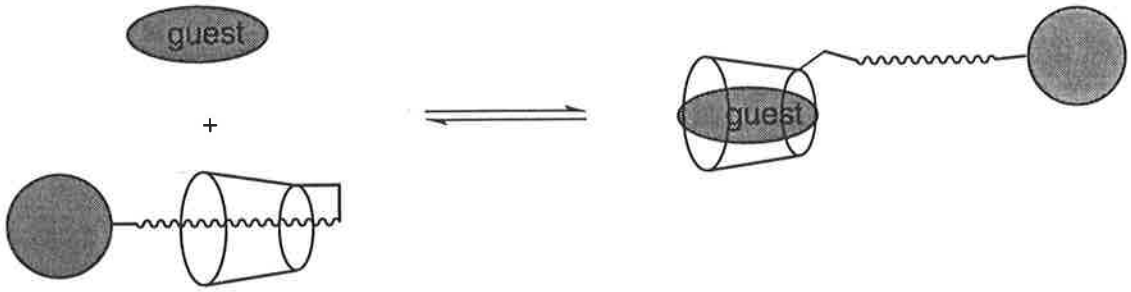
out-isomer will be formed when the reaction occurs while the substituent chain is located outside the annulus. Provided that the blocking group is sufficiently large to prevent its passage through the cyclodextrin, these isomers are not interconvertible. However, if the blocking group can pass through the cyclodextrin then the two isomers are interconvertible and represent two components of an equilibrium. The *in*-isomer is simply a self-included conformer of the cyclodextrin derivative.



Scheme 4.1. Proposed formation of a molecular knot. Y and X represent two reactive functional groups which can react with each other to form a covalent bond.

These two isomers may be differentiated using 2D-ROESY NMR spectroscopy to examine the competition for inclusion within the annulus between the substituent and an added guest molecule. The 2D-ROESY spectrum of the product of such a reaction will show the NOE interactions between the protons of the substituent and the annular protons H3 and H5, if they are included within the annulus. This will not differentiate between self-inclusion and a mechanically held knot. One test for knot formation would be the inability of a molecule, known to be a strong binder in the cyclodextrin annulus, to displace the chain and blocker from

the annulus (Scheme 4.2). If such a displacement occurs it will result in observable NOE interactions between the protons of the guest molecule and the annular protons H3 and H5 of the cyclodextrin, and the diminution, or loss, of the NOE interactions between those of the chain or blocking group and the annular protons H3 and H5. This behaviour is that of a self-included substituent. Should no NOE interactions between the protons of the added molecule and the annular protons be observed in the 2D-ROESY spectrum, this indicates that the self-inclusion of the substituent is more favoured than inclusion of the added molecule and may indicate the formation of a knot.



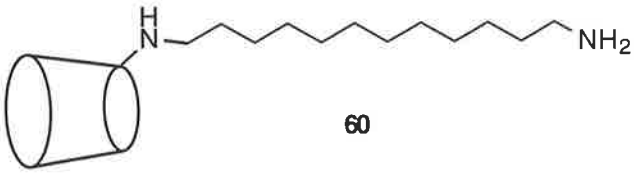
Scheme 4.2. Competitive host-guest chemistry. Displacement of a substituent from the annulus by an added guest molecule indicates that the substituent is simply included within the annulus.

A suitable molecule to use as a competitive guest to examine the effects of size and hydrophobicity on the self-inclusion of a substituent is adamantane-1-carboxylate **59**, which was used as a competitive inhibitor in the work discussed in the previous chapter. It was shown that the self-included 6-aminohexyl chain of the cyclodextrin **24** was readily displaced from the annulus in the presence of this guest. Other workers have shown that once the guest **59** is included within the annulus there is no room for further complexation of additional molecules, including water.[161] The formation constant, K, for the complex between adamantane-1-carboxylate **59** and β-cyclodextrin **2** is 1.8×10^4 dm^3 mol^{-1} at pH 8.5.[161] It was decided to use the carboxylate **59** as a probe to determine whether or not a self-included substituent could be displaced from the annulus.

4.2. Self-inclusion of hydrophobic substituents

4.2.1. Effect of increasing the length of the alkyl chain

It was expected that increasing the length of the alkyl chain linking the two nitrogens of an ω-aminoalkylamino substituent would increase the hydrophobicity of the substituent and hence the tendency for self-inclusion of the substituent. The 12-aminododecylamino substituted cyclodextrin **60** was prepared in order to examine its host guest chemistry with the carboxylate **59**.



60

Reaction of the mono-tosylate **32** with 1,12-diaminododecane in 1-methyl-pyrrolidin-2-one (NMP) under the standard conditions described in Chapter 2 gave the cyclodextrin **60** in 43% yield. This product was surprisingly soluble in water at pH 9. The water solubility of the aminoalkylamino-substituted cyclodextrins decreased as the number of carbons in the alkyl chain of the substituent increased from 2 to 6 and it was expected that the twelve carbon chain of the cyclodextrin **60** might limit the water solubility of this compound, particularly as its behaviour on TLC (R_c = 0.90) implied that it was considerably more non-polar than the 6-aminohexyl-amino derivative **24** (R_c = 0.75). Despite this, aqueous solutions of the cyclodextrin **60** at a concentration of 0.06 mol dm^{-3} were readily prepared over the full range of pH.

The 600 MHz ^{1}H NMR spectrum of the cyclodextrin **60** at pH ≥ 12 was poorly resolved by comparison with the ^{1}H NMR spectra of the cyclodextrin derivatives with shorter

alkyl chains in the substituent discussed in Chapter 2, perhaps due to the slow tumbling time of this larger derivative. Only the triplet resonance due to the protons ddnH12 was well resolved. This may be due to an increased freedom of movement towards the end of the dodecyl chain.

The 600 MHz 2D-ROESY spectrum of a solution of the cyclodextrin **60** at pH ≥ 12 shows that the 12-aminododecylamino chain is included within the annulus of the cyclodextrin moiety (Figure 4.1) It was not possible to assign the annular protons H3 and H5 to specific resonances in the region δ 3.4-3.7 but, generally, the resonances due to the protons H3 are found downfield (δ ~3.6) from those due to the protons H5 (δ ~ 3.4). The resonance due to the proton $H5^A$ occurs around δ 3.7 (dependent on pH). There are intense cross-peaks between the resonances due to the protons ddnH2-ddnH11 and those due to the annular protons H3 and H5. One of the diastereotopic protons ddnH1 shows a strong NOE interaction with $H5^A$ while the protons ddnH12 appear to have a strong NOE interaction with H3 and little else. This suggests that the 12-aminododecylamino substituent passes through the annulus in such a fashion that the primary amino group is located towards the secondary face of the cyclodextrin moiety.

In contrast to the observations with the 6-aminohexylamino derivative **24** at pH 1, the 2D-ROESY spectrum of the cyclodextrin **60** at pH 1 shows that the 12-aminododecylamino substituent remains partially included within the annulus of the cyclodextrin moiety. Although they are considerably weaker in intensity than those observed for the spectrum recorded at pH ≥ 12, there are cross-peaks between the resonances due to the protons ddnH3-ddnH10 and those due to the annular protons H5 and, to a lesser extent, the protons H3. There are no NOE interactions between the protons ddnH12 and the annular protons H3 and H5. The intra-chain interactions are more evident in the spectrum recorded at pH 1 than that recorded at pH ≥ 12. This suggests that the protonated amino groups are positioned away from the annulus so that these positively charged groups are solvated by water while the middle part of the hydrophobic chain of the substituent sits within the rim of the primary face of the cyclodextrin moiety.

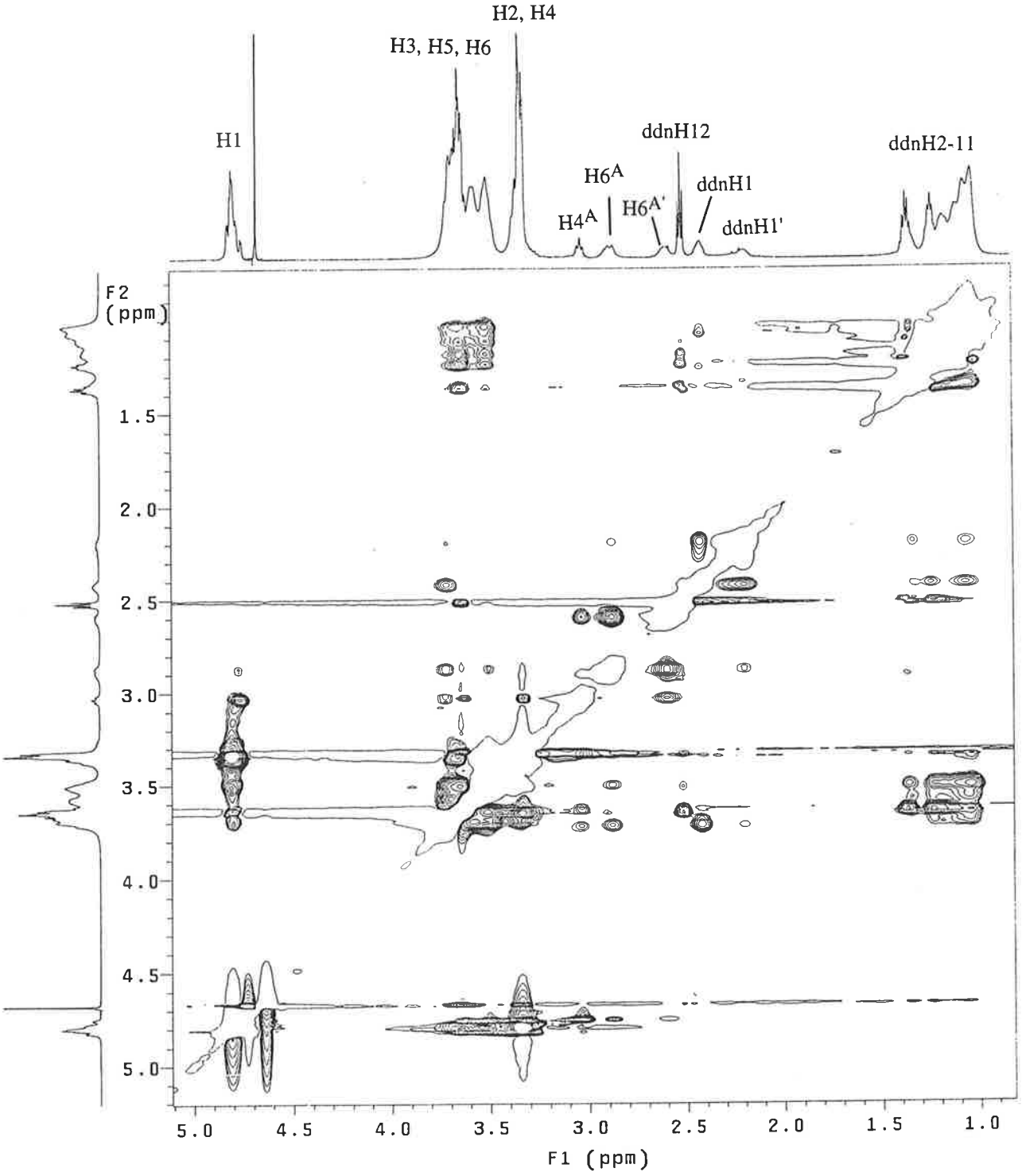
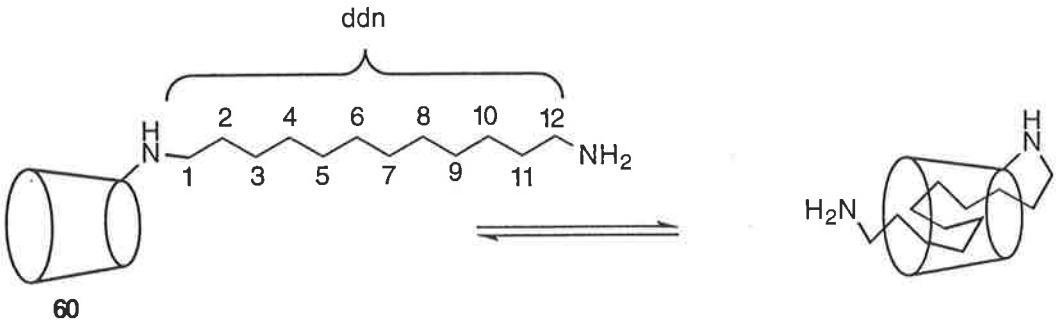


Figure 4.1. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} of the cyclodextrin **60**. The protons are labelled as shown in Scheme 4.3.



Scheme 4.3. Schematic representation of the self-inclusion of the substituent of the cyclodextrin **60** in aqueous solution at pH ≥ 12.

When one equivalent of 4-methylbenzoate **37** was added to a solution of the cyclodextrin **60** in D_2O at pH ≥ 12, no complexation of the guest **37** was observed. In contrast, addition of one equivalent of the carboxylate **59** to a solution of the cyclodextrin **60** in D_2O at pH ≥ 12 causes most of the 12-aminododecylamino chain to be pushed out of the annulus of the cyclodextrin moiety as the adamantyl group is included within the annulus. The 600 MHz 2D-ROESY spectrum of this solution shows that the adamantyl protons have NOE interactions with the annular protons H3 and H5 (Figure 4.2). There are also weak NOE interactions between the protons ddnH3-ddnH10 and the annular protons H5 suggesting that the middle part of the 12-aminododecylamino chain remains inside the primary face of the cyclodextrin moiety. There are no NOE interactions between the adamantyl protons and those of the 12-aminododecylamino chain.

Thus, adamantane-1-carboxylate **59** can displace the substituent from the annulus of the cyclodextrin **60** to some extent. However, the increased hydrophobicity of the dodecyl chain allows it to compete with the adamantyl group for binding in the annulus. This is in contrast to the observations made with the complex formed between the cyclodextrin **24** and adamantane-1-carboxylate **59**, where the 6-aminohexyl substituent appeared to be completely excluded from the annulus by the inclusion of the guest **59**. While it was not possible to assign the resonances in the region δ 3.2-3.7 to specific protons it appears that the adamantyl protons are interacting more strongly with the annular protons H3 than the protons H5 while the protons of the substituent appear to be interacting more strongly with the annular protons H5 than the

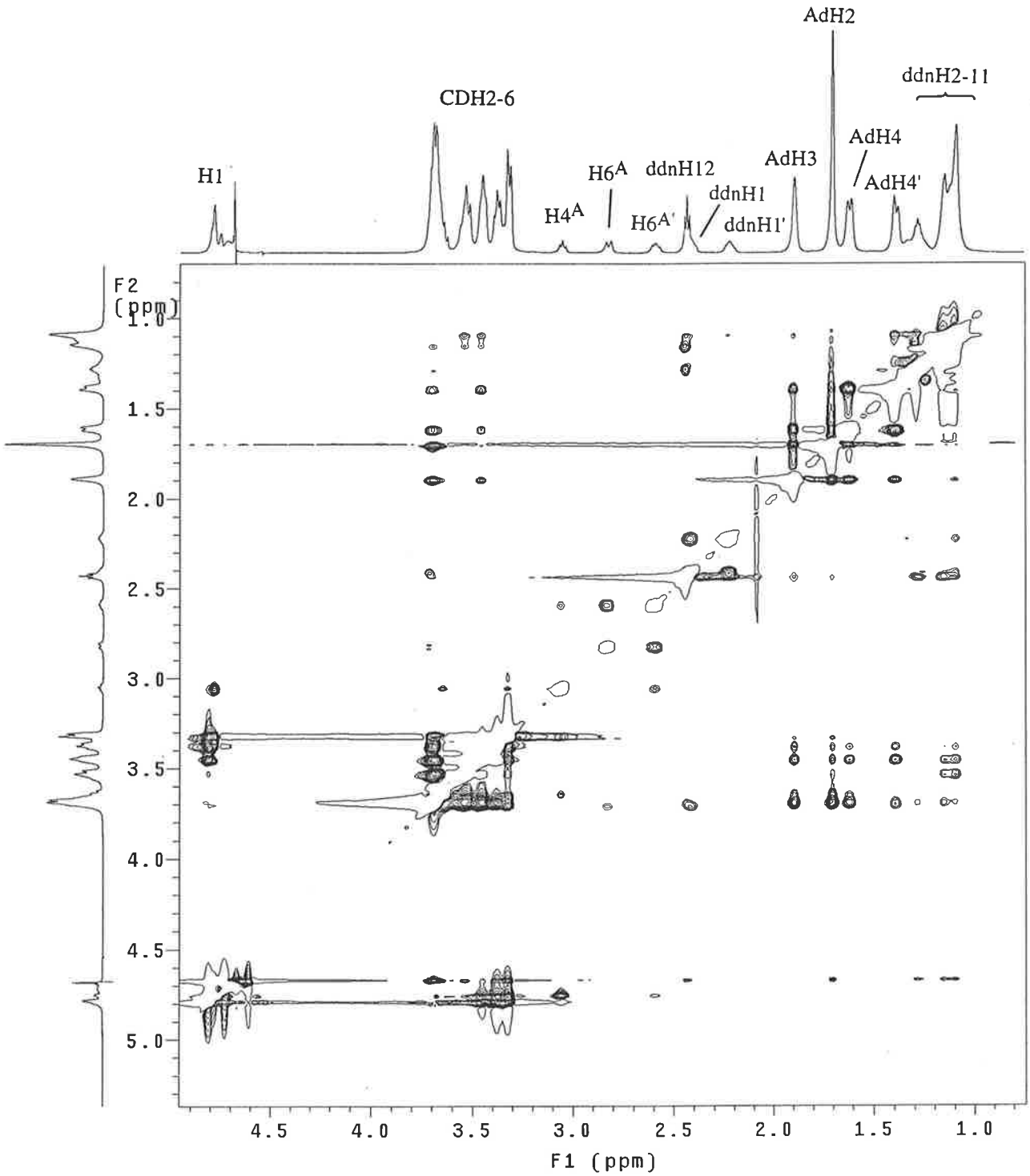
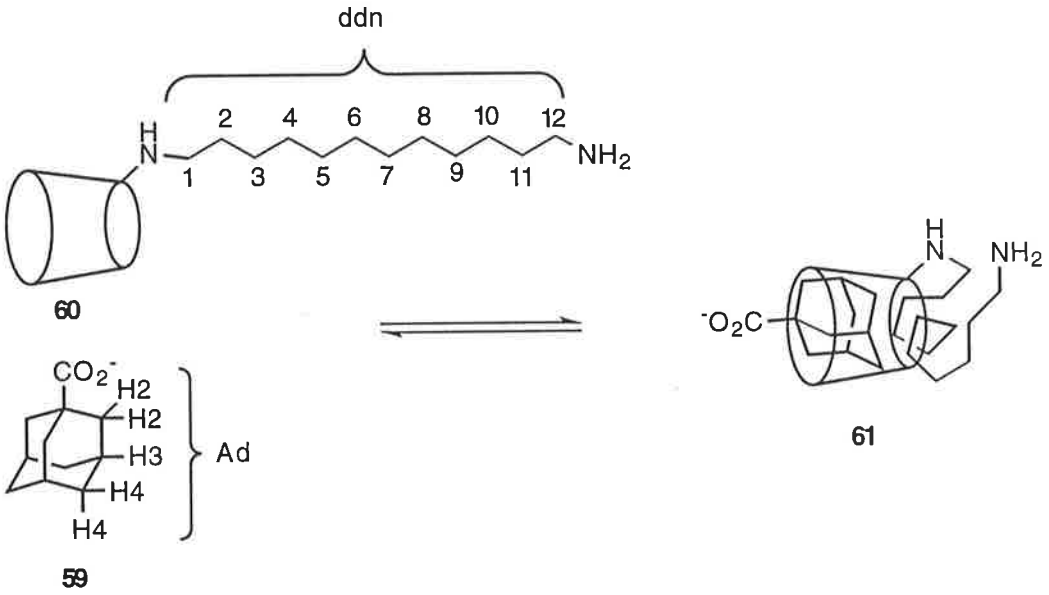


Figure 4.2. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~×10⁻³~~ mol dm^{-3} each of the cyclodextrin **60** and adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 4.4.



Scheme 4.4. Schematic representation of the inclusion of adamantane-1-carboxylate **59** in the cyclodextrin **60** in aqueous solution at pH ≥ 12.

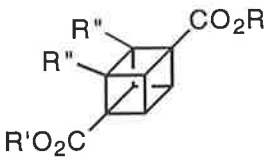
protons H3. This suggests that there is only a shallow inclusion of the adamantyl group of the guest **59**, with the dodecyl substituent sitting around the primary end of the annulus. The lack of NOE interactions between the adamantyl protons and those of the alkyl chain implies that these protons are further than 4 Å apart in the complex **61** that is formed between the guest **59** and the cyclodextrin **60**.

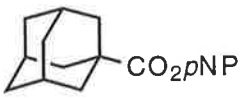
The 2D-ROESY spectrum of this solution represents a picture of the dynamic equilibrium that exists in this solution. It is possible that if a fast exchange process was occurring between the self-inclusion of the substituent and the total exclusion of the substituent on formation of a complex with the guest **59**. The NOE interactions between the protons of the guest **59** and the protons of the alkyl substituent with the annular protons would both give rise to cross-peaks between the resonances of the interacting protons. If this were the case, then the relative intensities of the cross-peaks due to the species where the substituent is fully included within the annulus would be the same as are observed in the absence of the guest **59**. This is not the case for the spectrum shown in Figure 4.2, where there are differences in the relative intensities of the cross-peaks due to NOE interactions between the methylene protons of the

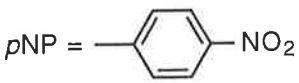
substituent and the protons H3 and the protons H5 which were not observed in the spectrum shown in Figure 4.1. It appears that the 2D-ROESY spectrum shown in Figure 4.2 is consistent with the formation of the complex **61** as the major species present in solution.

4.2.2. Effect of the addition of a bulky substituent

The reactions of 4-nitrophenyl acetate **48** with ω-aminoalkyamino-cyclodextrins occur with inclusion of the 4-nitrophenyl group within the annulus of the cyclodextrin moiety and involve acylation of the primary nitrogen (Chapter 3). It was believed that if the 4-nitrophenyl ester of a bulky acid was allowed to react with an ω-aminoalkylamino-cyclodextrin then the resultant ω-amidoalkylamino-cyclodextrin chain may be strongly self-included within the annulus of the cyclodextrin. A series of amido-cyclodextrins bearing cubanyl or adamantyl groups at the end of the linking chain were prepared by the reaction of the cyclodextrin **24** with the 4-nitrophenyl esters of the appropriate acids.







62 R = R" = H; R' = Me
63 R = H; R' = R" = Me
64 R = *p*NP; R' = Me; R" = H
65 R = *p*NP; R' = R" = Me

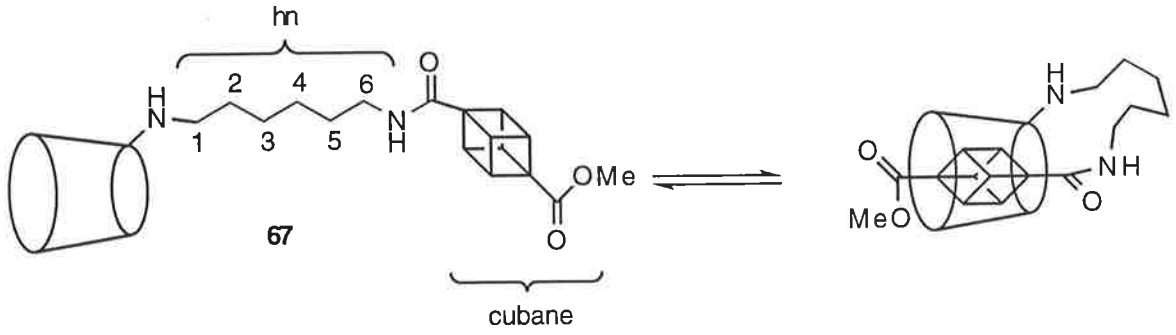
66

The cubane esters **64** and **65** were prepared by treating the corresponding acids **62** and **63** with one equivalent each of 4-nitrophenol **49** and dicyclohexylcarbodiimide (DCC) in dichloromethane at room temperature.[157] (The acids **62** and **63** were a gift from Dr. J. Tsanaksidis.[163 -168]) The pure esters **64** and **65** were obtained in 50% and 95% yields, respectively, after a standard work-up followed by purification by either flash

chromatography[169] or squat column chromatography.[170, 171] The adamantyl ester **66** was prepared from the corresponding acid **59** in 90% yield by the same method.

When the ester **64** was added to a solution of the 6-aminohexylamino-substituted cyclodextrin **24** in DMF, the hydrochloride salt of the cubane amide **67** was obtained in 51% yield after precipitation with 1:1 ethanol/ether, followed by an acidic work-up and treatment with a weak anion-exchange resin (AG4-X4, BioRad Laboratories). The isolated product gave a molecular ion at *m/z* 1421 with electrospray-ms and gave a satisfactory elemental analysis.

The 300 MHz ^{1}H NMR spectrum of the cyclodextrin amide **67** shows sets of multiplets near δ 4.1 due to the cubanyl protons. Resonances for the protons hnH6, adjacent to the amido nitrogen, overlap with those due to H4^A and H6^A but the diastereotopic protons hnH1, adjacent to the secondary nitrogen, give rise to separate signals at δ 2.6 and δ 2.4 indicating that the alkyl chain of the substituent is held in a rigid conformation. The 75 MHz ^{13}C NMR spectrum shows signals due to the two carbonyl carbons at δ 176.5 and δ 175.6.



Scheme 4.5. Schematic representation of the self-inclusion of the cubanyl substituent of the cyclodextrin amide **67** in aqueous solution at pH ≥12.

The 600 MHz 2D-ROESY spectrum of the cyclodextrin amide **67** in D_2O at pH ≥ 12 shows that the cubanyl group is included within the annulus of the cyclodextrin (Figure 4.3). Strong NOE interactions are observed between the resonances of the cubanyl protons and the annular protons H3 and H5. No cross-peaks are observed between the resonances due to the protons of the alkyl chain (hnH1-hnH6) and the annular protons H3 and H5, indicating that the alkyl chain is not included within the annulus.

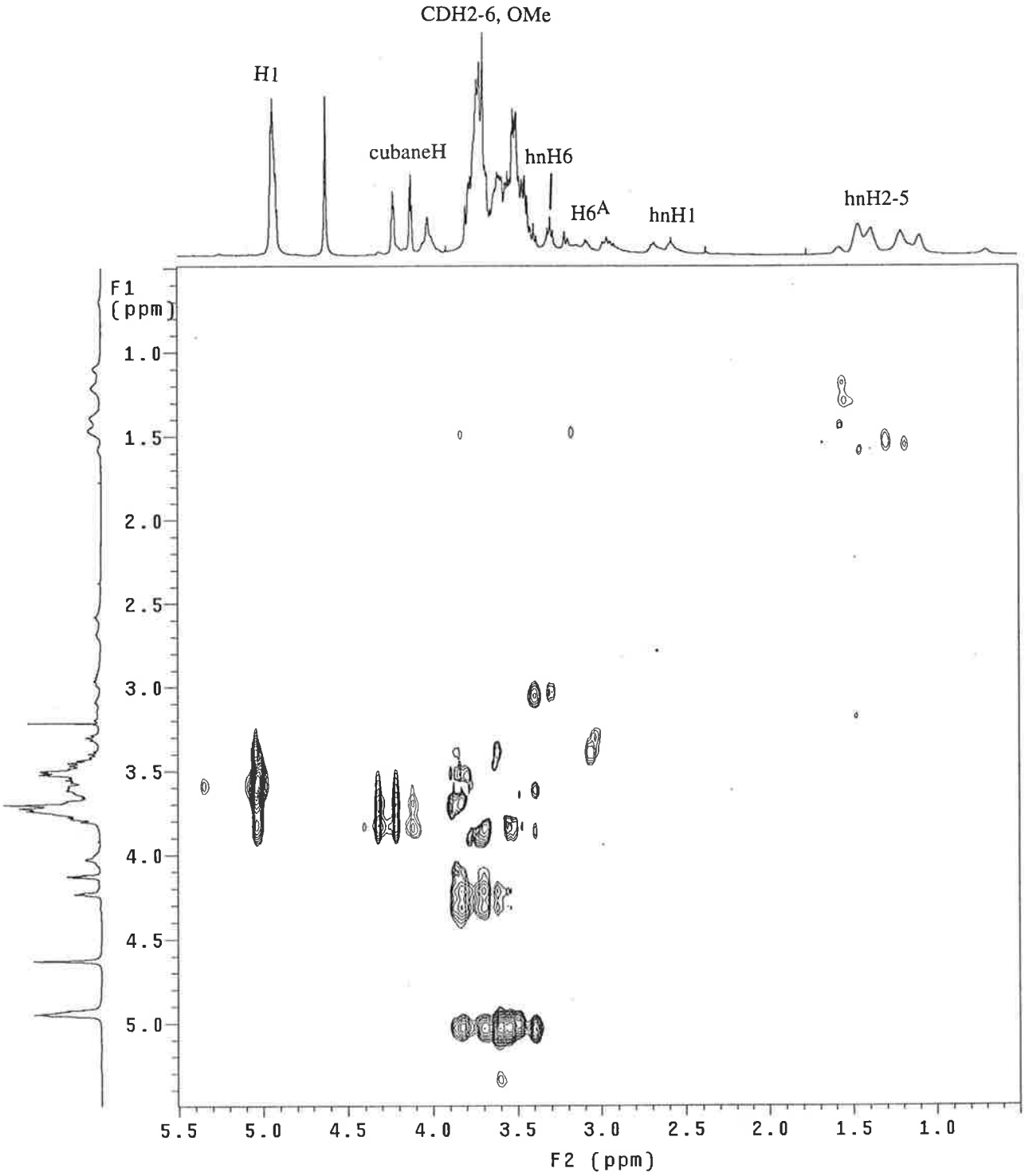


Figure 4.3. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10~~$^{-3}$ mol dm^{-3} of the cyclodextrin **67**. The protons are labelled as shown in Scheme 4.5.

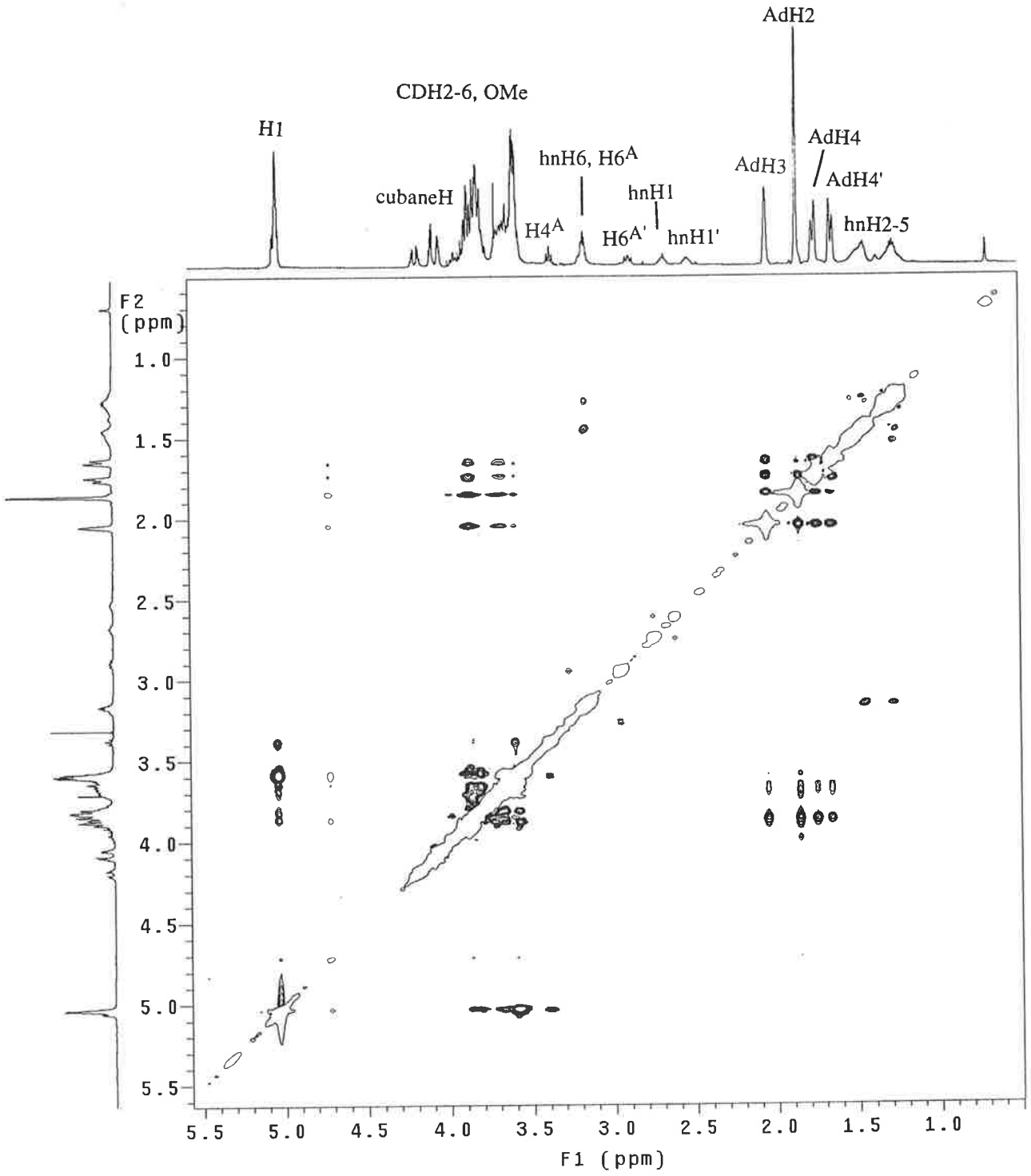
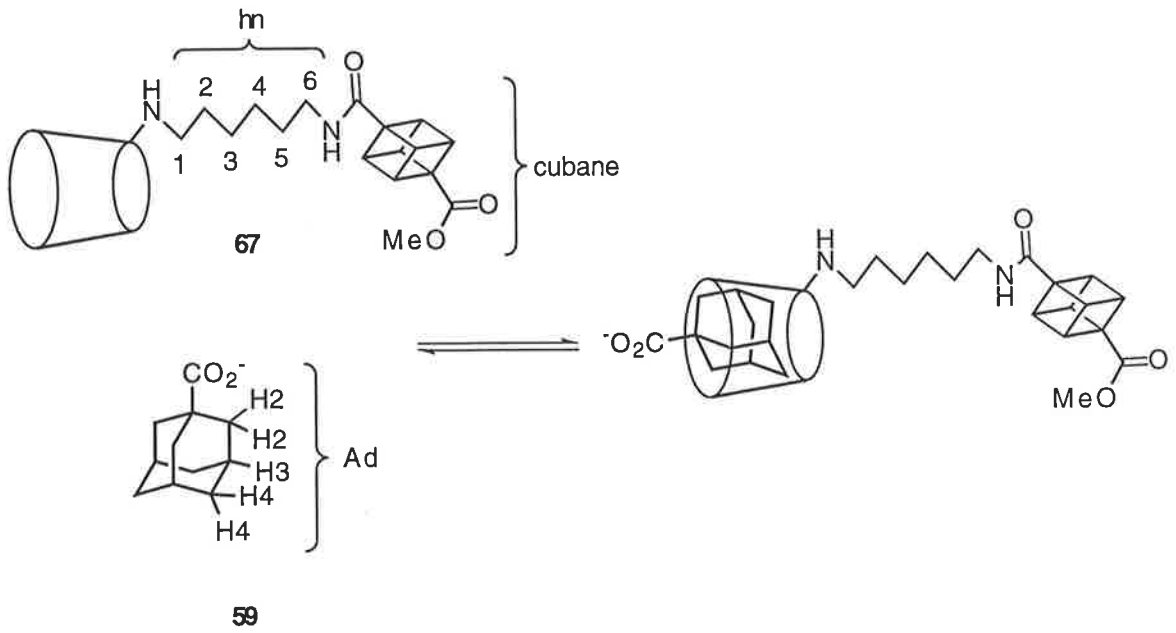


Figure 4.4. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} each of the cyclodextrin amide **67** and adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 4.6.

Addition of adamantane-1-carboxylate **59** to the solution of the amide **67** causes the cubanyl group to be displaced from the annulus of the cyclodextrin moiety as the adamantyl group is included within the annulus, as shown by the 600 MHz 2D-ROESY spectrum of this solution (Figure 4.4). Strong NOE interactions are observed between the adamantyl protons and the annular protons H3. Weaker NOE interactions are seen between the adamantyl protons and the annular protons H5, suggesting that the inclusion of the adamantyl group is not as deep within the annulus as was observed with the inclusion of this guest with the cyclodextrin **24**.



Scheme 4.6. Schematic representation of the inclusion of adamantane-1-carboxylate **59** in the cyclodextrin amide **67** in aqueous solution at pH ≥ 12.

When the ester **65** was allowed to react with the cyclodextrin **24** as described above for the ester **64** the amide **68** was obtained in 63% yield. The 300 MHz ^{1}H NMR spectrum of this product shows signals for the cubanyl protons at δ 4-4.2 and for the cubane methyl groups at δ 1.3, overlapping with the protons hnH2-hnH5 of the alkyl chain. The 75 MHz ^{13}C NMR spectrum of this product shows two signals at δ 175.8 and δ 175.5 due to the carbonyl carbons.

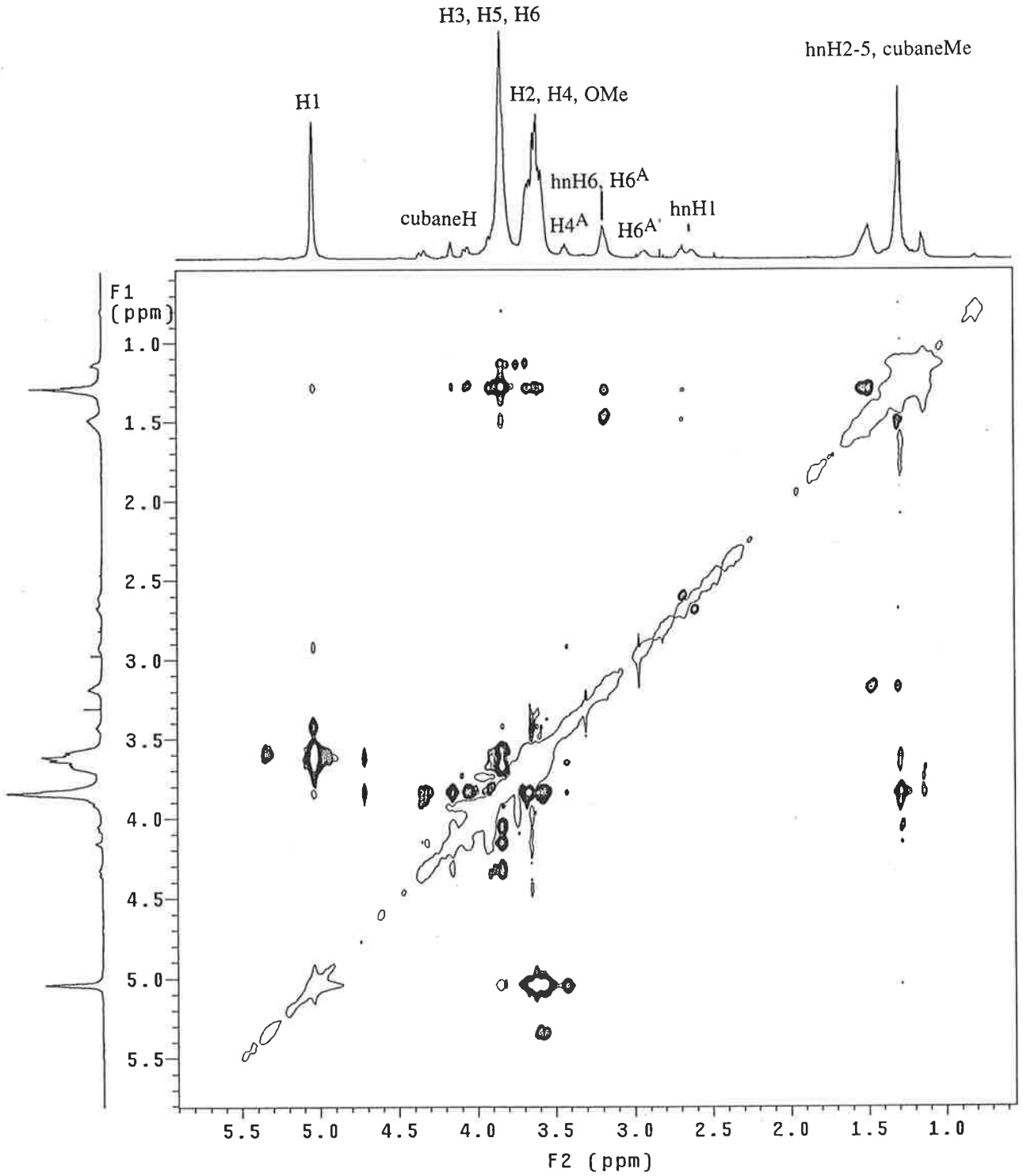
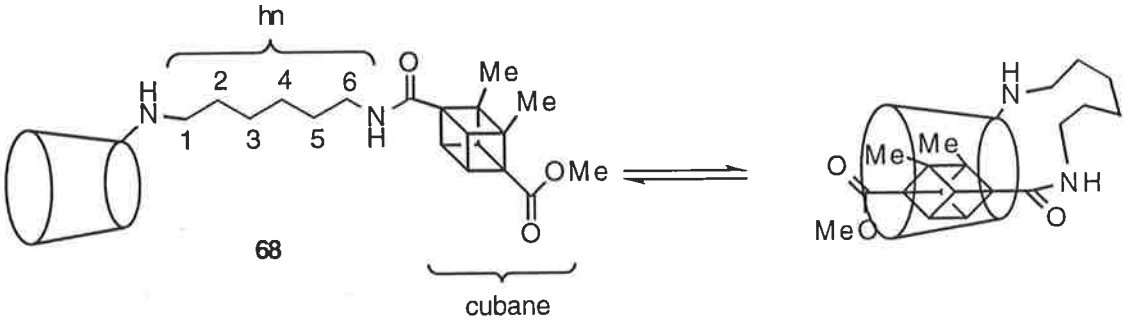


Figure 4.5. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} of the cyclodextrin **68**. The protons are labelled as shown in Scheme 4.7.



Scheme 4.7. Schematic representation of the self-inclusion of the cubanyl substituent of the cyclodextrin amide **68** in aqueous solution at pH ≥ 12 .

The 600 MHz 2D-ROESY spectrum of a solution of the amide **68** in D_2O at pH ≥ 12 indicates that the cubanyl group is included within the annulus of the cyclodextrin moiety (Figure 4.5). Strong cross-peaks are observed between the signals due to the cubanyl protons and the annular protons H3 and H5 which also show strong interactions with the cubane methyl groups. There are no interactions between the protons of the alkyl chain and the annular protons H3 and H5.

When adamantane-1-carboxylate **59** is added to the above solution the cubanyl group is displaced from the cyclodextrin annulus as the adamantyl group is included, as shown by 600 MHz 2D-ROESY spectroscopy (Figure 4.6). There are only weak NOE interactions between the adamantyl protons and the annular protons H5 and there are residual weak NOE interactions between the cubane methyl groups and the protons H5. The stronger NOE interactions between the adamantyl protons and the annular protons H3 show that the adamantyl group is only partially included within the cyclodextrin **68** with the cubane methyl groups remaining partially included at the primary end of the annulus. Increasing the hydrophobicity of the cubanyl skeleton by the addition of two methyl groups increases the tendency for this group to self-include within the annulus such that it can compete, to some extent, with the guest **59** for binding within the annulus. While this spectrum may also represent a dynamic equilibrium between inclusion of the guest **59** and self-inclusion of the substituent it is most consistent with the equilibrium shown in Scheme 4.8.

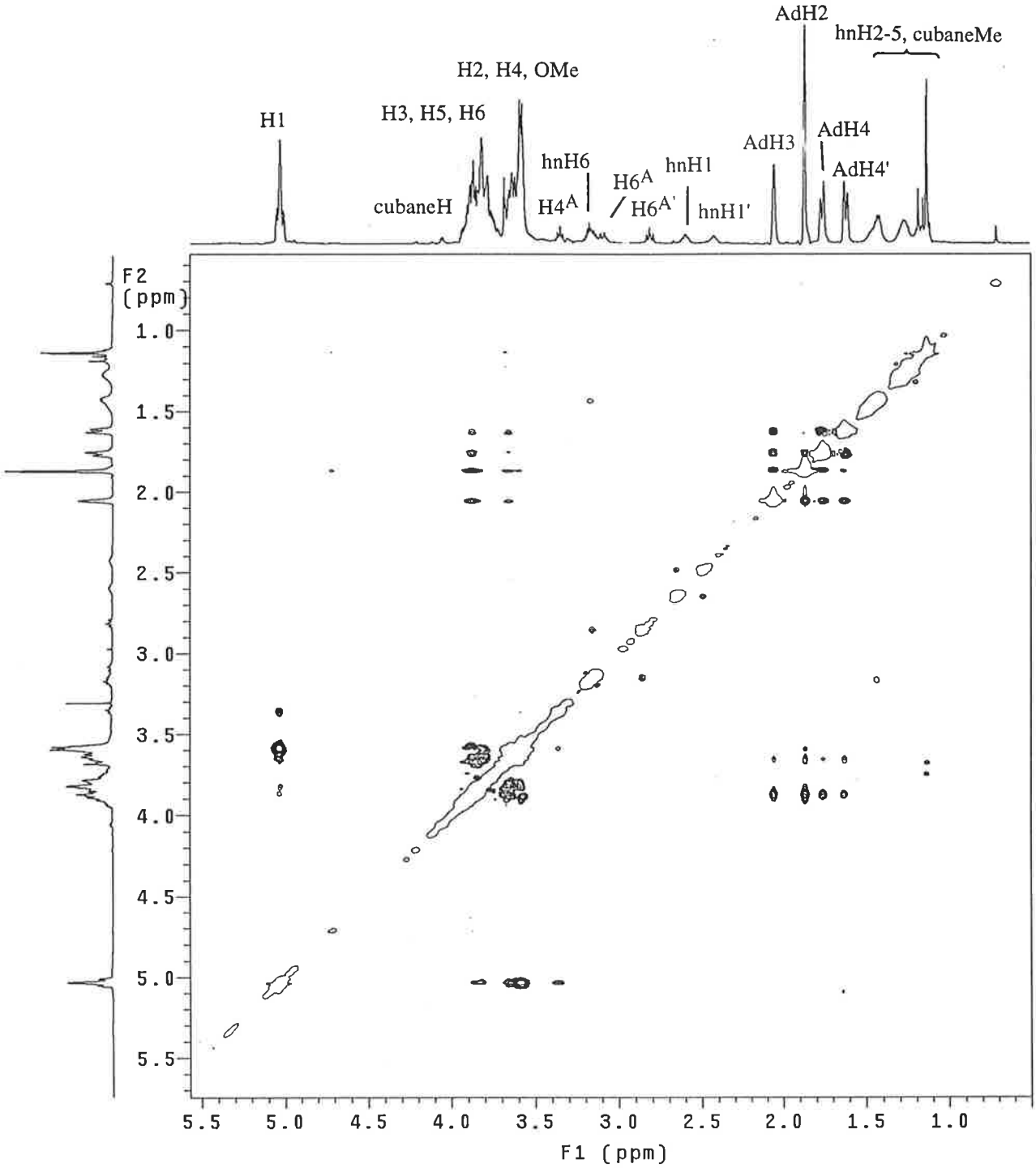
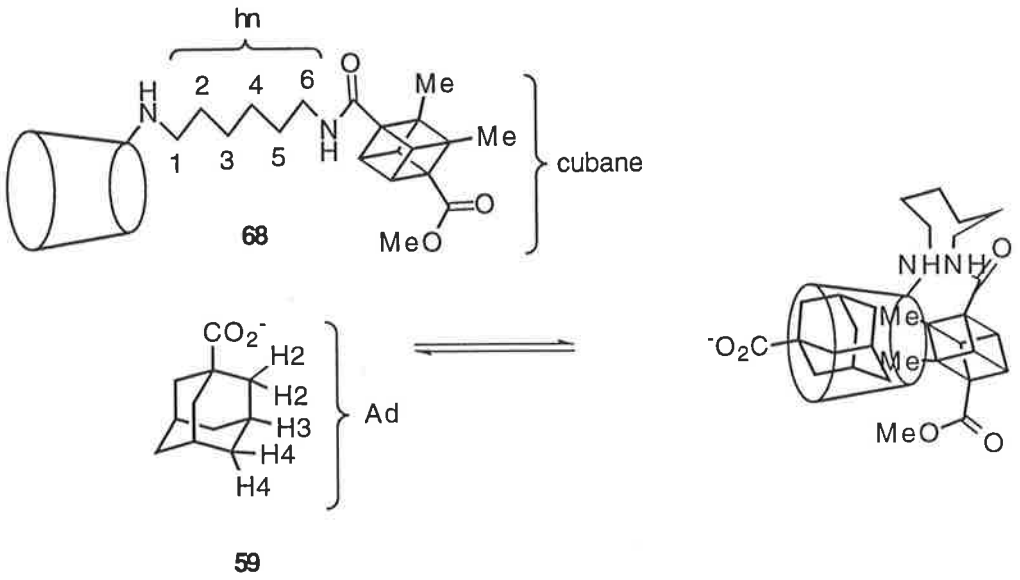
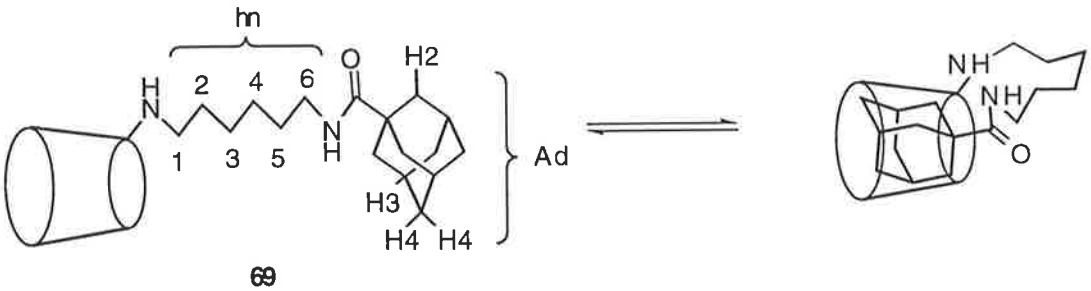


Figure 4.6 Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} each of the cyclodextrin **68** and adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 4.8.



Scheme 4.8. Schematic representation of the inclusion of adamantane-1-carboxylate **59** in the cyclodextrin amide **68** in aqueous solution at pH ≥ 12.

When the adamantyl ester **66** was allowed to react in DMF with one equivalent of the cyclodextrin **24** the amide **69** was obtained in a yield of 70 % following a similar work-up to that described above for the other amide derivatives. The 600 MHz ^{1}H NMR spectrum of the product suggests that the substituent is held in a rigid conformation with the protons hnH6 and hnH1 each giving rise to two resonances due to differentiation of the diastereotopic protons. The resonances due to the protons hnH2-hnH5 were likewise well resolved from each other. The 75 MHz ^{13}C NMR spectrum of the product showed a signal at δ 182.4 due to the carbonyl carbon.



Scheme 4.9. Schematic representation of the self-inclusion of the adamantyl substituent of the cyclodextrin amide **69** in aqueous solution at pH ≥ 12.

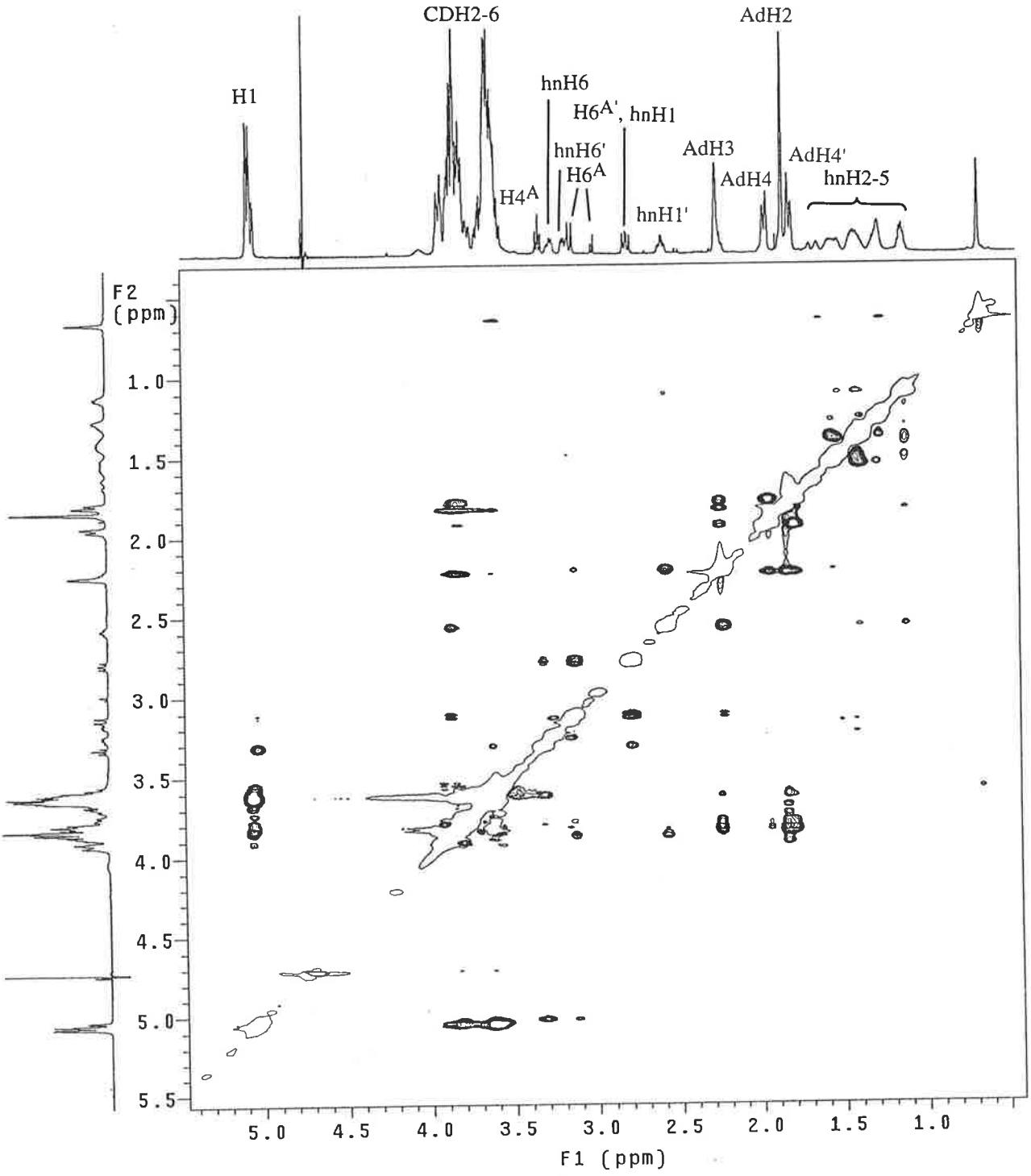
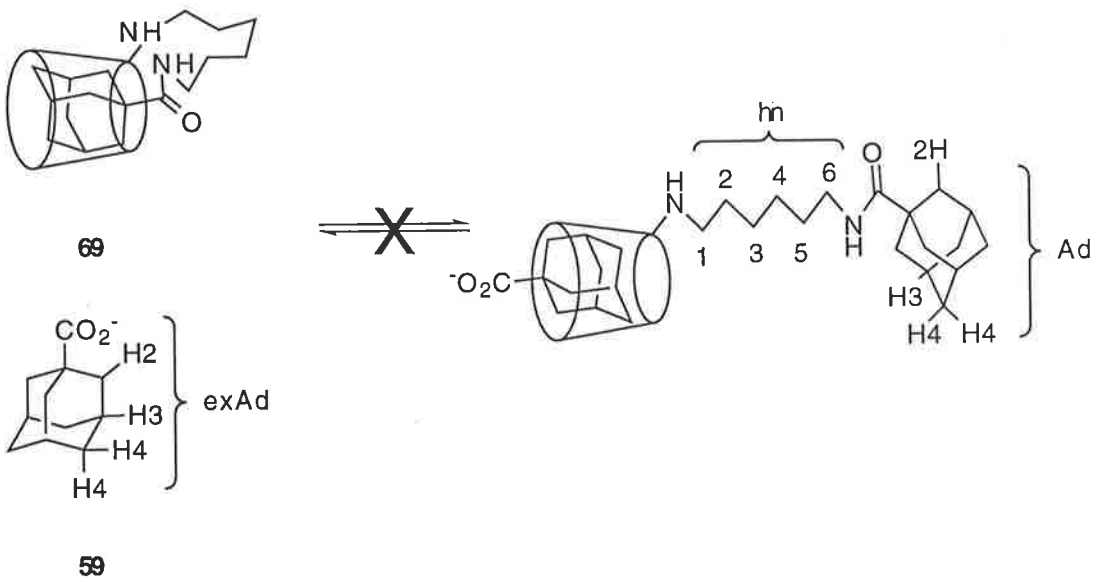


Figure 4.7. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10^{-3}~~ mol dm^{-3} of the cyclodextrin **69**. The protons are labelled as shown in Scheme 4.9.

The 600 MHz 2D-ROESY spectrum of a solution of the product in D_2O at pH ≥ 12 shows that there are strong interactions between the protons of the adamantyl group and the annular protons H3 and H5 (Figure 4.7). The protons AdH4 give rise to two separate doublets at δ 1.94 and 1.79. One set of protons AdH4 do not show any NOE interactions with the annular protons H3 and H5 while the protons giving rise to the signal at δ 1.79 show a strong NOE interaction with the annular protons H3 and H5 which suggests that the adamantyl group is restricted in its motions within the annulus of the cyclodextrin moiety. There are no NOE interactions between the protons of the alkyl chain and the annular protons H3 and H5.

On addition of two equivalents of adamantane-1-carboxylate **59** (in order to help differentiate between the signals due to the covalently attached adamantyl group and those of the added adamantyl protons) there is little significant change to the 1D ^1H NMR spectrum of the product. The signals of the added adamantane-1-carboxylate **59** are clearly distinguishable from those of the covalently attached adamantyl group and give rise to three broad singlets in the ratio of 3:6:6 as is expected for an adamantyl group which is not included within the annulus of a cyclodextrin, i.e. there is no differentiation of the H4 protons in the added adamantane-1-carboxylate **59**.



Scheme 4.10. Adamantane-1-carboxylate **59** does not form a detectable host-guest complex with the cyclodextrin amide **69** in aqueous solution at pH ≥ 12. The adamantyl substituent remains included within the annulus on addition of two equivalents of added guest.

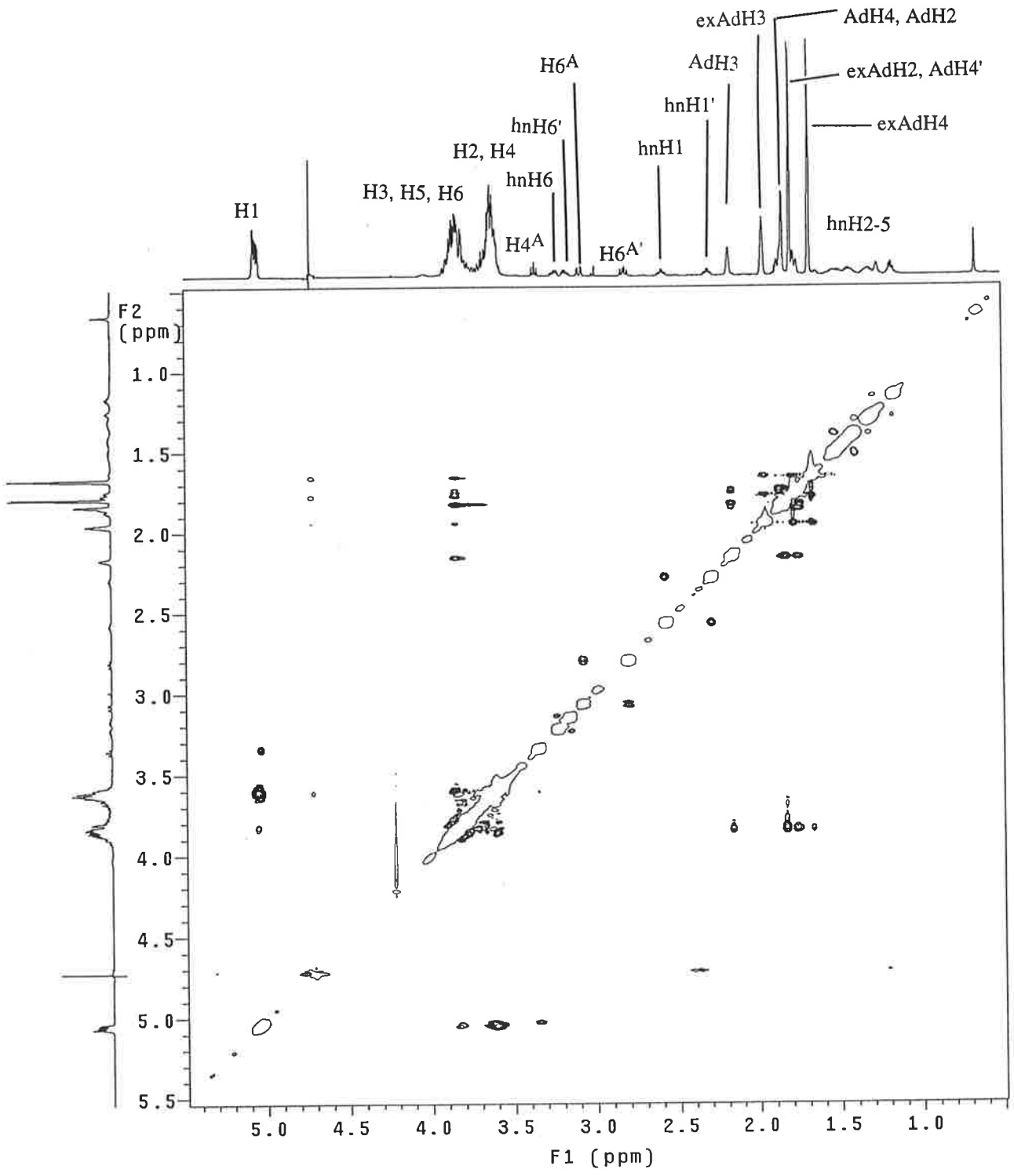


Figure 4.8. Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10~~$^{-3}$ mol dm^{-3} of the cyclodextrin **69** and 0.12 × 10^{-3} mol dm^{-3} of adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 4.10.

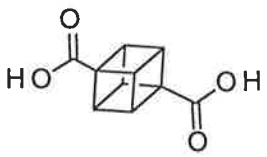
The 2D-ROESY spectrum of this solution shows that there is no change in the degree of the interactions between the covalently attached adamantyl group and the annulus of the cyclodextrin, that is, the added adamantane-carboxylate **59** is not able to push the covalently attached adamantyl group out of the annulus. The hydrophobicity of the adamantyl group of the substituent is probably the same as that of the added adamantane-1-carboxylate **59**. The covalent attachment of this group gives it an entropic advantage for complexation within the annulus over that of the added adamantane-1-carboxylate **59**, so favouring the self-inclusion of the substituent over the inclusion of adamantane-1-carboxylate **59**. Given that adamantyl compounds form the most stable host-guest complexes with cyclodextrins[161] it is unlikely that any other added molecule would be able to displace the substituent from the annulus of the cyclodextrin **69**. Although the adamantyl-substituted group may be able to pass through the cyclodextrin annulus it is not pushed out by adamantane-1-carboxylate **59**and so can be considered to be a molecular knot, held together mainly by non-covalent forces if the mechanical (steric) ones are not effective.

The dimethyl cubane group of the cyclodextrin **68** contains the same number of carbons as the adamantyl group of the cyclodextrin **69** and so might be expected to have a similar "level of hydrophobicity". If this is the case, then the shape of the adamantyl group may be the major factor that makes the self-inclusion of this substituent so favoured. The adamantyl group is known to fit snugly within the annulus of β-cyclodextrin **2**.

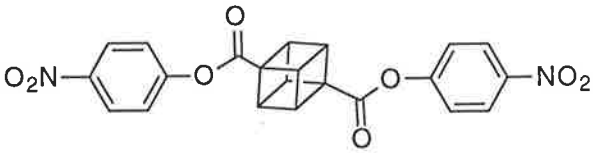
4.3. A novel cyclodextrin dimer

The cubanyl groups of the cyclodextrins **67** and **68** are able to include within the annulus of the cyclodextrin moiety. If a similar self-included cubanyl derivative was able to undergo a reaction which incorporated a large group onto the end of the substituent, then the substituent of the product of such a reaction may not be able to pass through the annulus. The product will be a molecular knot. The reaction of the diester **71**, prepared from the diacid **70**[172], with two equivalents of the cyclodextrin **24** was expected to give a cyclodextrin dimer

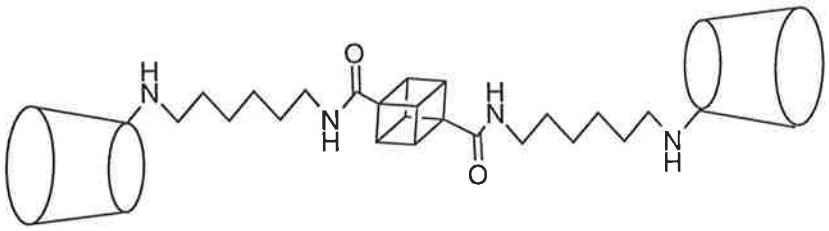
analogous to those reported in earlier work.[159] Three isomeric dimers **72**, **73** and **74** could be formed in this reaction. If either the dimer **73** or the dimer **74** was formed this would give a molecular knot, where a cyclodextrin moiety was acting as the blocking group.



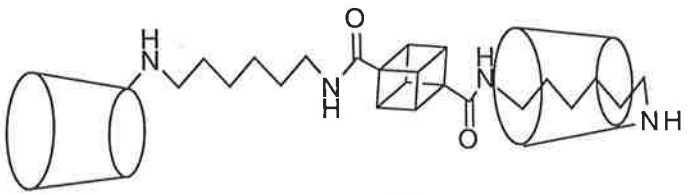
70



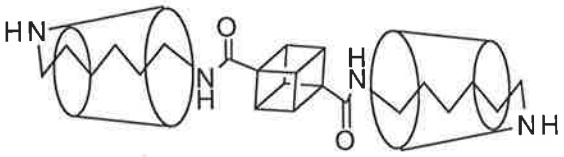
71



72



73



74

The diester **71** was prepared in 46% yield by the reaction of the diacid **70** with two equivalents each of 4-nitrophenol **49** and DCC in dichloromethane. Treatment of the diester **71** with two equivalents of the cyclodextrin **24** in DMF gave the dimer **72** (the identification of this isomer is discussed below) in 38% yield after a similar work-up procedure to that described for the amide **67** above, with the addition of a gel-filtration step using Sephadex G10 to

separate the dimer **72** from monomeric cyclodextrins. The isolated dimer **72** gave a clean electrospray-mass spectrum with a molecular ion at *m/z* 2622.

The 300 MHz ^{1}H NMR spectrum of the dimer **72** shows a complex set of resonances due to the cubanyl protons but all of the other signals were too broad and poorly resolved to give much structural information. Similarly, the 75 MHz ^{13}C NMR spectrum gives little structural information. The presence of two carbonyl resonances at δ 175 9 and δ 175.6 suggests that the product is asymmetric.

The 600 MHz 2D-ROESY spectrum of a solution of the dimer **72** in D_2O at pH ≥ 12 shows that the cubanyl substituent is included in one of the cyclodextrin moieties (Figure 4.9). Very strong cross-peaks are seen between the resonances due to the cubanyl protons and the annular protons H3 and H5. Weaker NOE interactions between protons on the alkyl chains and the annular protons H3 and H5 are also observable.

The 300 MHz ^{1}H NMR spectrum of a solution of the dimer **72** containing two equivalents of adamantane-1-carboxylate **59** indicates the formation of a highly symmetric species. The cubanyl protons give rise to a sharp singlet at δ 4.15, protons $H4^{A}$ give a sharp triplet at δ 3.40, protons $H6^{A}$ and $H6^{A'}$ give rise to a sharp doublet at δ 3.29 and a triplet at δ 3.00 respectively, a multiplet appears at δ 3.18 due to protons hnH1 and protons hnH6 give rise to two multiplets at δ 2.80 and δ 2.70. Additionally, the resonance due to protons $H5^{A}$ appears as a triplet at δ 4.03. The 75 MHz ^{13}C NMR spectrum of this solution shows only one signal for an amide carbonyl at δ 176.7 together with the carbonyl of the carboxylate **59** at δ 189.0 and the region where the signals due to carbons hnC2-hnC5 appear is simplified.

The 600 MHz 2D-ROESY spectrum of this solution shows that adamantane-1-carboxylate is included in the annuli of the cyclodextrin moieties of the dimer **72** (Figure 4.10,). There are strong cross-peaks between the adamantyl protons and the annular protons H3 and H5. There are no observable NOE interactions between the cubanyl protons and the annular protons H3 and H5.

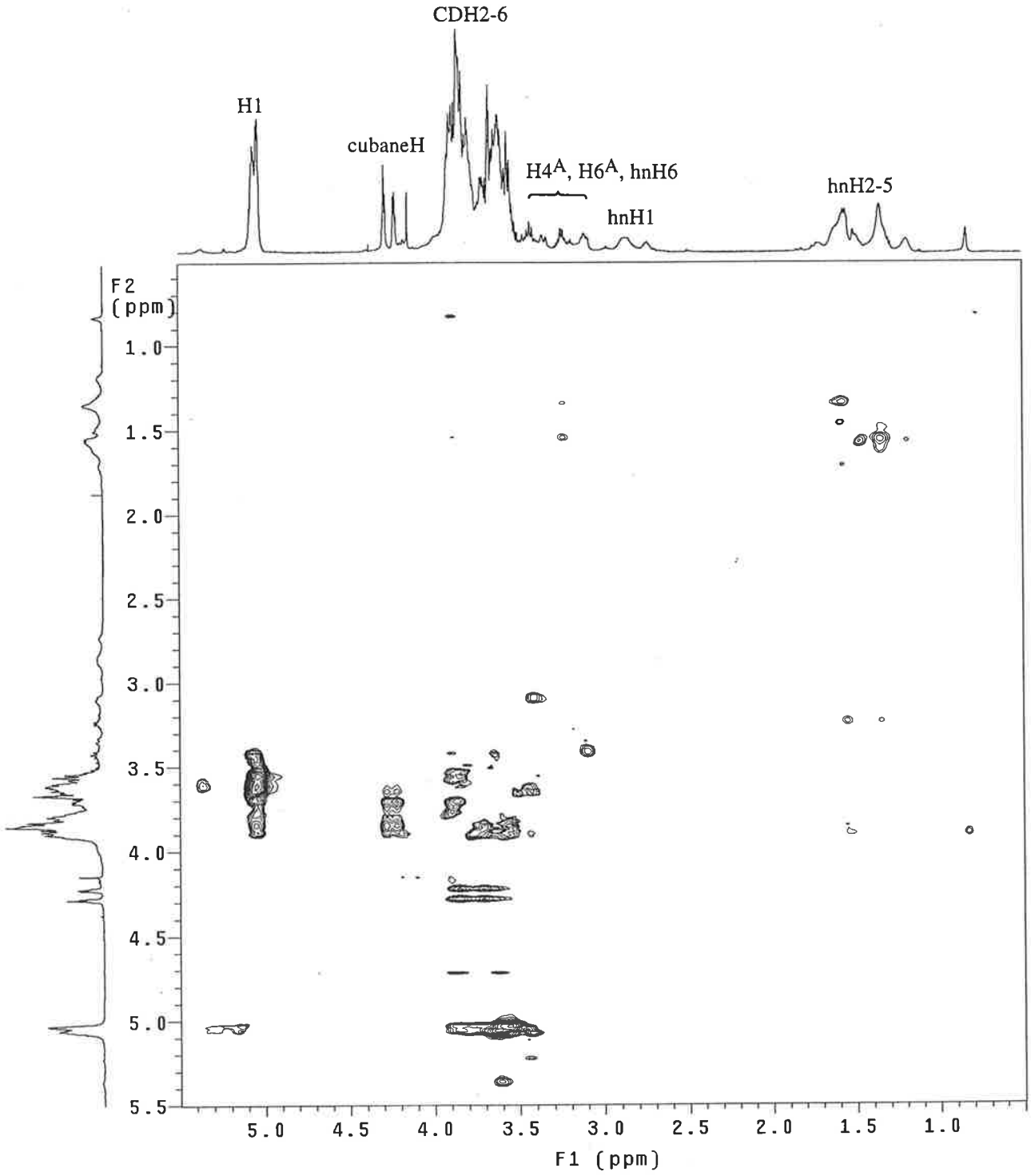


Figure 4.9 Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~$\times 10^{-3}$~~ mol dm^{-3} of the cyclodextrin dimer **72**. The protons are labelled as shown in Scheme 4.11.

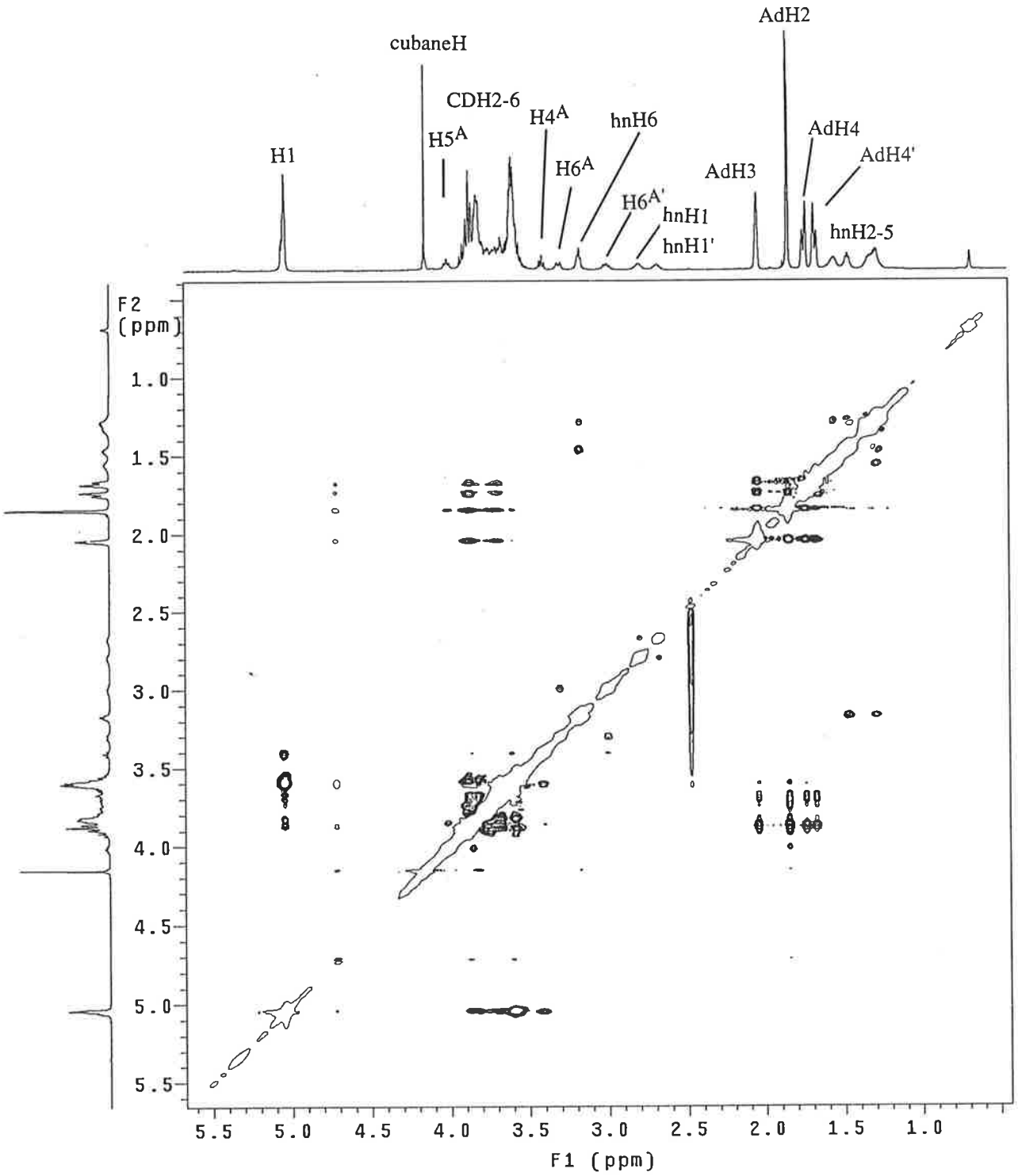
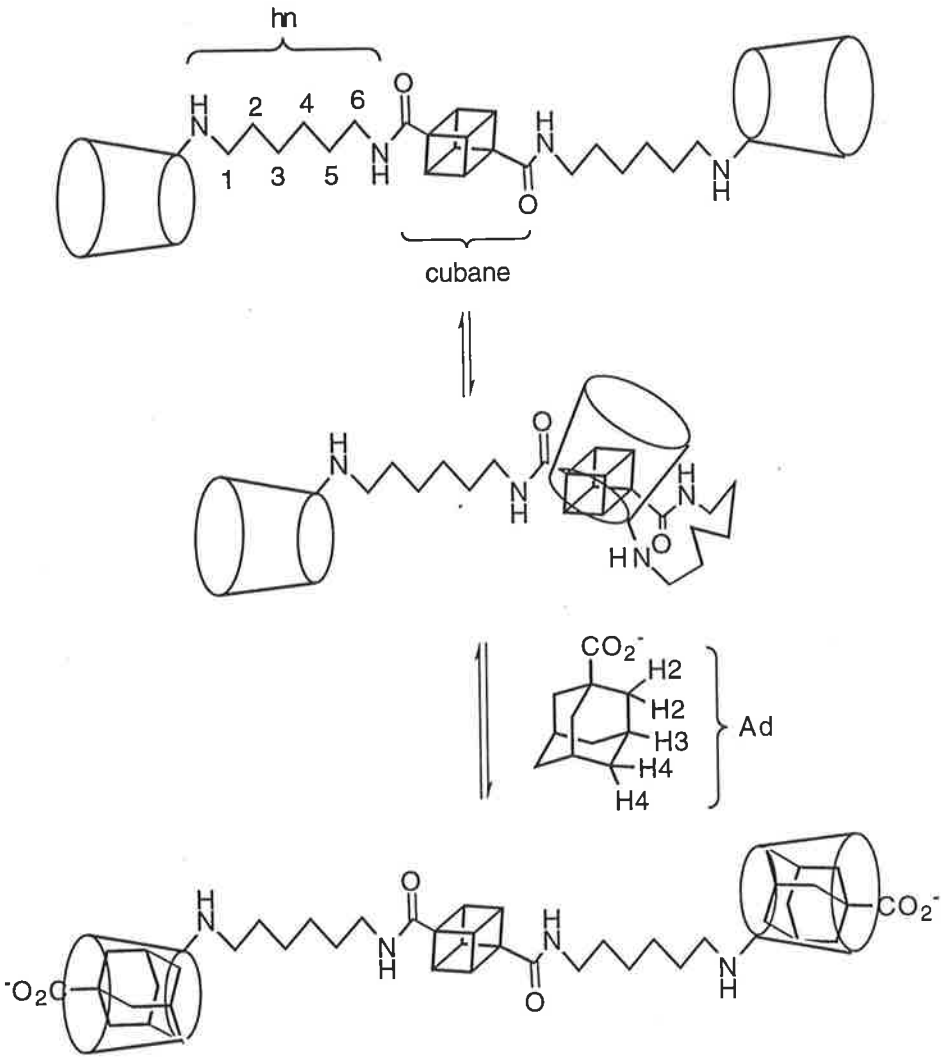


Figure 4.10 Contour plot of ROESY experiment (D_2O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 ~~× 10⁻³~~ mol dm^{-3} of cyclodextrin dimer **72** and 0.12×10^{-3} mol dm^{-3} of adamantane-1-carboxylate **59**. The protons are labelled as shown in Scheme 4.11.



Scheme 4.11. Schematic representation of the inclusion of the cubanyl group within one of the cyclodextrin moieties of the dimer **72** to give an asymmetric system. Addition of two equivalents of adamantane-1-carboxylate gives rise to a symmetric 1:2 host guest complex.

From the above data it is clear that the product from the reaction of the diester **71** with the cyclodextrin **24** is the dimer **72**. It is not possible for adamantane-1-carboxylate **59** to be included within either of the annuli of the dimer **74** as there is not enough room within the annuli while the alkyl chain is included. The dimer **73** could include adamantane-1-carboxylate **59** within one of the annuli but this would not give rise to the symmetric system described above. In solution, the dimer **72** exists as an asymmetric species with the cubanyl bridging group partially, or wholly, included within one of the cyclodextrin annuli. Only the dimer **72** is able to form a symmetric 1:2 host-guest complex with adamantane-1-carboxylate **59**.

4.4. Conclusion

The self-inclusion of a hydrophobic substituent attached to a cyclodextrin occurs readily in aqueous solution. Depending on the shape and the hydrophobicity of the substituent relative to that of an added guest, this self-inclusion can either increase or reduce, and in some cases prevent, the inclusion of the added guest. While 4-methylbenzoate **37** is readily accommodated within the annulus of the cyclodextrin **24** when the 6-aminohexyl substituent is included within the annulus, no complexation of the guest **37** is observed with the cyclodextrin **60**. Complete inclusion of adamantane-1-carboxylate **59** occurs with the total exclusion of the 6-aminohexyl substituent of the cyclodextrin **24** but when the cyclodextrin **60** is the host, the guest **59** is only partially included within the annulus and the 12-aminododecyl substituent is also partially included.

The reactions of the cyclodextrin **24** with the 4-nitrophenyl esters of a series of bulky aliphatic acids yielded amide products where the substituent was found to be included within the annulus of the cyclodextrin in aqueous solution. The substituent of the cubane amides **67** and **68** was readily displaced from the annulus of the cyclodextrin on addition of adamantane-1-carboxylate **59** to a solution containing either of the amides **67** and **68**. The adamantyl cyclodextrin **69** formed an extremely stable self-inclusion complex. The substituent could not be displaced by added adamantane-1-carboxylate **59**. The cyclodextrin **69** may be a molecular knot, held together by mainly non-covalent forces, although there may also be a steric interaction holding the substituent inside the annulus.

In solution, the linking group of the cyclodextrin dimer **72** is partially included within the annulus of one of the cyclodextrin moieties. When two equivalents of adamantane-1-carboxylate **59** are added to this solution the linking group is displaced from the annulus and a symmetrical 1:2 host-guest complex is formed between the cyclodextrin dimer and adamantane-1-carboxylate **59**.

Conclusion

Chapter 2 describes the development of a clean, simple and reproducible synthesis of 6^A-amino substituted β-cyclodextrins. The key improvement over the previous methods that have been used to prepare these derivatives is the use of 1-methyl-pyrrolidin-2-one (NMP) as the solvent for the reaction. The use of this solvent allows the rapid nucleophilic substitution of a 6^A-*O*-4-methylbenzenesulfonate by a wide variety of primary and secondary amines at moderate temperature. The use of this solvent avoids the need for high pressure or sealed tube reactions and the use of large excesses of the amine reagents, some of which may be expensive to obtain and have often been difficult to separate from the desired product. The amino-cyclodextrin products are obtained as pure materials after a simple and inexpensive ion-exchange step.

A series of amino-substituted β-cyclodextrins has been prepared by this procedure and systematic studies of their pH dependent solution structures and host-guest chemistry have been carried out using titrometric and 2D-NMR techniques. At high pH the hydrophobic, linear substituents are included within the annulus of the cyclodextrin moiety and remain included within the annulus when small aromatic guests are bound inside the cyclodextrin. Cyclic substituents form a tight cap over the primary face of the cyclodextrin at high pH resulting in the enhanced binding of aromatic guests. At lower pH both types of substituents move away from the annulus as the charged ammonium groups are better solvated by water, allowing easier dissociation of host-guest complexes.

The esterase activity of the Zn(II) complexes of some of these amino-cyclodextrins was investigated and it was found that the reaction of the cyclodextrin **30** with 4-nitrophenyl acetate **48** is inhibited by the presence of Zn(II) ion in the reaction mixture. The reaction between the cyclodextrin **31** and the ester **48** is marginally enhanced in the presence of Zn(II) at neutral pH

but is inhibited by Zn(II) at higher pH. The observed rate enhancement is most likely due to the increased polarisation of the carbonyl bond of the ester in the presence of Zn(II) and not due to the formation of a metallo-cyclodextrin hydroxy species.

The trans-acylation reactions of the ω-aminoalkylamino-cyclodextrins **21**, **22** and **24** with the esters **48** and **52** involve the nucleophilic attack of the primary nitrogen on the ester carbonyl to give *N*-acetylated derivatives. The site of reaction was confirmed by comparison of the reaction products with authentic samples of the amides **53-55** prepared by an independent synthesis. The non-protonated ω-aminoalkylamino-cyclodextrin species is the major reactive species in the trans-acylation reaction with the ester **48** as shown by pH dependence studies for the reaction of the cyclodextrin **24**.

The reactions of the cyclodextrin **24** with the esters **48** and **52** lead to both *N*- and *O*-acetylated cyclodextrin products. These reactions involve the prior inclusion of the esters **48** and **52** within the cyclodextrin annulus and indicate that both "head-first" and "tail-first" inclusion may occur. The product ratios for the reaction between the cyclodextrin **24** and the ester **48** are in accord with the ratio of the rates for the reactions at a secondary hydroxyl and at the primary nitrogen of the substituent of the cyclodextrin **24**. The product ratio suggests that there is a 1:1 ratio of the two inclusion modes in solution at pH 9.1.

Addition of adamantane-1-carboxylate **59** to these reactions inhibits the trans-acylation by competitive inhibition. 2D-ROESY NMR spectroscopy confirms that the adamantyl group is included within the annulus of the cyclodextrin **24** at pH ≥ 12.

The self-inclusion of a hydrophobic substituent attached to a cyclodextrin occurs readily in aqueous solution. Depending on the shape and the hydrophobicity of the substituent relative to that of an added guest, this self-inclusion can either increase or reduce, and in some cases prevent, the inclusion of the added guest. The guest 4-methylbenzoate **37** is readily accommodated within the annulus of the cyclodextrin **24** while the 6-aminohexyl substituent is included within the annulus, but no complexation of the guest **37** is observed with the 12-aminododecyl amino-cyclodextrin **60**. Complete inclusion of adamantane-1-carboxylate **59**

occurs with the total exclusion of the 6-aminohexyl substituent of the cyclodextrin **24** but when the cyclodextrin **60** is the host, the guest **59** is only partially included within the annulus and the 12-aminododecyl substituent is also partially included.

The reactions of the cyclodextrin **24** with the 4-nitrophenyl esters of a series of bulky aliphatic acids yielded amide products where the substituent was found to be included within the annulus of the cyclodextrin in aqueous solution. The substituent of the cubane amides **67** and **68** was readily displaced from the annulus of the cyclodextrin on addition of adamantane-1-carboxylate **59** to a solution containing either of the amides **67** and **68**. The dimethyl cubane substituent of the cyclodextrin **68** remained partially included in the presence of the guest **59**. The adamantyl cyclodextrin **69** formed an extremely stable self-inclusion complex. The substituent could not be displaced by added adamantane-1-carboxylate **59**. The cyclodextrin **69** may be a molecular knot, held together by mainly non-covalent forces, although there may be a steric interaction holding the substituent inside the annulus. The effect of self-inclusion on the host-guest chemistry of a substituted cyclodextrin depends on the relative size, shape and hydrophobicity of the substituent and the added guest.

In solution, the linking group of the cyclodextrin dimer **72** is partially included within the annulus of one of the cyclodextrin moieties. This self-inclusion is of such high stability that the dimer **72** is asymmetric on the 600 MHz NMR time-scale When two equivalents of adamantane-1-carboxylate **59** are added to this solution the linking group is displaced from the annulus and a symmetrical 1:2 host-guest complex is formed between the cyclodextrin dimer and adamantane-1-carboxylate **59**.

Experimental

E.1. General

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 either a Hitachi 270-30 grating spectrometer or an ATI Mattson Genesis FT-IR. The abbreviations strong (s), medium (m), weak (w) and broad (b) are used in reporting the infrared data.

Unless stated otherwise ^{1}H and ^{13}C NMR were recorded on a Bruker ACP-300 spectrometer operating at 300.145 MHz (^{1}H) or 75.4 MHz (^{13}C). During the course of this work the ACP-300 was modified by Varian to a Gemini 2000 system using the original Bruker magnet. Other spectrometers used were a Varian Gemini 200 operating at 199.953 MHz (^{1}H) and 50.4 MHz (^{13}C) and a Varian Inova 600 operating at 599.975 MHz (^{1}H) and 150.7 MHz (^{13}C). The NMR spectra of cyclodextrin derivatives were recorded in D_2O at concentrations of 0.06 mol dm^{-3} and the signals were referenced to aqueous trimethylsilylpropiosulfonic acid as an external standard. For the pH dependence studies the spectra were initially recorded at pH ~9 (sample dissolved in D_2O), NaOH was then added to give solutions pH $\geq$ 12 and finally HCl was added to give solutions of pH $\leq$ 2. Protons and carbons of the substituents are labelled as shown in Figure E.1.

All 2D-ROESY NMR spectra were recorded on a Varian Inova 600 Spectrometer operating at 599.957 MHz. using a standard sequence with a mixing time of 0.3 seconds. Under these conditions cross peaks could be observed due to TOCSY interactions as well as those due to nuclear Overhauser relaxation effects. During the course of this work new

sequences which avoided these additional cross-peaks were tested but most of the 2D-spectra reported below were obtained under the original conditions. The cyclodextrin (and the 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 pH ≥ 12. The resultant solutions were filtered (0.22 μm) and degassed by freeze-thawing before the spectra were recorded.

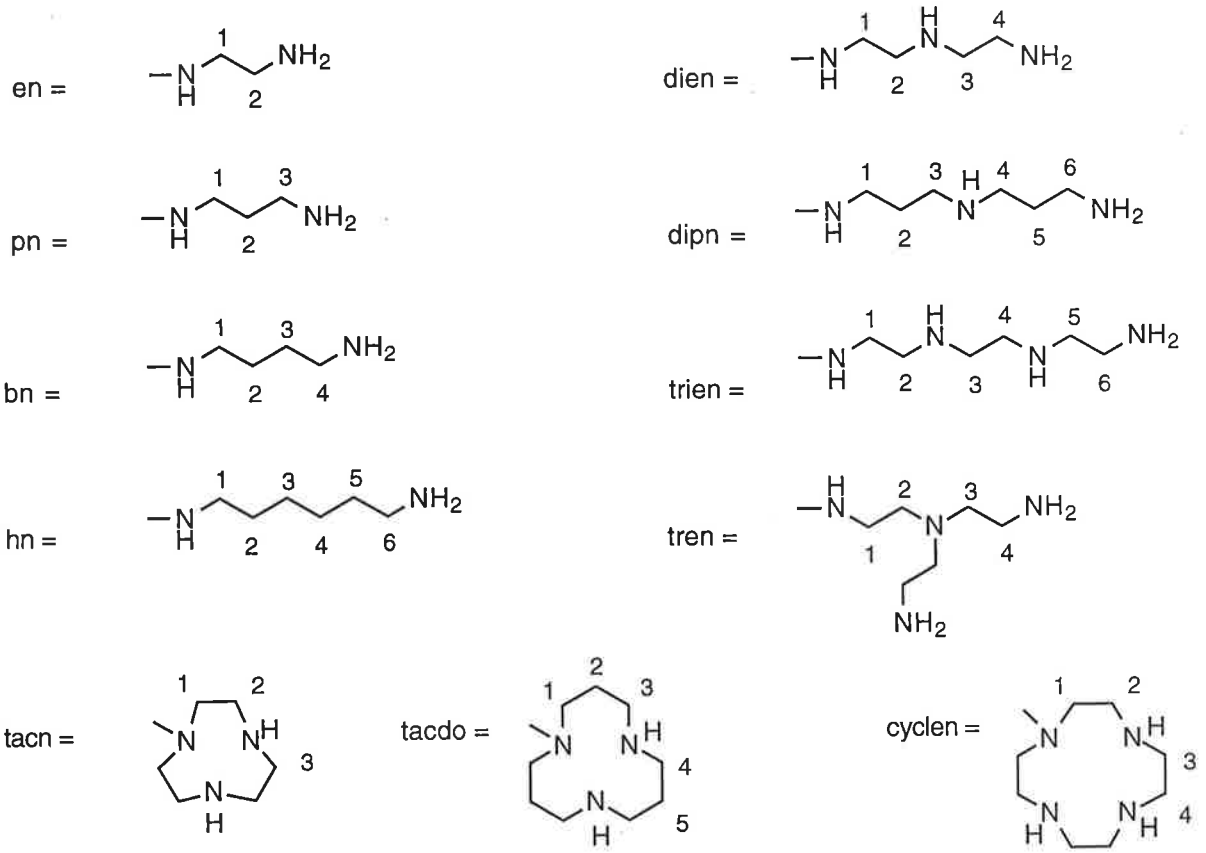


Figure E.1. Examples of the labelling of the protons and carbons of the substituents of the modified cyclodextrins described below.

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.

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 0.10 mol dm^{-3} $NaClO_4$. 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. 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 0.100 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 95 x 10^{-3} 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 0.100 mol dm^{-3} NaOH. Values of pK_a were determined using the program SUPERQUAD.[173] 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.

Ultra-violet spectroscopy was carried out with a Varian Cary 2200 spectrophotometer with a cell block that was held at 298.2 K.

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. 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.

Flash chromatography was carried out using Merck Kieselgel 60 (230-400 mesh ASTM) as described in the literature.[169]

Squat column chromatography was carried out using Merck Kieselgel 60 PF254 thin layer chromatography silica as described in the literature.[170, 171]

Unless stated otherwise, reagents were obtained from Aldrich and were used without further purification. 2-(2-(2-Aminoethyl)aminoethyl)aminoethylamine tetrahydrochloride (trien.4HCl, Aldrich) was purified by two recrystallisations from ethanol/water. β-

Cyclodextrin was a gift from Nihon Shokuhin Kako Co. and was dried by heating at 100 °C under vacuum (< .01 Torr) for 18 hours. 6^A-O-(4-Methylbenzenesulfonyl)-β-cyclodextrin **32** was prepared by the method of Matsui.[85] The cubane derivatives **62**, **63** and **70** were a gift from Dr. John Tsanaktsidis. *N,N*-Dimethyl-formamide (DMF) was dried by distillation from calcium hydride under reduced pressure and stored over freshly prepared 4Å molecular sieves. Pyridine and 1-methylpyrrolidin-2-one (NMP) were dried by distillation from calcium hydride but were not stored over molecular sieve as both solvents tend to extract material from the sieves. Ether refers to diethyl ether.

E.2. Experimental for Chapter 2

E.2.1. Preparation of 1,4,7-triazacyclononane

1,2-Bis-(4-methylbenzenesulfonato)ethane[103]

To a stirred solution of 1,2-ethanediol (13.25 g, 0.213 mol) in dry pyridine (150 cm^3) was added 4-methylbenzenesulfonic acid (85.61 g, 0.451 mol) in portions, such that the reaction temperature was kept at 0 °C (ice-salt bath). The resultant mixture was left to stir at room temperature for 18 hours during which time a thick precipitate had formed. The mixture was shaken with an equal volume of ice and left to stand at 4 °C for 6 hours. The solid was collected by vacuum filtration, washed successively with water (4 × 200 cm^3), ethanol (2 × 100 cm^3) and ether (2 × 100 cm^3) and air dried. the crude product was recrystallised from acetone to give the ditosylate (58.9 g, 74.8%) as white needles. (mp 131-132 °C, lit[103] 123-125 °C). $\delta_H(CDCl_3)$ 7.46 (d, J = 8 Hz, 4H); 7.36 (d, J = 8 Hz, 4H); 4.18 (s, 4H); 2.46 (s, 6H). I.R.(nujol) 1596 (w),1496 (w), 1374 (s), 1362 (s), 1310 (s), 1298 (w), 1192 (s), 1178 (s), 1094 (m), 1036 (m), 1018 (m), 978 (s), 816 (m), 798 (m), 770 (m), 668 (s), 592 (s) cm^{-1}.

N,N',N''-*tri(4-methylbenzenesulfonyl)diethylenetriamine* [103]

A solution of 4-methylbenzenesulfonyl chloride (146 g, 0.768 mol) in dry ether (700

cm^3) was added slowly over 5 hours to a mechanically stirred solution of diethylenetriamine (25.1 g, 0.243 mol) and sodium hydroxide (29.2 g, 0.730 mol) in water (250 cm^3). The reaction mixture was left to stir at room temperature for 16 hours. Methanol (400 cm^3) was added and the resultant precipitate was collected by vacuum filtration, washed with water (3 × 200 cm^3) and methanol (2 × 200 cm^3) and dried under vacuum to give the crude product (82.46 g) which was recrystallised from either methanol (~12 g/800 cm^3) or acetone (~36 g/250 cm^{-3}) to give the pure compound (54 g, 39%) as white needles. (mp 180-181 °C, lit[103] 173-175 °C). $\delta_H(CDCl_3)$ 7.76 (d, J = 8.3 Hz, 4H); 7.61 (d, J = 8.3 Hz, 2H); 7.3 (overlapping doublets, J = 8.3 Hz, 6H); 5.15 (t, J = 5.4 Hz, 2H NH_2); 3.17 (multiplet, 8H); 2.43 (s, 9H). I.R.(nujol) 3288 (s), 1596 (s), 1496 (s), 1322 (s), 1308 (s), 1154 (s), 1092 (s), 1078 (s), 992 (s), 940 (m), 908 (m), 830 (w), 814 (s), 748(m), 732 (s), 696 (s), 668 (s) cm^{-1}

1,4,7-tri(4-methylbenzenesulfonyl)-1,4,7-triazacyclononane [103]

Sodium hydride (2.2 g, 60% dispersion in oil, 0.055 mol) was added in one portion under nitrogen to a stirred solution of *N*,*N*',*N*''-tri(4-methyl-benzenesulfonyl)-diethylenetriamine (14.16 g, 0.025 mol) in dry DMF (250 cm^3). After the initial vigorous reaction was over the mixture was heated to 70 °C and left to stir for 2 hours. The mixture was then heated to 105 °C and a solution of 1,2-bis-4-methylbenzenesulfonatoethane (9.26 g, 0.025 mol) in DMF (100 cm^3) was added dropwise over 90 minutes. The resultant brown solution was stirred at 105 °C for 5 hours then evaporated to dryness under vacuum. The residue was triturated with water (750 cm^3) and the solid which formed was collected by vacuum filtration and washed successively with water (500 cm^3), ethanol (3 × 25 cm^3) and ether (50 cm^3) and air dried to give 15.2 g of a tan powder. This was suspended in boiling ethanol (150 cm^3) and boiling chloroform (~70 cm^3) was added until all of the solid had dissolved. The product crystallised as white needles (11.1 g, 75%). (mp 222 °C, lit[103] 218-220 °C). $\delta_H(CDCl_3)$ 7.67 (d, J = 8.4 Hz, 6H); 7.33 (d, J = 8.4 Hz, 6H); 3.42 (s, 12H); 2.44 (s, 9H). $\delta_C(CDCl_3)$ 143.93, 134.58, 129.90, 127.51, 51.88, 21.54. I.R. (nujol) 1596 (m), 1496 (m), 1342 (s), 1322 (s), 1186 (m), 1160 (s), 1120 (m), 1088 (s), 994 (s), 930 (m), 900 (m), 882 (m), 868 (m), 818 (s), 710 (s), 690 (s), 642 (s) cm^{-1}.

1,4,7-Triazacyclononane Trihydrochloride [103]

A stirred suspension of 1,4,7-tri(4-methylbenzenesulfonyl)-1,4,7-triazacyclononane (11.06 g, 0.019 mol) in 98% sulfuric acid (30 cm^3) was heated at 100 °C for 72 hours. The resultant dark brown solution was cooled to 0 °C and ethanol (100 cm^3) was added slowly. On addition of ether (200 cm^3) a gelatinous precipitate formed. This was collected by filtration under nitrogen and washed with ether (3 × 25 cm^3). The solid was dissolved in water (60 cm^3) and the resultant solution was heated on a steam-bath and treated with charcoal (12 g). The charcoal was removed by filtration through Celite and the clear, tan coloured solution was diluted to ~1 L with water to give a solution at pH 2.0. This solution was passed through a column of BioRad AG50W-X2, H^+form (3 × 18 cm). The amine band appeared as a gold band at the top of the column. The column was washed with water (400 cm^3) and 0.5 mol dm^{-3} HCl (400 cm^3) and the amine was eluted with 1.5 mol dm^{-3} HCl taking 100 cm^3 fractions. The fractions were analysed by TLC (8:1:1 acetic acid: chloroform: water) and fractions containing the pure product were combined and concentrated to about 20 cm^3, when crystallisation began. Addition of ethanol (150 cm^3) gave the product as a white solid (3.168 g, 71%). (mp 255-258 °C dec). $\delta_H(D_2O)$ 3.58 (s). $\delta_C(D_2O)$ 44.26.

An amount of this material (1.756 g) was dissolved in 1 mol dm^{-3} potassium hydroxide in brine (30 cm^3) and this solution was extracted with dichloromethane (3 × 30 cm^3). The combined organic solutions were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to leave 1,4,7-triazacyclononane as a yellow oil. Bulb to bulb distillation (130 °C/6 mmHg) gave pure 1,4,7-triazacyclononane (0.741 g, 78%).

E.2.2. Preparation of 1,5,9-triazacyclododecane

1,3-Bis-(4-methylbenzenesulfonato)propane[103]

A solution of propan-1,3-diol (10 g, 0.136 mol) in dry pyridine (25 cm^3) was added dropwise over 10 minutes to a vigorously stirred solution of 4-methylbenzenesulfonyl chloride (75 g, 0.394 mol) in pyridine (160 cm^3) cooled in an ice/salt bath (-10 °C) such that the temperature did not rise above -5 °C. After the addition was complete stirring was continued

for another 4 hours as the mixture slowly warmed to 5 °C. The reaction mixture was then poured onto ice (1.3 dm^3) and the resultant mixture was stirred vigorously to coagulate the precipitate that had formed. The precipitate was left to stand at 0 °C for 18 hours and was then collected by vacuum filtration, rinsed thoroughly with water (1 dm^3) and air dried. The crude product was recrystallised from ethanol (~300 cm^3) to give the product as white needles (41.6 g, 82%). (mp. 93-94 °C, lit[103] 91-93 °C). $\delta_H(CDCl_3)$ 7.85 (d, J = 8.1 Hz, 4H); 7.45 (d, J = 8.1 Hz, 4H); 4.17 (t, J = 6.0 Hz, 4H); 2.56 (s, 6H); 2.10 (pent, J = 6.0 Hz, 2H). $\delta_C(CDCl_3)$ 144.99, 132.51, 129.88, 127.76, 65.80, 28.56, 21.54. I.R. (nujol) 1599 (m), 1499 (w), 1190 (m), 1174 (s), 1096 (w), 946 (s), 856 (s), 812 (s), 742 (s), 664 (s) cm^{-1}.

N,N',N''-*tri(4-methylbenzenesulfonyl)-di(3-aminopropyl)amine*[104]

Freshly purified 4-~~methlybenzenesulfonyl~~ methylbenzenesulfonyl chloride (29.4 g. 0.155 mol) was added in portions over 3 hours to a stirred solution of bis(3-aminopropyl)amine (5.2 g, 0.040 mol) and potassium carbonate (12.4 g, 0.090 mol) in water (250 cm^3) heated at 60 °C. The reaction mixture was stirred at 60 °C for a further 3 hours and then left to stand at room temperature for 18 hours. The precipitated solid was collected by vacuum filtration and washed with water. The solid was then dissolved in dichloromethane (100 cm^3) and the solution was then washed successively with 1 mol dm^{-3} hydrochloric acid (100 cm^3), water (2 × 100 cm^3) and brine (50 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure to give the product as an oily solid. This was crystallised from ethanol to give the title compound as a white solid (16.5 g, 70%). (mp 117- 120 °C, lit[104] 119-120 °C). $\delta_H(CDCl_3)$ 7.71 (d, J = 8.3 Hz, 4H); 7.61 (d , J = 8.3 Hz, 2H); 7.27 (d, J = 8.3 Hz, 6H); 4.98 (broad singlet, 2H); 3.07 (t, J = 6.9 Hz, 4H); 2.91 (t, J = 6.2 Hz, 4H); 2.40 (s, 9H); 1.68 (tt, J = 6.9, 6.2 Hz, 4H). $\delta_C(CDCl_3)$ 143.76, 143.44, 136.70, 135.39, 129.95, 129.92, 129.78, 127.07, 126.98, 46.64, 40.17, 29.01,21.50. I.R. (nujol) 3291 (s), 3247 (s), 1597 (s), 1495 (s), 1337(s), 1309 (s), 1157 (s), 1091 (s), 1059 (w), 1005 (w), 950 (s), 943 (s), 821 (s), 777 (s), 687 (s) 659 (s) cm^{-1}.

1,5,9-tri(4-methylbenzenesulfonyl)-1,5,9-triazacyclododecane [104]

A mixture of *N,N',N''*-tri(4-methylbenzenesulfonyl)-di(3-aminopropyl)amine (9.109 g, 0.0154 mol) and sodium hydride (2.72 g, 60% dispersion in oil, 0.068 mol) in dry DMF (150 cm^3) was stirred at room temperature until the first, vigorous reaction had slowed and then at 70 °C for 1 hour. The mixture was then cooled to room temperature and filtered under nitrogen through Celite to remove the excess sodium hydride. The resultant clear, light yellow solution was heated to 110 °C and a solution of 1,3-bis(4-methyl-benzene-sulfonato)propane (6.00 g, 0.0156 mol) in dry DMF (75 cm^3) was added dropwise over 1 hour. The resultant clear, yellow solution was left to stir at 110 °C for a further 4 hours and then at room temperature for 18 hours. The solution was concentrated to 50 cm^3 under reduced pressure when crystals began to form. Water (400 cm^3) was added and the resultant precipitate was collected by vacuum filtration, washed with water (100 cm^3), ethanol (10 cm^3) and ether (50 cm^3) and air dried to give the crude product (9.125 g, 93.9%). A small amount of this material was recrystallised from chloroform/ethanol (~1:4) as needles m.p. 172-173 °C (lit 171 °C). $\delta_H(CDCl_3)$ 7.64 (d, J = 8.1 Hz, 6H); 7.31 (d, J = 8.1 Hz, 6H); 3.20 (t, J = 6.9 Hz, 12H); 2.43 (s, 9H); 1.90 (quintet, J = 6.9 Hz, 6H). $\delta_C(CDCl_3)$ 143.58, 135.09, 129.78, 127.24, 45.46, 26.30, 21.47. I.R. (nujol) 1598, 1496, 1378, 1304, 1158 (s), 1090, 1036, 1020,968, 944, 920, 846, 816, 748, 724, 708, 692, 680, 658 cm^{-1}.

1,5,9-triazacyclododecane trihydrochloride

The crude 1,5,9-tri(4-methylbenzenesulfonyl)-1,5,9-triazacyclododecane from above (9.0 g) was stirred in 98% sulfuric acid (25 cm^3) at 110 °C for 80 hours then cooled to room temperature and diluted with ethanol (75 cm^3) and ether (200 cm^3). The resultant black precipitate was collected by filtration under nitrogen and washed with ether (2 × 30 cm^3). The crude product was dissolved in boiling water (60 cm^3), treated with charcoal (2 g) and the mixture filtered through Celite to give a clear, pale yellow solution. This was diluted to ~1.600 L with water and loaded onto a column of BioRex AG50W-X8 (3 × 18 cm). The column was washed with water (500 cm^3) and then eluted successively with 0.5 mol dm^{-3} HCl (500 cm^3), 1.5 mol dm^{-3} HCl (500 cm^3) and 3 mol dm^{-3} HCl (700 cm^3) taking 100 cm^3

fractions. The fractions were analysed by TLC and fractions containing pure amine (#11-18) were combined and concentrated under reduced pressure to ~2 cm^3. The solution was diluted with ethanol (100 cm^3) and the resultant precipitate was collected by vacuum filtration and rinsed with ethanol and ether to give the product as a white powder (1.8g, 44.6%). (m.p. 230-231°C, lit 286°C). $\delta_H(D_2O)$ 3.42 (t, J = 6.7 Hz, 12H); 2.29 (quint, J = 6.7 Hz, 6H). $\delta_C(D_2O)$ 43.87, 21.93.

An amount of this material (1.685 g) was dissolved in 1 mol dm^{-3} potassium hydroxide in brine (30 cm^3) and this solution was extracted with dichloromethane (3 × 30 cm^3). The combined organic solutions were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to leave 1,5,7-triazacyclododecane as a yellow oil. Bulb to bulb distillation (150 °C/3 mmHg) gave pure 1,5,7-triazacyclododecane (0.817 g, 80%).

E.2.3. Preparation of 6^A- amino-substituted β-cyclodextrins

General procedure for preparation of amino-substituted cyclodextrins.

A solution of 6^A-*O*-(4-methylbenzenesulfonyl)-β-cyclodextrin **32** (2.0 g, 1.55 × 10^{-3} mol), potassium iodide (0.025 g, 0.15 × 10^{-3} mol) and the amine (5 × 10^{-3} mol) in dry N-methylpyrrolidin-2-one (NMP) (5 cm^3) was stirred at 70 °C in a lightly stoppered flask for 4-8 hours. The resultant light yellow solution was cooled to room temperature and diluted with ethanol (100 cm^3). The resulting precipitate was collected by vacuum filtration, washed successively with ethanol (100 cm^3) and ether (50 cm^3) and dried under vacuum to give the crude product. This material was dissolved in water (10 cm^3) and loaded onto a column (4.5 × 4.5 cm) of BioRex 70 (H^+ form). The column was washed with water (400 cm^3) and the amino-cyclodextrin product was eluted with 1 mol dm^{-3} NH_4OH. Fractions containing the product were combined and evaporated to dryness under vacuum. The residue was dissolved in water and the solution evaporated under reduced pressure to remove excess ammonia (this procedure was repeated several times). The product was dried under vacuum over P_2O_5 to give the amino-cyclodextrin in yields of 30-55%.

All of the syntheses of the amino-cyclodextrins described below were carried out by the

procedure described in the general method. However, it was later found that the addition of potassium iodide was not essential for obtaining good yields and so this reagent was omitted in reactions carried in the later part of this work. Yields and reaction times were not affected by the omission of potassium iodide.

6^A-(2-(bis(2-aminoethyl)amino)ethyl)amino-6^A-deoxy-β-cyclodextrin **28**[90]

A mixture of the tosylate **32** (2.048 g, 1.59×10^{-3} mol), tris-(2-aminoethyl)amine (0.74 g, 5.07×10^{-3} mol) and KI (0.024 g) in NMP (5 cm^3) was treated according to the general procedure to give the title compound as a white powder (1.192 g, 59%). R_c 0.31. Electrospray-ms *m/z* 1263 (M^+). (Found C, 43.84; H, 7.58; N, 4.40. Calculated for **28**.3H_2O ($C_{48}H_{92}N_4O_{34}$) C, 43.76; H, 7.04; N, 4.25%.) δ_H(D_2O/NaOH, pH ~ 14) 5.00 (bs, 7H + solvent, H1); 3.5 - 3.8 (m, 26H, H3, H5, H6); 3.1 - 3.4 (m, 13H, H2, H4); 3.02 (t, J = 9.0 Hz, 1H, $H4^A$); 2.85 (d, J = 12.0 Hz, 1H, $H6^A$); 2.2 - 2.7 (m, 13H, $H6^{A'}$, trenH). δ_H(D_2O, pH ~ 9) 5.05 (bs, 7H, H1); 3.8 - 4.0 (m, 26H, H3, H5, H6); 3.5 - 3.7 (m, 13H, H2, H4); 3.41 (t, J = 9.0 Hz, 1H, $H4^A$); 3.05 (d, J = 11.4 Hz, 1H, $H6^A$); 2.4 - 2.9 (m, 13H, H6A', trenH). δ_H(D_2O/HCl, pH ~ 1) 5.00 (s, 7H, H1); 4.10 (t, J = 9.0 Hz, 1H, $H5^A$); 3.6 - 4.0 (m, 25H, H3, H5, H6); 3.4 - 3.6 (m, 14H, H2, H4); 2.9 - 3.4 (m, 14H, $H6^A$, trenH). δ_C(D_2O/NaOH, pH ~ 14) 107.0, 106.6, 106.4, 105.2 (C1); 87.6 ($C4^A$); 85.0, 84.8, 84.5, 83.9 (C4); 77.3, 76.4, 76.3, 75.2, 74.9 (C2, C3, C5); 70.9 ($C5^A$); 63.0 (C6); 59.8 (trenC3,3'); (56.9); 55.1 ($C6^A$); 50.5 (trenC2); 46.2 (trenC1); 41.0 (trenC4,4'). δ_C(D_2O, pH ~ 9) 104.7, 104.3 (C1); 86.4 ($C4^A$), 84.0, 83.6 (C4); 75.9 (C2); 74.9 (C3), 74.7 (C5); 73.3 ($C5^A$); 63.1 (C6); 58.7 (trenC3,3'); 55.7 (trenC2); 52.0 ($C6^A$); 48.7 (trenC1); 40.7 (trenC4,4'). δ_C(D_2O/HCl, pH ~ 1) 104.5, 103.8 (C1); 85.8 ($C4^A$); 84.2, 83.8, 83.4 (C4); 75.8, 75.5, 75.0, 74.8, 74.5 (C2, C3, C5); 70.2 ($C5^A$); 63.6, 63.1 (C6); 52.8 (trenC3,3'); 51.5 ($C6^A$); 51.3 (trenC2); 47.0 (trenC1); 38.6 (trenC4,4').

6^A-(2-aminoethyl)amino-6^A-deoxy-β-cyclodextrin **21**

A mixture of the tosylate **32** (1.981 g, 1.53×10^{-3} mol, 1,2-diaminoethane (0.305 g, 5.08×10^{-3} mol) and KI (0.024 g) in NMP (5 cm^3) was treated according to the general

procedure except that the crude product, obtained from the ethanol precipitation, was dissolved in water (10 cm^3) and loaded onto a column (4.5 × 4.5 cm) of BioRex 70 (NH_4^+ form). The column was washed with water (120 cm^3) and the product was then eluted with 0.05 mol dm^{-3} NH_4HCO_3. Fractions containing the product were combined and evaporated to dryness to give the title compound as a white powder (1.005 g, 56%). R_c 0.62. Electrospray-ms *m/z* 1177 (M^+). (Found C, 42.70; H, 6.67; N, 2.18. Calculated for **21**.$3H_2O$ ($C_{45}H_{84}N_2O_{40}$) C, 42.92; H, 6.71; N, 2.27%.) δ_H(D_2O/NaOH, pH ~ 14) 4.87 (s, 7H + solvent, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.17 (t, *J* = 9.4 Hz, 1H, $H4^A$); 2.94 (d, *J* = 12.1 Hz, 1H, $H6^A$); 2.5-2.7 (m, 5H, $H6^{A'}$, enH1, enH2). δ_H(D_2O, pH ~ 10) 5.07 (s, 7H, H1); 3.8-4.0 (m, 26H, H3, H5, H6); 3.5 - 3.7 (m, 13H, H2, H4); 3.48 (t, *J* = 9.2 Hz, 1H, $H4^A$); 3.09 (d, *J* = 11.9 Hz, 1H, $H6^A$); 2.7-2.9 (m, 5H, $H6^{A'}$, enH1, enH2). δ_H(D_2O/HCl, pH ~ 1) 5.0 (m, 7H, H1); 4.17 (t, *J* = 7.0 Hz, 1H, $H5^A$); 3.7 (m, 25H, H3, H5, H6); 3.5 (m, 20H, H2, H4, $H6^A$, enH). δ_C(D_2O/NaOH, pH ~ 14) 106.08, 105.80, 105.51 (C1); 87.37 ($C4^A$); 84.70, 84.63, 84.51 (C4); 76.79, 76.69, 76.00, 74.58 (C2, C3, C5); 72.74 ($C5^A$); 63.16 (C6); 53.45 ($C6^A$); 52.21 (enC2); 42.50 (enC1). δ_C(D_2O, pH ~ 10) 104.57, 104.28 (C1); 86.25($C4^A$); 83.85, 83.68 (C4); 75.79, 75.73, 74.77, 74.51 (C2, C3, C5); , 73.17 ($C5^A$); 62.99(C6); 52.43 ($C6^A$); 51.96 (enC2); 42.21 (enC1). δ_C(D_2O/HCl, pH ~ 1) 104.53, 103.75 (C1); 85.74 ($C4^A$); 84.20, 83.82, 83.05 (C4); 75.83, 75.72, 75.53, 75.48, 75.08, 74.75, 74.65,74.49 (C2, C3, C5); 70.13 ($C5^A$); 63.51, 63.17,63.07 (C6); 51.45 ($C6^A$); 47.55 (enC2); 37.96 (enC1).

6^A-(3-aminopropyl)amino-6^A-deoxy-β-cyclodextrin **22**

A mixture of the tosylate **32** (2.052 g, 1.59 × 10^{-3} mol), 1,3-diaminopropane (0.420 g, 5.67 × 10^{-3} mol) and KI (0.026 g) in NMP (5 cm^3) was treated according to the general procedure to give the title compound as a white powder (0.792 g, 42%). R_c 0.50. Electrospray-ms *m/z* 1191 (M^+). (Found C, 43.65; H, 6.85; N, 2.39. Calculated for **22**.$3H_2O$ ($C_{45}H_{84}N_2O_{37}$) C, 43.40; H, 6.80; N, 2.24%.) δ_H(D_2O/NaOH, pH ~ 14) 4.97 (s, 7H, H1); 3.8 (m, 26H, H3, H5, H6); 3.5 (m, 13H, H2, H3, H4); 3.30 (t, *J* = 9.1 Hz, 1H, $H4^A$); 3.02 (d, *J* = 12.4 Hz, 1H, $H6^A$); 2.72 (dd, *J* = 9.8, 12.4 Hz, 1H, $H6^{A'}$); 2.58

(overlapping triplets, J = 7.0, 7.4 Hz, 4H, pnH1, pnH3); 1.59 (tt, J = 7.0, 7.4 Hz, 2H, pnH2). δ_H(D_2O, pH ~ 9) 5.06 (s, 7H, H1); 3.8-4.0 (m, 26H , H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.41 (t, J = 9.2 Hz, 1H, $H4^A$); 3.05 (d, J = 12.0 Hz, 1H, $H6^A$); 2.7-2.8 (m, 3H, $H6^{A'}$, pnH1); 2.62 (d, J = 7.0 Hz, 2H, pnH3); 1.67 (tt, J = 7.0, 7.3 Hz, 2H, pnH2). δ_H(D_2O/HCl, pH ~ 1) 5.13 (s, 7H, H1); 4.20 (t, J = 8.1 Hz, 1H, $H5^A$); 3.8 - 4.0 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 14H, H2, H4); 3.41 (m, 2H, $H6^A$); 3.22 (t, J = 7.1 Hz, 2H, pnH1); 3.13 (t, J = 7.4 Hz, 2H, pnH3); 2.18 (tt, J = 7.1, 7.3 Hz, 2H, pnH2). δ_C(D_2O/NaOH, pH ~ 14) 105.40, 105.27, 104.96 (C1); 86.99 ($C4^A$); 84.31, 84.27, 84.02 (C4); 76.35, 75.54, 74.58 (C2, C3, C5); 72,87 ($C5^A$); 63.14, 62.97 (C6); 52.27 ($C6^A$); 49.10 (pnC3); 41.40 (pnC1); 34.39 (pnC2). δ_C(D_2O, pH ~ 9) 104.59, 104.27 (C1); 86.40 ($C4^A$); 83.89, 83.61 (C4) 75.82, 75.75, 74,78, 74.54 (C2, C3, C5); 73.12 ($C5^A$); 63.00, 62.88 (C6); 52.21 ($C6^A$); 49.18 (pnC3); 41.15 (pnC1); 32.93 (pnC2). δ_C(D_2O/HCl, pH ~ 1) 103.79, 103.05 (C1); 85.12 ($C4^A$); 83.40, 83.06, 82.34 (C4); 75.09, 74.99, 74.81, 74.36, 74.05, 74.01, 73.91, 73,77, 73.70 (C2, C3, C5); 69.46, 62.68, 62.32 (C6); 50.41 ($C6^A$); 47.28 (pnC3); 38.57 (pnC1); 25.57 (pnC2).

6^A-(4-aminobutyl)amino-6^A-deoxy-β-cyclodextrin **23**

A mixture of the tosylate **32** (1.403 g, 1.088×10^{-3} mol), 1,4-diaminobutane (0.340 g, 3.86×10^{-3} mol) and KI (0.020 g) in NMP (3.5 cm^3) was treated according to the general procedure to give the title compound as a white powder (0.679 g, 52%). R_c 0.63. Electrospray-ms m/z 1205 (M^+). (Found C, 44.88; H, 7.17; N, 2.17. Calculated for **23**.$2H_2O$ ($C_{46}H_{84}N_2O_{36}$) C, 44.51; H, 6.82; N, 2.25%.) δ_H(D_2O/NaOH, pH ~ 14) 4.73 (s, 7H, H1); 3.6-3.7 (m, 26H, H3, H5, H6); 3.2 - 3.3 (m, 13H, H2, H4); 3.03 (t, J = 9.0 Hz, 1H, $H4^A$); 2.79 (d, J = 11.8 Hz, 1H, $H6^A$); 2.3 - 2.6 (m, 5H, $H6^{A'}$, bnH1, bnH4); 1.25 (bs, 4H, bnH2, bnH3). δ_H(D_2O, pH ~ 10) 5.03 (s, 7H, H1); 3.8-4.0 (m, 26H, H3, H5, H6); 3.5 - 3.7 (m, 13H, H2, H4); 3.39 (t, J = 9.3 Hz, 1H, $H4^A$); 3.00 (m, 2H, $H6^A$); 2.75 (m, 2H, bnH1); 2.58 (m, 2H, bnH4); 1.52 (bs, 4H, bnH2, bnH3). δ_H(D_2O/HCl, pH ~ 1) 4.9 (bs, 7H + solvent, H1); 4.06 (t, J = 9.0 Hz, 1H, $H5^A$); 3.6 - 3.9 (m, 25H, H3, H5, H6); 3.5-3.6 (m, 14H, H2, H4); 3.40 (m, 2H, $H6^A$); 3.02 (bs, 2H, bnH1); 2.92 (bs, 2H, bnH4); 1.66

(bs, 4H, bnH2, bnH3). δ_C(D_2O/NaOH, pH ~ 14) 106.73, 106.44, 106.36, 106.27, 106.20, 106.05, 105.43 (C1); 87.63 ($C4^A$); 84.87, 84.67, 84.57, 84.18 (C4); 77.28, 77.15, 77.00, 76.21, 76.12, 74.77, 74.64 (C2, C3, C5); 71.87 ($C5^A$); 62.92 (C6); 51.86 ($C6^A$); 50.59 (bnC4); 43.19 (bnC1); 32.47 (bnC3); 28.53 (bnC2); δ_C(D_2O, pH ~ 9) 104.57, 104.21 (C1); 86.30 ($C4^A$); 83.88, 83.62 (C4); 75.81, 75.66, 74.73, 74.55 (C2, C3, C5); 73.08 ($C5^A$); 62.99 (C6); 52.13 ($C6^A$); (51.64), (51.32), 51.16 (bnC4); (43.69), 42.52 (bnC1); (30.13), (29.56), (29.05), 28.48 (bnC2, bnC3). δ_C(D_2O/HCl, pH ~ 1) 104.53, 104.43, 103.75 (C1); 85.81 ($C4^A$); 84.04, 83.73, 83.04 (C4); 75.73, 75.64, 75.46, 75.01, 74.67, 74.59, 74.43, 74.33 (C2, C3, C5); 70.14 ($C5^A$); 63.31, 63.08, 62.97 (C6); 50.87 ($C6^A$); 50.28 (bnC4); 41.47 (bnC1); 26.67, 25.27 (bnC2, bnC3).

6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin **24**

A mixture of the tosylate **32** (1.432 g, 1.111×10^{-3} mol), 1,6-diaminohexane (0.460 g, 3.97×10^{-3} mol) and KI (0.016 g) in NMP (4 cm^3) was treated according to the general procedure to give the title compound as a white powder (0.700 g, 51%). R_c 0.75. Electrospray-ms *m/z* 1233 (M^+). (Found C, 44.95; H, 7.27; N, 1.88. Calculated for **24**.3H_2O ($C_{48}H_{90}N_2O_{37}$) C, 44.79; H, 7.04; N, 2.17%.) δ_H(D_2O/NaOH, pH ~ 14) 4.80 (s, 7H+ solvent, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.2-3.4 (m, 13H, H2, H4); 3.11 (t, J = 9.3 Hz, 1H, $H4^A$); 2.93 (d, J = 12.4 Hz, 1H, $H6^A$); 2.65 (m, 3H, $H6^{A'}$, hnH6); 2.46 (m, 2H, hnH1); 1.40 (m, 4H, hnH2, hnH5); 1.26 (m, 4H, hnH3, hnH4). δ_H(D_2O, pH ~ 9) 5.09 (s, 7H, H1); 3.8 - 1 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.48 (t, J = 9.3 Hz, 1H, $H4^A$); 3.28 (d, J = 12.0 Hz, 1H, $H6^A$); 2.99 (m, 3H, $H6^{A'}$, hnH1); 2.81 (bs, 2H, hnH6);1.65 (m, 4H, hnH2, hnH5); 1.41 (m, 4H, hnH3, hnH4). δ_H(D_2O/HCl, pH ~ 1) 4.80 (s, 7H + solvent, H1); 4.07 (t, J = 9.5 Hz, 1H, $H5^A$); 3.65-3.9 (m, 25H, H3, H5, H6); 3.4 - 3.6 (m, 14H, H2, H4); 3.22 (m, 2H, $H6^A$); 2.97 (m, 2H, hnH1); 2.88 (m, 2H, hnH6); 1.59 (m, 4H, hnH2, hnH5); 1.30 (bs, 4H , hnH3, hnH4). δ_C(D_2O/NaOH, pH ~ 14) 106.64, 106.29, 106.05, 105.91, 105.17 (C1); 87.42 ($C4^A$); 84.76, 84.68, 84.55, 84.38, 84.18, 83.72 (C4); 77.38, 77.25, 77.15, 76.26, 76.14, 75.11, 74.95, 74.86, 74.74 (C2, C3, C5); 71.16 ($C5^A$); 62.85, 62.68 (C6); (51.48), (50.27), (44.09), 43.29, 34.71, (32.26), (30.84),

(29.16), 28.96, (28.85), 28.56. δ_C(D_2O, pH ~ 9) 104.31, 103.71 (C1); 85.39 ($C4^A$); 83.72, 83.61, 82.94 (C4); 75.60, 75.42, 75.35, 75.30, 75.25, 74.49, 74.33 (C2, C3, C5); 71.66 ($C5^A$), 62.76 (C6); 51.32 ($C6^A$); 50.74 (hnC6); 41.90 (hnC1); 29.45, 29.28, 28.19, 27.91 (hnC2-5). δ_C(D_2O/HCl, pH ~ 1) 104.54, 104.43, 103.71 (C1); 85.80 ($C4^A$); 83.97, 83.72, 83.00 (C4); 75.74, 75.65, 75.45, 75.05, 74.69, 74.60, 74.43, 74.33 (C2, C3, C5); 70.11 ($C5^A$), 63.27, 62.98 (C6); 50.84 ($C6^A$); 50.72 (hnC6); 42.02 (hnC1); 29.13, 27.93, 27.82, 27.75 (hnC2-5).

6^A-(2-(2-aminoethyl)aminoethyl)amino-6^Adeoxy-β-cyclodextrin **25**

A mixture of the tosylate **32** (2.026 g, 1.57×10^{-3} mol), 2-(2-aminoethyl)-aminoethylamine (0.538 g, 5.27×10^{-3} mol) and KI (0.038 g) in NMP (5 cm^3) was treated according to the general procedure to give the title compound as a white powder (1.033 g, 54%). R_c 0.62. Electrospray-ms *m/z* 1220 (M^+). (Found C, 44.88; H, 6.75; N, 4.05. Calculated for **25**.H_2O ($C_{46}H_{83}N_3O_{35}$) C, 44.62; H, 6.75; N, 3.39%.) δ_H(D_2O/NaOH, pH ~ 14) 5.06 (s, 7H + solvent, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.1-3.3 (m, 13H, H2, H4); 3.01 (t, J = 9.6, 1H, $H4^A$); 2.82 (d, J = 12.6, 1H, $H6^A$); 2.3-2.6 (m, 9H, $C6^{A'}$, dienH). δ_H(D_2O, pH ~ 9) 5.07 (s, 7H, H1); 3.8-4.1 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.44 (t, J = 9.2 Hz, 1H, $H4^A$); 3.07 (d, J = 11.9 Hz, 1H, $H6^A$); 2.7 (m, 9H). δ_H(D_2O/HCl, pH ~ 1) 4.9 (m, 7H + solvent, H1); 4.08 (t, J = 8.2 Hz, 1H, $H5^A$); 3.6-3.9 (m, 25H, H3, H5, H6); 3.6 - 2.5 (m, 24H). δ_C(D_2O/NaOH, pH ~ 14) 106.8, 106.5, 106.4, 106.35, 106.3, 105.9 (C1); 88.0 ($C4^A$); 85.4, 85.3, 85.1, 84.9 (C4); 77.5, 77.4, 77.3 (C2); 76.6 (C3); 75.34, 75.2, 75.1 (C5); 72.8 ($C5^A$); 63.7 (C6); 53.9 (dienC3); 52.3 ($C6^A$); 50.5, 50.3 (dienC1,2); 43.1 (dienC4). δ_C(D_2O, pH ~ 9) 104.6, 104.3 (C1); 86.4 ($C4^A$); 83.9, 83.7 (C4); 75.8, 75.7, 74.8, 74.6 (C2, C3, C5); 73.2 ($C5^A$); 63.0 (C6); 52.9 ($C6^A$); 52.1 (dienC3); 50.6, 50.2 (dienC1, dienC2); 42.4 (dienC4). δ_C(D_2O/HCl) 104.45, 103.65(C1); 85.68 ($C4^A$); 84.15, 83.74, 82.96 (C4); 75.78, 75.73, 75.66, 75.48, 75.42, 75.03, 74.76, 74.72, 74.61, 74.44, 74.35 (C2, C3, C5); 70.10 ($C5^A$); 63.53, 63.17, 63.06 (C6); 51.58 ($C6^A$); 47.34(dienC3); 46.33 (dienC1); 46.00 (dienC2); 38.04 (dienC4).

6^A-(3-(3-aminopropyl)aminopropyl)amino-6^A-deoxy-β-cyclodextrin **26**

A mixture of the tosylate **32** (1.997 g, 1.55×10^{-3} mol), 3-(3-aminopropyl)-aminopropylamine (0.460 g, 3.97×10^{-3} mol) and KI (0.026 g) in NMP (5 cm^3) was treated according to the general procedure to give the title compound as a white powder (971 mg, 50%) . R_c 0.38. Electrospray-ms *m/z* 1248 (M^+). (Found C, 45.17; H, 6.52; N, 3.12. Calculated for **26**.$2H_2O$ ($C_{48}H_{89}N_3O_{36}$) C, 44.89; H, 6.98; N, 3.27%.) δ_H(D_2O/NaOH, pH ~ 14) 4.87 (bs, 7H + solvent, H1); 3.7-4.0 (m, 26H , H3, H5, H6); 3.4-3.6 (m, 13H, H2, H4); 3.27 (t, *J* = 9.6 Hz, 1H, $H4^A$); 2.98 (d, *J* = 12.6 Hz, 1H, $H6^A$); 2.4-2.7 (m, 9H, $H6^{A'}$, dipnH1, dipnH3, dipnH4, dipnH6); 1.4-1.6 (m, 4H, dipnH2, dipnH5). δ_H(D_2O, pH ~ 9) 4.91 (s, 7H, H1); 3.7 - 3.9 (m, 26H, H3, H5, H6); 3.4-3.6 (m, 13H, H2, H4); 3.26 (t, *J* = 8.9 Hz, 1H, $H4^A$); 2.90 (d, *J* = 11.9 Hz, 1H, $H6^A$); 2.4 - 2.7 (m, 9H ($H6^{A'}$, dipnH1, dipnH3, dipnH4, dipnH6); 1.53 (m, 4H, dipnH2, dipnH5). δ_H(D_2O/HCl, pH ~ 1) 5.10 (s, 7H+ solvent; H1); 4.22 (t, *J* = 9.2 Hz, 1H, $H5^A$); 3.8 - 4.1 (m, 25H, H3, H5, H6), 3.5-3.7 (m, 15H, H2, H4, $H6^A$); 3.42 (dd, *J* = 12.8, 9.2 Hz, 1H, $H6^{A'}$); 3.20 (m, 8H, dipnH1, dipnH3, dipnH4, dipnH6); 2.18 (m, 4H, dipnH3, dipnH5). δ_C(D_2O/NaOH, pH ~ 14) 105.3, 105.1, 104.8 (C1); 86.9 ($C4^A$); 84.3, 84.2, 83.9 (C4); 76.3, 76.1 (C2); 75.4 (C3); 74.7 (C5); 72.8 ($C5^A$); 63.1 (C6); 52.0 ($C6^A$); 49.3, 49.2, 48.9 (dipnC1, dipnC3, dipnC4); 41.5 (dipnC6); 34.5, 31.0 (dipnC2, dipnC5). δ_C(D_2O, pH ~ 9) 104.57, 104.16 (C1); 86.34 ($C4^A$); 83.88, 83.53 (C4); 75.78, 74.78, 74.72, 74.60 (C2, C3, C5); 73.02 ($C5^A$); 63.01 (C6); 51.97($C6^A$); 49.41, 49,29, (49.07), 48.79, (48.48), 41.27, (41.18) (dipnC1, dipnC3, dipnC4, dipnC6); 33.30, (31.52), 30.75, (30.24) (dipnC2, dipnC5). δ_C(D_2O/HCl, pH ~ 1) 104.49, 103.82 (C1); 85.83 ($C4^A$); 84.15, 83.84, 83.20 (C4); 75.83, 75.75, 75.55, 75.09, 74.77, 74.69, 74.54, 74.46 (C2, C3, C5); 70.19 ($C5^A$); 63.48, 63.14 (C6); 51.14 ($C6^A$); 47.96, 47.45, 47.34 (dipnC1, dipnC3, dipnC4); 39.32 (dipnC6); 26.44, 25.18 (dipnC2, dipnC5).

6^A-(2-(2-(2-aminoethyl)aminoethyl)aminoethyl)amino-6^A-deoxy-β-cyclodextrin **27**

A mixture of 2-(2-(2-aminoethyl)aminoethyl)aminoethylamine .4HCl (0.855 g, 2.92×10^{-3} mol) and sodium hydroxide (0.474 mg, 11.85×10^{-3} mol in ethanol (30 cm^3) was

stirred at room temperature for 15 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm^3). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm^3) and the tosylate **32** (1.119 g, 0.868×10^{-3} mol) and KI (0.024 g) were added to the solution. The resultant mixture was treated according to the general procedure to give the title compound as a white powder (0.446 g, 41%). R_c 0.28. Electrospray-ms *m/z* 1263 (M^+). (Found C, 44.83; H, 6.89; N, 4.42. Calculated for **27**.H_2O ($C_{48}H_{88}N_4O_{35}$) C, 44.99; H, 6.92; N, 4.37%.) δ_H(D_2O/NaOH, pH ~ 14) 5.0 (s, 7H + solvent, H1); 3.5-3.9 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.13 (t, J = 9.0 Hz, 1H, $H4^A$); 2.93 (d, J = 12 Hz, 1H, $H6^A$); 2.5 - 2.7 (m, 13H, $H6^{A'}$, trienH). δ_H(D_2O, pH ~ 9) 5.08 (s, 7H, H1); 3.8-4.0 (m, 26H, H3, H5, H6); 3.5 - 3.7 (m, 13H, H2, H4); 3.46 (t, J = 8.1 Hz, 1H, $H4^A$); 3.06 (d, J = 11.6 Hz, 1H, $H6^A$); 2.4 - 2.9 (m, 13H, $H6^{A'}$, trienH). δ_H(D_2O/HCl, pH ~ 1) 4.95 (bs, 7H + solvent, H1), 4.08 (t, J = 9.0 Hz, 1H, $H5^A$); 3.6-3.9 (m, 25H, H3, H5, H6); 3.2-3.6 (m, 28H). δ_C(D_2O/NaOH, pH ~ 14) 106.7, 106.3, 106.2, 105.8 (C1); 87.9 ($C4^A$); 85.2, 85.1, 85.0, 84.8 (C4); 77.3, 77.1 (C2); 76.5 (C3); 75.3, 75.2, 75.1 (C5); 73.0 ($C5^A$); 63.6 (C6); 53.9 (trienC1?); 52.2 ($C6^A$); 50.6, 50.2 (trienC2-5), 43.1 (trienC6). δ_C(D_2O, pH ~ 9) 104.55, 104.19 (C1); 86.31 ($C4^A$); 83.88, 83.63 (C4); 75.81, 75.74, 74.75, 74.56 (C2, C3, C5); 73.08 ($C5^A$); 63.01 (C6); 52.69 ($C6^A$); 52.01, (51.32), 50.51, 50.30, 50.21, (49.95), (43.02), 42.35. δ_C(D_2O/HCl, pH ~ 1) 104.48, 103.62 (C1); 85.71 ($C4^A$); 84.25, 83.82, 83.76, 82.95 (C4); 75.73, 75.65, 75.47, 75.37, 75.07, 74.69, 74.47, 74.40 (C2, C3, C5); 70.11 ($C5^A$); 63,57, 63.03 (C6); 51.59 ($C6^A$); 47.31, 46.19, 45.98, 45.88, 37.94.

6^A-(1,4,7-triazacyclonon-1-yl)-6^A-deoxy-β-cyclodextrin **29**

A mixture of 1,4,7 triazacyclononane.3HCl (1.014 g, 4.28×10^{-3} mol) and potassium hydroxide (0.261 g, 4.65×10^{-3} mol) in ethanol (40 cm^3) was stirred at room temperature for 15 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm^3). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm^3) and the tosylate **32** (1.945 g, 1.51×10^{-3} mol) and KI (0.025 g) were added to the solution. The resultant mixture was

treated according to the general procedure except that the crude product isolated after the ethanol precipitation step was dissolved in a solution of triethylamine (1.5 cm3) in water (10 cm^3) and the cyclodextrins were then precipitated by addition of ethanol (100 cm^3). The crude product was then purified by ion exchange as for the general procedure to give the title compound as a white powder (0.630 g, 33%). R_c 0.38. Electrospray-ms *m/z* 1246 (M^+). (Found C, 44.59; H, 6.83; N, 3.30. Calculated for **29**.3H_2O ($C_{48}H_{89}N_3O_{37}$) C, 44.34; H, 6.90; N, 3.23%.) δ_H(D_2O/NaOH, pH ~ 14) 5.0 (bs, 7H + solvent, H1); 3.5-3.9 (m, 26H, H3, H5, H6); 3.2-4.4 (m, 13H, H2, H4); 2.9-3.1 (m, 2H, $H4^A$, $H6^A$); 2.3-2.7 (m, 7H, $H6^{A'}$, tacnH). δ_H(D_2O, pH ~ 9) 5.0 (s, 7H, H1); 3.7-4.0 (m, 26H, H3, H5, H6); 2.7-3.7 (m, 23H, $H4^A$, $H6^A$, tacnH). δ_H(D_2O/HCl, pH ~ 1) 3.96 (bt, 1H, $H5^A$); 3.5-3.8 (m, 25H, H3, H5, H6); 3.0-3.4 (m, 23H, H2, H4, $H6^A$, tacnH). δ_C(D_2O/NaOH, pH ~ 14) 107.0, 106.6, 106.3, 106.0, 104.8 (C1); 87.8 ($C4^A$); 85.2, 85.1, 84.9, 83.7 (C4); 77.5, 77.2, 76.7, 76.4, 76.3, 75.6, 75.0, 74.8, (C2, C3, C5); 73.7 ($C5^A$); 63.3 (C6); 61.7, 60.5, 58.1, 57.4, 55.5, 55.3, 54.6, 49.8, 49.4, 47.2. δ_C(D_2O, pH ~ 9) 104.7, 104.6, 104.0 (C1); 86.3 ($C4^A$); 83.9, 83.3, 83.1 (C4); 75.9, 75.6, 74.7 (C2, C3, C5); 72.9 ($C5^A$); 63.0 (C6); 59.4, 58.6, 56.2, 54.4, 53.2, 52.8, 50.6, 49.0, 48.3, 46.5, 45.6. δ_C(D_2O/HCl, pH ~ 1) 104.4, 103.8 (C1); 86.5 ($C4^A$); 84.0, 83.8, 83.7, 83.1 (C4); 75.8, 75.7, 75.4, 75.2, 74.7, 74.5 (C2, C3, C5); 71.6 ($C5^A$); 63.4, 63.0 (C6); 56.7 ($C6^A$); 50.0 (tacnC1); 45.8, 44.4 (tacnC2 tacnC3).

When this reaction was carried out using 1,4,7-triazacyclononane that had been purified as described above (5.2.1) and without addition of potassium iodide to the reaction mixture a yield of 52% was obtained. The product of this reaction was identical to that described above in all respects.

6^A-(1,5,9-triazacyclododecan-1-yl)-6^A-deoxy-β-cyclodextrin **30**

A mixture of 1,5,9-triazacyclododecane.3HCl (1.451 g, 5.18 × 10^{-3} mol) and sodium hydroxide (0.625 g, 15.62 ×10^{-3} mol) in ethanol (30 cm^3) was stirred room temperature for 90 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm^3). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm^3) and the tosylate **32** (2.081 g,

1.61×10^{-3} mol) and KI (0.030 g) were added to the solution. The resultant mixture was treated according to the general procedure to give the title compound as a white powder (0.709 g, 34%). R_c 0.75 Electrospray-ms *m/z* 1288 (M^+). (Found C, 45.28; H, 7.34; N, 3.15. Calculated for **30**.$4H_2O$ ($C_{51}H_{97}N_3O_{38}$) C, 45.03; H, 7.18; N, 3.08%.) δ_H(D_2O/NaOH, pH ~ 14) 4.9 (bs, 7H+solvent, H1); 4.14 (t, J = 6.0 Hz, 1H, $H5^A$); 3.7-4.0 (m, 25H, H3, H5, H6); 3.17 (t, J = 6.0 Hz, 1H, $H4^A$); 2.88 (d, J = 15 Hz, 1H, $H6^A$); 2.64 (m, 13H, $H6^{A'}$, tacdoH1, tacdoH3, tacdoH4); 1.66 (m, 6H, tacdoH2, tacdoH5). δ_H(D_2O/~ 1 eq HCl, pH ~ 8.5) 5.09 (s, 7H + solvent , H1); 4.26 (t, J = 9.0 Hz, 1H, $H5^A$); 3.8-4.2 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.39 (t, J = 9.0 Hz, 1H, $H4^A$); 2.5-3.2 (m, 14H, $H6^A$, tacdoH1, tacdoH3, tacdoH4); 1.6 - 2.0 (m, 6H, tacdoH2, tacdoH5). δ_H(D_2O/~ 2 eq HCl, pH ~ 6.0) 5.07 (bs, 7H, H1); 4.25 (t, J = 9.0 Hz, 1H, $H5^A$); 3.8-4.1 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.43 (t, J = 9.0 Hz, 1H, $H4^A$); 2.7-3.3 (m, 14H, $H6^A$, tacdoH1, tacdoH3, tacdoH4); 1.7-2.2 (m, 6H, tacdoH2, tacdoH5). δ_H(D_2O/HCl, pH ~ 1) 5.0 (bs, 7H+solvent, H1); 4.33 (bt, 1H, $H5^A$); 3.7-4.0 (m 25H, H3, H5, H6); 3.2-3.6 (m, 27H, H2, H4, $H6^A$, tacdoH1, tacdoH3, tacdoH4); 2.2 (broad, 6H, tacdoH2, tacdoH5). δ_C(D_2O/NaOH, pH ~ 14) 106.9, 106.6, 106.4, 106.3, 105.8, 105.7, 104.3 (C1); 87.7 ($C4^A$); 85.2, 85.1, 85.0, 84.9, 84.5, 84.3, 82.9 (C4); 77.4, 77.2, 77.1, 77.0, 76.9, 76.8, 76.7, 76.5, 76.3, 76.1, 75.4, 75.1, 74.9 (C2, C3, C5); 72.5 ($C5^A$); 63.4, 63.1 (C6); 55.9 ($C6^A$); 54.6 (tacdoC1); 48.7, 48.6 (tacdoC3, tacdoC4); 28.0, 26.5 (tacdoC2, tacdoC5). δ_C(D_2O/~1 eq HCl, pH ~ 8.5) 104.8, 104.5, 103.4, 103.0 (C1); 86.4 ($C4^A$); 84.1, 83.9, 83.7, 83.6, 82.5 (C4); 76.1, 76.0, 75.9, 75.6, 74.9, 74.7 (C2, C3, C5); 70.5 ($C5^A$); 63.3, 63.2 (C6); 54.3 ($C6^A$); 51.9, 49.0 (tacdoC1, tacdoC3, tacdoC4); 26.6, 25.4 (tacdoC2, tacdoC5). δ_C(D_2O/~2 eq HCl, pH ~ 6) 104.8, 104.7,103.7 (C1); 86.5 ($C4^A$); 84.1, 83.9, 82.6 (C4); 76.1, 76.0, 75.9, 75.8, 75.6, 74.9, 74.8 (C2, C3, C5); 70.7 ($C5^A$); 63.4, 63.2 (C6); 53.8, (53.1), 47.5 (broad), (45.7) ($C6^A$, tacdoC1, tacdoC3, tacdoC4); 25.1 (broad), 23.9 (broad) (tacdoC2, tacdoC5). δ_C(D_2O/HCl, pH ~ 1) 104.9. 104.7, 104.6, 103.7 (C1); 86.3 ($C4^A$); 84.3, 84.0, 82.8 (C4); 76.1, 76.0, 75.9, 75.6, 75.2, 75.0, 74.7, 74.6 (C2, C3, C5); 70.0 ($C5^A$); 63.8, 63.3 (C6); 58.6, 50.9 (broad), 47.5, 45.2, 43.9 ($C6^A$, tacdoC1, tacdoC3, tacdoC4); (25.4), 23.7, 19.9 (tacdoC2, tacdoC5).

When this reaction was carried out using 1,5,9-triazacyclododecane purified as described above (5.2.2) and without addition of potassium iodide to the reaction mixture a yield of 50% was obtained. The product of this reaction was identical to that described above in all respects.

6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecan-1-yl)-β-cyclodextrin **31**

A mixture of 1,4,7,10-tetraazacyclododecane sulfate (1.845 g, 5.0×10^{-3} mol) and sodium hydroxide (0.784 g, 19.6×10^{-3} mol) was stirred in ethanol (40 cm^3) at room temperature for 90 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm^3). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm^3) and the tosylate **32** (2.177 g, 1.69×10^{-3} mol) and KI (0.024 g) were added to the solution. The resultant mixture was treated according to the general procedure to give the title compound as a white powder (0.759 g, 34.8%). R_c 0.31. Electrospray-ms *m/z* 1289 (M^+). (Found C, 44.76; H, 7.10: N, 4.36. Calculated for **31**.$3H_2O$ ($C_{50}H_{94}N_4O_{37}$) C, 44.71; H, 7.05; N, 4.17%.) δ_H(D_2O/NaOH, pH ~ 14) 4.9 (s, 7H+solvent, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.2-3.5 (m, 13H, H2, H4); 3.09 (t, J = 8.7 Hz, 1H, $H4^A$); 2.2-2.8 (m, 18H, $H6^A$, cyclenH). δ_H(D_2O, pH ~ 10) 5.03 (bs, 7H, H1); 3.7-4.0 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.41 (t, J = 9.3 Hz, 1H, $H4^A$); 2.4 - 2.9 (broad, 18H, $H6^A$, cyclenH). δ_H(D_2O/HCl, pH ~ 1) 4.9 (s, 7H + solvent, H1); 4.07 (t, J = 9.4 Hz, 1H, $H5^A$); 3.4-3.9 (m, 25H, H3, H5, H6); 2.9-3.5 (m, 32H, $H6^A$, cyclenH). δ_C(D_2O/NaOH, pH ~ 14) 106.9, 106.8, 106.6, 106.4, 105.7, 105.3, 104.4 (C1); 86.35 ($C4^A$); 85.2, 85.0, 84.7, 84.6, 84.0, 82.6 (C4); 77.9, 77.7, 77.5, 77.4, 77.2, 77.1, 76.8, 76.6, 76.4, 76.1, 76.0, 75.8, 75.6, 75.2, 75.1 (C2, C3, C5); 74.6 ($C5^A$); 63.1, 62.9, 62.8 (C6); 59.0 ($C6^A$); 55.0, (50.2), 48.0, 47.1, 46.1 (cyclenC). δ_C(D_2O, pH ~ 10) 104.9, 104.6, 104.3, 104.2, 103.8(C1); 85.6 ($C4^A$); 84.1, 83.6, 83.5, 82.2 (C4); 76.4, 76.1, 76.0, 75.8, 75.5, 75.4, 75.2, 75.1, 74.9, 74.6, 74.5 (C2, C3, C5); 73.5 ($C5^A$); 63.1, 63.0, 62.8 (C6); 58.3 ($C6^A$), 54.3, (50.2), 48.2, 46.9, 46.2 (cyclenC). δ_C(D_2O/HCl, pH ~ 1) 104.5, 104.4. 104.3, 103.6 (C1); 86.4 ($C4^A$); 84.0, 83.7, 83.6, 82.6 (C4); 75.9, 75.8, 75.6, 75.5, 75.3, 75.2, 74.9, 74.8, 74.7, 74.6, 74.4, 74.3, 74.2

(C2, C3, C5); 70.9 ($C5^A$); 63.6, 63.2, 63.0 (C6); 56.2 ($C6^A$); 51.5 (cyclenC1); 45.7, 45.4, 44.9 (cyclenC2-4).

E.2.4. 2D-ROESY spectroscopy of inclusion complexes.

E.2.4.1. Self-inclusion of the substituent

Inclusion of the substituent in 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin **24**

1D proton spectrum data: δ_H 4.80 (s, 7H+ solvent, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.2-3.4 (m, 13H, H2, H4); 3.11 (t, J = 9.3 Hz, 1H, $H4^A$); 2.93 (d, J = 12.4 Hz, 1H, $H6^A$); 2.70 (m, 1H, $H6^{A'}$); 2.65 (m, 2H, hnH6); 2.46 (m, 2H, hnH1); 1.40 (bs, 4H, hnH2, hnH5); 1.26 (bs, 4H, hnH3, hnH4).

2D ROESY cross-peaks: δ_H 1.26 (hnH3,4) shows cross-peaks with 1.40 (hnH2,5), 2.46 (hnH1), 2.65 (hnH6), 3.7 (H5), 3.8 (H3); 1.40 (hnH2,5) shows cross-peaks with 1.26 (hnH3,4), 2.46 (hnH1), 2.65 (hnH6), 3.7 (H5), 3.8 (H3); 2.46 (hnH1) shows cross peaks with 1.26 (hnH3,4), 1.4 (hnH2,5), 3.9 ($H5^A$?); 2.65 (hnH6) shows cross-peaks with 1.26 (hnH3,4), 1.4 (hnH2,5), 3.7 (H5), 3.8 (H3).

Inclusion of the substituent in 6^A-(3-(3-aminopropyl)aminopropyl)amino-6^A-deoxy-β-cyclodextrin **26**

1D proton spectrum data: δ_H 4.87 (bs, 7H + solvent, H1); 3.7-3.9 (m, 26H , H3, H5, H6); 3.4-3.6 (m, 13H, H2, H4); 3.27 (t, J = 9.6 Hz, 1H, $H4^A$); 2.98 (d, J = 12.6 Hz, 1H, $H6^A$); 2.65 (dd, J = 9.0, 12.6 Hz, 1H, $H6^{A'}$); 2.58 (t, J = 6.6 Hz, 2H, dipnH6) 2.48 (m, 6H, dipnH1, dipnH3, dipnH4); 1.5 (m, 4H, dipnH2, dipnH5).

2D ROESY cross-peaks: δ_H 1.5 (dipnH2, dipnH5) shows cross-peaks with 2.6-2.4 (dipnH1, dipnH3, dipnH4, dipnH6), 2.65 ($H6^A$), 3.7-3.9 (H3, H5); 2.48 (dipnH1, dipnH3, dipnH4) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.65 ($H6^{A'}$), 2.98 ($H6^A$), 3.7-3.9 (H3, H5); 2.58 (dipnH6) shows cross-peaks with 1.5 (dipnH2, dipnH5), 3.7-3.9 (H3, H5); 2.65 ($H6^{A'}$) shows cross-peaks with 2.48 (dipnH1, dipnH3, dipnH4), 2.98 ($H6^A$), 3.27

($H4^A$), 3.9 ($H5^A$); 2.98 ($H6^A$) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.48 (dipnH1, dipnH3, dipnH4), 2.65 ($H6^{A'}$), 3.7-3.9 (H3, H5); 3.27 ($H4^A$) shows cross-peaks with 2.65 ($H6^{A'}$); 3.7-3.9 (H3, H5) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.48 (dipnH1, dipnH3, dipnH4), 2.58 (dipnH6), 2.98 ($H6^A$).

E.2.4.2. Host-guest complexes of substituted cyclodextrins.

Inclusion of 4-methylbenzoate **37** *in* 6^A*-(6-aminohexyl)amino-*6^A*-deoxy-β-cyclodextrin* **24**

1D proton spectrum data: δ_H 7.80 (d, J = 7.8 Hz, 2H, ArH2); 7.29 (d, J = 7.8 Hz, 2H, ArH3); 5.00 (m, 7H + solvent, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.4-3.6 (m, 13H, H2, H4); 3.26 (t, J = 9.6 Hz, 1H, $H4^A$); 2.99 (d, J = 13.2 Hz, 1H, $H6^A$); 2.71 (m, 1H, $H6^{A'}$); 2.64 (t, J = 7.2 Hz, 2H, hnH6); 2.45 (t, J = 7.2 Hz, 2H, hnH1); 2.41 (s, 3H, Me); 1.4-1.5 (m, 4H, hnH2, hnH5); 1.2-1.3 (m, 4H, hnH3, hnH4).

2D ROESY cross-peaks: δ_H 1.2-1.3 (hnH3,4) shows cross-peaks with 1.4-1.5 (hnH2,5), 2.45 (hnH1), 2.64 (hnH6), 3.8 (H5), 3.9 (H3); 1.4-1.5 (hnH2,5) shows cross-peaks with 1.2-1.3 (hnH3,4), 2.45 (hnH1), 2.64 (hnH6), 3.8 (H5), 3.9 (H3); 2.41 (Me) shows a cross-peak with 7.29 (ArH3); 2.45 (hnH1) shows cross-peaks with 1.2-1.3 (hnH3,4), 1.4-1.5 (hnH2,5), 3.8 (H5), 3.9 (H3); 2.64 (hnH6) shows cross-peaks with 1.2-1.3 (hnH3,4), 1.4-1.5 (hnH2,5), 3.8 (H5), 3.9 (H3); 2.71 ($H6^{A'}$) shows a cross-peak with 3.26 ($H4^A$); 3.26 ($H4^A$) shows a cross-peak with 2.71 ($H6^{A'}$); 3.8 (H5) shows cross-peaks with 1.2-1.3 (hnH3,4), 1.4-1.5 (hnH2,5), 7.29 (ArH3), 7.80 (ArH2); 3.9 (H3) shows cross-peaks with 1.2-1.3 (hnH3,4), 1.4-1.5 (hnH2,5), 2.45 (hnH1), 2.64 (hnH6), 7.29 (ArH3), 7.80 (ArH2); 7.29 (ArH3) shows cross-peaks with 2.41 (Me), 3.8 (H5), 3.9 (H3), 7.80 (ArH2); 7.80 (ArH2) shows cross-peaks with 3.8 (H5), 3.9 (H3), 7.29 (ArH3).

Inclusion of (S)*-2-phenylpropionate* (S)-**38** *in* 6^A*-(6-aminohexyl)amino-*6^A*-deoxy-β-cyclodextrin* **24**

1D proton spectrum data: δ_H 7.30 (m, 4H, ArH2,2', ArH3,3'); 7.22 (m, 1H, ArH4); 4.92 (bs, 7H + solvent, H1); 3.6-4.0 (m, 26H, H3, H5, H6); 3.58 (q, J = 7.2 Hz, αCH);

3.2-3.5 (m, 13H, H2, H4); 3.19 (t, J = 9.6 Hz, 1H, $H4^A$); 2.93 (d, J = 12.6 Hz, 1H, $H6^A$); 2.67 (dd, J = 12.6, 9.5 Hz, 1H, $H6^{A'}$); 2.62 (t, J = 7.2 Hz, 2H, hnH6); 2.45 (t, J = 7.8 Hz, 2H, hnH1); 1.39 (m, 4H, hnH2, hnH5); 1.36 (d, J = 7.2 Hz, 3H, αMe); 1.25 (m, 4H, hnH3, hnH4).

2D ROESY cross-peaks: δ_H 1.25 (hnH3, hnH4) shows cross-peaks with 1.39 (hnH2, hnH5), 2.45 (hnH1), 2.62 (hnH6), 3.7(H5), 3.75 (H3); 1.36 (αMe) shows a cross-peak with 3.58 (αCH); 1.39 (hnH2, hnH5) shows cross-peaks with 1.25 (hnH3, hnH4), 2.45 (hnH1), 2.62 (hnH6), 3.7(H5), 3.75 (H3); 2.45 (hnH1) shows cross-peaks with 1.25 (hnH3, hnH4), 1.39 (hnH2, hnH5), 3.7(H5), 3.8 ($H5^A$); 2.62 (hnH6) shows cross-peaks with 1.25 (hnH3, hnH4), 1.39 (hnH2, hnH5), 3.7(H5), 3.75 (H3); 2.67 ($H6^{A'}$) shows cross-peaks with 2.93 ($H6^A$), 3.19 ($H4^A$); 2.93 ($H6^A$) shows cross-peaks with 2.67 ($H6^{A'}$), 3.7 (H5), 3.8 ($H5^A$); 3.19 ($H4^A$) shows cross-peaks with 2.67 ($H6^{A'}$), 3.75 (H3), 3.8 ($H5^A$); 3.58 (αCH) shows cross-peaks with 1.36 (αMe), 7.30 (ArH2,2', ArH3,3'); 3.7 (H5) shows cross-peaks with 1.25 (hnH3, hnH4), 1.39 (hnH2, hnH5), 2.62 (hnH6), 7.30 (ArH2,2', ArH3,3'); 3.75 (H3) shows cross-peaks with 1.25 (hnH3, hnH4), 1.39 (hnH2, hnH5), 2.62 (hnH6), 7.30 (ArH2,2', ArH3,3'); 3.8 ($H5^A$) shows cross-peaks with 2.45 (hnH1), 2.93 ($H6^A$), 3.19 ($H4^A$); 7.30 (ArH2,2', ArH3,3') shows cross-peaks with 3.58 (αCH), 3.75 (H3).

Identical spectra were recorded for the complex formed between (*R*)-2-phenylpropionate (*R*)-**38** and the cyclodextrin **24**.

Inclusion of 4-methylbenzoate **37** *in* 6^A*-(2-(2-aminoethyl)aminoethyl)amino-6A-deoxy-β-cyclodextrin* **25**

1D proton spectrum data: δ_H 7.68 (d, J = 7.8 Hz, 2H, H_o); 7.18 (d, J = 7.8 Hz, 2H, H_m); 4.83 (m, 7H, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.3-3.5 (m, 13H, H2, H4); 3.14 (t, J = 9.6 Hz, 1H, $H4^A$); 2.83 (d, J =13.2 Hz, 1H, $H6^A$); 2.4-2.6 (m, 9H, $H6^{A'}$, dienH2-4); 2.31 (s, 3H, Me).

2D ROESY cross-peaks: δ_H 2.31 (Me) shows cross-peaks with 3.5-3.8 (H3, H5), 7.18 (H_m); 2.6 ($H6^{A'}$) shows cross-peaks with 2.83 ($H6^A$), 3.14 ($H4^A$); 2.83 ($H6^A$) shows cross-peaks with 2.6 ($H6^{A'}$), 3.65 (H5); 3.14 ($H4^A$) shows cross-peaks with 2.6 ($H6^{A'}$); 3.65

(H5) shows cross-peaks with 2.31 (Me), 2.83 ($H6^A$), 7.18 (H_m), 7.68 (H_o); 3.8 (H3) shows cross-peaks with 2.31 (Me), 7.18 (H_m), 7.68 (H_o); 7.16 shows cross-peaks with 2.31 (Me), 3.6-3.8 (H3, H5); 7.68 shows cross-peaks with 3.6-3.8 (H3, H5).

Inclusion of 4-methylbenzoate **37***in* *6^A-(3-(3-aminopropyl)aminopropyl)amino-6^A-deoxy-β-cyclodextrin* **26**

1D proton spectrum data: δ_H 7.71 (d, J = 7.8 Hz, 2H, H_o); 7.20 (d, J =7.8 Hz, 2H, H_m); 4.87 (m, 7H, H1); 3.6-3.9 (m, 26H, H3, H5, H6); 3.2-3.5 (m, 13H, H2, H4); 3.18 (t, J = 9.0 Hz, 1H, $H4^A$); 2.89 (d, J = 12.0 Hz, 1H, $H6^A$); 2.62 (dd, J = 9.0, 12.0 Hz, 1H, $H6^{A'}$); 2.55 (t, J = 6.6 Hz, 2H, dipnH6); 2.46 (t, J = 7.8 Hz, 2H, dipnH4); 2.40 (t, J = 7.8 Hz, 2H, dipnH3); 2.34 (m, 5H, dipnH1, Me); 1.52 (m, 4H, dipnH2, dipnH5).

2D ROESY cross-peaks: δ_H 1.52 (dipnH2, dipnH5) shows cross-peaks with 2.34 (dipnH1), 2.40 (dipnH3), 2.46 (dipnH4), 2.55 (dipnH6); 2.34 (Me) shows cross-peaks with 3.6-3.9 (H3, H5), 7.20 (H_m); 2.40 (dipnH3) shows cross-peaks with 1.52 (dipnH2, dipnH5), 2.46 (dipnH4); 2.46 (dipnH4) shows cross-peaks with 1.52 (dipnH2, dipnH5), 2.40 (dipnH3), 2.55 (dipnH6); 2.55 (dipnH6) shows cross-peaks with 1.52 (dipnH2, dipnH5), 2.46 (dipnH4); 2.62 ($C6^{A'}$) shows cross-peaks with 2.89 ($C6^A$), 3.18 ($C4^A$); 2.89 ($C6^A$) shows cross-peaks with 2.62 ($C6^{A'}$), 3.7 ($H5^A$); 3.18 ($C4^A$) shows cross-peaks with 2.62 ($C6^{A'}$); 3.6-3.9 (H3, H5) shows cross-peaks with 2.34 (Me), 7.20 (H_m), 7.71 (H_o); 7.20 (H_m) shows cross-peaks with 2.34 (Me), 3.6-3.9 (H3, H5); 7.71 (H_o) shows cross-peaks with 3.6-3.9 (H3, H5).

E.3 Experimental for Chapter 3

E.3.1. Preparation of solutions

Table E.1. Buffer compositions of 0.05 mol dm^{-3} HEPES solutions (I = 0.1 mol dm^{-3})[a]

pH	x	y	pH	x	y
6.6	5	9.5	7.6	26.4	7.36
6.9	9.1	9.09	7.8	32.0	6.80
7.1	13.1	8.69	8.0	36.9	6.31
7.2	15.4	8.46	8.2	40.9	5.91
7.3	18.0	8.20	8.4	43.8	5.62

[a] 1.192 g of HEPES + **x** cm^3 0.1 mol dm^{-3} NaOH + **y** cm^3 1.0 mol dm^{-3} $NaClO_4$ made up to 100 cm^3 with deionised water.

Table E.2. Composition of 0.05 mol dm^{-3} borate buffer (I = 0.1 mol dm^{-3})[a]

pH	x	pH	x
7.9	3.9	9.1	23.6
8.1	4.9	9.5	34.6

[a] Stock boric acid solution prepared by dissolving 6.184 g H_3BO_3 and 12.244 g $NaClO_4$ in water to 1 dm^3. Buffer prepared by adding **x** cm^3 0.1 mol dm^{-3} NaOH to 50 cm^3 of boric acid stock and diluting to 100 cm^3 with water.

Buffers were prepared from analytical grade reagents. Boric acid and sodium perchlorate were obtained from BDH. Sodium perchlorate was dried under vacuum over P_2O_5 prior to use. HEPES was obtained from Aldrich and used as supplied. Buffers were prepared according to Perrin and Boyd[174] except that $NaClO_4$ was used to maintain the ionic strength at I =0.1 mol dm^{-3} rather than KCl or NaCl.

Solutions containing cyclodextrins were prepared by dissolving the appropriate weight

of the cyclodextrin in buffer to give stock solutions of 1.03×10^{-3} mol dm^{-3} in cyclodextrin. The molecular weight of the cyclodextrin was assumed to be that of the hydrate determined by elemental analysis.

The final pH of all buffered solutions was measured using an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode that was filled with 0.10 mol dm^{-3} $NaClO_4$. There was some variation with the nominal pH of the buffer and that found for some of the final solutions. The reported pH values are those measured in this way.

Fresh stock solutions of 4-nitrophenyl acetate **48** were prepared each day by dissolving 74.2 mg of the ester **48** in dry acetonitrile (10 cm^3). For initial rate studies 0.05 cm^3 of this solution was added to 2.0 cm^3 of solution to give a final ester concentration of 1.0×10^{-3} mol dm^{-3}. For the first order studies this stock solution was diluted 1:100 with dry acetonitrile and 0.05 cm^3 of this solution was added to 2.0 $cm^3$of solution to give a final ester concentration of 1.0×10^{-5} mol dm^{-3}.

E.3.2. Reactions of Zn(II) complexes of amino-cyclodextrins

The variation of molar absorbance ε_{400} of 4-nitrophenol **49** with pH was determined by measuring the absorbance at 400 nm of solutions of phenol **49** at concentrations over the range $0.337\text{-}8.41 \times 10^{-5}$ mol dm^{-3} in buffer over the range pH 6.9-8.5. Solutions were prepared by addition of 0.05 cm^3 of stock solutions of the phenol to 2.0 cm^3 of buffer (Table E.3). The values of ε_{400} were used to convert the measured rate of change of absorbance at 400 nm to the rate of formation of 4-nitrophenol **49** for the buffer reaction and the reaction with complex **43**.

The reactions of the ester **48** with the complexes **43** and **47** were examined by the initial rate method. Reactions were carried out at 298.2 K by pipetting 2.0 cm^3 of the appropriate solution (buffer or buffer + catalyst) into a quartz cell (1 cm pathlength) and placing this in the heated cell block of a Cary 2200 spectrophotometer. The solution was allowed to equilibrate and then 0.05 cm^3 of a stock solution of 4-nitrophenyl acetate **48** in acetonitrile (0.041 mol dm^{-3}) was added to give a final solution that was 2.5% acetonitrile and contained each reactant at a concentration of 1.0×10^{-3} mol dm^{-3}. The solution was mixed quickly and

the increase in absorbance at 400 nm was recorded digitally for the first 2% of reaction. The absorbance was referenced against a solution of buffer placed in the reference beam. Each run was carried out in triplicate and the results averaged. Variations between runs were less than 5%. The data collected for the reactions of the ester **48** in the presence and the absence of the complex **43** are given in Tables E.4 and E.5.

Table E.3. Experimental data used to determine the variation of molar absorbance at 400 nm with pH for 4-nitrophenol **49**[a]

	A_{400}						
10^5[**49**] /mol dm^{-3}	pH 6.9	pH 7.1	pH 7.3	pH 7.6	pH 7.8	pH 8.0	pH 8.5
0.337	0.033	0.037	0.039	0.048	0.052	0.054	0.059
0.520	0.047	0.056	0.066	0.074	0.075	0.079	0.091
0.841	0.085	0.090	0.108	0.120	0.119	0.116	0.129
1.27	0.125	0.147	0.174	0.176	0.196	0.202	0.229
2.58	0.247	0.273	0.295	0.349	0.359	0.400	0.438
6.34	0.684	0.725	0.817	0.0936	0.862	1.023	
8.41	0.872	0.943	1.038	1.199		1.252	1.390
ε_{400}[b]	10600	11300	12500	13500	14500	15300	16500

[a] In 0.05 mol dm^{-3} HEPES buffer (I = 0.1 mol dm^{-3}) 2.5% v/v acetonitrile in water at 298.2 K. [b] Molar absorbance calculated by determining slope of the line from plots of A_{400} vs [**49**].

Table E.4. Experimental data used to obtain the values for the initial rate of formation of 4-nitrophenol **49** from the reaction of 4-nitrophenyl acetate **48** (1.0×10^{-3} mol dm^{-3}) in aqueous buffered solutions (I = 0.1 mol dm^{-3}) at 298.2 K.[a]

pH	ε_{400}	10^4slope[b] /AU s^{-1}	10^4average /AU s^{-1}	$10^8 k_o$[c] /mol dm^{-3} s^{-1}
6.9	10600	0.588 0.580 0.569	0.579	0.546
7.1	11300	0.771 0.774 0.770	0.772	0.689
7.3	12500	1.11 1.20 1.10	1.14	0.912
7.6	13500	1.92 1.80 1.88	1.87	1.39
7.8	14500	3.00 2.87 2.90	2.92	2.01
8.0	15300	4.27 4.29 4.22	4.26	2.78
8.2	15900[d]	7.90 7.89 7.83	7.87	4.95

[a] 0.05 mol dm^{-3} HEPES. [b] Determined from the least squares fit of A_{400} against time for the first 2% of reaction. [c] Rate of formation of the phenol **49** calculated by dividing the average slope by the value of ε_{400}. [d] From interpolation of a plot of ε_{400} against pH.

Table E.5. Experimental data used to obtain the values for the initial rate of formation of 4-nitrophenol **3.7** from the reaction of 4-nitrophenyl acetate **48** (1.0×10^{-3} mol dm^{-3}) in the presence of the complex **43** (1.0×10^{-3} mol dm^{-3}) in aqueous buffered solutions ($I = 0.1$ mol dm^{-3}) at 298.2 K.[a]

pH	ε_{400}	10^4slope[b] /AU s^{-1}	10^4average /AU s^{-1}	$10^8 k_{obs}$[c] /mol dm^{-3} s^{-1}
6.9	10600	1.45 1.41 1.40	1.42	1.34
7.1	11300	2.14 2.06 2.13	2.11	1.87
7.3	12500	4.64 4.60 4.50	4.58	3.66
7.6	13500	6.24 6.29 6.04	6.19	4.59
7.8	14500	6.44 6.40 6.30	6.38	4.40
8.0	15300	10.8 11.2 10.7	10.9	7.12
8.2	15900[d]	14.4 15.2 14.9	14.8	9.31

[a] 0.05 mol dm^{-3} HEPES. [b] Determined from the least squares fit of A_{400} against time for the first 2% of reaction. [c] Rate of formation of the phenol **49** calculated by dividing the average slope by the value of ε_{400}. [d] From interpolation of a plot of ε_{400} against pH.

The molar absorbance ε_{400} of 4-nitrophenol **49** is affected by the presence of cyclodextrins. Therefore it was necessary to determine the value of ε_{400} for each solution containing a cyclodextrin. The molar absorbance ε_{400} of 4-nitrophenol **49** in solutions containing cyclodextrins was calculated from the observed absorbance at 400 nm measured by adding 0.05 cm^3 of a stock solution of phenol **49** (1.5×10^{-3} mol dm^{-3}) in acetonitrile to the cyclodextrin solution (or a solution of the buffer) to give a final concentration of 3.65×10^{-5}

mol dm^{-3} phenol **49** in 2.5% acetonitrile. These values of ε_{400} were used to convert the measured rate of change of absorbance at 400 nm to the rate of formation of 4-nitrophenol **49** for reactions involving cyclodextrins. In order to minimise the systematic errors that may arise from using this method to calculate ε_{400}, the reactions of the ester **48** in buffer alone were repeated, and the initial rate values, k_o, were calculated using a value of ε_{400} determined by addition of the stock solution of the phenol **49** to a solution of buffer as described above. The data collected for the reactions of the ester **48** in the presence and the absence of the complex **47** are given in Tables E.6 and E.7.

Table E.6. Experimental data used to obtain the values for the initial rate of formation of 4-nitrophenol **49** from the hydrolysis of 4-nitrophenyl acetate **48** (1.0×10^{-3} mol dm^{-3}) in aqueous buffered solutions (I = 0.1 mol dm^{-3}) at 298.2 K.

pH	A_{400}[a]	ε_{400}	10^4slope[b] /AU s^{-1}	10^4average /AU s^{-1}	$10^8 k_o$[c] /mol dm^{-3} s^{-1}
6.6[d]	0.285	7800	0.173 0.178 0.168	0.173	0.218
7.2[d]	0.550	15000	0.835 0.805 0.776	0.805	0.417
7.8[d]	0.655	18000	1.92 1.95 1.95	1.94	1.08
8.1[d,e]	0.704	19300	4.73 4.80	4.77	2.47
8.4[d]	0.730	20000	6.58 6.50	6.54	3.27
9.1[e]	0.757	20700	38.5 39.1 39.6	39.1	18.9

[a] Measured from a solution of 4-nitrophenol **49** (3.65×10^{-5} mol dm^{-3}) in buffer and used to calculate the value for ε_{400}. [b] Determined from the least squares fit of A_{400} against time for the first 2% of reaction. [c] Rate of formation of the phenol **49** calculated by dividing the average slope by the calculated values of ε_{400}. [d] 0.05 mol dm^{-3} HEPES. [e] 0.05 mol dm^{-3} borate.

Table E.7. Experimental data used to obtain the values for the initial rate of formation of 4-nitrophenol **49** from the reactions of 4-nitrophenyl acetate **48** (1.0×10^{-3} mol dm^{-3}) in the presence of the complex **47** (1.0×10^{-3} mol dm^{-3}) in aqueous buffered solutions (I = 0.1 mol dm^{-3}) at 298.2 K.

pH	A_{400}[a]	ε_{400}	10^4slope[b] /AU s^{-1}	10^4average /AU s^{-1}	$10^8 k_{obs}$[c] /mol dm^{-3} s^{-1}
6.6[d]	0.325	8900	0.634 0.639 0.614	0.629	0.707
7.2[d]	0.538 (0.481)[g]	14700 (13200)[g]	3.34 3.23 3.28 (3.16, 3.22)[g]	3.28 (3.19)[g]	2.23 (2.42)[g]
7.8[d]	0.705	19300	16.6 17.0 15.9	16.5	8.55
8.1[d]	0.755	20700	33.2 32.0 34.1	33.1	16.0
8.1[e]	0.776	21300	49.2 48.8	49.0	23.0
8.4[d, f]	0.775	21300	54.5 52.2 53.6	53.4	24.9
9.1[e, f]	0.770 (0.691)[g]	21100 (18900)[g]	274 269 283 (321, 322)[g]	275 (322)[g]	130 (170)[g]

[a] Measured from a solution of 4-nitrophenol **49** (3.65×10^{-5} mol dm^{-3}) in buffer containing the Zn(II) complex of the cyclodextrin **30** and used to calculate the value for ε_{400}. [b] Determined from the least squares fit of A_{400} against time for the first 2% of reaction. [c] Rate of formation of the phenol **49** calculated by dividing the average slope by the calculated values of ε_{400}. [d] 0.05 mol dm^{-3} HEPES. [e] 0.05 mol dm^{-3} borate. [f] Some precipitate was observed in the solutions containing Zn(II). [g] Reaction in the absence of Zn(II).

The reactions of the ester **48** with the cyclodextrin **31** in the presence and absence of Zn(II) were carried out as described above. The experimental data collected for these reactions is given in Table E.8.

Table E.8. Experimental data used to obtain the values for the initial rate of formation of 4-nitrophenol **49** from the reactions of 4-nitrophenyl acetate **48** (1.0×10^{-3} mol dm^{-3}) in the presence of the cyclodextrin **31** (1.0×10^{-3} mol dm^{-3}) in aqueous buffered solutions ($I = 0.1$ mol dm^{-3}) at 298.2 K in the presence and absence of $Zn(ClO_4)_2$.

10^3[Zn(II)] /mol dm^{-3}	pH	A_{400}[a]	ε_{400}	10^4slope /AU s^{-1}[b]	$10^8 k_{obs}$ /mol dm^{-3} s^{-1}[c]
0	7.2[d]	0.468	12800	2.69 2.70 2.49	2.05
1.0	7.2[d]	0.464	12700	3.91 3.71	3.00
0	9.1[e]	0.678	18600	191 193 186	102
1.0	9.1[e]	0.685	18800	141 139	74.5

[a] Measured from a solution of 4-nitrophenol **49** (3.65×10^{-5} mol dm^{-3}) in buffer containing the cyclodextrin **31** or the cyclodextrin **31** and Zn(II), and used to calculate the value for ε_{400}. [b] Determined from the least squares fit of A_{400} against time for the first 2% of reaction. [c] Rate of formation of the phenol **49** calculated by dividing the average slope by the calculated values of ε_{400}. [d] 0.05 mol dm^{-3} HEPES. [e] 0.05 mol dm^{-3} borate.

E.3.3. Kinetics of reactions of 6^A-ω-aminoalkylamino-β-cyclodextrins

These reactions were studied by the first order method. Reactions were carried out at 298.2 K by pipetting 2.0 cm^3 of a stock solution (1.03×10^{-3} mol dm^{-3}) of the amino-alkylamino-β-cyclodextrin **21**, **22** or **24** (or the corresponding free amine $NH_2(CH_2)_xNH_2$) in 0.05 mol dm^{-3} borate buffer pH 9.1 into a quartz cell (1 cm pathlength) and placing this in the heated cell block of a Cary 2200 spectrophotometer. The solution was allowed to equilibrate and then 0.05 cm^3 of a stock solution of 4-nitrophenyl acetate **48** in acetonitrile (4.1×10^{-4} mol dm^{-3}) was added to give a final solution that was 2% acetonitrile and contained the ester **48** at a

concentration of 1.0×10^{-5} mol dm^{-3} and the amine at a concentration of 1.0×10^{-3} mol dm^{-3}. The solution was mixed quickly and the increase in absorbance at 400 nm was recorded digitally for at least eight reaction half-lives. The absorbance of the reaction solution was referenced against a solution of buffer placed in the reference beam. The rate of hydrolysis of ester **48** in buffer alone was determined by a similar method.

Table E.9. Experimental data for the first -order rates for the formation of 4-nitrophenol **49** from the reaction of the ester **48** in the presence of cyclodextrins or diaminoalkanes in 0.05 mol dm^{-3} borate buffer pH 9.1 (I = 0.1 mol dm^{-3}) at 298.2 K.[a]

Added reactant[b]	10^3observed rate/s^{-1}[c]	10^3 average rate/s^{-1}
nil	0.190, 0.208	0.200
$NH_2(CH_2)_2NH_2$	0.893, 0.877, 0.900	0.890
$NH_2(CH_2)_3NH_2$	1.33, 1.35, 1.37	1.35
$NH_2(CH_2)_6NH_2$	1.27, 1.30, 1.26	1.28
2	0.339, 0.341, 0.351	0.344
21	7.17, 7.46	7.32
22	3.79, 3.81, 3.89	3.83
24	1.13, 1.13	1.13

[a] Initial concentration of the ester **48** 1.0×10^{-5} mol dm^{-3}. [b] Initial concentration of added reactant 1.0×10^{-3} mol dm^{-3}. [c] Calculated from a least squares fit of a plot of $\ln(A_{inf}-A_t)$ against time.

The first order rate constants were calculated by fitting the collected data to a first order rate equation ($A_{inf}-A_t = A_{inf}.e^{-kt}$). The amount of 4-nitrophenyl acetate **48** remaining in the reaction mixture at ant time, t, is related to the absorbance at infinite time, A_{inf}, minus the absorbance at that time, A_t. Plots of $\ln(A_{inf}-A_t)$ against time give straight lines with a slope equal to the first order rate constant, k. Each run was carried out in triplicate and the results averaged. Variations in the calculated first order rates between runs were less than 5%. The obtained data from these reactions is given in Table E.9.

E.3.4. pH dependence study

These reactions were carried out as described above in Section E.3.3. Solutions of the cyclodextrin **24** (1.03×10^{-3} mol dm^{-3}) were prepared in 0.05 mol dm^{-3} borate buffers over the range pH 9.1-10.3. The reactions were carried out in triplicate and the results were averaged. Variations in the calculated first order rates were less than 5% between runs. The obtained experimental data is given in Table E.10.

Table E.10. Experimental data for the variation of first order rate constant for the reaction of 4-nitrophenyl acetate **48** with the cyclodextrin **24** and concentrations of non- and mono-protonated species **24** in 0.05 mol dm^{-3} borate buffers (I =0.1 mol dm^{-3}) at 298.2 K.[a]

	buffer reaction		added **24**				
pH	$10^3 k_o$/s^{-1}	average $10^3 k_o$/s^{-1}	$10^3 k_{obs}$ /s^{-1}	average $10^3 k_{obs}$ /s^{-1}	10^3 (k_{obs}-k_o) /s^{-1}	10^4[**24**] /mol dm^{-3} non-protonated[b]	10^4[**24**] /mol dm^{-3} mono-protonated[b]
10.3	1.87 1.91 1.83	1.87	10.3 11.1 10.9	10.8	8.93	5.2	4.7
10.0	0.994 1.05 1.08	1.02	5.91 5.91 6.17	6.00	4.98	3.5	6.2
9.7	0.577 0.575 0.584	0.579	3.61 3.66	3.64	3.06	2.0	7.2
9.4	0.290 0.280 0.280	0.283	1.86 1.84 1.86	1.85	1.57	1.1	7.5
9.1	0.191 0.208	0.200	1.13 1.13 1.13	1.13	0.93	0.44	6.7

[a] Initial concentration of the ester **48** is 1.0×10^{-5} mol dm^{-3}. Initial concentration of the cyclodextrin **24** is 1.0×10^{-3} mol dm^{-3}. [b] Calculated from the measured pK_as = 10.47 and 8.72.

E.3.5. Preparation of *N*-acetyl-aminoalkyl amino β-cyclodextrins

6^A-(2-acetamidoethylamino-6^A-deoxy-β-cyclodextrin **53**

A mixture of the cyclodextrin 21 (0.100 g, 0.085×10^{-3} mol) and 4-nitrophenyl acetate **48** (0.015 g, 0.082×10^{-3} mol) in NMP (2 cm^3) was stirred at room temperature for 18 hours. TLC analysis showed the presence of β-cyclodextrin **2** and a new spot (R_c 1.06). The yellow solution was diluted with 1 mol dm^{-3} HCl (30 cm^3) and washed with dichloromethane (5×20 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions containing the product were combined and dried to give the title compound as a white powder (0.021 g, 21%). R_c 1.06. Electrospray-ms 1219 (M^+). (Found C, 40.43; H, 6.38; N, 1.86. Calculated for **53**.HCl.$6H_2O$ ($C_{46}H_{91}ClN_2O_{41}$) C, 40.52; H, 6.72; N, 2.05%). δ_H (D_2O) 5.07 (s, 7H, H1); 3.8-4.0 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.43 (t, J = 9.3 Hz, 1H, $H4^A$); 3.31 (m, 2H, CH_2NAc); 3.06 (d, J = 11.7 Hz, 1H, $H6^A$); 2.77 (m, 3H, $H6^{A'}$, CH_2NH); 1.99 (s, 3H, Me). δ_C-(D_2O) 177.05 (C=O); 104.57, 104.21 (C1); 86.32 ($C4^A$); 83.90, 83.56 (C4); 75.84, 74.83, 74.60 (C2, C3, C5); 73.11 ($C5^A$); 63.04 (C6); 51.64 ($C6^A$); 50.14 (enC1); 41.26 (enC2); 24.63 (Me).

6^A-(3-acetamidopropyl)amino-6^A-deoxy-β-cyclodextrin **54**

A mixture of the cyclodextrin **22** (0.096 g, 0.086×10^{-3} mol) and 4-nitrophenyl acetate **48** (0.016 g, 0.088×10^{-3} mol) in NMP (2 cm^3) was stirred at room temperature for 18 hours. TLC analysis showed the presence of β-cyclodextrin **2** and a new spot (R_c 1.1). The yellow solution was diluted with 1 mol dm^{-3} HCl (30 cm^3) and washed with dichloromethane (5×10 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions 4-14, containing the product, were combined and dried to give the title compound as a white powder

(0.048 g, 45%). *Rc* 1.1. Electrospray-ms 1233 (M^+). (Found C, 41.63; H, 6.81; N, 2.23. Calculated for 54.HCl.5H_2O ($C_{47}H_{91}ClN_2O_{40}$) C, 41.52; H, 6.74; N, 2.06%). δ_H (D_2O, 25 °C) 5.06 (s, 7H, H1); 3.8-4.0 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 14H, H2, H4);3.0-3.5 (m, 5H); 2.80 (m, 0.3H); 2.58 (t, J = 6.9 Hz, 0.6H); 2.00, 1.98 (s, 3H, Me ratio 2:1); 1.68 (m, 2H, pnH2). δ_C (D_2O) 176.69, 167.46 (C=O); 104.82, 104.33 (C1); 86.57 ($C4^A$); 83.92, 83.52 (C4); 75.94, 74.95, 74.67 (C2, C3, C5); 72.67 ($C5^A$); 65.38, 63.03 (C6); 51.86 ($C6^A$); 48.52 (pnC1); 39.95 (pnC3); 30.93 (pnC2); 24.64 (Me). δ_H (D_2O, 50 °C) 5.35 (s, 7H, H1); 4.0-4.3 (m, 26H, H3, H5, H6); 3.8-4.0 (m, 13H, H2, H4); 3.68 (t, J = 8.9 Hz, 1H, $H4^A$); 3.47 (t, J = 6.9 Hz, 2H, CH_2NAc); 3.30 (d, J = 11.9 Hz, 1H, $H6^A$); 3.04 (dd, J = 11.9, 7.1 Hz, 1H, $H6^{A'}$); 2.87 (t, J-= 6.9 Hz, 2H, CH_2NH); 2.26 (s, 3H, Me); 1.94 (bs, 2H, pnH2).

6^A-(6-acetamidohexylamino-6^A-deoxy-β-cyclodextrin **55**

A mixture of the cyclodextrin **24** (0.101 g, 0.082 × 10^{-3} mol) and 4-nitrophenyl acetate **48** (0.014 g, 0.078 × 10^{-3} mol) in NMP (2 cm^3) was stirred at room temperature for 18 hours. TLC analysis showed the presence of β-cyclodextrin **2** and a new spot (R_c 1.2). The yellow solution was diluted with 1 mol dm^{-3} HCl (30 cm^3) and washed with dichloromethane (5 × 20 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions containing the product were combined and dried to give the title compound as a white powder (0.018 g, 18%). R_c 1.2. Electrospray-ms 1275 (M^+). (Found C, 42.84; H, 6.64; N, 1.99. Calculated for 55.HCl.5H_2O ($C_{50}H_{97}ClN_2O_{40}$) C, 42.84; H, 6.97; N, 2.00%). δ_H (D_2O) 5.07 (s, 7H, H1); 3.5-4.0 (m, 39H, H2, H3, H4, H5, H6); 3.40 (t, J = 9.0 Hz, 1H, $H4^A$); 3.16 (t, J =7.2 Hz, 2H, CH_2NAc); 3.05 (d, J = 12.6 Hz, 1H, $H6^A$); 2.76 (m, 1H, $H6^{A'}$); 2.58 (t, J =7.2 Hz, 2H, CH_2NH); 1.99 (s, 3H, Me); 1.2-1.6 (m, 8H, hnH2, hnH3, hnH4, hnH5). δ_C-(D_2O)-176.51 (C=O); 104.81, 104.73, 104.61, 104.43, 103.36 (C1); 85.72 ($C4^A$); 84.03, 83.89, 83.75, 82.83 (C4); 76.48, 76.04, 75.85, 75.65, 74.79, 74.45 (C2, C3, C5); 71.24

($C5^A$); 63.04, 62.90 (C6); 50.47 ($C6^A$); 49.20 (hnC1); 42.11 (hnC6); 31.26, 29.92, 28.81, 28.70 (hnC2, hnC3, hnC4, hnC5); 24.76 (Me).

Identification of the products of the reaction between the cyclodextrin **24** *and the esters* **48** *and* **52**.

A solution of 4-nitrophenyl acetate **48** (3.0 mg, 0.016×10^{-3} mol) in dry acetonitrile (0.1 cm^3) was added to a stirred solution of the cyclodextrin **24** (15 mg, 0.012×10^{-3} mol) in 0.05 mol dm^{-3} borate buffer pH 9.1 (5 cm^3). The resultant yellow solution was left to stir at room temperature for 18 hours after which time the solution was freeze-dried and the residue was dissolved in D_2O (0.8 cm^3). The 300 MHz ^1H NMR spectrum of this solution showed the presence of a complex mixture of products. Signals due to acyl methyl groups appeared at δ 2.11 and 1.94 and were integrated as 10 units and 27 units respectively. The signal for protons H1 integrated as 117 units. The solution was made pH ≥ 12 by the addition of sodium hydroxide and left to stand at room temperature for 2 hours. The 300 MHz ^1H NMR spectrum of this solution showed that the signal at δ 2.11 had disappeared suggesting that this signal was due to an *O*-acyl group. The spectrum appeared to be that of a mixture of the cyclodextrins **24** and **55**

The above procedure was repeated using 3-nitrophenyl acetate **52** (3.0 mg, 0.016×10^{-3} mol) in place of 4-nitrophenyl acetate **48**. The 300 MHz ^1H NMR spectrum of the solution after work-up showed signals at δ 2.11 and 1.94 which integrated as 23 and 7 units respectively. The signal for protons H1 integrated as 95 units.

E.3.6. Inhibition by adamantane-1-carboxylate

Stock solutions of adamantane-1-carboxylate **59** and 4-nitrophenyl acetate **48** were prepared by dissolving either 0.0182 g or 0.072 g of adamantane-1-carboxylate **59** in acetonitrile, adding 0.039 cm^3 of 0.0534 mol dm^{-3} 4-nitrophenyl acetate **48** in acetonitrile and making the resultant solution up to 5 cm^3 with acetonitrile to give solutions which were 4.22×10^{-4} mol dm^{-3} in ester **48** and either 2.0×10^{-2} mol dm^{-3} or 8.0×10^{-2} mol dm^{-3} in adamantane-1-carboxylate **59**. For the inhibition studies 0.05 cm^3 of either of these solutions

was added to 2.0 cm^3 of a solution of one of the cyclodextrins **21**, **22** or **24** (1.05×10^{-3} mol dm^{-3}) in 0.05 mol dm^{-3} borate buffer pH 9.1 and the reactions monitored as described above. The experimental data collected is given in Table E.11.

Table E.11. Experimental data for the effect of adamantane-1-carboxylate **59** on the first order rate of the reaction between the ester **48** and the cyclodextrins **21-24** in 0.05 mol dm^{-3} borate buffer pH 9.1 (I = 0.1 mol dm^{-3}) at 298.2 K.[a]

cyclodextrin	$10^3 k_{obs}/s^{-1}$	10^3average k_{obs}/s^{-1}
21	4.24, 4.49, 4.59	4.44
22	2.17, 2.19 (0.704, 0.606, 0.678)[b]	2.18 (0.663)[b]
24	0.943, 0.916, 0.889	0.916

[a] Initial concentrations of the ester **48** is 1.0×10^{-5} mol dm^{-3}. Initial concentration of the cyclodextrin is 1.0×10^{-3} mol dm^{-3}. The concentration of the added carboxylate **59** is 0.48×10^{-3} mol dm^{-3}. [b] Concentration of the carboxylate **59** is 2.0×10^{-3} mol dm^{-3}.

Inclusion of 1-adamantane carboxylate **59** *in* 6^A*-(6-aminohexyl)amino-*6^A*-deoxy-β-cyclodextrin* **24**

1D proton spectrum data: δ_H 4.65 (m, 7H, H1); 3.81 (t, J = 9.6 Hz, 1H, $H5^A$); 3.5-3.8 (m, 25H, H3, H5, H6); 3.2-3.4 (m, 13H, H2, H4); 3.06 (t, J = 9.6 Hz, 1H, $H4^A$); 2.92 (d, J = 14.0 Hz, 1H, $H6^A$); 2.58 (dd, J = 14.0, 9.6 Hz, 1H, $H6^{A'}$); 2.42 (m, 2H, hnH6); 2.23 (dt, J = 5.4, 10.8 Hz, 1H, hnH1); 2.14 (d, J = 5.4 Hz, 1H, hnH1'); 1.99 (bs, 3H, AdH3); 1.76 (bs, 6H, AdH2); 1.69 (bd, J = 10.8 Hz, 3H, AdH4); 1.45 (bd, J = 10.8 Hz, 3H, AdH4'); 1.0-1.4 (m, 8H, hnH2-5).

2D ROESY cross-peaks: δ_H 1.45 (AdH4') shows cross-peaks with 1.69 (AdH4), 1.99 (AdH2), 3.53 (H5?), 3.7 (H3?); 1.69 (AdH4) shows cross-peaks with 1.45 (AdH4'), 1.99 (AdH2), 3.53 (H5?), 3.7 (H3?); 1.76 (AdH2) shows cross-peaks with 1.99 (AdH2), 3.53 (H5?), 3.7 (H3?);1.99 (AdH3) shows cross-peaks with 1.45 (AdH4'), 1.69 (AdH4), 1.76 (AdH2), 3.53 (H5?), 3.7 (H3?); 2.23 (hnH1) shows cross-peaks with 2.42 (hnH6), 2.92 ($H6^A$), 3.81 ($H5^A$); 2.42 (hnH6) shows cross-peaks with 2.23 (hnH1), 3.81 ($H5^A$); 2.58 ($H6^{A'}$) shows cross-peaks with 2.92 ($H6^A$), 3.06 ($H4^A$); 2.92 ($H6^A$) shows cross-peaks

with 2.58 ($H6^{A'}$), 3.81 ($H5^{A}$); 3.53 (H5?) shows cross-peaks with 1.45 (AdH4'), 1.69 (AdH4), 1.76 (AdH2), 1.99 (AdH3); 3.7 (H3?) shows cross-peaks with 1.45 (AdH4'), 1.69 (AdH4), 1.76 (AdH2), 1.99 (AdH3); 3.81 ($H5^{A}$) shows cross-peaks with 2.23 (hnH1), 2.42 (hnH6), 2.92 ($H6^{A}$).

E.4. Experimental for Chapter 4

E.4.1. Preparation and inclusion chemistry of 6^{A}-(12-aminododecyl)amino-6^{A}-deoxy-β-cyclodextrin

6^{A}-(12-aminododecyl)amino-6^{A}-deoxy-β-cyclodextrin **60**

A solution of the tosylate **32** (1.256 g, 0.974×10^{-3} mol) and 1,12-diaminododecane (0.620 g, 3.10×10^{-3} mol) in dry N-methylpyrrolidin-2-one (2 cm^3) was stirred at 75 °C in a lightly stoppered flask for 18 hours. The resultant light yellow solution was cooled to room temperature and diluted with 2:1 ethanol/ether (100 cm^3). The resulting precipitate was collected by vacuum filtration, washed successively with 2:1 ethanol/ether (50 cm^3) and ether (50 cm^3) and dried under vacuum to give the crude product. This material was suspended in water (20 cm^3), insoluble material was removed by filtration and the filtrate was loaded onto a column (4.5 × 4.5 cm) of BioRex 70 (H^+ form). The column was washed with water (400 cm^3) and the product was eluted with 1 mol dm^{-3} NH_4OH. Fractions containing the product were combined and evaporated to dryness under vacuum. The residue was dissolved in water and the solution evaporated under reduced pressure to remove excess ammonia (this procedure was repeated several times). Final drying under vacuum over P_2O_5 gave the title compound as a white powder (0.570 g, 43%). R_c 0.90. Electrospray-ms *m/z* 1317 (M^+). (Found C, 47.50; H, 7.70; N, 2.15. Calculated for **60**.$3H_2O$ ($C_{54}H_{102}N_2O_{37}$) C, 47.29; H, 7.49; N, 2.04%.) δ_H(D_2O, pH ~ 10) 5.07 (s, 7H, H1); 3.8-4.0 (bs, 26H, H3, H5, H6); 3.5 - 3.7 (bs, 13H, H2, H4); 3.48 (m 1H, $H4^{A}$); 3.09 (m, 1H, $H6^{A}$); 2.3-2.9 (m, 5H, $H6^{A'}$, ddnH1, ddnH12); 1.0-1.7 (m, 20H, ddnH). δ_H(D_2O, pH ~ 14, 600 MHz) 4.78 (bs, 7H + solvent, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.2-3.4 (m, 13H, H2, H4); 3.03 (t, J = 9.6 Hz,

1H, $H4^A$); 2.87 (m, 1H, $H6^A$); 2.58 (m, 1H, $H6^{A'}$); 2.52 (t, J = 7.2 Hz, 2H, ddnH12); 2.42 (m, 1H, ddnH1); 2.19 (m, 1H, ddnH1'); 1.00-1.40 (m, 20H, ddnH). δ_C(D_2O, pH ~ 10, 50 MHz) 104.9 (C1); 87.0 ($C4^A$); 83.7 (C4); 76.2, 74.8 (C2, C3, C5); 70.0 ($C5^A$); 62.6 (C6); 51.2, 49.4, 47.3 ($C6^A$, ddnC1); 42.9 (ddnC12); 32.3, 31.9, 30.9, 29.7, 28.6, 27.5, 26.6 (ddnC). δ_C(D_2O, pH ~ 14, 75 MHz) 106.3, 106.2, 106.1, 105.8 (C1); 87.9 ($C4^A$); 84.6, 84.4, 84.3 (C4); 77.2, 77.0 (C2); 76.4 (C3); 75.3, 75.1 (C5); 71.2 ($C5^A$); 63.1 (C6); 51.9, 50.2 (ddnC1, $C6^A$); 43.7 (ddnC12); 34.9, 31.3, 31.1, 29.4, 28.8 (ddnC).

Self-inclusion of the substituent in 6^A-(12-aminododecyl)amino-6^A-deoxy-β-cyclodextrin **60**

1D proton spectrum data: δ_H 4.78 (bs, 7H + solvent, H1); 3.5-3.8 (m, 26H, H3, H5, H6); 3.2-3.4 (m, 13H, H2, H4); 3.03 (t, J = 9.6 Hz, 1H, $H4^A$); 2.87 (m, 1H, $H6^A$); 2.58 (m, 1H, $H6^{A'}$); 2.52 (t, J = 7.2 Hz, 2H, ddnH12); 2.42 (m, 1H, ddnH1); 2.19 (m, 1H, ddnH1'); 1.00-1.40 (m, 20H, ddnH2-ddnH11).

2D ROESY cross-peaks: δ_H 1-1.4 (ddnH2-ddnH11) shows cross-peaks with 2.52 (ddnH12), 3.5-3.8 (H3, H5); 2.42 (ddnH1) shows a cross-peak with 3.7 ($H5^A$?); 2.52 (ddnH12) shows cross-peaks with 1-1.4 (ddnH2-ddnH11), 3.63 (H3?); 2.58 ($H6^{A'}$) shows cross-peaks with 2.87 ($H6^A$), 3.03 ($H4^A$); 2.87 ($H6^A$) shows cross-peaks with 2.58 ($H6^{A'}$), 3.5 (H5?), 3.7 ($H5^A$); 3.03 ($H4^A$) shows cross-peaks with 2.58 ($H6^{A'}$), 3.63 (H3?), 3.7 ($H5^A$); 3.5-3.8 (H3, H5) shows cross-peaks with 1-1.4 (ddnH2-ddnH11), 2.42 (ddnH1), 2.52 (ddnH12), 2.87 ($H6^A$), 3.03 ($H4^A$).

Inclusion of 1-adamantane-1-carboxylate **59** *in 6^A-(12-aminododecyl)amino-6^A-deoxy-β-cyclodextrin* **60**

1D proton spectrum data: δ_H 4.76 (m, 7H, H1); 3.2-3.8 (m, 39H, H2-H6); 3.05 (t, J = 9.0 Hz 1H, $H4^A$); 2.81 (d, J = 13.2 Hz, 1H, $H6^A$); 2.58 (dd, J = 9.0, 13.2 Hz, 1H, $H6^{A'}$), 2.43 (m, 3H, ddnH12, ddnH1); 2.21 (m, 1H, ddnH1'); 1.89 (bs, 3H, AdH3); 1.69 (bs, 6H, AdH2); 1.61 (d, J = 10.8 Hz, 3H, AdH4); 1.38 (d, J =10.8 Hz, 3H, AdH4'); 1-1.35 (m, 20H, ddnH2-ddnH11).

2D ROESY cross-peaks: δ_H 1.0-1.2 (ddnH3-ddnH10) shows cross-peaks with 3.4-

3.44 (H5?), 3.55 (H6?); 1.38 (AdH4') shows cross-peaks with 1.61 (AdH4), 1.89 (AdH3), 3.44 (H5?), 3.7 (H3?); 1.61 (AdH4) shows cross-peaks with 1.38 (AdH4'), 1.89 (AdH3), 3.44 (H5?), 3.7 (H3?); 1.69 (AdH2) shows cross-peaks with 1.89 (AdH3), 3.7 (H3?); 1.89 (AdH3) shows cross-peaks with 1.38 (AdH4'), 1.61 (AdH4), 3.44 (H5?), 3.7 (H3?); 3.44 (H5?) shows cross-peaks with 1.0-1.2 (ddnH3-ddnH10), 1.38 (AdH4'), 1.61 (AdH4), 1.89 (AdH3); 3.55 (H6?) shows cross-peaks with 1.0-1.2 (ddnH3-ddnH10); 3.7 (H3?) shows cross-peaks with 1.38 (AdH4'), 1.61 (AdH4), 1.89 (AdH3).

E.4.2. Preparation and inclusion chemistry of 6-amidohexylamino-substituted cyclodextrins

E.4.2.1. Synthesis

1-Methoxycarbonyl-4-(4-nitrophenoxy)carbonyl-cubane **64**

A mixture of 4-nitrophenol **49** (0.139 g, 1×10^{-3} mol), 1-methoxycarbonyl-cubane-4-carboxylic acid **62** (0.207 g, 1×10^{-3} mol) and dicyclohexylcarbodiimide (0.216 g, 1.02×10^{-3} mol) in dry dichloromethane (5 cm^3) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite which was washed with dichloromethane (3×5 cm^3) and the combined filtrate was washed successively with 5% sodium bicarbonate solution (3×25 cm^3) and brine (25 cm^3) and dried over sodium sulfate. The filtered solution was evaporated under reduced pressure and the residue was dissolved in dichloromethane (2 cm^3) and subjected to flash chromatography (column 1.5 cm i.d., eluted with dichloromethane).[171] Fractions containing the product were combined and evaporated under reduced pressure to give the title compound as colourless needles (0.163 g, 50%). This material was used in later steps without further purification. A small portion of this material was recrystallised from dichloromethane-hexane mp 150-151 °C δ_H ($CDCl_3$) 8.27 (d, J = 6.8 Hz, 2H, ArH); 7.33 (d, J = 6.8 Hz, 2H, ArH); 4.4 (m, 6H, cubaneH); 3.75 (s, 3H, MeO). δ_C ($CDCl_3$) 171.6, 168.7, 155.3, 145.2, 125.1, 122.3, 55.7, 55.4, 51.5, 47.1, 47.0. I.R. ($CDCl_3$) 3114 (w), 3083 (w), 3019 (w), 2996 (m), 2948 (w), 1735 (s), 1722 (s), 1589 (m), 1521 (m), 1490 (s), 1430 (s), 1342 (s),,1305 (s), 1222 (m), 1199 (s), 1052 (s), 863 (m), 844

(m), 736 (m) cm^{-1}.

1-Methoxycarbonyl-4-(4-nitrophenoxy)carbonyl-2,3-dimethyl-cubane **65**

A mixture of 4-nitrophenol **49** (0.142 g, 1.02×10^{-3} mol), 1-methoxycarbonyl-2,3-dimethyl-cubane-4-carboxylic acid **63** (0.245 g, 1.05×10^{-3} mol) and dicyclohexylcarbodiimide (0.220 g, 1.07×10^{-3} mol) in dry dichloromethane (5 cm^3) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite which was washed with dichloromethane (3×5 cm^3) and the combined filtrate was evaporated under reduced pressure. The residue was dissolved in chloroform (2 cm^3) and subjected to flash chromatography (column 1.5 cm i.d., eluted with chloroform).[171] Fractions containing the product were combined and evaporated under reduced pressure to give a viscous oil (0.417 g, 115%) which solidified on standing. This material was used in later steps without further purification. A small portion of this material was recrystallised from dichloromethane-hexane mp 79-80 °C. δ_H ($CDCl_3$) 8.29 (d, J = 6.7 Hz, 2H, ArH); 7.31 (d, J = 6.7 Hz, 2H, ArH); 4.25 (m, 2H, cubaneH); 4.17 (m, 2H, cubaneH); 3.73 (s, 3H, MeO); 1.32 (s, 3H, Me); 1.31 (s, 3H, Me). δ_C ($CDCl_3$) 171.0, 168.3, 155.4, 145.3, 125.1, 122.4, 57.4, 56.8, 55.9, 55.3, 55.1, 51.25, 48.2, 44.9, 12.2, 12.0. I.R. (film) 3116 (w), 3085 (w), 2996 (m), 2915 (m), 2856 (m), 1745 (s), 1722 (s), 1614 (m), 1592 (m), 1525 (s), 1490 (m), 1436 (m), 1348 (s), 1324 (s), 1203 (s), 1160 (s), 1110 (s), 1091 (s), 1002 (m), 862 (m), 756 (m) cm^{-1}.

1-(4-nitrophenylcarboxy)-adamantane **66**

A mixture of 4-nitrophenol **49** (0.139 g, 1.0×10^{-3} mol), adamantane-1-carboxylic acid **59** (0.182 g, 1.0×10^{-3} mol) and dicyclohexylcarbodiimide (0.218 g, 1.1×10^{-3} mol) in dichloromethane (5 cm^3) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite and the pad was washed with dichloromethane (10 cm^3). The filtrate was loaded onto a squat column (30 g, 4.5 cm i.d.) and eluted with dichloromethane (100 cm^3).[172, 173] Fractions containing the product were combined and evaporated under reduced pressure to give the title compound as an off-white powder (0.270 g, 90%). This material was used in later steps without further purification. A small portion of

this material was recrystallised from dichloromethane-hexane mp 130-132 °C. δ_H ($CDCl_3$) 8.21 (d, J = 9.0 Hz, 2H, ArH); 7.22 (d, J = 9.0 Hz, 2H, ArH); 2.04 (bs, 10H); 1.91 (bs, 2H); 1.70 (bs, 3H). δ_C ($CDCl_3$) 175.1, 156.0, 145.1, 124.9, 122.3, 41.0, 38.4, 38.1, 27.5. I.R. (nujol) 1747 (s) (C=O); 1614 (m), 1589 (m), 1488 (m) (Ar); 1519 (s), 1342 (s) (NO_2); 1199 (s), 1049 (s) (C-O).

*6^A-deoxy-6^A-(6-*N*-(4-methoxycarbonyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin* **67**

A solution of the cyclodextrin **24** (0.128 g, 1.0×10^{-4} mol) and 1-methoxycarbonyl-4-(4-nitrophenoxy)carbonyl-cubane **64** (0.036 g, 1.1×10^{-4} mol) in dry DMF (2 cm^3) was stirred at room temperature for 20 hours. The resultant yellow solution was added dropwise with stirring to 1:1 ether:ethanol (50 cm^3) cooled to 0 °C and the resultant fine yellow precipitate was collected by vacuum filtration on a Celite pad. The product was dissolved in water (5 cm^3) and the Celite was removed by filtration. The filtrate was acidified with dilute hydrochloric acid and extracted with dichloromethane (3×20 cm^3). The aqueous layer was treated with AG 4-X4 weak anion exchanger (5 g, Bio Rad Laboratories, 100-200 Mesh, free base form) for 1 hour. The resin was removed by filtration and the filtrate was freeze dried to give the title compound as a colourless powder (0.072 g, 51%). R_c = 1.11. Electrospray-ms *m/z* 1421 (M^+). (Found C, 45.71; H, 6.79; N, 1.75. Calculated for **67**.HCl.5H_2O ($C_{59}H_{103}ClN_2O_{42}$) C, 45.77; H, 6.70; N, 1.80%.) δ_H (D_2O) 4.76 (bs, 7H, H1); 4.2 (m, 6H, cubaneH); 3.4-4.0 (m, 42H, CDH, MeO); 3.0-3.4 (m, 4H, $C4^A$, $C6^A$, hnH6); 2.8 (m, 1H, $C6^{A'}$); 2.55 (m, 1H, hnH1); 2.40 (m, 1H, hnH1'); 1.1-1.5 (m, 8H, hnH2-5). δ_C (D_2O) 176.5, 175.6 (C=O); 105.0 (C1); 87.3 ($C4^A$); 83.9 (C4); 76.0, 74.9, 72.4, 70.6 (C2, C3, C5); 63.0 (C6); 60.6, 58.7, 55.3, 52.3, 51.6, 49.5, 49.1, 40.3 ($C6^A$, cubaneC, hnC1, hnC6, MeO); 25-31 (broad lump, hnC2-5).

*6^A-deoxy-6^A-(6-*N*-(4-methoxycarbonyl-2,3-dimethyl-cuban-1-oyl)aminohexyl)amino-β-*

cyclodextrin **68**

A solution of the cyclodextrin **24** (0.123 g, 1.0×10^{-4} mol) and 1-methoxycarbonyl-4-(4-nitrophenoxy)carbonyl-2,3-dimethylcubane **65** (0.058 g, 1.6×10^{-4} mol) in dry DMF (2 cm^3) was stirred at room temperature for 20 hours. This solution was diluted with ether (50 cm^3) and the resultant precipitate was collected on a pad of Celite. The pad was washed with ether (3×10 cm^3) and then suspended in water (25 cm^3) to dissolve cyclodextrins. The Celite was filtered off and the filtrate was acidified with dilute hydrochloric acid and extracted with dichloromethane (3×20 cm^3). The aqueous layer was treated with AG 4-X4 weak anion exchanger (5 g) for 1 hour and the resin was removed by vacuum filtration. The colourless filtrate was freeze-dried to give the product as a white powder (0.092 g, 63%). R_c = 1.11. Electrospray-ms *m/z* 1449 (M^+). (Found C, 46.94; H, 6.68; N, 1.79. Calculated for **68**.HCl.4H_2O ($C_{61}H_{105}ClN_2O_{41}$) C, 47.03; H, 6.79; N, 1.80%.) δ_H (D_2O) 5.04 (bs, 7H, H1); 3.5-4.4 (m, 46H, CDH, MeO); 3.0-3.4 (m, 4H, $C4^A$, $C6^A$, hnH6); 2.6 (m, 3H, $C6^{A'}$, hnH1) 1.1-1.5 (m, 14H, cubaneMe, hnH2-5). δ_C (D_2O) 175.8, 175.5 (C=O); 105.1 (C1); 86.7 ($C4^A$); 84.2 (C4); 76.0, 74.8, 72.3 (C2, C3, C5); 62.8 (C6); 60.9, 59.9, 59.7, 59.1, 57.5, 57.4, 54.5 51.5, 49.6, 46.9, 46.1, 45.6, 41.7 ($C6^A$, cubaneC, hnC1, hnC6, MeO); 28.5-31.4 (broad lump, hnC2-5); 15.4, 14.5, 14.2 (cubaneMe).

*6^A-deoxy-6^A-(6-*N*-(adamantan-1-oyl)aminohexyl)amino-β-cyclodextrin* **69**

A solution of the cyclodextrin **24** (0.130 g, 1.05×10^{-4} mol) and 1-(4-nitrophenylcarboxy)-adamantane **66** (0.037 g, 1.22×10^{-4} mol) in dry DMF (4 cm^3) was stirred at room temperature for 20 hours. TLC showed two new spots at R_c 1.2 and 1.0. The reaction mixture was diluted with ether (40 cm^3) and the resultant precipitate was collected by vacuum filtration and washed with ether (3×10 cm^3). The crude product was dissolved in water (20 cm^3) and the solution was acidified with dilute hydrochloric acid and extracted with dichloromethane (3×15 cm^3). The aqueous layer was treated with AG 4-X4 weak anion exchanger (5 g) for 1 hour and the resin was removed by filtration and the colourless filtrate was freeze-dried to give the product as a white powder (0.103 g, 70%). R_c = 1.2, 1.0. Electrospray-ms *m/z* 1395 (M^+). δ_H (D_2O) 5.03 (bs, 7H, H1); 2.5-4.1 (m, 65H, CDH,

hnH1, hnH6); 1.1-2.4 (m, 31H, AdH, hnH2-5). δ_C (D_2O) 182.4, (177.1) (C=O); 105.2, 105.0, 104.8, 102.3 (C1); 87.85, 86.7, 86.0, 84.5, 84.3, 84.2, 84.1, 83.8 (C4); 79.5, 76.0, 75.9, 75.8, 75.5, 75.2, 75.1, 75.0, 74.8, 74.7, 74.6, 74.5, 73.9, 72.3, 72.2, 72.1, 70.2, 69.6 (C2, C3, C5); 63.2, 63.0, 62.8, 62.7 (C6); 57.1, 51.1, 50.6, 48.8, 45.5, 43.3, 43.2, 42.0, 41.5, 39.4, 39.2, 38.6, ($C6^A$, hnC1, hnC6, AdC1-3); 34.9, 33.9, 31.8, 31.3, 30.7, 30.3, 30.0, 29.4, 28.5, 28.0, 27.8, 27.4, 26.0, 25.0 (hnC2-5, AdC4).

E.4.2.2. Inclusion chemistry

Self-inclusion of the cubanyl group in 6^A-deoxy-6^A-(6-N-(4-methoxycarbonyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin **67**

1D proton spectrum data: δ_H 4.76 (bs, 7H, H1); 4.30 (m, 3H, cubaneH); 4.16 (m, 3H, cubaneH'); 3.4-4.0 (m, 42H, CDH, MeO); 3.33 (m, 2H, $C4^A$, hnH6);3.06 (m, 2H, $H6^A$, hnH6'); 2.8 (m, 1H, $C6^{A'}$); 2.55 (m, 1H, hnH1); 2.40 (m, 1H, hnH1'); 1.1-1.5 (m, 8H, hnH2-5).

2D-ROESY spectrum data: δ_H 3.4-4.0 (CDH) shows cross-peaks with 4.16 (cubaneH'), 4.30 (cubaneH); 4.16 shows cross-peaks with 3.4-4.0 (CDH); 4.30 (cubaneH) shows cross-peaks with 3.4-4.0 (CDH).

Inclusion of adamantane-1-carboxylate **59** *in 6^A-deoxy-6^A-(6-N-(4-methoxycarbonyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin* **67**

1D proton spectrum data: δ_H 5.04 (bs, 7H, H1); 4.20 (m, 2H, cubaneH); 4.12 (m, 4H, cubaneH'); 3.5-4.0 (m, 42H, CDH, MeO); 3.37 (t, J = 9.6 Hz, 1H, $H4^A$); 3.17 (m, 3H, $H6^A$, hnH6); 2.87 (m, 1H, $H6^{A'}$); 2.67 (m, 1H, hnH1); 2.47 (m, 1H, hnH1'); 2.04 (bs, 3H, AdH3); 1.86 (bs, 6H, AdH2); 1.75 (d, J = 12.0 Hz, 3H, AdH4); 1.64 (d, J = 12.0 Hz, 3H, AdH4'); 1.2-1.5 (m, 8H, hnH2-5).

2D-ROESY spectrum data: δ_H 1.64 (AdH4') shows cross-peaks with 3.5-4.0 (CDH); 1.75 (AdH4) shows cross-peaks with 3.5-4.0 (CDH); 1.86 (AdH2) shows cross-peaks with 3.5-4.0 (CDH); 2.04 (AdH3) shows cross-peaks with 3.5-4.0 (CDH); 3.54 (CDH) shows cross-peaks with 1.64 (AdH4'), 1.75 (AdH4), 1.86 (AdH2), 2.04 (AdH3).

Self-inclusion of the dimethylcubanyl group in 6^{A}-deoxy-6^{A}-(6-N-(4-methoxycarbonyl-2,3-dimethyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin **68**

1D proton spectrum data: δ_H 5.04 (bs, 7H, H1); 3.5-4.4 (m, 46H, cubaneH, CDH, MeO); 3.42, (t, J = 9.0 Hz, 1H, H4^{A}); 3.19 (m, 3H, H6^{A}, hnH6); 2.92 (m, 1H, H6$^{A'}$); 2.64 (m, 2H, hnH1); 1.1-1.5 (m, 14H, hnH2-5, Me).

2D-ROESY spectrum data: δ_H 1.3 (Me) shows cross-peaks with 3.5-4.0 (CDH), 4.0-4.2 (cubaneH); 3.5-4.0 (CDH) shows cross-peaks with 1.3 (Me), 4.0-4.2 (cubaneH); 4.0-4.2 (cubaneH) shows cross-peaks with 1.3 (Me), 3.5-4.0 (CDH).

*Inclusion of adamantane-1-carboxylate***59** *in 6^{A}-deoxy-6^{A}-(6-N-(4-methoxycarbonyl-2,3-dimethyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin* **68**

1D proton spectrum data: δ_H 5.05 (bs, 7H, H1); 3.5-4.2 (m, 46H, cubaneH, CDH, MeO); 3.36 (t, J = 9.0 Hz, 1H, H4^{A}); 3.10 (m, 3H, H6^{A}, hnH6); 2.81 (dd, J = 7.2, 14.3 Hz, 1H, H6$^{A'}$); 2.59 (m, 1H, hnH1); 2.42 (m, 1H, hnH1'); 2.05 (bs, 3H, AdH3); 1.87 (bs, 6H, AdH2); 1.76 (d, J = 12 Hz, 3H, AdH4); 1.61 (d, J = 12 Hz, 3H, AdH4'); 1.1-1.4 (m, 14H, hnH2-5, Me).

2D-ROESY spectrum data: δ_H 1.61 (AdH4') shows cross-peaks with 3.8 (H3); 1.76 (AdH4) shows cross-peaks with 3.8 (H3); 1.87 (AdH2) shows cross-peaks with 3.8 (H3); 2.05 (AdH3) shows cross-peaks with 3.8 (H3); 3.8 (H3) shows cross-peaks with 1.61 (AdH4'), 1.76 (AdH4), 1.87 (AdH2), 2.05 (AdH3).

Self-inclusion of the adamantyl group in 6^{A}-deoxy-6^{A}-(6-N-(adamantan-1-oyl)-amino-hexyl)amino-β-cyclodextrin **69**

1D proton spectrum data: δ_H 5.1 (bs, 7H, H1); 3.5-4.0 (m, 39H, CDH); 3.32 (t, J = 9.0 Hz, 1H, H4^{A}); 3.25 (m, 1H, hnH6); 3.0 (m, 2H, hnH6', H6^{A}); 2.79 (dd, J = 9.0, 12.0 Hz, 1H, H6^{A}); 2.56 (m, 1H, hnH1); 2.28 (m, 4H, hnH1', AdH3); 1.94 (d, J = 12.6 Hz, 3H, AdH4); 1.84 (s, 6H, AdH2); 1.79 (d, J = 12.6 Hz, 3H, AdH4'); 1.0-1.8 (m, 8H, hnH2-5).

2D-ROESY spectrum data: δ_H 1.79 (AdH4') shows cross-peaks with 3.7-4.0 (CDH);

1.84 (AdH2) shows cross-peaks with 3.5-4.0 (CDH); 2.28 (AdH3) shows cross-peaks with 3.7-4.0 (CDH).

Addition of 2 equivalents of adamantane-1-carboxylate **59** *to* 6^A*-deoxy-*6^A*-(6-*N*-(adamantan-1-oyl)aminohexyl)amino-β-cyclodextrin* **69**

1D proton spectrum data: δ_H 5.1 (bs, 7H, H1); 3.5-4.0 (m, 39H, CDH); 3.37 (t, J = 9.0 Hz, 1H, $H4^A$); 3.23 (m, 1H, hnH6); 3.16 (m, 1H, hnH6'); 3.07 (d, J = 12.0 Hz, 1H, $H6^A$); 2.80 (dd, J = 9.0, 12.0 Hz, 1H, $H6^A$); 2.57 (m, 1H, hnH1); 2.29 (m, 1H, hnH1'); 2.17 (bs, 3H, AdH3); 1.95 (bs, 6H, exADH3); 1.84 (m, 9H, AdH4, AdH2); 1.79 (m, 15H, exADH2, AdH4'); 1.67 (bs, 6H, exAdH4); 1.0-1.8 (m, 8H, hnH2-5).

2D-ROESY spectrum data: δ_H 1.67 (exADH4) shows cross-peaks with 3.8-4.0 (CDH); 1.8 (AdH4') shows cross-peaks with 3.8-4.0 (CDH); 1.84 (AdH2) shows cross-peaks with 3.5-4.0 (CDH); 2.17 (AdH3) shows cross-peaks with 3.8-4.0 (CDH).

E.4.3. Preparation of a cyclodextrin dimer

1,4-bis (4-nitrophenoxycarbonyl)-cubane **71**

A mixture of 4-nitrophenol **49** (0.282 g, 2.00×10^{-3} mol), cubane-1,4-dicarboxylic acid **70** (0.193 g, 1.00×10^{-3} mol) and dicyclohexylcarbodiimide (0.411 g, 2.0×10^{-3} mol) in dichloromethane (10 cm^3) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite and the pad was washed with dichloromethane (30 cm^3). The combined filtrates were washed with 5% sodium bicarbonate (3×20 cm^3), water (20 cm^3) and brine (20 cm^3) and dried over sodium sulfate. The filtered solution was evaporated to about 20 cm^3 and loaded onto a squat column (30 g, 4.5 cm i.d.) which was eluted with dichloromethane.[172, 173] Fractions containing the product were combined and evaporated under reduced pressure to give the title compound as a white powder (0.263 g, 60%). A small portion of this material was recrystallised from dichloromethane-hexane mp 215-217 °C. δ_H ($CDCl_3$) 8.30 (d, J = 9.2 Hz, 4H, ArH2); 7.33 (d, J = 9.2 Hz, 4H, ArH3); 4.53 (s, 6H, cubaneH). δ_H ($CDCl_3$) 168.5, 155.3, 145.5, 125.3, 122.4, 47.4. I.R. (nujol) 1741 (s) (C=O); 1612 (m), 1589 (m), 1488 (m) (Ar); 1521 (s), 1339 (s) (NO_2); 1199 (s), 1049

(s) (C-O).

*1,4-bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocarbonyl)-cubane* **72**

A solution of the cyclodextrin **24** (0.128 g, 1.0×10^{-4} mol) and 1,4-bis(4-nitrophenoxycarbonyl)-cubane **71** (0.020 g, 4.61×10^{-5} mol) in dry DMF (2 cm^3) was stirred at room temperature for 20 hours. The reaction mixture was diluted with ether (40 cm^3) and the resultant precipitate was collected by vacuum filtration and washed with ether (3×10 cm^3). The crude product was dissolved in water (20 cm^3) and the solution was acidified with dilute hydrochloric acid and extracted with dichloromethane (3×15 cm^3). The aqueous layer was treated with AG 4-X4 weak anion exchanger (5 g) for 1 hour and the resin was removed by filtration and the colourless filtrate was freeze-dried to give the crude product as a white powder. This was dissolved in water (5 dm^3) and loaded onto a column of Sephadex G-10 (600×20 mm i.d.). The column was eluted with 10% aqueous ethanol and 10 cm^3 fractions were collected. Fractions containing the product were combined and freeze-dried to give the title compound as a white powder (0.046 g, 38%). $R_c = 0.71$. Electrospray-ms *m/z* 2622 (M^+). δ_H (D_2O) 5.00 (bs, 14H, H1); 2.5-4.4 (m, 142H, cubaneH, CDH, hnH1, hnH6); 1.1-1.8 (m, 16H, hnH2-5). δ_C (D_2O) 175.9, 175.6 (C=O); 105.1, 104.6, 104.1 (C1); 87.8, 86.9, 85.8, 84.4, 84.3, 84.0, 83.8, 83.3, 83.1 (C4); 75.8, 75.6, 75.3, 75.2, 74.9, 74.7, 74.5, 72.3, 72.2, 72.1, 70.4, 69.6 (C2, C3, C5); 63.2, 63.0, 60.5, 60.1 (C6); 51.5, 51.0, 50.8, 49.4, 49.1, 49.0, 48.8, 42.0, 41.9, 39.7 (C6^A, cubaneC, hnC1, hnC6); 34.7, 34.0, 31.8, 31.4, 31.1, 30.8, 29.6, 29.5, 29.2, 28.6, 28.3, 28.2, 27.9, 27.5, 25.6, 25.0 (broad lump, hnC2-5).

Addition of two equivalents of adamantane-1-carboxylate **59** *to 1,4-bis((6-*N*-(6^A-deoxy-β-cyclodextrin-6^A-yl)aminohexyl)aminocarbonyl)-cubane* **72**

600 MHz 1D proton spectrum data: δ_H 5.06 (s, 14H, H1); 4.16 (s, 6H, cubaneH); 4.01 (t, J = 9.2 Hz, 2H, H5^A); 3.9-3.5 (m, 76H, H2-H6), 3.40 (t, J = 9.2 Hz, 2H, H4^A); 3.30 (d, J = 13.2 Hz, 2H, H6^A); 3.18 (m, 4H, hnH6); 3.01 (m, 2H, H6$^{A'}$); 2.80 (m, 2H, hnH1); 2.68 (m, 2H, hnH1'); 2.04 (s, 6H, AdH3); 1.84 (s, 12H, AdH2); 1.74 (d, J = 11 Hz,

6H, AdH4); 1.67 (d, J = 11 Hz, 6H, AdH4'); 1.2-1.6 (m, 16H, hnH2-hnH5).

2D-ROESY spectrum data: δ_H 1.2-1.6 shows cross-peaks with 3.18 (hnH6); 1.67 (AdH4') shows cross-peaks with 3.6-3.9 (H3, H5); 1.74 (AdH4) shows cross-peaks with 3.6-3.9 (H3, H5); 1.84 (AdH2) shows cross-peaks with 3.6-3.9 (H3, H5); 2.04 (AdH3) shows cross-peaks with 3.6-3.9 (H3, H5);3.18 (hnH6) shows cross-peaks with 1.2-1.6 (hnH2-hnH5); 3.6-3.9 (H3, H5) shows cross-peaks with 1.67 (AdH4'), 1.74 (AdH4), 1.84 (AdH2), 2.04 (AdH3).

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Appendix 1: Publications arising from this thesis

1. B.L. May, S.D. Kean, C.J. Easton and S.F. Lincoln, *J. Chem. Soc. Perkin Trans. 1*, 1997, 3157.

2. S.D. Kean, B.L. May, P. Clements, S.F. Lincoln and C.J. Easton, *J. Chem. Soc. Perkin Trans. 2*, 1999, 000 (accepted for publication).

1 PERKIN

Preparation and characterization of 6^A-polyamine-mono-substituted β-cyclodextrins

Bruce L. May,[a] Suzanna D. Kean,[a] Christopher J. Easton[b] and Stephen F. Lincoln*[,a]

[a] *Department of Chemistry, University of Adelaide, Adelaide, SA 5005, Australia*
[b] *Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia*

General syntheses for eleven β-cyclodextrins (cyclomaltoheptaoses) mono-substituted at the C6 position by a polyamine are described. The basis of the synthesis is the reaction of 6^A-*O*-(4-methylphenylsulfonyl)-β-cyclodextrin in the presence of KI in 1-methylpyrrolidin-2-one solution. This produces a clean product and obviates the substantial purification procedures which other preparative methods often entail. Systematic studies of the variations of the pK_as of the protonated amine groups and the ^{13}C NMR spectra of the modified β-cyclodextrins with pH are reported.

Introduction

The ability of the naturally occurring cyclodextrins (cyclomaltopolyoses) to form host–guest complexes where a guest molecule enters the annulus of the host cyclodextrin is well established.[1–3] These complexing abilities may be modified by substitution at one or more of the C2, C3 and C6 sites;[1–6] the 6^A-polyamine-substituted β-cyclodextrins (β-CDX) discussed below and shown in Fig. 1 exemplify such substitutions at C6. Some of these β-CDXs have been studied because of their ability to form host–guest complexes,[2,7–10] and also because they coordinate metal ions to form binary metallocyclodextrins which sometimes show enantioselective and biomimetic characteristics in their interaction with guests in ternary metallocyclodextrins.[3,5–19]

We require a range of β-CDXs which can be produced in reasonable yield and high purity for our host–guest complex and metallocyclodextrin studies. Some of these β-CDXs have been reported previously. However, in our hands, the products obtained through these preparations usually required lengthy purification and this provided the impetus for our quest for an improved general synthetic method. Two major routes have been previously reported for the syntheses of the required β-CDXs. For the liquid polyamines, heating either β-cyclodextrin (β-CD),[18] 6^A-*O*-(4-methylphenylsulfonyl)-β-cyclodextrin (β-CDtos)[12] or 6^A-deoxy-6^A-iodo-β-cyclodextrin (β-CDI)[19] in excess polyamine in a sealed tube yields β-CDX which requires purification by lengthy chromatographic separation. For either the more expensive liquid or solid polyamines, reaction of β-CDtos[10–13,16,20] with the polyamine in *N*,*N*-dimethylformamide (DMF) under similar conditions yields β-CDX, but we found it difficult to avoid some formylation of the β-CDX which necessitated tedious separations using this method. We now report a simple general procedure for the synthesis of some reported β-CDXs where X is either the 2-aminoethylamino,[11,18] 3-aminopropylamino,[7–9] 2-(2-aminoethylamino)ethylamino,[12,14,19] 2-[2-(2-aminoethylamino)ethylamino]ethylamino,[12] 2-[bis(2-aminoethyl)amino]ethylamino[10] or 1,4,7,10-tetraazacyclododecan-1-yl[13,15] group bonded through nitrogen to the β-CD C6 carbon which in most cases have not been fully characterised, and some new β-CDXs that yield clean products under mild conditions.

The β-CDX's protonated amine groups exhibit a wide range of pK_as which are likely to have a major influence on host–guest complexation and metal ion coordination reactions. Accordingly, a systematic study of pK_a variation with the nature of X has been carried out in parallel with a study of the ^{13}C NMR spectral variation of β-CDXs with pH to gain an insight into the factors influencing these characteristics.

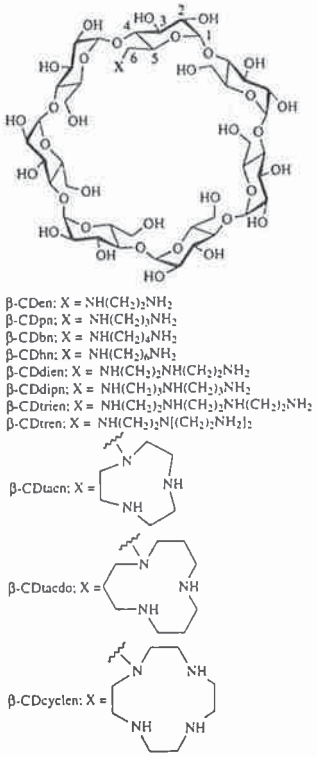

Fig. 1 Schematic representations of the β-CDXs prepared. The individual C and H atoms of the polyamino substituent are labelled 1, 2, ... *n* as distance from the β-CD moiety increases.

Results and discussion

Preparative aspects

The synthesis of 6^A-{2-[bis(2-aminoethyl)amino]ethylamino}-6^A-deoxy-β-cyclodextrin (β-CDtren) serves to illustrate pre-

Table 1 Reaction times, yields and analytical data for the preparation of β-CDXs

β-CDX	Reaction time *t*/h	Yield (%)	Elemental analyses (%)	C	H	N
β-CDen·3H_2O	6	55	Found:	42.70	6.67	2.18
			Calc.:	42.92	6.71	2.27
β-CDpn·3H_2O	4.5	42	Found:	43.65	6.85	2.39
			Calc.:	43.40	6.80	2.24
β-CDbn·2H_2O	4.5	52	Found:	44.88	7.17	2.17
			Calc.:	44.51	6.82	2.25
β-CDhn·3H_2O	5	51	Found:	44.95	7.27	1.88
			Calc.:	44.79	7.04	2.17
β-CDtrien·H_2O	7	40	Found:	44.83	6.89	4.42
			Calc.:	44.99	6.92	4.37
β-CDtren·3H_2O	4	57	Found:	43.84	7.58	4.40
			Calc.:	43.76	7.04	4.25
β-CDdien·H_2O	4.5	54	Found:	44.88	6.75	4.05
			Calc.:	44.62	6.75	3.39
β-CDdipn·2H_2O	6	50	Found:	45.17	6.52	3.12
			Calc.:	44.89	6.98	3.27
β-CDtacn·3H_2O	5	33	Found:	44.59	6.83	3.30
			Calc.:	44.34	6.90	3.23
β-CDtacdo·4H_2O	7	34	Found:	45.28	7.34	3.15
			Calc.:	45.03	7.18	3.08
β-CDcyclen·3H_2O	14	35	Found:	44.76	7.10	4.36
			Calc.:	44.71	7.05	4.17

parative aspects which generally apply to the other β-CDX considered. Heating a mixture of β-CDtos and one equivalent of tris(2-aminoethyl)amine in DMF at 70 °C in a loosely stoppered flask for 24 h gave the expected β-CDtren in low yield. This product was contaminated with *N*-formylated material formed by transacylation between primary amino groups and the DMF solvent. Reaction of 6^A-deoxy-6^A-iodo-β-cyclodextrin (β-CDI) under the same conditions gave a more rapid conversion to the product but again there was a significant amount of the formylated product formed. When pyridine was used as the solvent in place of DMF, a much cleaner β-CDtren product was obtained, but it was isolated largely as a very stable host–guest complex of pyridine with β-CDtren. Pure β-CDtren was obtained from all three of the above preparative routes, but only after lengthy purification.

NMP is a dipolar aprotic solvent that has been shown to be superior to DMF for nucleophilic substitutions of toluene-*p*-sulfonates[25] but is more stable than DMF under either acid or base conditions.[26] When β-CDtos was heated at 70 °C for 4 h with 3.3 equiv. of tris(2-aminoethyl)amine and 0.1 equiv. of KI (to generate β-CDI *in situ*) in NMP, pure β-CDtren was obtained in 60% yield following a single precipitation with ethanol and product separation through ion exchange chromatography. There was no evidence for reaction between tris(2-aminoethyl)amine or β-CDtren and NMP. The formation of β-CDI in the reaction was shown by TLC of the reaction mixture during the course of the reaction. A series of β-CDXs, having either linear, branched or cyclic polyamine substituents, was prepared under the same conditions (Table 1). All of the β-CDXs prepared by this procedure were shown to be pure by TLC, ^{1}H and ^{13}C NMR spectroscopy and microanalysis. (A referee has pointed out that the cyclic solvent, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone, has been employed in the nucleophilic substitution of a modified cyclodextrin.[27])

pK_a Variations

The two pK_as of β-CDXs increase as X is systematically changed from 1,2-diaminoethane (en) to 1,6-diaminohexane (hn) while the difference between the two pK_as decreases, and a similar trend is seen for the free diamine analogues (Table 2). The latter observation is attributable to increases in charge separation in the diprotonated species decreasing electrostatic repulsion as the diamine increases in size. The increase in pK_a magnitude coincides with increases in hydrophobicity as the aliphatic chain lengthens and indicates a decrease in the ability of surrounding water to accept a proton from the protonated amine as overall hydration decreases. The two pK_as of β-CDXs are less than those of the analogous free diamine.

Table 2 pK_as[a] for some protonated 6^A-polyamine-substituted β-cyclodextrins and the corresponding free polyamines in aqueous $NaClO_4$ (I = 0.10 mol dm^{-3}) at 298.2 K

Species	pK_a	Species	pK_a
β-CDenH_2^{2+}	9.42 ± 0.01	enH_2^{2+}	9.97 ± 0.03
	5.70 ± 0.02		7.16 ± 0.02
β-CDpnH_2^{2+}[b]	9.90 ± 0.1	pnH_2^{2+}	10.56 ± 0.02
	7.39 ± 0.04		8.97 ± 0.01
β-CDbnH_2^{2+}	10.26 ± 0.02	bnH_2^{2+}	10.91 ± 0.02
	8.06 ± 0.01		9.49 ± 0.01
β-CDhnH_2^{2+}	10.27 ± 0.03	hnH_2^{2+}	11.01 ± 0.06
	8.72 ± 0.01		10.04 ± 0.03
β-CDdienH_3^{3+}	9.52 ± 0.02	dienH_3^{3+}	9.78 ± 0.01
	7.63 ± 0.03		8.99 ± 0.03
	3.88 ± 0.07		4.32 ± 0.03
β-CDdipnH_3^{3+}	10.06 ± 0.02	dipnH_3^{3+}	10.56 ± 0.05
	8.44 ± 0.03		9.44 ± 0.06
	6.72 ± 0.03		7.54 ± 0.06
β-CDtrienH_4^{4+}	9.33 ± 0.02	trienH_4^{4+}	9.83 ± 0.04
	8.22 ± 0.03		8.93 ± 0.05
	5.61 ± 0.03		5.40 ± 0.05
	3.13 ± 0.07		3.0 ± 0.1
β-CDtrenH_4^{4+}[c]	9.85 ± 0.02	trenH_4^{4+}[d]	10.14
	8.99 ± 0.09		9.43
	6.89 ± 0.05		8.41
	2.6 ± 0.3		
β-CDtacnH_3^{3+}	10.0 ± 0.1	tacnH_3^{3+}	10.69 ± 0.02
	5.89 ± 0.07		7.01 ± 0.01
	2.4 ± 0.2		
β-CDtacdoH_3^{3+}	11.24 ± 0.04	tacdoH_3^{3+}[e]	12.60
	5.85 ± 0.03		7.57
	2.8 ± 0.1		2.41
β-CDcyclenH_4^{4+}	10.40 ± 0.01	cyclenH_4^{4+}[f]	10.6
	8.62 ± 0.02		9.6

[a] Errors represent one standard deviation. [b] Ref. 7. [c] Ref. 10. [d] Ref. 28. [e] Ref. 29. [f] Ref. 30.

The increased acidity of the protonated diamine moiety of β-CDX, by comparison with that of the free diamine analogue (Table 2), may partially arise from either the electronic and steric effects of the substitution of an amine nitrogen by β-CD or the difference in solvation experienced by the protonation sites in β-CDX and the free diamine or a combination of both. In addition, the diamine moiety in β-CDX is bound adjacent to the ring of six primary hydroxy groups delineating the narrow end of the cyclodextrin annulus such that hydrogen bonding between them and the amine nitrogens may decrease the basicity of the latter. This is supported to some extent through the observation that in basic solution more fine structure is seen in the ^{13}C NMR spectra of β-CDX (see Experimental) than is seen in acidic solution, consistent with the unprotonated diamine moiety hydrogen-bonding to the β-CD hydroxy groups more effectively than does its protonated analogue. (This is illustrated by the spectra of β-CDtacdo and β-CDcyclen in Figs. 2 and 3.) A similar interpretation has been presented for β-CDdien (where pK_a magnitude increases in the sequence -NH_3^+ < β-CD-NH_2^+- < -$(CH_2)_2$-NH_2^+-$(CH_2)_2$- as identified by ^{13}C NMR spectroscopy[19]) which together with its β-CDdipn homologue shows similar trends (Table 2) to those discussed above. Generally, similar trends in pK_a magnitudes are observed for the polyamine β-CDX as for their diamine analogues and their origins are probably similar.

^{13}C NMR Spectra

The substituent X on the β-CDX C6 carbon of the A glucopyranose unit renders it and the other six glucopyranoses (often labelled B–G) inequivalent, and as a result they may each exhibit six ^{13}C unique resonances to give a total of 42 resonances when the magnetic inequivalence is sufficiently large.

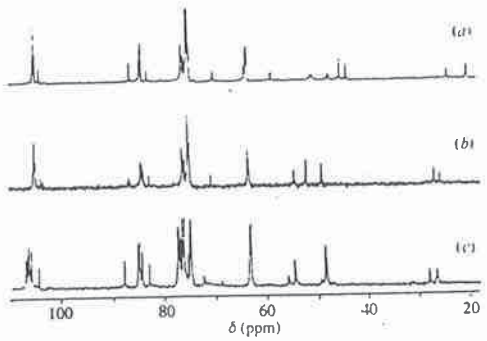

Fig. 2 75.47 MHz ^{13}C NMR spectra of β-CDtacdo in (*a*) 0.1 mol dm^{-3} HCl–D_2O, (*b*) HCl–D_2O, pH ~8.5 and (*c*) 0.1 mol dm^{-3} NaOH–D_2O‡

‡ δ_C(0.1 mol dm^{-3} HCl–D_2O) 104.9, 104.7, 104.6, 103.7 (C1), 86.3 (C4^), 84.3, 84.0, 82.8 (C4), 76.1, 76.0, 75.9, 75.6, 75.2, 75.0, 74.7, 74.6 (C2, C3, C5), 70.0 (C5^), 63.8, 63.3 (C6), 58.6, 50.9 (broad), 47.5, 45.2, 43.9 (C6^, tacdoC1, tacdoC3, tacdoC4), (25.4), 23.7, 19.9 (tacdoC2, tacdoC5); δ_C(HCl–D_2O, pH ~8.5) 104.8, 104.5, 103.4, 103.0 (C1), 86.4 (C4^), 84.1, 83.9, 83.7, 83.6, 82.5 (C4), 76.1, 76.0, 75.9, 75.6, 74.9, 74.7 (C2, C3, C5), 70.5 (C5^), 63.3, 63.2 (C6), 54.3 (C6^), 51.9, 49.0 (tacdoC1, tacdoC3, tadcoC4), 26.6, 25.4 (tacdoC2, tacdoC5); δ_C(0.1 mol dm^{-3} NaOH–D_2O) 106.9, 106.6, 106.4, 106.3, 105.8, 105.7, 104.3 (C1), 87.7 (C4^), 85.2, 85.1, 85.0, 84.9, 84.5, 84.3, 82.9 (C4), 77.4, 77.2, 77.1, 77.0, 76.9, 76.8, 76.7, 76.5, 76.3, 76.1, 75.4, 75.1, 74.9 (C2, C3, C5), 72.5 (C5^), 63.4, 63.1 (C6), 55.9 (C6^), 54.6 (tacdoC1), 48.7, 48.6 (tacdoC3, tacdoC4), 28.0, 26.5 (tacdoC2, tacdoC5).

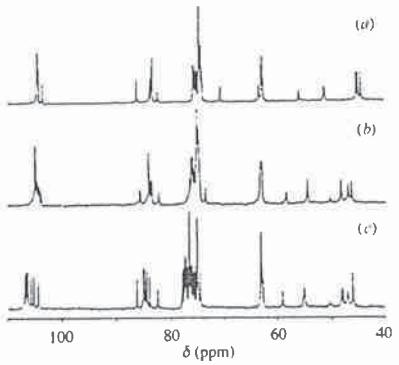

Fig. 3 75.47 MHz ^{13}C NMR spectra of β-CDcyclen in (*a*) 0.1 mol dm^{-3} HCl–D_2O, (*b*) HCl–D_2O, pH ~9 and (*c*) 0.1 mol dm^{-3} NaOH–D_2O§

§ δ_C(0.1 mol dm^{-3} HCl–D_2O) 104.5, 104.4, 104.3, 103.6 (C1), 86.4 (C4^), 84.0, 83.7, 83.6, 82.6 (C4), 75.9, 75.8, 75.6, 75.5, 75.3, 75.2, 74.9, 74.8, 74.7, 74.6, 74.4, 74.3, 74.2 (C2, C3, C5), 70.9 (C5^), 63.6, 63.2, 63.0 (C6), 56.2 (C6^), 51.5 (cyclenC1), 45.7, 45.4, 44.9 (cyclenC2-4); δ_C(HCl–D_2O, pH ~9) 104.9, 104.6, 104.3, 104.2, 103.8 (C1), 85.6 (C4^), 84.1, 83.6, 83.5, 82.2 (C4), 76.4, 76.1, 76.0, 75.8, 75.5, 75.4, 75.2, 75.1, 74.9, 74.6, 74.5, 73.5 (C2, C3, C5), 63.1, 63.0, 62.8 (C6), 58.3 (C6^), 54.3, (50.2), 48.2, 46.9, 46.2 (cyclenC); δ_C(0.1 mol dm^{-3} NaOH–D_2O) 106.9, 106.8, 106.6, 106.4, 105.7, 105.3, 104.4 (C1), 86.35 (C4^), 85.2, 85.0, 84.7, 84.6, 84.0, 82.6 (C4), 77.9, 77.7, 77.5, 77.4, 77.2, 77.1, 76.8, 76.6, 76.4, 76.1, 76.0, 75.8, 75.6, 75.2, 75.1, 74.6 (C2, C3, C5), 63.1, 62.9, 62.8 (C6), 59.0 (C6^), 55.0, (50.2), 48.0, 47.1, 46.1 (cyclenC).

Usually the ^{13}C NMR chemical shift differences between the seven glucopyranose units are insufficient for all 42 ^{13}C resonances to be separately observed. As the polyamine nitrogens of β-CDX protonate as the solution pH decreases, concomitant changes in the β-CDX ^{13}C NMR spectrum occur as has been briefly discussed above and as shown in the Experimental.

The ^{13}C NMR spectra of β-CDtacdo and β-CDcyclen at different pHs appear in Figs. 2 and 3, respectively, and illustrate the substantial spectral changes which occur with change in pH. At pH 1 resolution of the ^{13}C resonances of fully protonated β-CDtacdoH_3^{3+} and β-CDcyclenH_4^{4+} is relatively small consistent with the polyamine substituent swinging out from the β-CD moiety so that it interacts weakly if at all with the primary hydroxy groups and the differentiation of the seven glucopyranose units is minimised. At the highest pH, where β-CDtacdo and β-CDcyclen exist as the deprotonated neutral species, all seven C1 and C4 resonances are observed consistent with the polyamine substituents hydrogen bonding with the primary hydroxy groups of β-CD and maximising the differentiation between the seven glucopyranose units. This interpretation is in agreement with that presented for the similarly pH dependent ^{13}C NMR spectra of β-CDdien.[19]

Experimental

Materials and instrumental methods

The polyamines 1,2-diaminoethane (en), 1,3-diaminopropane (pn), 1,4-diaminobutane (bn), 1,6-diaminohexane (hn), 2-(2-aminoethylamino)ethylamine (dien), 3-(3-aminopropylamino)-propylamine (dipn), tris(2-aminoethyl)amine (tren) and 1,4,7,10-tetraazacyclododecane bis(dihydrogen sulfate) (cyclen·$2H_2SO_4$) were purchased from Aldrich and used without further purification. 2-[2-(2-Aminoethylamino)ethylamino]ethylamine tetrahydrochloride (trien·4HCl, Aldrich) was purified by two recrystallisations from ethanol–water.[21] 1,4,7-Triazacyclononane·3HCl and 1,5,9-triazacyclododecane·3HCl were prepared as in the literature.[22,23] HPLC grade 1-methylpyrrolidin-2-one (NMP, Aldrich) was dried by distillation from CaH_2 at reduced pressure. β-Cyclodextrin was a gift from Nihon Shokhuin Kako Co. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} silica on aluminium sheets and samples were eluted using a mixture of propan-2-ol–ethyl acetate–water–ammonium hydroxide (7:7:5:4). Compounds containing amino groups were detected by dipping the developed plate into a solution of 1% ninhydrin in ethanol and heating the plate. Cyclodextrins were detected by dipping the developed plate into a solution of 1.5% H_2SO_4 in ethanol and heating the plate. R_f values are reported as R_c (retention relative to β-CD).

Titrations were carried out using a Metrohm Dosimat E665 titrimator, an Orion SA 720 potentiometer, and an Orion 8172 Ross Sureflow combination pH electrode which was filled with 0.10 mol dm^{-3} $NaClO_4$. During all titrations a stream of fine nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} NaOH to remove any last traces of CO_2 and then 0.10 mol dm^{-3} $NaClO_4$ to ensure a constant water vapour pressure) was passed through the titration solution which was magnetically stirred and thermostatted at 298.2 ± 0.1 K in a water-jacketted 20 cm^3 titration vessel which was closed to the atmosphere with the exception of a small exit for the nitrogen stream. Deionised water, purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm, was used in the preparation of all solutions after boiling to remove CO_2. Standardised 0.100 mol dm^{-3} NaOH was titrated against 10.00 cm^3 aliquots of solutions (0.002 mol dm^{-3} in the species of interest, 0.005 mol dm^{-3} in $HClO_4$ and 0.095 mol dm^{-3} in $NaClO_4$ in all titrations). The pK_as were determined using the programme SUPERQUAD[24] on a Digital Venturis 575 computer.

NMR spectra were recorded on a Bruker ACP300 spectrometer operating at 300 (^1H) and 75.47 MHz (^{13}C) for all β-CDXs except for 6^-{2-[bis(2-aminoethyl)amino]-ethylamino}-6^-deoxy-β-cyclodextrin (β-CDtren) where a Varian Gemini 200 spectrometer operating at 200 (^1H) and 50.29 MHz (^{13}C) was used.

General procedure for preparation of amino-substituted β-cyclodextrins

A solution of β-CDtos[11] (2.0 g, 1.55×10^{-3} mol), KI (0.025 g, 0.15×10^{-3} mol) and the amine (5×10^{-3} mol) in dry NMP (5

cm^3) was stirred at 70 °C in a lightly stoppered flask for 4–8 h. The resultant light yellow solution was cooled to room temperature and diluted with ethanol (100 cm^3). The resulting precipitate was collected by vacuum filtration, washed successively with ethanol (100 cm^3) and diethyl ether (50 cm^3) and dried under vacuum to give the crude product. This material was dissolved in water (10 cm^3) and loaded onto a column (4.5 × 4.5 cm) of H^+ form BioRex 70, 100–200 mesh (Biorad). The column was washed with water (400 cm^3) and β-CDX was eluted with 1 mol dm^{-3} NH_4OH. Fractions containing β-CDX were combined and evaporated to dryness under vacuum. The residue was dissolved in water and the solution evaporated under reduced pressure to remove excess ammonia (this procedure was repeated several times). The product was dried under vacuum over P_2O_5 to give β-CDX in yields of 25–60%. Specific preparative descriptions and characterisation data of β-CDtren, previously prepared by other methods,[10] and previously unreported β-CDtacdo are provided below. Similarly detailed preparative and characterisation data for the remaining β-CDXs shown in Fig. 1 are provided as supplementary data.†

6^A^-{2-[Bis(2-aminoethyl)amino]ethylamino}-6^A^-deoxy-β-cyclodextrin (β-CDtren)

A mixture of β-CDtos (2.048 g, 1.59×10^{-3} mol), tris(2-aminoethyl)amine (0.74 g, 5.07×10^{-3} mol) and KI (0.024 g) in NMP (5 cm^3) was treated according to the general procedure to give β-CDtren as a white powder (1.192 g, 59%). R_c 0.31; Electrospray-MS *m/z* 1263 (M^+) [Found: C, 43.84; H, 7.58; N, 4.40. Calc. for β-CDtren·$3H_2O$ ($C_{48}H_{92}N_4O_{34}$): C, 43.76; H, 7.04; N, 4.25%]; δ_H(D_2O–NaOH, pH ~14) 5.00 (br s, 7H + solvent, H1), 3.5–3.8 (m, 26H, H3, H5, H6), 3.1–3.4 (m, 13H, H2, H4), 3.02 (t, *J* 9.0, 1H, H4^A^), 2.85 (d, *J* 12.0, 1H, H6^A^), 2.2–2.7 (m, 13H, H6^A^, trenH); δ_H(D_2O, pH ~9) 5.05 (br s, 7H, H1), 3.8–4.0 (m, 26H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.41 (t, *J* 9.0, 1H, H4^A^), 3.05 (d, *J* 11.4, 1H, H6^A^), 2.4–2.9 (m, 13H, H6^A^, trenH); δ_H(D_2O–HCl, pH ~1) 5.00 (s, 7H, H1), 4.10 (t, *J* 9.0, 1H, H5^A^), 3.6–4.0 (m, 25H, H3, H5, H6), 3.4–3.6 (m, 14H, H2, H4), 2.9–3.4 (m, 14H, H6^A^, trenH); δ_C(D_2O–NaOH, pH ~14), 107.0, 106.6, 106.4, 105.2 (C1), 87.6 (C4^A^), 85.0, 84.8, 84.5, 83.9 (C4), 77.3, 76.4, 76.3, 75.2, 74.9 (C2, C3, C5), 70.9 (C5^A^), 63.0 (C6), 59.8 (trenC3,3'), (56.9), 55.1 (C6^A^), 50.5 (trenC2), 46.2 (trenC1), 41.0 (trenC4,4'); δ_C(D_2O, pH ~9) 104.7, 104.3 (C1), 86.4 (C4^A^), 84.0, 83.6 (C4), 75.9 (C2), 74.9 (C3), 74.7 (C5), 73.3 (C5^A^), 63.1 (C6), 58.7 (trenC3,3'), 55.7 (trenC2), 52.0 (C6^A^), 48.7 (trenC1), 40.7 (trenC4,4'); δ_C(D_2O–HCl, pH ~1) 104.5, 103.8 (C1), 85.8 (C4^A^), 84.2, 83.8, 83.4 (C4), 75.8, 75.5, 75.0, 74.8, 74.5 (C2, C3, C5), 70.2 (C5^A^), 63.6, 63.1 (C6), 52.8 (trenC3,3'), 51.5 (C6^A^), 51.3 (trenC2), 47.0 (trenC1), 38.6 (trenC4,4').

6^A^-(1,5,9-Triazacyclododecan-1-yl)-6^A^-deoxy-β-cyclodextrin (β-CDtacdo)

A mixture of 1,5,9-triazacyclododecane·3HCl[31] (1.451 g, 5.18×10^{-3} mol) and sodium hydroxide (0.625 g, 15.62×10^{-3} mol) in ethanol (30 cm^3) was stirred at room temp. for 90 min. The mixture was filtered and the collected solid was washed with ethanol (10 cm^3). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm^3) and β-CDtos (2.081 g, 1.61×10^{-3} mol) and KI (0.030 g) were added to the solution. The resultant mixture was treated according to the general procedure to give β-CDtacdo as a white powder (0.709 g, 34%). R_c 0.75; Electrospray-MS *m/z* 1288 (M^+) [Found: C, 45.28; H, 7.34; N, 3.15. Calc. for β-CDtacdo·$4H_2O$ ($C_{51}H_{97}N_3O_{38}$): C, 45.03; H, 7.18; N, 3.08%]; δ_H(D_2O–NaOH, pH ~14) 4.9 (br s, 7H + solvent, H1), 4.14 (t, *J* 6.0, 1H, H5^A^), 3.7–4.0 (m, 25H, H3, H5, H6), 3.17 (t, *J* 6.0, 1H, H4^A^), 2.88 (d, *J* 15, 1H, H6^A^), 2.64 (m, 13H, H6^A^, tacdoH1, tacdoH3, tacdoH4), 1.66 (m, 6H, tacdoH2, tacdoH5); δ_H[D_2O–HCl (1:1), pH ~8.5] 5.09 (s, 7H + solvent, H1), 4.26 (t, *J* 9.0, 1H, H5^A^), 3.8–4.2 (m, 25H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.39 (t, *J* 9.0, 1H, H4^A^), 2.5–3.2 (m, 14H, H6^A^, tacdoH1, tacdoH3, tacdoH4), 1.6–2.0 (m, 6H, tacdoH2, tacdoH5); δ_H[D_2O–HCl (1:2), pH ~6.0] 5.07 (br s, 7H, H1), 4.25 (t, *J* 9.0, 1H, H5^A^), 3.8–4.1 (m, 25H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.43 (t, *J* 9.0, 1H, H4^A^), 2.7–3.3 (m, 14H, H6^A^, tacdoH1, tacdoH3, tacdoH4), 1.7–2.2 (m, 6H, tacdoH2, tacdoH5); δ_H(D_2O–HCl, pH ~1) 5.0 (br s, 7H + solvent, H1), 4.33 (br t, 1H, H5^A^), 3.7–4.0 (m, 25H, H3, H5, H6), 3.2–3.6 (m, 27H, H2, H4, H6^A^, tacdoH1, tacdoH3, tacdoH4), 2.2 (br, 6H, tacdoH2, tacdoH5).

† Available as supplementary material (SUP 57281; 9 pp.) deposited with the British Library. Details are available from the editorial office.

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Host–guest complexation of aromatic carboxylic acids and their conjugate bases by 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins† in aqueous solution

Suzanna D. Kean,[a] Bruce L. May,[a] Philip Clements,[a] Stephen F. Lincoln,*[a] and Christopher J. Easton[b]

[a] *Department of Chemistry, University of Adelaide, South Australia 5005, Australia*
[b] *Research School of Chemistry, The Australian National University, ACT 0200, Australia*

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A potentiometric titration study of the complexation of the guests benzoic acid, 4-methylbenzoic acid and (*RS*)-2-phenylpropanoic acid and their conjugate bases by host 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrins, where the ω-aminoalkylamino groups are -NH(CH$_2$)$_n$NH$_2$ (n = 2, 3, 4 and 6), is reported. Of the 64 host–guest complexes whose formation is statistically possible over the pH range 2.0–12.0 studied, 35 were detected. Their stability constants range from 140 ± 35 dm^3 mol^{-1} for the [βCDNH(CH$_2$)$_2$NH$_2$·4-methyl benzoate] complex to 1760 ± 150 dm^3 mol^{-1} for the [βCDNH(CH$_2$)$_4$NH$_2$·(*S*)-2-phenylpropanoate]$^-$ complex at 298.2 K and I = 0.10 mol dm^{-3} (NaClO$_4$). The charge and hydrophobicity of both host and guest appear to be significant factors in the variation of host–guest complex stability. Qualitative structural information on the host–guest complexes obtained from 600 MHz ^{1}H NMR ROESY spectroscopy and is generally consistent with the structures generated by molecular modelling.

Introduction

The range of cyclodextrins (CDs), their modified forms and the host–guest complexes formed by them is extensive.[1–16] In most cases, entry of a hydrophobic moiety of the guest into the hydrophobic region of the host CD annulus occurs during host–guest complexation, and the CD may discriminate between guests on the basis of their size, hydrophobicity, charge and chirality. We are particularly interested in the effects of variation of charge and hydrophobicity of both the host CD and the guest on complex stability. Our reported syntheses of 6^A-(ω-aminoalkylamino)-6^A-deoxy-βCDs (βCDNH(CH$_2$)$_n$NH$_2$) (Fig. 1) and the determination of the pK_as of their protonated forms[17] facilitate a systematic study of the effects of the simultaneous variation of these factors in both host and guest on complex stability. We have studied the relative importance of these factors in βCDNH(CH$_2$)$_n$NH$_2$ (n = 2, 3, 4 and 6) where the hydrophobicity of the -NH(CH$_2$)$_n$NH$_2$ substituent increases with n, and a variation of charge occurs through amine protonation.[14] The guests selected for this study, benzoic acid, 4-methylbenzoic acid and (*R*) or (*S*)-2-phenylpropanoic acid and their conjugate bases, also exhibit differences in hydrophobicity and charge depending on protonation, and in the case of the (*R*) or (*S*)-2-phenylpropanoic acids and their conjugate bases, differences in chirality. We have previously reported[2] the complexation of these guests by βCD and 6^A-amino-6^A-deoxy-βCD which provides a comparison with our new studies.

Experimental

The modified βCDs were prepared as previously described,[17] and were dried to constant weight and stored over P_2O_5 prior to use. The carboxylic acids were high quality commercial grade materials. Deionized water was purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm and, after boiling to remove CO_2, was used in the preparation of all solutions. 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$_4$ was used in all titrations. During each titration a fine stream of nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} NaOH to remove any CO_2 traces, and then through aqueous 0.10 mol dm^{-3} NaClO$_4$) was passed through the titration solution which was magnetically stirred and thermostatted at 298.2 ± 0.1 K in a water-jacketed 20 cm^3 titration vessel which was closed to the atmosphere with the exception of a small vent for the nitrogen stream. In each titration, 10 cm^3 of a solution 1.0 × 10^{-3} mol dm^{-3} in [βCDNH(CH$_2$)$_n$NH$_2$] and [carboxylic acid] and 2.0 × 10^{-3} mol dm^{-3} in [HClO$_4$] and I = 0.10 mol dm^{-3} (NaClO$_4$) was measured into the titration vessel and allowed 30 min to reach thermal equilibrium. Sodium hydroxide solution (0.10 mol dm^{-3}) was the titrant. All titrations were carried out in duplicate at least and a typical titration curve is shown in Fig. 2. Complex stability constants were derived from the titration data using the program SUPERQUAD.[18]

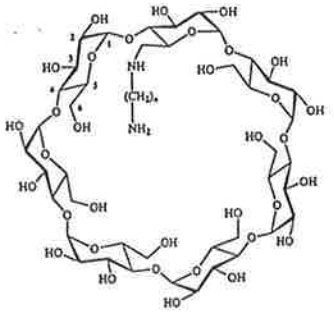

Fig. 1 6^A-(ω-Aminoalkylamino)-6^A-deoxy-β-cyclodextrins where n = 2, 3, 4 or 6.

† β-Cyclodextrin = cycloheptamaltose.

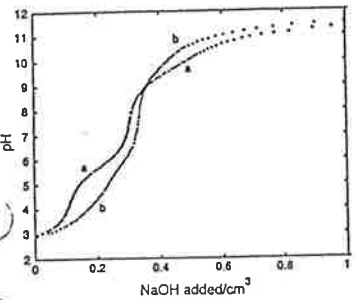

Fig. 2 The titration curves for a) a 0.010 mol dm^{-3} solution of βCDNH(CH$_2$)$_4$NH$_2$ in 0.020 mol dm^{-3} in [HClO$_4$], and b) a 0.010 mol dm^{-3} solution of βCDNH(CH$_2$)$_4$NH$_2$ and 4-methylbenzoic acid, in 0.020 mol dm^{-3} in [HClO$_4$] against 0.100 mol dm^{-3} NaOH, at 298.2 K where I = 0.10 mol dm^{-3} (NaClO$_4$).

The ^{1}H NMR 1D and 2D ROESY (mixing time of 0.35 s)[19] experiments were run on a Varian Inova 600 spectrometer. Either βCDNH(CH$_2$)$_4$NH$_2$ alone or equimolar amounts of the guest species and βCDNH(CH$_2$)$_4$NH$_2$ were dissolved in D_2O to give total concentrations of 0.06 mol dm^{-3} of each species, and the pH was adjusted to ≥11.5. The resultant solutions were filtered and degassed by freeze–thawing before the spectra were recorded. The spectral assignments below are according to the glucopyranose numbering system H1–H6 (where a superscript A specifies the glucopyranose unit bearing the 1,6-diaminohexyl substituent) in Fig. 1. The hexyl protons are labelled Ha–Hf as distance increases from the secondary amine group, and aromatic guest protons are labelled as H*o* and H*m* where the former is adjacent to the carboxylate group.

The spectral assignments for βCDNH(CH$_2$)$_4$NH$_2$ alone at pH ≥ 11.5 are: 1D ^{1}H spectrum: δ_H 4.80 (s, 7H + solvent, H1); 3.5–3.8 (m, 26H, H3, H5, H6); 3.2–3.4 (m, 13H, H2, H4); 3.11 (t, J = 9.3 Hz, 1H, H4^); 2.93 (d, J = 12.4 Hz, 1H, H6^); 2.70 (m, 1H, H6^'); 2.65 (m, 2H, Hf); 2.46 (m, 2H, Ha); 1.40 (br s, 4H, Hb, He); 1.26 (br s, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.26 (Hc,d) shows cross-peaks with 1.40 (Hb,e), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 1.40 (Hb,e) shows cross-peaks with 1.26 (Hc,d), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 2.46 (Ha) shows cross-peaks with 1.26 (Hc,d), 1.4 (Hb,e), 3.9 (H5^); 2.65 (Hf) shows cross-peaks with 1.26 (Hc,d), 1.4 (Ha,e), 3.7 (H5), 3.8 (H3). These cross-peaks are absent from the 2D-ROESY spectrum obtained after acidification of the sample solution to pH 1 with hydrochloric acid.

The stability constants discussed below indicate that the [βCDNH(CH$_2$)$_4$NH$_2$·4-methylbenzoate]$^+$ complex constitutes 85% of the total [βCDNH(CH$_2$)$_4$NH$_2$] and [4-methylbenzoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ^{1}H spectrum: δ_H 7.80 (d, J = 7.8 Hz, 2H, H*o*); 7.29 (d, J = 7.8 Hz, 2H, H*m*); 5.00 (m, 7H + solvent, H1); 3.6–3.9 (m, 26H, H3, H5, H6); 3.4–3.6 (m, 13H, H2, H4); 3.26 (t, J = 9.6 Hz, 1H, H4^); 2.99 (d, J = 13.2 Hz, 1H, H6^); 2.71 (m, 1H, H6^'); 2.64 (t, J = 7.2 Hz, 2H, Hf); 2.45 (t, J = 7.2 Hz, 2H, Ha); 2.41 (s, 3H, Me-); 1.4–1.5 (m, 4H, Hb, He); 1.2–1.3 (m, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.2–1.3 (Hc,d) shows cross-peaks with 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 1.4–1.5 (Hb,e) shows cross-peaks with 1.2–1.3 (Hc,d), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 2.41 (Me) shows a cross-peak with 7.29 (H*m*); 2.45 (Ha) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.64 (Hf) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.71 (H6^') shows a cross-peak with 3.26 (H4^); 3.26 (H4^) shows a cross-peak with 2.71 (H6^'); 3.8 (H5) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 7.29 (H*o*), 7.80 (H*m*); 3.9 (H3) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 7.29 (H*o*), 7.80 (H*m*); 7.29 (H*o*) shows cross-peaks with 2.41 (Me-), 3.8 (H5), 3.9 (H3), 7.80 (H*m*); 7.80 (H*m*) shows cross-peaks with 3.8 (H5), 3.9 (H3), 7.29 (H*o*).

The stability constants discussed below indicate that the [βCDNH(CH$_2$)$_4$NH$_2$]·(*S*)-2-phenylpropanoate complex constitutes 90% of the total [βCDNH(CH$_2$)$_4$NH$_2$] and [(*S*)-2-phenylpropanoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ^{1}H spectrum: δ_H 7.30 (m, 4H, H*m*, H*o*); 7.22 (m, 1H, H*p*); 4.92 (br s, 7H + solvent, H1); 3.6–4.0 (m, 26H, H3, H5, H6); 3.58 (q, J = 7.2 Hz, αCH); 3.2–3.5 (m, 13H, H2, H4); 3.19 (t, J = 9.6 Hz, 1H, H4^); 2.93 (d, J = 12.6 Hz, 1H, H6^); 2.67 (dd, J = 12.6, 9.5 Hz, 1H, H6^'); 2.62 (t, J = 7.2 Hz, 2H, Hf); 2.45 (t, J = 7.8 Hz, 2H, Ha); 1.39 (m, 4H, Hb,e); 1.36 (d, J = 7.2 Hz, 3H, Me); 1.25 (m, 4H, Hc,d). 2D ROESY cross-peaks: δ_H 1.25 (Hc,d) shows cross-peaks with 1.39 (Hb,e), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 1.36 (Me) shows a cross-peak with 3.58 (αCH); 1.39 (Hb,e) shows cross-peaks with 1.25 (Hc,d), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 2.45 (Ha) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.8 (H5^); 2.62 (Hf) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.75 (H3); 2.67 (H6^') shows cross-peaks with 2.93 (H6^), 3.19 (H4^); 2.93 (H6^) shows cross-peaks with 2.67 (H6^'), 3.7 (H5), 3.8 (H5^); 3.19 (H4^) shows cross-peaks with 2.67 (H6^'), 3.75 (H3), 3.8 (H5^); 3.58 (αCH) shows cross-peaks with 1.36 (Me), 7.30 (H*o*, H*m*); 3.7 (H5) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (H*o*, H*m*); 3.75 (H3) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (H*o*, H*m*); 3.8 (H5^) shows cross-peaks with 2.45 (Ha), 2.93 (H6^), 3.19 (H4^); 7.30 (H*o*, H*m*) shows cross-peaks with 3.58 (αCH), 3.75 (H3). Very similar spectra were recorded for the βCDNH(CH$_2$)$_4$NH$_2$·(*R*)-2-phenylpropanoate complex.

Molecular modelling[20] was carried out using a Silicon Graphics Iris Indigo X2 400 Unix workstation. Computational results were obtained using the force-field programme CVFF with the 6-12 ε function with geometric averages for the heteronuclear interactions. Energy minimisations were performed with the Discover programme, using a steepest descents algorithm until the root mean square of the residuals (RMS) derived ≤10, whereafter a conjugate gradients algorithm was used until RMS <1 and the global minimisation was obtained using a quasi Newton–Raphson algorithm. Several local energy minima were found before the global minimum was reached. Graphical displays were printed through the Insight II molecular modelling programme.

Results and discussion

Complex stabilities

The various weak secondary bonding interactions between the CD host and the guest can sum to produce quite high stabilities in CD complexes. It has been concluded from a wide range of studies dominated by natural CDs that the complexation process involves conformational change in the CD host and the guest accompanied by dehydration of both to an extent depending on the nature of both entities.[1,11,13,15,16] These aspects of CD complexation have been extensively discussed, and as a consequence only those interactions which appear to dominate the variations in stability observed in this study are discussed in detail here. On a statistical basis, 64 different host–guest complexes could be formed between the four host βCDNH$_3$·(CH$_2$)$_n$NH$_2^{3+}$ (n = 2, 3, 4 and 6) and the four guest carboxylic acids and their respective conjugate bases, but only 35 of these complexes were detected under the conditions of this study. The complex stability range is encompassed by stability constants K = 140 and 1760 dm^3 mol^{-1} for equilibria (7) and (16) when n = 2 and 6, respectively, as shown in Table 1. While each pos-

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Table 1 Equilibria, K and pK_a^a values determined in aqueous solution at $I = 0.10$ mol dm^{-3} ($NaClO_4$) and 298.2 K

Equilibrium	K/dm^3 mol^{-1}			
	$n = 2$	$n = 3$	$n = 4$	$n = 6$
(1) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + benzoic acid ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·benzoic acid]$^{3+}$	820 ± 170	350 ± 80	740 ± 100	545 ± 140
(2) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + benzoate$^-$ ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·benzoate]$^+$	870 ± 70			
(3) βCDNH(CH$_2$)$_n$NH$_3^+$ + benzoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·benzoate]	180 ± 15		305 ± 35	275 ± 30
(4) βCDNH(CH$_2$)$_n$NH$_2$ + benzoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·benzoate]$^-$		915 ± 60	950 ± 120	1000 ± 120
(5) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + 4-methylbenzoic acid ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·4-methylbenzoic acid]$^{3+}$		420 ± 60	415 ± 65	
(6) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + 4-methylbenzoate$^-$ ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·4-methylbenzoate]$^+$	1000 ± 170			
(7) βCDNH(CH$_2$)$_n$NH$_3^+$ + 4-methylbenzoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·4-methylbenzoate]	140 ± 35			180 ± 20
(8) βCDNH(CH$_2$)$_n$NH$_2$ + 4-methylbenzoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·4-methylbenzoate]$^-$		885 ± 65	535 ± 50	750 ± 20
(9) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + (*R*)-2-phenylpropanoic acid ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·(*R*)-2-phenylpropanoic acid]$^{3+}$	850 ± 170	395 ± 50	420 ± 65	
(10) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + (*R*)-2-phenylpropanoate$^-$ ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·(*R*)-2-phenylpropanoate]$^+$	790 ± 80			
(11) βCDNH(CH$_2$)$_n$NH$_3^+$ + (*R*)-2-phenylpropanoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·(*R*)-2-phenylpropanoate]				250 ± 35
(12) βCDNH(CH$_2$)$_n$NH$_2$ + (*R*)-2-phenylpropanoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·(*R*)-2-phenylpropanoate]$^-$		760 ± 75	630 ± 30	1150 ± 295
(13) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + (*S*)-2-phenylpropanoic acid ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·(*S*)-2-phenylpropanoic acid]$^{3+}$	345 ± 40	690 ± 70	570 ± 80	
(14) βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ + (*S*)-2-phenylpropanoate$^-$ ⇌ [βCDNH$_3$(CH$_2$)$_n$NH$_3$·(*S*)-2-phenylpropanoate]$^+$	630 ± 30			
(15) βCDNH(CH$_2$)$_n$NH$_3^+$ + (*S*)-2-phenylpropanoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·(*S*)-2-phenylpropanoate]				285 ± 80
(16) βCDNH(CH$_2$)$_n$NH$_2$ + (*S*)-2-phenylpropanoate$^-$ ⇌ [βCDNH(CH$_2$)$_n$NH$_3$·(*S*)-2-phenylpropanoate]$^-$		465 ± 60	630 ± 105	1760 ± 150
(17) βCD + benzoic acid ⇌ [βCD·benzoic acid]	590[b]			
(18) βCD + benzoate$^-$ ⇌ [βCD·benzoate]$^-$	60[b]			
(19) βCDNH$_3^+$ + benzoic acid ⇌ [βCDNH$_3$·benzoic acid]$^+$	340[b]			
(20) βCDNH$_3^+$ + benzoate$^-$ ⇌ [βCDNH$_3$·benzoate]	120[b]			
(21) βCDNH$_2$ + benzoate$^-$ ⇌ [βCDNH$_2$·benzoate]$^-$	50[b]			
(22) βCD + 4-methylbenzoic acid ⇌ [βCD·4-methylbenzoic acid]	1680[b]			
(23) βCD + 4-methylbenzoate$^-$ ⇌ [βCD·4-methylbenzoate]$^-$	110[b]			
(24) βCDNH$_3^+$ + 4-methylbenzoic acid ⇌ [βCDNH$_3$·4-methylbenzoic acid]$^+$	910[b]			
(25) βCDNH$_3^+$ + 4-methylbenzoate$^-$ ⇌ [βCDNH$_3$·4-methylbenzoate]	330[b]			
(26) βCDNH$_2$ + 4-methylbenzoate$^-$ ⇌ [βCDNH$_2$·4-methylbenzoate]$^-$	100[b]			

[a] The pK_as of βCDNH$_3$(CH$_2$)$_n$NH$_3^{3+}$ are 9.42 and 5.70 when $n = 2$, 9.90 and 7.39 when $n = 3$, 10.26 and 8.06 when $n = 4$ and 10.26 and 8.72 when $n = 6$ under the conditions of this study.[17] The pK_a of βCDNH$_3^+$ = 8.49.[2] The pK_as of benzoic, 4-methylbenzoic and (*R*)-or (*S*)-2-phenylpropanoic acid are 4.06, 4.20 and 4.23, respectively.[17] [b] From reference 2.

sible type of complex was detected for each guest species, each type of complex was not detected for every value of n for the βCDNH(CH$_2$)$_n$NH$_2$ host. There are systematic absences in the detected species about the diagonal of data sets for $n = 2$, 3, 4 and 6 in Table 1. The negatively charged complex was not detected when $n = 2$, the neutral and unipositively charged complexes were not detected when $n = 3$ and 4, except when the guest was benzoate in the last case. When $n = 6$, the dipositively charged complex was only detected when benzoic acid was the guest. (It should be noted that complexes present at concentrations at ≤ 5% of total [βCDNH(CH$_2$)$_n$NH$_2$] are not reliably detected by the potentiometric method used in this study and are listed as not detected.)

A broad pattern emerges in the variation of complex stability in Table 1. This may be represented through Fig. 3 where the rectangle represents the set of stability constants for a carboxylic acid and its carboxylate being complexed by βCDNH(CH$_2$)$_n$NH$_2$ and its protonated analogues. Generally, the most stable complexes occur in the top left and bottom right corners of the rectangle (with the exception of [βCDNH$_3$(CH$_2$)$_2$NH$_3$·4-methyl benzoic acid]$^{3+}$ which was not detected), while there is an absence of detected complexes about the diagonal running from the bottom right to the top left of the rectangle. This

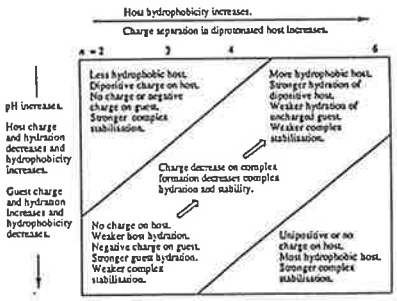

Fig. 3 A schematic representation of the variation of complex stability and the factors contributing to it.

pattern of variation in stability may be attributed to the effects of changes in i) the charge, ii) the hydrophobicity iii) the stereochemistry of both host and guest, and iv) changes in CD host,

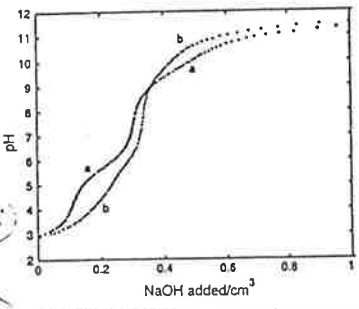

Fig. 2 The titration curves for a) a 0.010 mol dm^{-3} solution of βCDNH(CH$_2$)$_4$NH$_2$ in 0.020 mol dm^{-3} [HClO$_4$], and b) a 0.010 mol dm^{-3} solution in 0.010 mol dm^{-3} βCDNH(CH$_2$)$_4$NH$_2$ and 4-methylbenzoic acid, respectively, in 0.020 mol dm^{-3} in [HClO$_4$] against 0.100 mol dm^{-3} NaOH, at 298.2 K where I = 0.10 mol dm^{-3} (NaClO$_4$).

The ^{1}H NMR 1D and 2D ROESY (mixing time of 0.35 s)[19] experiments were run on a Varian Inova 600 spectrometer. Either βCDNH(CH$_2$)$_4$NH$_2$ alone or equimolar amounts of the guest species and βCDNH(CH$_2$)$_4$NH$_2$ were dissolved in D_2O to give total concentrations of 0.06 mol dm^{-3} of each species, and the pH was adjusted to ≥11.5. The resultant solutions were filtered and degassed by freeze–thawing before the spectra were recorded. The spectral assignments below are according to the glucopyranose numbering system H1–H6 (where a superscript A specifies the glucopyranose unit bearing the 1,6-diaminohexyl substituent) in Fig. 1. The hexyl protons are labelled Ha–Hf as distance increases from the secondary amine group, and aromatic guest protons are labelled as H*o* and H*m* where the former is adjacent to the carboxylate group.

The spectral assignments for βCDNH(CH$_2$)$_4$NH$_2$ alone at pH ≥ 11.5 are: 1D ^{1}H spectrum: δ_H 4.80 (s, 7H + solvent, H1); 3.5–3.8 (m, 26H, H3, H5, H6); 3.2–3.4 (m, 13H, H2, H4); 3.11 (t, J = 9.3 Hz, 1H, H4^A); 2.93 (d, J = 12.4 Hz, 1H, H6^A); 2.70 (m, 1H, H6$^{A'}$); 2.65 (m, 2H, Hf); 2.46 (m, 2H, Ha); 1.40 (br s, 4H, Hb, He); 1.26 (br s, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.26 (Hc,d) shows cross-peaks with 1.40 (Hb,e), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 1.40 (Hb,e) shows cross-peaks with 1.26 (Hc,d), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 2.46 (Ha) shows cross-peaks with 1.26 (Hc,d), 1.4 (Hb,e), 3.9 (H5^A); 2.65 (Hf) shows cross-peaks with 1.26 (Hc,d), 1.4 (Ha,e), 3.7 (H5), 3.8 (H3). These cross-peaks are absent from the 2D-ROESY spectrum obtained after acidification of the sample solution to pH 1 with hydrochloric acid.

The stability constants discussed below indicate that the [βCDNH(CH$_2$)$_4$NH$_2$·4-methylbenzoate]$^-$ complex constitutes 85% of the total [βCDNH(CH$_2$)$_4$NH$_2$] and [4-methylbenzoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ^{1}H spectrum: δ_H 7.80 (d, J = 7.8 Hz, 2H, H*o*); 7.29 (d, J = 7.8 Hz, 2H, H*m*); 5.00 (m, 7H + solvent, H1); 3.6–3.9 (m, 26H, H3, H5, H6); 3.4–3.6 (m, 13H, H2, H4); 3.26 (t, J = 9.6 Hz, 1H, H4^A); 2.99 (d, J = 13.2 Hz, 1H, H6^A); 2.71 (m, 1H, H6$^{A'}$); 2.64 (t, J = 7.2 Hz, 2H, Hf); 2.45 (t, J = 7.2 Hz, 2H, Ha); 2.41 (s, 3H, Me-); 1.4–1.5 (m, 4H, Hb, He); 1.2–1.3 (m, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.2–1.3 (Hc,d) shows cross-peaks with 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 1.4–1.5 (Hb,e) shows cross-peaks with 1.2–1.3 (Hc,d), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 2.41 (Me) shows a cross-peak with 7.29 (H*m*); 2.45 (Ha) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.64 (Hf) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.71 (H6$^{A'}$) shows a cross-peak with 3.26 (H4^A); 3.26 (H4^A) shows a cross-peak with 2.71 (H6$^{A'}$); 3.8 (H5) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 7.29 (H*o*), 7.80 (H*m*); 3.9 (H3) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 7.29 (H*o*), 7.80 (H*m*); 7.29 (H*o*) shows cross-peaks with 2.41 (Me-), 3.8 (H5), 3.9 (H3), 7.80 (H*m*); 7.80 (H*m*) shows cross-peaks with 3.8 (H5), 3.9 (H3), 7.29 (H*o*).

The stability constants discussed below indicate that the [βCDNH(CH$_2$)$_4$NH$_2$·(*S*)-2-phenylpropanoate] complex constitutes 90% of the total [βCDNH(CH$_2$)$_4$NH$_2$] and [(*S*)-2-phenylpropanoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ^{1}H spectrum: δ_H 7.30 (m, 4H, H*m*, H*o*); 7.22 (m, 1H, H*p*); 4.92 (br s, 7H + solvent, H1); 3.6–4.0 (m, 26H, H3, H5, H6); 3.58 (q, J = 7.2 Hz, αCH); 3.2–3.5 (m, 13H, H2, H4); 3.19 (t, J = 9.6 Hz, 1H, H4^A); 2.93 (d, J = 12.6 Hz, 1H, H6^A); 2.67 (dd, J = 12.6, 9.5 Hz, 1H, H6$^{A'}$); 2.62 (t, J = 7.2 Hz, 2H, Hf); 2.45 (t, J = 7.8 Hz, 2H, Ha); 1.39 (m, 4H, Hb,e); 1.36 (d, J = 7.2 Hz, 3H, Me); 1.25 (m, 4H, Hc,d). 2D ROESY cross-peaks: δ_H 1.25 (Hc,d) shows cross-peaks with 1.39 (Hb,e), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 1.36 (Me) shows a cross-peak with 3.58 (αCH); 1.39 (Hb,e) shows cross-peaks with 1.25 (Hc,d), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 2.45 (Ha) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.8 (H5^A); 2.62 (Hf) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.75 (H3); 2.67 (H6$^{A'}$) shows cross-peaks with 2.93 (H6^A), 3.19 (H4^A); 2.93 (H6^A) shows cross-peaks with 2.67 (H6$^{A'}$), 3.7 (H5), 3.8 (H5^A); 3.19 (H4^A) shows cross-peaks with 2.67 (H6$^{A'}$), 3.75 (H3), 3.8 (H5^A); 3.58 (αCH) shows cross-peaks with 1.36 (Me), 7.30 (H*o*, H*m*); 3.7 (H5) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (H*o*, H*m*); 3.75 (H3) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (H*o*, H*m*); 3.8 (H5^A) shows cross-peaks with 2.45 (Ha), 2.93 (H6^A), 3.19 (H4^A); 7.30 (H*o*, H*m*) shows cross-peaks with 3.58 (αCH), 3.75 (H3). Very similar spectra were recorded for the βCDNH(CH$_2$)$_4$NH$_2$·(*R*)-2-phenylpropanoate complex.

Molecular modelling[20] was carried out using a Silicon Graphics Iris Indigo X2 400 Unix workstation. Computational results were obtained using the force-field programme CVFF with the 6-12 ε function with geometric averages for the heteronuclear interactions. Energy minimisations were performed with the Discover programme, using a steepest descents algorithm until the root mean square of the residuals (RMS) derived ≤10, whereafter a conjugate gradients algorithm was used until RMS <1 and the global minimisation was obtained using a quasi Newton–Raphson algorithm. Several local energy minima were found before the global minimum was reached. Graphical displays were printed through the Insight II molecular modelling programme.

Results and discussion

Complex stabilities

The various weak secondary bonding interactions between the CD host and the guest can sum to produce quite high stabilities in CD complexes. It has been concluded from a wide range of studies dominated by natural CDs that the complexation process involves conformational change in the CD host and the guest accompanied by dehydration of both to an extent depending on the nature of both entities.[1,3,11,12,15,16] These aspects of CD complexation have been extensively discussed, and as a consequence only those interactions which appear to dominate the variations in stability observed in this study are discussed in detail here. On a statistical basis, 64 different host–guest complexes could be formed between the four hosts βCDNH$_2$-(CH$_2$)$_n$NH$_2^{2+}$ (n = 2, 3, 4 and 6) and the four guest carboxylic acids and their respective conjugate bases, but only 35 of these complexes were detected under the conditions of this study. The complex stability range is encompassed by stability constants K = 140 and 1760 dm^3 mol^{-1} for equilibria (7) and (16) when n = 2 and 6, respectively, as shown in Table 1. While each pos-

guest and complex hydration superimposing on the stabilising effect of entry of the hydrophobic phenyl group of the guest into the hydrophobic centre of the βCD annulus.

The βCD annulus and the two amine groups represent constant structural features in the four βCDNH$(CH_2)_nNH_2$ hosts. Hence, variations in effects i)–iv) generated by the CD hosts arise predominantly from the changes in the length of the -NH$(CH_2)_nNH_2$ substituent indicated by *n*. The phenyl ring and the carboxylic acid group are invariant in the four guest carboxylic acids, and therefore differences in their complexation characteristics arise from differences in hydrophobicity and stereochemistry engendered by the CH_3- and $CH_3CH<$ moieties in 4-methylbenzoic acid and (*R*) or (*S*)-2-phenylpropanoic acid, respectively, when compared with those of benzoic acid.

Descending the vertical axis of Fig. 3 is equivalent to increasing solution pH so that the host's charge decreases with decreasing protonation and its hydrophobicity increases as a consequence. Correspondingly, the guest becomes negatively charged with the probable result that its hydration increases and its hydrophobicity decreases. On the horizontal axis, host hydrophobicity increases and the charge separation in the diprotonated host increases as *n* in βCDNH$(CH_2)_nNH_2$ increases. Thus, in the upper left hand corner of Fig. 3, the host has a dipositive charge and the guest has either no charge or a negative charge, and it appears that either a charge–dipole or a charge–charge interaction stabilises the complex. The intensity of this interaction probably diminishes as *n* increases and the charges in βCDNH$_2(CH_2)_nNH_3^{2+}$ move further apart so that the stabilities of the complexes diminish in the upper right hand corner of Fig. 3. (The orientation of the carboxylic acid or carboxylate guest within the annulus may also change with variation of βCDNH$(CH_2)_nNH_2$ charge as its protonation changes as indicated by the molecular modelling studies discussed below.)

At the lower left hand corner of Fig. 3, the host is uncharged, the guest is negatively charged and no complexes are observed. (This is consistent with the observation that the analogous complexes formed between βCD and βCDNH$_2$ and the same carboxylates as those studied here are characterised by stability constants in the range 13–63 dm^3 mol^{-1} while the complexes formed by βCD, βCDNH$_2$ and βCDNH$_3^+$ with the analogous acids are characterised by stability constants in the range 110–1680 dm^3 mol^{-1}.[3]) However, as hydrophobicity increases with *n* as Fig. 3 is traversed from left to right, complexation of the carboxylate guest strengthens to give more stable complexes in the lower right hand corner of Fig. 3. While it is possible that the increase in host hydrophobicity is the main source of complex stabilisation, it also appears (from the NMR and molecular modelling studies discussed below) that the -NH-$(CH_2)_nNH_2$ substituent enters the annulus in basic solution both in βCDNH$(CH_2)_nNH_2$ and in its carboxylate complexes. As the βCDNH$(CH_2)_nNH_2$ carboxylate complexes are some of the more stable species, it seems that this latter effect stabilises the host–guest complex while the short -NH$(CH_2)_2$-NH_2 substituent is less effective in this role. Similar self-complexations are observed for the pendant naphthyl group and pendant dansyl group of 6^A-*N*-(*N*'-(5-dimethylamino-1-naphthylsulfonyl)diaminoethane]-6^A-deoxy-β-cyclodextrin and 3^A-*O*-naphthylmethyl-β-cyclodextrin, respectively.[22,23] The charged -NH$_2(CH_2)_nNH_3^{2+}$ substituent does not appear to enter the βCDNH$_2(CH_2)_nNH_3^{2+}$ annulus as is discussed below.

Superimposed on these effects is that of hydration. The hydration of βCDNH$(CH_2)_nNH_2$ and its protonated analogues probably has two main components: water occupying the annulus but interacting weakly with the methylene, methine and ether oxygen moieties defining the hydrophobic centre of the annulus, and water hydrogen bonding with the hydroxy groups at either end of the annulus. (From 6–6.5 H_2O have been observed in the βCD annulus in solid state neutron and X-ray diffraction studies.[24–27]) The displacement of water from the annulus by the hydrophobic moiety of the guest during complexation represents a major hydration change and probably makes a significant contribution to the free energy of complexation.[1,3,6,13,16] Depending on whether it is βCDNH$(CH_2)_nNH_2$ or one of its protonated analogues which acts as the host, and whether the carboxylic acid or its carboxylate acts as the guest, the extent of hydration may either increase or decrease on formation of the complex. The complete or partial cancellation of host charge by that of the guest is likely to produce a decrease in the overall hydration of the complex compared with that of its charged components, and a consequent lessening of complex stability. This occurs in the centre of Fig. 3 and is exemplified by equilibria (2) and (3), (6) and (7), (10) and (11), and (14) and (15) in Table 1. Hydration effects on stability are likely to be less for equilibria (1), (4), (5), (8), (9), (12), (13) and (16) where no change in overall charge occurs on complexation. Thus, a degree of complex stabilisation may be achieved through minimising the interactions of the hydrophobic moieties of the host and guest with water through partial encapsulation of the hydrophobic guest moiety in the hydrophobic annulus of the host while retaining the hydration of their hydrophilic moieties.

There is no apparent systematic stereochemical effect of the variation of *n* in βCDNH$(CH_2)_nNH_2$ and its conjugate acids and of the stereochemistry of the carboxylic acids and the conjugate bases other than those subsumed into the discussion of factors i)–iv) above. However, there is a small chiral discrimination in equilibria (9) and (13) when $n = 2$ and 3, where [βCDNH$_2(CH_2)_nNH_3$·(*R*)-2-phenylpropanoic acid]$^{2+}$ is more stable than its (*S*)-analogue when $n = 2$ and *vice versa* when $n = 3$. Although small, these differences are consistent with *n* of the -NH$_2(CH_2)_nNH_3^{2+}$ substituent influencing guest orientation as is also the case for the -NH$(CH_2)_nNH_2$ substituent where $n = 3$ and 6 in equilibria (12) and (16).

NMR structural studies

The detailed assignment of the ^{1}H NMR spectrum of βCD-NH$(CH_2)_nNH_2$ at pH ≥ 11.5 (Fig. 4) is presented in the Experimental section, and the cross-peaks observed in the ROESY spectrum (Table 2) provide structural information. The cross-peaks arising from interaction between Hb–Hf of the -NH-$(CH_2)_nNH_2$ substituent and H3 and H5 of the βCD annulus and Ha–He and H5^A are consistent with complexation of -NH$(CH_2)_nNH_2$ inside the annulus as shown schematically in Fig. 5. These cross-peaks are absent from the ROESY spectrum obtained after acidification of the sample solution to pH 1 with hydrochloric acid consistent with the protonated -NH$_2(CH_2)_n$-NH_3^{2+} substituent being excluded from the annulus as a result of its decreased hydrophobicity.

The titration data discussed above indicate that the [βCD-NH$(CH_2)_nNH_2$·4-methylbenzoate] complex constitutes 85% of the total [βCDNH$(CH_2)_nNH_2$] and [4-methylbenzoate] at the pH ≥ 11.5 of the NMR study. The ^{1}H chemical shifts and the spectral resolution of the -NH$(CH_2)_nNH_2$ substituent methylene protons of βCDNH$(CH_2)_nNH_2$ differ from those observed for the [βCDNH$(CH_2)_nNH_2$·4-methylbenzoate] complex (Fig. 5 and Experimental) consistent with the methylene chain of -NH$(CH_2)_nNH_2$ being inside the βCD annulus and parallel to the face of the aromatic ring where they experience an anisotropic field arising from the high π electron density of the guest. Cross-peaks between Ha–Hf of -NH$(CH_2)_nNH_2$ and H3 and H5, and Ho and Hm of the 4-methylbenzoate and H3 and H5 (Fig. 6 and Table 2) are consistent with the simultaneous complexation of both entities in the βCD annulus. However, there are no cross-peaks due to interactions between the -NH$(CH_2)_n$-NH_2 substituent and 4-methylbenzoate. These data are consistent with either a single complex where the carboxylate protrudes from either the primary or the secondary face (Fig. 5) of the βCD annulus (respectively delineated by primary and

Table 2 ^{1}H NMR cross-peaks[a] observed for βCDNH$_2$(CH$_2$)$_4$NH$_2$ and its 4-methylbenzoate and (*S*)-2-phenylpropanoate complexes

	βCDNH$_2$(CH$_2$)$_4$NH$_2$					
Annular protons	6-Aminohexyl substituent protons					
	Ha	Hb	Hc	Hd	He	Hf
H3		+	++	++	++	++
H5	+	++	++	++	++	+
H5^	++	+	+			

	[βCDNH$_2$(CH$_2$)$_4$NH$_2$·4-methylbenzoate]$^-$								
Annular protons	6-Aminohexyl substituent and 4-methylbenzoate protons								
H	Ha	Hb	Hc	Hd	He	Hf	Ho	Hm	Me
H3	++	++	++	++	++	++	+	++	+
H5	++	++	++	++	++	+	+	++	+

	[βCDNH$_2$(CH$_2$)$_4$NH$_2$·(*S*)-2-phenylpropanoate]$^-$								
Annular protons	6-Aminohexyl substituent and (*S*)-2-phenylpropanoate protons								
	Ha	Hb	Hc	Hd	He	Hf	Ho	Hm	Me
H3		+	+	+	+	++	+	+	
H5	+	+	+	+	+	+			
H5^	++								

[a] The intensity of the cross peaks increases from + to ++. The concentrations of βCDNH$_2$(CH$_2$)$_4$NH$_2$ and either 4-methylbenzoate or (*S*)-2-phenylpropanoate, when present, were 0.06 mol dm^{-3}.

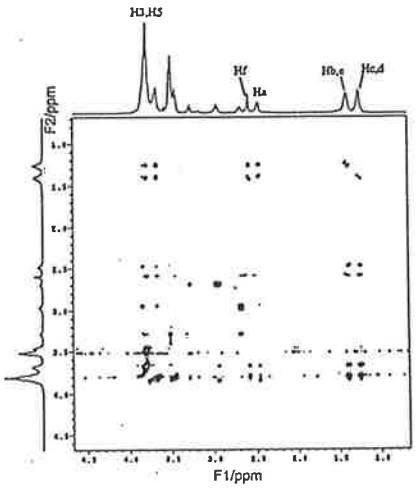

Fig. 4 The ^{1}H 600 MHz ROESY NMR spectrum of βCDNH-(CH$_2$)$_4$NH$_2$. Cross-peaks are formed between Hb–Hf and H3, Ha–Hf and H5 and Ha–Hc and H5^.

secondary hydroxy groups), or the coexistence of both complex isomers.

The titration data above also indicate that the [βCDNH-(CH$_2$)$_4$NH$_2$]·(*S*)-2-phenylpropanoate complex constitutes 90% of the total [βCDNH(CH$_2$)$_4$NH$_2$] and [(*S*)-2-phenylpropanoate] at the pH ≥ 11.5 of the NMR study. In the ROESY spec-

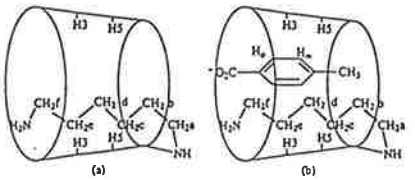

Fig. 5 Schematic representations of the structures of (a) the intramolecular complex formed by βCDNH(CH$_2$)$_4$NH$_2$ and (b) the intermolecular [βCDNH(CH$_2$)$_4$NH$_2$·4-methylbenzoate] complex.

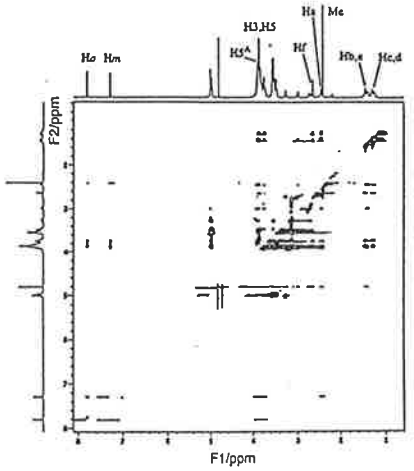

Fig. 6 The ^{1}H 600 MHz ROESY NMR spectrum of the [βCDNH(CH$_2$)$_4$NH$_2$·4-methylbenzoate]$^-$ complex showing cross-peaks formed between Ha to Hf and H3 and H5, Ho and Hm of 4-methylbenzoate and H3 and H5, and Me of 4-methylbenzoate and H3 and H5.

trum cross-peaks between Ha–Hf of -NH(CH$_2$)$_6$NH$_2$ and H3 and H5, and Ho and Hm of (*S*)-2-phenylpropanoate and H3 and H5 (Fig. 5 and Table 2) are consistent with the simultaneous complexation of both entities in the βCD annulus. However, there are no cross-peaks due to interactions between the -NH(CH$_2$)$_6$NH$_2$ substituent and (*S*)-2-phenylpropanoate. The cross-peak between Hm and H3 and the lack of cross-peaks between the aryl protons of (*S*)-2-phenylpropanoate and H5 is consistent with shallow complexation of the aromatic ring in the βCD annulus. No cross-peaks are observed between the methyl group of the (*S*)-2-phenylpropanoate and either H3 or H5 consistent with the (*S*)-2-phenylpropanoate moiety protruding from the secondary face of the β-CD annulus. A very similar spectrum was recorded for the [βCDNH(CH$_2$)$_4$NH$_2$·(*R*)-2-phenylpropanoate] complex.

Molecular modelling

Gas phase force-field molecular modelling[20] produced the global energy minimised (877.1 kJ mol^{-1}) structure of βCDNH-(CH$_2$)$_4$NH$_2$ with the -NH(CH$_2$)$_6$NH$_2$ substituent complexed inside the βCD annulus as shown in Fig. 7. Similar modelling showed that the -NH$_2$(CH$_2$)$_6$NH$_3^{2+}$ substituent of βCDNH$_2$-(CH$_2$)$_6$NH$_3^{2+}$ does not enter the βCD annulus. Both of these

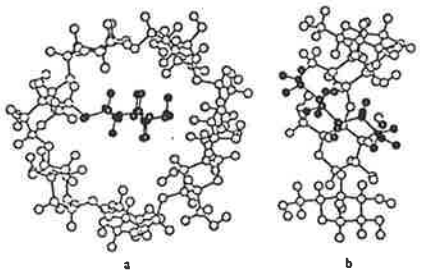

Fig. 7 The global energy minimised structure of $\beta CDNH(CH_2)_6NH_2$ viewed from a) the primary face of the annulus and b) from the side with three glucopyranose units cut away. The $-NH(CH_2)_6NH_2$ substituent is shown in dark shading.

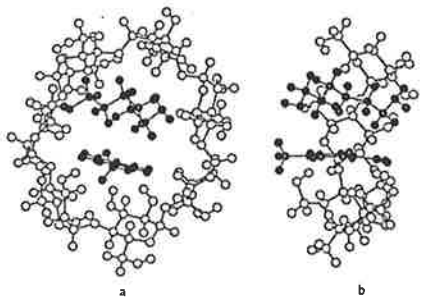

Fig. 8 The global energy minimised structure of $[\beta CDNH(CH_2)_6NH_2\cdot$4-methyl benzoate$]^-$ viewed from a) the primary face of the βCD annulus and b) from the side with three glucopyranose units cut away. The $-NH(CH_2)_6NH_2$ substituent and 4-methylbenzoate are shown in dark shading.

models are consistent with the deductions made from the NMR data discussed above.

Modelling the $[\beta CDNH(CH_2)_6NH_2\cdot$4-methylbenzoate$]^-$ complex produced a global energy minimum (797.4 kJ mol^{-1}) for the structure shown in Fig. 8 where 4-methylbenzoate is oriented with its carboxylate group towards the secondary face of the βCD annulus and the $-NH(CH_2)_6NH_2$ substituent is also complexed inside the βCD annulus consistent with the NMR data. This orientation of the carboxylate towards the secondary face is also found in adamantane-1-carboxylate complexes of αCD and βCD[10] and the cyclohexanecarboxylate complex of βCD.[11] When the 4-methylbenzoate orientation is reversed so that the carboxylate group is oriented towards the primary face the complex energy is 870.4 kJ mol^{-1} consistent with this being a less favoured orientation. The modelled structure of the $[\beta CDNH_3(CH_2)_6NH_2\cdot$4-methylbenzoate$]^+$ complex shows the 4-methylbenzoate guest to have its carboxylate group in the vicinity of the primary face of the βCD annulus. This reversal of orientation, compared with that in the $[\beta CDNH(CH_2)_6NH_2\cdot$4-methylbenzoate$]^-$ complex, is consistent with the charge of the $-NH_{(1+m)}(CH_2)_2NH_{(2+n)}^{(m+n)+}$ substituent (where m = 0 or 1 and n = 0 or 1) substantially influencing guest orientation through electrostatic interactions. This has also been found to occur in modelling studies of the 4-methylbenzoate complex of protonated heptakis(6-amino-6-deoxy)-β-cyclodextrin[12] and also its amino acid complexes.[14] While our modelling studies show the probable orienting effects of charge in $[\beta CDNH_3(CH_2)_6NH_2\cdot$4-methylbenzoate$]^-$ and $[\beta CDNH(CH_2)_6NH_2\cdot$4-methylbenzoate$]^-$, the latter complex was not detected in solution as discussed above.

Molecular modelling also shows that both the $-NH(CH_2)_6NH_2$ substituent and (*S*)-phenylpropanoate are complexed within the βCD annulus in the $[\beta CDNH(CH_2)_6NH_2\cdot$(*S*)-phenylpropanoate$]^-$ complex. The carboxylate group is oriented towards the secondary face of the βCD annulus. The $[\beta CDNH(CH_2)_6NH_2\cdot$(*R*)-phenylpropanoate$]^-$ complex is found to have a similar structure to that of its (*S*)-analogue with some differences in orientation of the guest within the βCD annulus. The globalised energy minima of the $[\beta CDNH(CH_2)_6NH_2\cdot$(*S*)-phenylpropanoate$]^-$ complex and its (*R*)-analogue are 738.5 and 812.3 kJ mol^{-1}, respectively, showing the (*S*)-diastereomer to be the more stable in the gas phase.

Conclusion

The stabilities of the host–guest complexes formed between the 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-cyclodextrin hosts and their protonated forms [where the ω-aminoalkylamino groups are $-NH(CH_2)_nNH_2$ and n = 2, 3, 4 and 6] and the guests, benzoic acid, 4-methylbenzoic acid and (*R*)- or (*S*)-2-phenylpropanoic acid and their conjugate bases, vary significantly. This is consistent with the charge and hydrophobicity of the host and the guest generating significant secondary interactions which affect complex stability. The ^{1}H NMR studies show that the $-NH(CH_2)_6NH_2$ substituent of $\beta CDNH(CH_2)_6NH_2$ self-complexes inside the βCD annulus, and that in $[\beta CDNH(CH_2)_6NH_2\cdot$4-methylbenzoate$]^-$ and its (*S*)-2-phenylpropanoate analogue both the guest and the $-NH(CH_2)_6NH_2$ substituent are simultaneously complexed within the βCD annulus. Gas phase force-field modelling also show this to occur for $\beta CDNH(CH_2)_6NH_2$ and these two complexes, where in the latter two cases the carboxylate group is oriented towards the secondary face of the annulus. The entry of the $-NH(CH_2)_6NH_2$ substituent into the βCD annulus may significantly affect complex stability, as may also be the case for the $-NH(CH_2)_nNH_2$ substituents where n = 2, 3 and 4 if they also enter the βCD annulus. In contrast the fully protonated $-NH_2(CH_2)_6NH_3^{2+}$ substituent does not enter the βCD annulus according to the ^{1}H NMR and molecular modelling studies.

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Appendix 2: Publications in this area from prior work

1. J.H. Coates, C.J. Easton, S.J. Van Eyk , S.F.Lincoln, B.L. May, C.B. Whalland and M.L. Williams, "A New Synthesis of Cyclodextrin Dimers.", *J. Chem. Soc. Perkin Transactions I*, 1990, 2619-2620.

2. J.H. Coates, C.J. Easton, S.J. Van Eyk, B.L. May, P. Singh and S.F. Lincoln, "Chiral differentiation in the Deacylation of 6A-O-(2-(4-(2-methylpropyl)phenyl)-propanoyl)-β-Cyclodextrin.", *J. Chem. Soc., Chem. Commun.*, 1991, 759-760.

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