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

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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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Bruce L. May

10/5/99
Abstract

This thesis describes the preparation and characterisation of a series of 6A-amino-substituted β-cyclodextrins. The reactions of 6A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin 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 pKa 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 dm³ 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 6A-(6-aminohexyl)amino-6A-deoxy-β-cyclodextrin and the carboxylates indicate that the 6-aminohexyl substituent is included simultaneously with the carboxylate.

The Zn(II) complexes of 6A-(1,5,9-triazacyclododecan-1-yl)- and 6A-(1,4,7,10-tetraazacyclododecan-1-yl)-6A-deoxy-β-cyclodextrin were examined as mimics of esterases. The reactions of 6A-(1,5,9-triazacyclododecan-1-yl)-6A-deoxy-β-cyclodextrin with 4-
nitrophenyl acetate are inhibited by the presence of Zn(II). The reactions of $6^\text{A}$-(1,4,7,10-tetraazacyclodecan-1-yl)-$6^\text{A}$-deoxy-$\beta$-cyclodextrin with 4-nitrophenyl 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 $\omega$-aminoalkylamino-substituted $\beta$-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 $6^\text{A}$-(6-aminohexyl)amino-$6^\text{A}$-deoxy-$\beta$-cyclodextrin with 4-nitrophenyl acetate. The reaction this ester with the $\omega$-aminoalkylamino-substituted $\beta$-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$_\text{N}2$ pathway. The solution structure of the complex formed between adamantane-1-carboxylate and $6^\text{A}$-(6-aminohexyl)amino-$6^\text{A}$-deoxy-$\beta$-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 $6^\text{A}$-(12-aminododecyl)amino-$6^\text{A}$-deoxy-$\beta$-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 $6^\text{A}$-(6-aminohexyl)amino-$6^\text{A}$-deoxy-$\beta$-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\textsuperscript{A}(6-N-(adamantan-1-oyl)amino)hexyl)amino-6\textsuperscript{A}-deoxy-\(\beta\)-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\textsuperscript{A}(6-N-(adamantan-1-oyl)amino)hexyl)amino-6\textsuperscript{A}-deoxy-\(\beta\)-cyclodextrin may be a molecular knot.

The reaction of 1,4-bis(4-nitrophenoxycarbonyl)-cubane with 6\textsuperscript{A}(6-aminohexyl)amino-6\textsuperscript{A}-deoxy-\(\beta\)-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.

![Diagram of cyclodextrins](image)

**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 oligomers 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 hydrolysates 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 worldwide 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 amylpectin. 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 \(^4\)C\(_1\) 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**

<table>
<thead>
<tr>
<th>Property</th>
<th>Cyclodextrin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. glucose units</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>anhydrous molecular weight</td>
<td>1</td>
<td>972.85</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1134.99</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1297.14</td>
</tr>
<tr>
<td>cavity length (Å)</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>cavity diameter (Å)</td>
<td>1</td>
<td>~5.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>~6.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>~8.4</td>
</tr>
<tr>
<td>solubility in water, 25 °C (mol dm(^{-3}))</td>
<td>1</td>
<td>0.121</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0163</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.168</td>
</tr>
<tr>
<td>pK(_a) (25 °C)</td>
<td>1</td>
<td>12.33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17, 18</td>
</tr>
</tbody>
</table>

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 hydrolysates 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\textsuperscript{19} and to the presence of intramolecular hydrogen bonding around the rim of β-cyclodextrin 2.\textsuperscript{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

\[
K = \frac{k_1}{k_1} = \frac{[CD,GUEST]}{[CD][GUEST]}
\]

\textbf{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).\textsuperscript{20 -30} Their ability to form host-guest complexes has lead to the use of cyclodextrins in the formulation of pharmaceuticals,\textsuperscript{31, 32} as stationary phases in gas liquid chromatography\textsuperscript{33} and high pressure liquid chromatography,\textsuperscript{34, 35} as a mobile phase modifier in capillary electrophoresis,\textsuperscript{26} and as catalysts for chemical reactions.\textsuperscript{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 \(\alpha\)-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 \(\alpha\)-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 homochiral 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.\textsuperscript{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.\textsuperscript{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 (\textit{E})-4 (Scheme 1.2).\textsuperscript{48} Under acidic conditions, in the absence of cyclodextrins, irradiation of the trans isomer (\textit{E})-4 yields mainly the \textit{cis} isomer (\textit{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 \textit{cis-trans} isomerization is possible. In the presence of the larger \textit{γ}-cyclodextrin 3, however, 2:1 guest:host complex formation is most favoured. This generates a high local concentration of the stilbene (\textit{E})-4 and consequently dimerization to give the cyclobutanes 6 proceeds readily. This chemistry has been extended to the formation of a \textit{[3]}-rotaxane made up of the cyclodextrins 2 and 3 threaded onto a poly-(\textit{trans}-stilbene).\textsuperscript{49}
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 \(\geq 10.5\))
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 (e.g., tyrosine, phenylalanine) and a hydroxyl group of a serine residue which is activated as a nucleophile through a charge relay network.\textsuperscript{55}

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

Generally, the greatest rate enhancement of the hydrolysis occurs when \( \alpha \)-cyclodextrin 1 is the catalyst and the phenyl ester substrate is \textit{meta}-substituted.\textsuperscript{56-58} With this catalyst, \textit{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 \textit{para}-substituted ester is positioned further away from the active hydroxyl group. This leads to a greater enhancement of the rate of reaction of \textit{meta}-substituted esters relative to that of the \textit{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. 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.

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.

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 6A-bromo-6A-deoxy-β-cyclodextrin.
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 2A-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 dimer62 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 3A-O-sulfonates 7 and 8 which when treated with mild bases are converted to allo-2A,3A-epoxycyclodextrins 9 and 10.68-71 The epoxides 9 and 10 react with nucleophiles to give mainly the corresponding C3A-substituted cyclodextrins with retention of stereochemistry at C2A and C3A (Scheme 1.4).67

The treatment of the C2A-O-sulfonates 11 and 12 with a mild base generates the manno-2A,3A-epoxycyclodextrins 13 and 14 which react with nucleophiles to give mainly the corresponding C3A-substituted cyclodextrins with inversion of the stereochemistry at C2A and C3A (Scheme 1.5).68, 72-74
Scheme 1.4. Preparation of cyclodextrins substituted at C3\textsuperscript{A} through the formation of an allo-2,3-epoxide. The stereochemistry at C2\textsuperscript{A} and C3\textsuperscript{A} is retained in this process.

Scheme 1.5. Preparation of cyclodextrins substituted at C3\textsuperscript{A} through the formation of a manno-2,3-epoxide. The stereochemistry at C2\textsuperscript{A} and C3\textsuperscript{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. 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.

![Mannan Epoxy Cyclodextrin](image)

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 ([(CH₃)₂NCHX]⁺X⁻), prepared either in situ or isolated as the crystalline salts. The product per(6-halo)-β-cyclodextrins are readily converted to the per(6-amino)- or per(6-thio)-β-cyclodextrins 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.

The selective substitution of a single C6 hydroxyl group is usually managed through the formation of the 6⁻-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⁻-O-4-methylbenzenesulfonyl-α-cyclodextrin and converted this to the corresponding halo-, azido-, amino- and deoxy-α-cyclodextrin derivatives. Matsui and Okimoto have developed a similar strategy for the preparation of derivatives of β-cyclodextrin 2.

A series of disulfonyl chloride reagents has been developed for the selective di-
sulfonation, and subsequent substitution, of primary face hydroxyl groups. The sulfonyl chlorides 17 and 18 give AB di-sulfonation, while the sulfonyl chlorides 19 and 20 give AC and AD disulfonation, respectively.

![Chemical Structures](image)

17 \( X = H \)
18 \( X = OMe \)
19
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. 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 outcome of a reaction.

The work discussed in the following chapters involves the preparation of a series of 6A-amino-6A-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 6A-ω-aminoalkylamino-6A-deoxy-β-cyclodextrins. The reactions of some 6A-ω-aminoalkylamino-substituted cyclodextrins with the 4-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 6A-amino-6A-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 guests89, 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 6A-(2-aminoethylamino)-6A-deoxy-β-cyclodextrin 21 to 6A-(6-aminohexylamino)-6A-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.

![Diagram of cyclodextrin and polyamines](image)

21 $X = \text{NH}_2 \text{NH}_2$

22 $X = \text{NH}_2 \text{NH}_2$

23 $X = \text{NH}_2 \text{NH}_2$

24 $X = \text{NH}_2 \text{NH}_2$

25 $X = \text{NH}_2 \text{NH}_2$

26 $X = \text{NH}_2 \text{NH}_2$

27 $X = \text{NH}_2 \text{NH}_2$

28 $X = \text{NH}_2 \text{NH}_2$

29 $X = \text{NHHHNHHH}$

30 $X = \text{NHHHNHHH}$

31 $X = \text{NHHHNHHH}$

**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](image_url)

**Scheme 2.1.** Schematic representation of the cyclodextrin 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.\textsuperscript{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\textsuperscript{A}-modified cyclodextrins begins with the preparation of the monotosylate derivative 32, which was prepared by the method of Matsui.\textsuperscript{85, 95} Slow addition of one equivalent of 4-methylbenzenesulfonyl chloride to a solution of dry \(\beta\)-cycloextrin 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 \(\beta\)-cycloextrin 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.
A new method for the preparation of the tosylate 32 from β-cyclodextrin using 4-methylbenzenesulfonyl anhydride 33 in aqueous solution has been reported. 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 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 6A-(2-(bis(2-aminoethyl)amino)ethyl)amino)-6A-deoxy-β-cyclodextrin 28 was used as a starting point for developing an improved method for the synthesis of amino-cyclodextrins. 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 Rf 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. 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. This effect has been used previously to separate mixtures of substituted cyclodextrins.

![Diagram of the N-formyl derivative 34](image)

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. 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. 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. In addition, NMP is more stable to acid and base than is DMF and it is also more stable to prolonged heating.

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\textsuperscript{A}-iodo-6\textsuperscript{A}-deoxy-β-cyclodextrin 35 \textit{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.\textsuperscript{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\textsuperscript{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\textsuperscript{A}amino-6\textsuperscript{A}-deoxy-β-
cyclodextrins 21-31.\textsuperscript{a}

<table>
<thead>
<tr>
<th>βCDX</th>
<th>Time (hr)</th>
<th>Yield (%)</th>
<th>βCDX</th>
<th>Time (hr)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>6</td>
<td>55</td>
<td>27</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>22</td>
<td>4.5</td>
<td>52</td>
<td>28</td>
<td>4</td>
<td>57</td>
</tr>
<tr>
<td>23</td>
<td>4.5</td>
<td>52</td>
<td>29</td>
<td>5</td>
<td>33 (52\textsuperscript{b})</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>51</td>
<td>30</td>
<td>7</td>
<td>34 (50\textsuperscript{b})</td>
</tr>
<tr>
<td>25</td>
<td>4.5</td>
<td>54</td>
<td>31</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>26</td>
<td>6</td>
<td>50</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Conditions: 3.3 equivalents of amine and catalytic KI in NMP at 70 °C. \textsuperscript{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 NH₄⁺ 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 and 1,5,9-triazacyclododecane, prepared by a modified Richman-Atkins procedure. 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-triazacyclododecane 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$^{-3}$.) 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 $\beta$-cyclodextrin 2) values of 0.75. In contrast to this, the other linear diamines ran at $R_c \sim 0.6$ while the other polyamines ran at $R_c \sim 0.3$. 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 $\beta$-cyclodextrin 2 are equivalent, consistent with complete conformational averaging and the $^1$H and $^{13}$C NMR spectra of $\beta$-cyclodextrin 2 are relatively simple. In particular, the $^{13}$C NMR spectrum of $\beta$-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 $\beta$-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 H6A.

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. This study was carried out by adding stoichiometric amounts of DCI 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 D₂O/H₂O 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-¹³C correlation spectroscopy. At pH ≥ 12 and pH 9, the two diastereotopic protons H₆ᴬ (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 H₅ᴬ) 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 H₄ᴬ 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 H₆ᴬ and the aminomethylene protons on the substituent often merge together and the resonance of the proton H₄ᴬ merges with those of the protons H₂ and H₄ of the unsubstituted rings while an apparent triplet (J ~ 9 Hz) due to the proton H₅ᴬ appears at around δ 5.1. Interestingly, for the cyclodextrin 30 the resonance of the proton H₅ᴬ is clearly resolved from the rest of the protons H₅ at each pH. If the shift of the resonance of H₅ᴬ 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 H₅ᴬ 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 H₅ᴬ.

The 75.5 MHz ¹³C 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 $\delta$ 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 $\beta$-carbons.\textsuperscript{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 \textsuperscript{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$_2$O at a concentration of 0.06 mol dm$^{-3}$ at (a) pH $\leq$ 2, (b) pH $\sim$ 9 and (c) pH $\geq$ 12.
Figure 2.3. 75.5 MHz $^{13}$C NMR spectra of the cyclodextrin 31 in D$_2$O 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. $pK_a$s for some protonated 6A-substituted-β-cyclodextrins and the corresponding protonated free polyamines in aqueous NaClO$_4$ ($I = 0.10 \text{ mol dm}^{-3}$) at 298.2 K.

<table>
<thead>
<tr>
<th>Cyclodextrin</th>
<th>$pK_a$</th>
<th>Free Amine</th>
<th>$pK_a$</th>
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<tbody>
<tr>
<td>21</td>
<td>9.42</td>
<td>1,2-diaminoethane</td>
<td>9.97</td>
</tr>
<tr>
<td></td>
<td>5.70</td>
<td></td>
<td>7.16</td>
</tr>
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</tr>
<tr>
<td>31</td>
<td>10.40</td>
<td>1,4,7,10-tetraazacyclododecane$^d$</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>8.62</td>
<td></td>
<td>9.6</td>
</tr>
</tbody>
</table>

$^a$ 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 $pK_a$s of 2-(methylamino)ethylamine are 10.21
and 7.27 while for 1,2-diaminoethane (en) the pKa's are 9.97 and 7.16. 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 C6B-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 $\Delta G^\circ_{\text{protonation}}$ than for the corresponding protonation of 2-(2-aminoethyl)aminoethylamine while the reverse is true for the enthalpic contributions. 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 pKa's across the series of cyclodextrins 21-31 parallels that of the parent polyamines. For example, for the 6- aminoalkylamino-substituted cyclodextrins 21-24 the two pKa's increase as the chain length increases while the difference between the two pKa's is decreased, and a similar trend is observed for the free diamine analogues.

The increase in the pKa 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. While there is a large difference in the pKa's for the first protonation of the cyclodextrins 21 and 22 there is little difference between the first pKa's of the cyclodextrins 23 and 24. The decrease in the difference between pKa1 and pKa2 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)\(^\text{113}\) 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.\(^\text{114, 115}\) The involvement of all nitrogens in the first protonation of polyamines has been disputed on the basis of \(^1\)H and \(^13\)C NMR evidence that suggested that protonation of these systems involved only the terminal primary amino nitrogens.\(^\text{116}\) 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.\(^\text{117}\)

A full study of the pH dependence of the \(^13\)C NMR spectra of the cyclodextrin \(^\text{25}\) 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.\(^\text{107}\) 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\(^\text{108}\) and such a shift was observed for C5^A after the first addition of acid to a solution of the cyclodextrin \(^\text{25}\). 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 \(^\text{21-24}\) 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). The likelihood of the self-inclusion of this substituent in the annulus in the solution phase lead 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.](image)

Recently, 2D-ROESY has been shown to be a powerful tool for examining the solution structure of host-guest complexes of cyclodextrins. 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\(_2\)O 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).

![Scheme 2.2](image)

**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 $\omega$-aminoalkylamino substituents and so increase their solvation by water through additional hydrogen bonding interactions.
Figure 2.5. Contour plot of ROESY experiment (D$_2$O, pH $\geq$ 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 24. The protons are labelled as shown in Scheme 2.2.
Figure 2.6. Contour plot of ROESY experiment (D$_2$O, pH $\geq$ 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.
Chapter 2

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.

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

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 \((R)-\text{ and } (S)\)-2-phenylpropionate 38. 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.\(^a\)

<table>
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<tr>
<th>Cyclodextrin</th>
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<td>(c)</td>
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<td>(c)</td>
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<td>(c)</td>
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<td>15000</td>
<td>6000</td>
<td>41000</td>
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\(^a\) 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 \(\leq 5\%\) 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 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-aminoheptylamino-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 (pKₐ − 12¹⁷).
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 benzylc 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 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 6A-deoxy-6A-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.

\[ \text{Scheme 2.6 } \text{Schematic representation of the inclusion of the 6-aminohexyl substituent and the 2-phenylpropionate 38 in the cyclodextrin 24 in aqueous solution at pH} \geq 12 \text{ indicating the two possible modes of inclusion of the guest 38.} \]
Figure 2.8. Contour plot of ROESY experiment (D$_2$O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 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 \(H_a\) or the \(\alpha\)-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 \(pH \geq 12\) 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$_2$O, pH $\geq$ 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 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 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. 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 6A-amino substituted $\beta$-cyclodextrins has been developed. The key improvement over previous methods is the use of 1-methylpyrrolidin-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 $\beta$-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.\(^\text{138-142}\) 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\(_a\)s of the water and hydroxyl bound to Zn(II) in the complexes 43 and 44 are 7.3 and 7.4 respectively.

![Diagram](image)

The Zn(II) complex 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).\(^\text{143,144}\)

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.\(^\text{145,146}\)
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.\(^\text{147}\) Control of enzyme activity is dependent on the requirement for the substrate to be bound to the enzyme before any reaction can occur.\(^\text{55}\) 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.\(^\text{50, 52, 54, 148, 149}\) 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.\textsuperscript{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.\textsuperscript{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.

![Chemical structure of complex 47](image)

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.\textsuperscript{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).
is by monitoring changes in absorbance at 400 nm that the reactions are followed.

![Scheme 3.1 Hydrolysis of 4-nitrophenyl acetate](image)

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. 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. 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. Complexation
of a metal by a C6 hydroxyl group has been proposed for the cobalt (III) complex of the cyclodextrin 31.151

![Image of a metal complex](image)

Solutions of the complexes 43 and 47 were prepared by dissolving the appropriate weight of the free ligand in buffer \((I = 0.1 \text{ mol dm}^{-3})\) over the range pH 6.6-9.1 and adding one equivalent of Zn(ClO₄)₂ from a stock solution to give a final concentration of \(1.03 \times 10^{-3} \text{ mol dm}^{-3}\) of complex, assuming that complete complexation of Zn(II) occurs. Solutions of pH \(\geq 8.5\) 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 cm³ 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 cm³ 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 \(1.0 \times 10^{-3} \text{ mol dm}^{-3}\). 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 \((I = 0.1 \text{ mol dm}^{-3})\) at 298.2 K.

<table>
<thead>
<tr>
<th>pH(^a)</th>
<th>(\varepsilon_{400} \text{ dm}^3 \text{ mol}^{-1}b)</th>
<th>(10^8 k_0/\text{mol dm}^{-3} \text{s}^{-1}c)</th>
<th>(10^8 k_{\text{obs}} - k_0/\text{mol dm}^{-3} \text{s}^{-1}d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>10600</td>
<td>0.546</td>
<td>0.74</td>
</tr>
<tr>
<td>7.1</td>
<td>11300</td>
<td>0.689</td>
<td>1.18</td>
</tr>
<tr>
<td>7.3</td>
<td>12500</td>
<td>0.912</td>
<td>2.75</td>
</tr>
<tr>
<td>7.6</td>
<td>13500</td>
<td>1.39</td>
<td>3.10</td>
</tr>
<tr>
<td>7.8</td>
<td>14500</td>
<td>2.01</td>
<td>2.39</td>
</tr>
<tr>
<td>8.0</td>
<td>15300</td>
<td>2.78</td>
<td>4.34</td>
</tr>
<tr>
<td>8.2</td>
<td>15900</td>
<td>4.95</td>
<td>4.36</td>
</tr>
</tbody>
</table>

\(^a\) 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 \(\varepsilon_{400}\) 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 \(\varepsilon_{400}\) at each pH in order to convert the kinetic data, obtained as \(\Delta \text{AU} \text{s}^{-1}\), into the rate of formation of the phenol 49 \((\text{mol dm}^{-3} \text{s}^{-1})\). The absorbance of seven solutions of 4-nitrophenol 49 over the concentration range 0.337-8.41 \times 10^{-5} \text{ mol dm}^{-3} was determined at each pH studied and the value of \(\varepsilon_{400}\) 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 \(\varepsilon_{400}\) being increased, most probably due to complexation of phenol 49 within the cyclodextrin annulus. Therefore, the value of \(\varepsilon_{400}\) 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 $\times$ 10$^{-5}$ mol dm$^{-3}$). The solution was allowed to equilibrate, the absorbance at 400 nm was recorded and a value for $\varepsilon_{400}$ 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.

<table>
<thead>
<tr>
<th>pH</th>
<th>$\varepsilon_{400} \text{ dm}^3 \text{ mol}^{-1}d$</th>
<th>$10^8 k_o/\text{mol dm}^{-3} \text{ s}^{-1}e$</th>
<th>$10^8 k_{obs} - k_o/\text{mol dm}^{-3} \text{ s}^{-1}f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6$^a$</td>
<td>8900</td>
<td>0.218</td>
<td>0.489</td>
</tr>
<tr>
<td>7.2$^a$</td>
<td>14700</td>
<td>0.417</td>
<td>1.81</td>
</tr>
<tr>
<td>7.8$^a$</td>
<td>19300</td>
<td>1.08</td>
<td>7.47</td>
</tr>
<tr>
<td>8.1$^a$</td>
<td>21300</td>
<td>2.47</td>
<td>13.5</td>
</tr>
<tr>
<td>8.1$^b$</td>
<td>21300</td>
<td>2.47</td>
<td>20.5</td>
</tr>
<tr>
<td>8.4$^a,c$</td>
<td>21300</td>
<td>3.27</td>
<td>21.6</td>
</tr>
<tr>
<td>9.1$^b,c$</td>
<td>21300</td>
<td>18.9</td>
<td>111</td>
</tr>
</tbody>
</table>

$^a$ 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 $\times$ 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 $4.36 \times 10^{-2}$ dm$^3$ mol$^{-1}$ s$^{-1}$ is obtained which compares well with the reported value $(4.1 \times 10^{-2}$ dm$^3$ mol$^{-1}$ s$^{-1}$).\textsuperscript{138}

![Figure 3.1](image)

**Figure 3.1.** Plot of variation of rate ($k_{\text{obs}} - k_0$) with pH for the reaction of the ester 3.6 with the complexes 3.1 (x) 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 $\leq 7.3$ 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

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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 $2.42 \times 10^{-8}$ mol dm$^{-3}$ s$^{-1}$ and $1.70 \times 10^{-6}$ 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. 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). 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 \((k_{\text{obs}}/k_0 = 2.58 \times 10^{-8} \text{ and } 1.63 \times 10^{-8} \text{ mol dm}^{-3} \text{ s}^{-1}\) 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 \((k_{\text{obs}}/k_0 = 5.56 \times 10^{-7} \text{ and } 8.31 \times 10^{-7} \text{ mol dm}^{-3} \text{ s}^{-1}\) 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 \(\geq 8.5\).

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.
Chapter 3

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 \(\beta\)-cyclodextrin \(^2\) at pH > 10 forms a transient C2-O-acyl cyclodextrin which is slowly hydrolysed back to \(\beta\)-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.
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 ([RNH₂]>[48]).

Reactions were carried out at 298.2 K by pipetting 2.0 cm³ 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 cm³ 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 \((k_{\text{obs}}-k_0)\) 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 \((k_0)\) was found to be \(2.00 \times 10^{-4}\) s\(^{-1}\).

### Table 3.3 Pseudo-first order rates for the reaction of the ester 48 with \(\omega\)-aminoalkylamines in 0.05 mol dm\(^{-3}\) borate buffer pH 9.1 at 298.2 K.\(^{a}\)

<table>
<thead>
<tr>
<th>(\beta)CDX</th>
<th>(10^3(k_{\text{obs}}-k_0)/\text{s}^{-1})</th>
<th>X</th>
<th>(10^3(k_{\text{obs}}-k_0)/\text{s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>7.11</td>
<td>(\text{NH}_2(\text{CH}_2)_2\text{NH}_2)</td>
<td>0.69</td>
</tr>
<tr>
<td>22</td>
<td>3.61</td>
<td>(\text{NH}_2(\text{CH}_2)_3\text{NH}_2)</td>
<td>1.15</td>
</tr>
<tr>
<td>24</td>
<td>0.93</td>
<td>(\text{NH}_2(\text{CH}_2)_6\text{NH}_2)</td>
<td>1.08</td>
</tr>
</tbody>
</table>

\(^{a}\) Initial concentration of the ester 48 and \(\omega\)-aminoalkylamine 1.00 \(\times\) \(10^{-5}\) and 1.00 \(\times\) \(10^{-3}\) mol dm\(^{-3}\) respectively. \(^{b}\) Difference in the observed first order rate, \(k_{\text{obs}}\), and the rate observed with buffer alone, \(k_0 = 2.00 \times 10^{-4}\) 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 24 a decrease in rate (0.86) is observed. However, such an observation ignores the effect of the different \(pK_a\)s 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 pKₐ's and speciation at pH 9.1 of the ω-aminoaalkylamino-cyclodextrins 21, 22 and 24 and the corresponding “free” diaminoalkanes.

<table>
<thead>
<tr>
<th>Amine</th>
<th>pKₐ</th>
<th>% Species at pH 9.1ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂(CH₂)₂NH₂</td>
<td>9.97, 7.16</td>
<td>11.8</td>
</tr>
<tr>
<td>NH₂(CH₂)₃NH₂</td>
<td>10.56, 8.97</td>
<td>2.0</td>
</tr>
<tr>
<td>NH₂(CH₂)₆NH₂</td>
<td>11.01, 10.04</td>
<td>0.13</td>
</tr>
<tr>
<td>21</td>
<td>9.42, 5.70</td>
<td>32.4</td>
</tr>
<tr>
<td>22</td>
<td>9.90, 7.39</td>
<td>13.7</td>
</tr>
<tr>
<td>24</td>
<td>10.27, 8.72</td>
<td>6.3</td>
</tr>
</tbody>
</table>

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 \((k_{\text{obs}}-k_0)\) 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 \((k_{\text{obs}}-k_0)\) 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 \((k_{\text{obs}} - k_0)\) 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\(_2\))\(_x\)NH\(_2\)
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 \( \omega \)-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.](image)
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 $\delta$ 176 and $\delta$ 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 $\omega$-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$_2$O 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 $\delta$ 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 \( ^1\text{H} \) NMR spectra of the amide 54 in D\( _2\)O 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 $R_e$ 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 $R_e$ 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 $2.4 \times 10^{-3}$ mol dm$^{-3}$, in 0.05 mol dm$^{-3}$ borate buffer pH 9.1 was freeze-dried and the residue was dissolved in D$_2$O. 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 $\delta$ 2.1 and $\delta$ 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 $\delta$ 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 (pKₐ = 12) 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

<table>
<thead>
<tr>
<th>ester</th>
<th>24</th>
<th>55</th>
<th>O-acetylated products</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>27</td>
<td>54</td>
<td>20</td>
</tr>
<tr>
<td>52</td>
<td>26</td>
<td>17</td>
<td>57</td>
</tr>
</tbody>
</table>

*aInitial 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 led 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 ($k_{obs}$-$k_0$) for the reaction of the ester 48 with β-cyclodextrin 2 at pH 9.1 is $3.4 \times 10^{-4}$ 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). Adamantane-1-carboxylate 59 was found to be an effective inhibitor of the reactions of ester 48 with the cyclodextrins 2 and 52. The formation constants for the complexes formed between ester 48 and these cyclodextrins was determined to be around 100-200 dm\(^3\) mol\(^{-1}\).

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 (\(k_{\text{obs}}/k_o\)) were \(4.24 \times 10^{-3}\) s\(^{-1}\), \(1.98 \times 10^{-3}\) s\(^{-1}\) and \(0.746 \times 10^{-3}\) s\(^{-1}\) for the cyclodextrins 21, 22 and 24, respectively. In the presence of two equivalents of the carboxylate 59, the first order rate (\(k_{\text{obs}}/k_o\)) for the reaction between the ester 48 and the cyclodextrin 22 was \(0.463 \times 10^{-3}\) 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<sup>-3</sup> in D<sub>2</sub>O 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<sub>A</sub>). 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 h<sub>n</sub>H1 and h<sub>n</sub>H6 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.** Contour plot of ROESY experiment (D$_2$O, pH ≥ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing $0.06 \times 10^3$ 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. A recent report described an attempt to limit the self-inclusion of ω-aminoalkylamino substituents attached to β-cyclodextrin through tert-butyloxycarbonylation of the ω-nitrogen. 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. 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
Chapter 4

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 interconvertable. However, if the blocking group can pass through the cyclodextrin then the two isomers are interconvertable and represent two components of an equilibrium. The in-isomer is simply a self-included conformer of the cyclodextrin derivative.

\[ \text{in-isomer} \quad \xrightarrow{\text{out-isomer}} \]

\[ \text{Blocking group} \]

\[ \text{in-isomer} \quad \text{out-isomer} \]

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. The formation constant, $K$, for the complex between adamantane-1-carboxylate 59 and $\beta$-cyclodextrin 2 is $1.8 \times 10^4$ dm$^3$ mol$^{-1}$ at pH 8.5. 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.

![Chemical structure of 60](image)

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 H5A occurs around δ 3.6 (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 H5A 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 $\text{mol dm}^{-3}$ 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 D2O 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 D2O 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.
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
Chapter 4

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.

\[ \text{RozR} \quad \text{COzFN} \]
\[ \text{pNP} = \begin{array}{c} \text{NO}_2 \\
\end{array} \]

62 \( R = R' = H; R'' = Me \)
63 \( R = H; R' = R'' = Me \)
64 \( R = pNP; R' = Me; R'' = H \)
65 \( R = pNP; R' = R'' = Me \)

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\textsuperscript{169} or squat column chromatography.\textsuperscript{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 \textit{m/z} 1421 with electrospray-ms and gave a satisfactory elemental analysis.

The 300 MHz \textsuperscript{1}H NMR spectrum of the cyclodextrin amide 67 shows sets of multiplets near \( \delta = 4.1 \) due to the cubanyl protons. Resonances for the protons \( \text{hnH6} \), adjacent to the amido nitrogen, overlap with those due to \( \text{H4A} \) and \( \text{H6A} \) but the diastereotopic protons \( \text{hnH1} \), adjacent to the secondary nitrogen, give rise to separate signals at \( \delta = 2.6 \) and \( \delta = 2.4 \) indicating that the alkyl chain of the substituent is held in a rigid conformation. The 75 MHz \textsuperscript{13}C NMR spectrum shows signals due to the two carbonyl carbons at \( \delta = 176.5 \) and \( \delta = 175.6 \).

\begin{center}
\textbf{Scheme 4.5.} Schematic representation of the self-inclusion of the cubanyl substituent of the cyclodextrin amide 67 in aqueous solution at pH \( \geq \)12.
\end{center}

The 600 MHz 2D-ROESY spectrum of the cyclodextrin amide 67 in D\textsubscript{2}O at pH \( \geq 12 \) 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$_2$O, pH $\geq$ 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 67. The protons are labelled as shown in Scheme 4.5.
Figure 4.4. Contour plot of ROESY experiment (D$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing 0.06 x 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 $^{hn}H_2$-$^{hn}H_5$ 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$_2$O, 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$_2$O 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing $0.06 \times 10^{-3}$ mol dm$^{-3}$ each of the cyclodextrin 68 and adamantane-1-carboxylate 59. The protons are labelled as shown in Scheme 4.8.
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 $h_n$H6 and $h_n$H1 each giving rise to two resonances due to differentiation of the diastereotopic protons. The resonances due to the protons $h_n$H2-$h_n$H5 were likewise well resolved from each other. The 75 MHz $^{13}$C NMR spectrum of the product showed a signal at $\delta$ 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 $\geq$ 12.
Figure 4.7. Contour plot of ROESY experiment (D$_2$O, pH $\geq$ 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 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$_2$O 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 $^1$H 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 (D₂O, 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\textsuperscript{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 $\beta$-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\textsuperscript{172}, with two equivalents of the cyclodextrin 24 was expected to give a cyclodextrin dimer.
analogous to those reported in earlier work. 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.

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$_2$O 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 H4A give a sharp triplet at δ 3.40, protons H6A and H6A' 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 hH1 and protons hH6 give rise to two multiplets at δ 2.80 and δ 2.70. Additionally, the resonance due to protons H5A 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 hC2-hC5 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$_2$O, pH $\geq$ 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$_2$O, pH $\geq$ 12, 298 K, 600 MHz, 0.3 s mixing time) performed on a sample containing $0.06 \times 10^{-3}$ mol dm$^{-3}$ of cyclodextrin dimer 72 and $0.12 \times 10^{-3}$ mol dm$^{-3}$ of adamantane-1-carboxylate 59. The protons are labelled as shown in Scheme 4.11.
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 6A-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 6A-\(O\)-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 \( \omega \)-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 \( \omega \)-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 \( \geq 12 \).

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$_2$O 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$_2$O), NaOH was then added to give solutions pH ≥ 12 and finally HCl was added to give solutions of pH ≤ 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 cycloextrin (and the guest when present) were dissolved in 0.1 mol dm\(^{-3}\) NaOH in D\(_2\)O to give final concentrations of 0.06 mol dm\(^{-3}\) of each component and a final pH \(\geq 12\). The resultant solutions were filtered (0.22 \(\mu\)m) and degassed by freeze-thawing before the spectra were recorded.

\[
\begin{align*}
  \text{en} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{dien} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{pn} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{dipn} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{bn} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{trien} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{hn} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{tren} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{tacn} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{tacdo} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} \\
  \text{cyclen} &= -\begin{array}{c}N \\ 1 \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array} - \begin{array}{c}N \end{array}
\end{align*}
\]

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 $E_0$ 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 $\chi^2$ 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 $\beta$-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.$4$HCl, Aldrich) was purified by two recrystallisations from ethanol/water. $\beta$-
Experimental

Cyclodextrin was a gift from Nihon Shokuhin Kako Co. and was dried by heating at 100 °C under vacuum (< .01 Torr) for 18 hours. 6A-O-(4-Methylbenzenesulfonyl)-β-cyclodextrin 32 was prepared by the method of Matsui. 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

To a stirred solution of 1,2-ethanediol (13.25 g, 0.213 mol) in dry pyridine (150 cm³) 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 mixture was collected by vacuum filtration, washed successively with water (4 × 200 cm³), ethanol (2 × 100 cm³) and ether (2 × 100 cm³) 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, lit103 123-125 °C). δH(CDCl3) 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

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

cm³) 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 cm³). The reaction mixture was left to stir at room temperature for 16 hours. Methanol (400 cm³) was added and the resultant precipitate was collected by vacuum filtration, washed with water (3 × 200 cm³) and methanol (2 × 200 cm³) and dried under vacuum to give the crude product (82.46 g) which was recrystallised from either methanol (~12 g/800 cm³) or acetone (~36 g/250 cm³) to give the pure compound (54 g, 39%) as white needles. (mp 180-181 °C, lit¹⁰³ 173-175 °C). δ_H(CDCl₃) 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 NH₂); 3.17 (multiplet, 8H); 2.43 (s, 9H). I.R.(nujol) 3288 (s), 1596 (s), 1496 (s), 1322 (s), 1308 (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 cm³). 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 cm³) 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 cm³) and the solid which formed was collected by vacuum filtration and washed successively with water (500 cm³), ethanol (3 × 25 cm³) and ether (50 cm³) and air dried to give 15.2 g of a tan powder. This was suspended in boiling ethanol (150 cm³) and boiling chloroform (~70 cm³) was added until all of the solid had dissolved. The product crystallised as white needles (11.1 g, 75%). (mp 222 °C, lit¹⁰³ 218-220 °C). δ_H(CDCl₃) 7.67 (d, J = 8.4 Hz, 6H); 7.33 (d, J = 8.4 Hz, 6H); 3.42 (s, 12H); 2.44 (s, 9H). δ_C(CDCl₃) 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.
**Experimental**

1,4,7-Triazacyclononane Trihydrochloride

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 cm³) was heated at 100 °C for 72 hours. The resultant dark brown solution was cooled to 0 °C and ethanol (100 cm³) was added slowly. On addition of ether (200 cm³) a gelatinous precipitate formed. This was collected by filtration under nitrogen and washed with ether (3 x 25 cm³). The solid was dissolved in water (60 cm³) 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 x 18 cm). The amine band appeared as a gold band at the top of the column. The column was washed with water (400 cm³) and 0.5 mol dm⁻³ HCl (400 cm³) and the amine was eluted with 1.5 mol dm⁻³ HCl taking 100 cm³ 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 cm³, when crystallisation began. Addition of ethanol (150 cm³) gave the product as a white solid (3.168 g, 71%). (mp 255-258 °C dec). δH(D₂O) 3.58 (s). δC(D₂O) 44.26.

An amount of this material (1.756 g) was dissolved in 1 mol dm⁻³ potassium hydroxide in brine (30 cm³) and this solution was extracted with dichloromethane (3 x 30 cm³). 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

A solution of propan-1,3-diol (10 g, 0.136 mol) in dry pyridine (25 cm³) was added dropwise over 10 minutes to a vigorously stirred solution of 4-methylbenzenesulfonyl chloride (75 g, 0.394 mol) in pyridine (160 cm³) 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...
Experimental

for another 4 hours as the mixture slowly warmed to 5 °C. The reaction mixture was then poured onto ice (1.3 dm³) 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 dm³) and air dried. The crude product was recrystallised from ethanol (~300 cm³) to give the product as white needles (41.6 g, 82%). (mp. 93-94 °C, lit¹⁰³ 91-93 °C). δH(CDCl₃) 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). δC(CDCl₃) 144.99, 132.51, 129.88, 127.76, 65.80, 28.56, 21.54. I.R. (nujol) 1599 (m), 1499 (w), 1170 (s), 1096 (w), 946 (s), 856 (s), 812 (s), 742 (s), 664 (s) cm⁻¹.

N,N',N"-tri(4-methylenesulfonyl)di(3-aminopropyl)amine¹⁰⁴

Freshly purified 4-methylenesulfonyl 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 cm³) 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 cm³) and the solution was then washed successively with 1 mol dm⁻³ hydrochloric acid (100 cm³), water (2 × 100 cm³) and brine (50 cm³), dried (Na₂SO₄) 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, lit¹⁰⁴ 119-120 °C). δH(CDCl₃) 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). δC(CDCl₃) 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), 3197 (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.

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Experimental

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-methylbenzene-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). \( \delta_H(\text{CDCl}_3) \) 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). \( \delta_C(\text{CDCl}_3) \) 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 (\(2 \times 30 \) 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 \( \times \) 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 cm³. The solution was diluted with ethanol (100 cm³) and the resultant precipitate was collected by vacuum filtration and rinsed with ethanol and ether to give the product as a white powder (1.8 g, 44.6%). (m.p. 230-231°C, lit 286°C). δH(D₂O) 3.42 (t, J = 6.7 Hz, 12H); 2.29 (quint, J = 6.7 Hz, 6H). δC(D₂O) 43.87, 21.93.

An amount of this material (1.685 g) was dissolved in 1 mol dm⁻³ potassium hydroxide in brine (30 cm³) and this solution was extracted with dichloromethane (3 x 30 cm³). 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 6A- amino-substituted β-cyclodextrins

General procedure for preparation of amino-substituted cyclodextrins.

A solution of 6A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin 32 (2.0 g, 1.55 x 10⁻³ mol), potassium iodide (0.025 g, 0.15 x 10⁻³ mol) and the amine (5 x 10⁻³ mol) in dry N-methylpyrrolidin-2-one (NMP) (5 cm³) 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 cm³). The resulting precipitate was collected by vacuum filtration, washed successively with ethanol (100 cm³) and ether (50 cm³) and dried under vacuum to give the crude product. This material was dissolved in water (10 cm³) and loaded onto a column (4.5 x 4.5 cm) of BioRex 70 (H⁺ form). The column was washed with water (400 cm³) and the amino-cyclodextrin product was eluted with 1 mol dm⁻³ NH₄OH. 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 P₂O₅ 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
Experimental

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.

6Å-(2-(bis(2-aminoethyl)aminooethyl)amino-6Å-deoxy-ß-cyclodextrin 2890

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 cm³) 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 28.3H₂O (C₄₈H₉₂N₄O₃₄) C, 43.76; H, 7.04; N, 4.25%.

δ_H(D₂O/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, H4A); 2.85 (d, J = 12.0 Hz, 1H, H6A); 2.2 - 2.7 (m, 13H, H6A', trenH).

δ_H(D₂O, 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, H4A); 3.05 (d, J = 11.4 Hz, 1H, H6A); 2.4 - 2.9 (m, 13H, H6A', trenH).

δ_H(D₂O/HCl, pH ~ 1) 5.00 (s, 7H, H1); 4.10 (t, J = 9.0 Hz, 1H, H5A); 3.6 - 4.0 (m, 25H, H3, H5, H6); 3.4 - 3.6 (m, 14H, H2, H4); 2.9 - 3.4 (m, 14H, H6A, trenH).

δ_C(D₂O/NaOH, pH ~ 14) 107.0, 106.6, 106.4, 105.2 (C1); 87.6 (C₄A); 85.0, 84.8, 84.5, 83.9 (C4); 77.3, 76.4, 76.3, 75.2, 74.9 (C2, C3, C5); 70.9 (C₅A); 63.0 (C6); 59.8 (trenC3,3'); 56.9; 55.1 (C₆A); 50.5 (trenC2); 46.2 (trenC1); 41.0 (trenC4,4').

δ_C(D₂O/HCl, pH ~ 1) 104.5, 103.8 (C1); 86.4 (C₄A); 84.0, 83.6 (C4); 75.9 (C2); 74.9 (C3); 74.7 (C5); 73.3 (C₅A); 63.1 (C6); 58.7 (trenC3,3'); 55.7 (trenC2); 52.0 (C₆A); 48.7 (trenC1); 40.7 (trenC4,4').

6Å-(2-aminoethyl)amino-6Å-deoxy-ß-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 cm³) was treated according to the general
Experimental

procedure except that the crude product, obtained from the ethanol precipitation, was dissolved in water (10 cm³) and loaded onto a column (4.5 x 4.5 cm) of BioRex 70 (NH₄⁺ form). The column was washed with water (120 cm³) and the product was then eluted with 0.05 mol dm⁻³ NH₄HCO₃. Fractions containing the product were combined and evaporated to dryness to give the title compound as a white powder (1.005 g, 56%). Rₜ 0.62. Electrospray-ms m/z 1177 (M⁺). (Found C, 42.70; H, 6.67; N, 2.18. Calculated for 21.3H₂O (C₄₅H₈₄N₂O₄₀) C, 42.92; H, 6.71; N, 2.27%). δ_H(D₂O/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, H4A); 2.94 (d, J = 12.1 Hz, 1H, H6A); 2.5-2.7 (m, 5H, H6A', enH1, enH2). δ_H(D₂O, 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, H4A); 3.09 (d, J = 11.9 Hz, 1H, H6A); 2.7-2.9 (m, 5H, H6A', enH1, enH2). δ_H(D₂O/HCl, pH ~ 1) 5.0 (m, 7H, H1); 4.17 (t, J = 7.0 Hz, 1H, H5A); 3.7 (m, 25H, H3, H5, H6); 3.5 (m, 20H, H2, H4, H6A, enH). δ_C(D₂O/NaOH, pH ~ 14) 106.08, 105.80, 105.51 (C1); 87.37 (C4A); 84.70, 84.63, 84.51 (C4); 76.79, 76.69, 76.00, 74.58 (C2, C3, C5); 72.74 (C5A); 63.16 (C6); 53.45 (C6A); 52.21 (enC2); 42.50 (enC1). δ_C(D₂O, pH ~ 10) 104.57, 104.28 (C1); 86.25 (C4A); 83.85, 83.68 (C4); 75.79, 75.73, 74.77, 74.51 (C2, C3, C5); 73.17 (C5A); 62.99 (C6); 52.43 (C6A); 51.96 (enC2); 42.21 (enC1). δ_C(D₂O/HCl, pH ~ 1) 104.53, 103.75 (C1); 85.74 (C4A); 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 (C5A); 63.51, 63.17, 63.07 (C6); 51.45 (C6A); 47.55 (enC2); 37.96 (enC1).

6₄-(3-aminopropyl)amino-6₄-deoxy-β-cyclodextrin 22

A mixture of the tosylate 32 (2.052 g, 1.59 x 10⁻³ mol), 1,3-diaminopropane (0.420 g, 5.67 x 10⁻³ mol) and KI (0.026 g) in NMP (5 cm³) was treated according to the general procedure to give the title compound as a white powder (0.792 g, 42%). Rₜ 0.50. Electrospray-ms m/z 1191 (M⁺). (Found C, 43.65; H, 6.85; N, 2.39. Calculated for 22.3H₂O (C₄₅H₈₄N₂O₃₇) C, 43.40; H, 6.80; N, 2.24%). δ_H(D₂O/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, H4A); 3.02 (d, J = 12.4 Hz, 1H, H6A); 2.72 (dd, J = 9.8, 12.4 Hz, 1H, H6A); 2.58
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, H4A); 3.05 (d, J = 12.0 Hz, 1H, H6A); 2.7-2.8 (m, 3H, H6A, pnH1); 2.62 (d, J = 7.0 Hz, 2H, pnH3); 1.67 (tt, J = 7.0, 7.3 Hz, 2H, pnH2).

δH(D2O/NaOH, pH ~ 1) 5.13 (s, 7H, H1); 4.20 (t, J = 8.1 Hz, 1H, H5A); 3.8 - 4.0 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 14H, H2, H4); 3.41 (m, 2H, H6A); 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).

δC(D2O/NaOH, pH ~ 14) 105.40, 105.27, 104.96 (C1); 86.99 (C4A); 84.31, 84.21, 84.02 (C4); 76.35, 75.54, 74.58 (C2, C3, C5); 72.87 (C5A); 63.14, 62.97 (C6); 52.27 (C6A); 49.10 (pnC3); 41.40 (pnC1); 34.39 (pnC2). δC(D2O, pH ~ 9) 104.59, 104.27 (C1); 86.40 (C4A); 83.89, 83.61 (C4) 75.82, 75.75, 74.78, 74.54 (C2, C3, C5); 73.12 (C5A); 63.00, 62.88 (C6); 52.21 (C6A); 49.18 (pnC3); 41.15 (pnC1); 32.93 (pnC2). δC(D2O/HCl, pH ~ 1) 103.79, 103.05 (C1); 85.12 (C4A); 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 (C6A); 47.28 (pnC3); 38.57 (pnC1); 25.57 (pnC2).

6A-(4-aminobutyl)amino-6A-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 cm³) was treated according to the general procedure to give the title compound as a white powder (0.679 g, 52%). Rf 0.63. Electrospray-ms m/z 1205 (M⁺). (Found C, 44.88; H, 7.17; N, 2.17. Calculated for 23.2H₂O (C₄₆H₈₄N₂O₃₆) C, 44.51; H, 6.82; N, 2.25%.)

δH(D₂O/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, H4A); 2.79 (d, J = 11.8 Hz, 1H, H6A); 2.3 - 2.6 (m, 5H, H6A, bnH1, bnH4); 1.25 (bs, 4H, bnH2, bnH3). δH(D₂O, 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, H4A); 3.00 (m, 2H, H6A); 2.75 (m, 2H, bnH1); 2.58 (m, 2H, bnH4); 1.52 (bs, 4H, bnH2, bnH3). δH(D₂O/HCl, pH ~ 1) 4.9 (bs, 7H + solvent, H1); 4.06 (t, J = 9.0 Hz, 1H, H5A); 3.6 - 3.9 (m, 25H, H3, H5, H6); 3.5-3.6 (m, 14H, H2, H4); 3.40 (m, 2H, H6A); 3.02 (bs, 2H, bnH1); 2.92 (bs, 2H, bnH4); 1.66
Experimental

(bis, 4H, bnH2, bnH3). \(\delta_c(D_2O/NaOH, \text{pH} \sim 14)\) 106.73, 106.44, 106.36, 106.27, 106.20, 106.05, 105.43 (C1); 87.63 (C4A); 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 (C5A); 62.92 (C6); 51.86 (C6A); 50.59 (borC4); 43.19 (bnC1); 32.47 (bnC3); 28.53 (bnC2); 17.28, 77.15, 71.00, 66.21, 76.12, 74.77, 74.64 (C2, C3, C5); 71.87 (C5A); 62.92 (C6); 51.86 (C6A); 50.59 (borC4); 43.19 (bnC1); 32.47 (bnC3); 28.53 (bnC2); \(\delta_c(D_2O, \text{pH} \sim 9)\) 104.57, 104.21 (C1); 86.30 (C4A); 83.88, 83.62 (C4); 75.81, 75.64, 75.46, 74.33 (C2, C3, C5); 70.14 (C5A); 62.99 (C6); 52.13 (borC4); 43.69, 42.52 (bnC1); 30.13, (29.56), (29.05), 28.48 (bnC2, bnC3). \(\delta_c(D_2O/HCl, \text{pH} \sim 1)\) 104.53, 104.43, 103.75 (C1); 85.81 (C4A); 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 (C5A); 63.31, 62.97 (C6); 50.87 (C6A); 50.28 (borC4); 41.47 (bnC1); 26.67, 25.27 (bnC2, bnC3).

6\(^{A}\)-(6-aminohexyl)amino-6\(^{A}\)-deoxy-\(\beta\)-cyclodextrin 24

A mixture of the tosylate 32 (1.432 g, 1.111 \times 10^{-3} \text{ mol}), 1,6-diaminohexane (0.460 g, 3.97 \times 10^{-3} \text{ 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\(_e\) 0.75. Electrospray-ms \(m/z\) 1233 (M\(^+\)). (Found C, 44.95; H, 7.27; N, 1.88. Calculated for \(C_{48}H_{90}N_{2}O_{37}\) C, 44.79; H, 7.04; N, 2.17%.) \(\delta_H(D_2O/NaOH, \text{pH} \sim 14)\) 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, \(J = 9.3\ \text{Hz}, 1H, H4A\)); 2.93 (d, \(J = 12.4\ \text{Hz}, 1H, H6A\)); 2.65 (m, 3H, H6\(^A\), HnH6); 2.46 (m, 2H, HnH1); 1.40 (m, 4H, HnH2, HnH5); 1.26 (m, 4H, HnH3, HnH4). \(\delta_H(D_2O, \text{pH} \sim 9)\) 5.09 (s, 7H, H1); 3.8 - 1 (m, 26H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.48 (t, \(J = 9.3\ \text{Hz}, 1H, H4A\)); 3.28 (d, \(J = 12.0\ \text{Hz}, 1H, H6A\)); 2.99 (m, 3H, H6\(^A\), HnH1); 2.81 (bs, 2H, HnH6); 1.65 (m, 4H, HnH2, HnH5); 1.41 (m, 4H, HnH3, HnH4). \(\delta_H(D_2O/HCl, \text{pH} \sim 1)\) 4.80 (s, 7H + solvent, H1); 4.07 (t, \(J = 9.5\ \text{Hz}, 1H, H5A\)); 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). \(\delta_c(D_2O/NaOH, \text{pH} \sim 14)\) 106.64, 106.29, 106.05, 105.91, 105.17 (C1); 87.42 (C4A); 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 (C5A); 62.85, 62.68 (C6); (51.48), (50.27), (44.09), 43.29, 34.71, (32.26), (30.84).
Experimental

(29.16), 28.96, (28.85), 28.56. δC(D2O, pH ~ 9) 104.31, 103.71 (C1); 85.39 (C4A); 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 (C5A), 62.76 (C6); 51.32 (C6A); 50.74 (hnC6); 41.90 (hnC1); 29.45, 29.28, 28.19, 27.91 (hnC2-5). δC(D2O/HCl, pH ~ 1) 104.54, 104.43, 103.71 (C1); 85.80 (C4A); 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 (C5A), 63.27, 62.98 (C6); 50.84 (C6A); 50.72 (hnC6); 42.02 (hnC1); 29.13, 27.93, 27.82, 27.75 (hnC2-5).

6A-(2-(2-aminoethyl)aminoethyl)amino-6Adeoxy-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 cm³) was treated according to the general procedure to give the title compound as a white powder (1.033 g, 54%). Rf 0.62. Electrospray-ms m/z 1220 (M⁺). (Found C, 44.88; H, 6.75; N, 4.05. Calculated for 25.H2O (C46H83N3O35) C, 44.62; H, 6.75; N, 3.39%). δH(D2O/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, H4A); 2.82 (d, J = 12.6, 1H, H6A); 2.3-2.6 (m, 9H, C6A, dienH). δH(D2O, 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, H4A); 3.07 (d, J = 11.9 Hz, 1H, H6A); 2.7 (m, 9H). δH(D2O/HCl, pH ~ 1) 4.9 (m, 7H + solvent, H1); 4.08 (t, J = 8.2 Hz, 1H, H5A); 3.6-3.9 (m, 25H, H3, H5, H6); 3.6 - 2.5 (m, 24H). δC(D2O/NaOH, pH ~ 14) 106.8, 106.5, 106.4, 106.35, 106.3, 105.9 (C1); 88.0 (C4A); 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 (C5A); 63.7 (C6); 53.9 (dienC3); 52.3 (C6A); 50.5, 50.3 (dienC1,2); 43.1 (dienC4). δC(D2O, pH ~ 9) 104.6, 104.3 (C1); 86.4 (C4A); 83.9, 83.7 (C4); 75.8, 75.7, 74.8, 74.6 (C2, C3, C5); 73.2 (C5A); 63.0 (C6); 52.9 (C6A); 52.1 (dienC3); 50.6, 50.2 (dienC1, dienC2); 42.4 (dienC4). δC(D2O/HCl) 104.45, 103.65(C1); 85.68 (C4A); 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 (C5A); 63.53, 63.17, 63.06 (C6); 51.58 (C6A); 47.34(dienC3); 46.33 (dienC1); 46.00 (dienC2); 38.04 (dienC4).
6\textsuperscript{A}-(3-(3-aminopropyl)aminopropyl)amino-6\textsuperscript{A}-deoxy-\textbeta-cyclodextrin 26

A mixture of the tosylate 32 (1.997 g, 1.55 × 10\textsuperscript{-3} mol), 3-(3-aminopropyl)-aminopropylamine (0.460 g, 3.97 × 10\textsuperscript{-3} mol) and KI (0.026 g) in NMP (5 cm\textsuperscript{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\textsuperscript{+}). (Found C, 45.17; H, 6.52; N, 3.12. Calculated for \( \text{C}_{36}\text{H}_{3}\text{eN}_{9}\text{O}_{3\text{e}} \) C, 44.89; H, 6.98; N, 3.21\%).

\[ \delta_{\text{H}}(\text{D}_2\text{O}/\text{NaOH}, \text{pH} \sim 14) \]
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, \( J = 9.6 \text{ Hz}, 1\text{H}, \text{H}_4^\text{A} \)); 2.98 (d, \( J = 12.6 \text{ Hz}, 1\text{H}, \text{H}_6^\text{A} \)); 2.4 - 2.7 (m, 9H, \text{H}_6^\text{A}, dipnH1, dipnH3, dipnH4, dipnH6); 1.4 - 1.6 (m, 4H, dipnH2, dipnH5). \( \delta_{\text{H}}(\text{D}_2\text{O}, \text{pH} \sim 9) \)
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, \( J = 8.9 \text{ Hz}, 1\text{H}, \text{H}_4^\text{A} \)); 2.90 (d, \( J = 11.9 \text{ Hz}, 1\text{H}, \text{H}_6^\text{A} \)); 2.4 - 2.7 (m, 9H (\text{H}_6^\text{A}, dipnH1, dipnH3, dipnH4, dipnH6); 1.53 (m, 4H, dipnH2, dipnH5). \( \delta_{\text{H}}(\text{D}_2\text{O}/\text{HCl}, \text{pH} \sim 1) \)
5.10 (s, 7H + solvent; H1); 4.22 (t, \( J = 9.2 \text{ Hz}, 1\text{H}, \text{H}_5^\text{A} \)); 3.8 - 4.1 (m, 25H, H3, H5, H6), 3.5 - 3.7 (m, 15H, H2, H4, \text{H}_6^\text{A}); 3.42 (dd, \( J = 12.8, 9.2 \text{ Hz}, 1\text{H}, \text{H}_6^\text{A} \)); 3.20 (m, 8H, dipnH1, dipnH3, dipnH4, dipnH6); 2.18 (m, 4H, dipnH3, dipnH5). \( \delta_{\text{C}}(\text{D}_2\text{O}/\text{NaOH}, \text{pH} \sim 14) \)
105.3, 105.1, 104.8 (C1); 86.9 (C4A); 84.3, 84.2, 83.9 (C4); 76.3, 76.1 (C2); 75.4 (C3); 74.7 (C5); 72.8 (C5A); 63.1 (C6); 52.0 (C6A); 49.3, 49.2, 48.9 (dipnC1, dipnC3, dipnC4); 41.5 (dipnC6); 34.5, 31.0 (dipnC2, dipnC5). \( \delta_{\text{C}}(\text{D}_2\text{O}, \text{pH} \sim 9) \)
104.57, 104.16 (C1); 86.34 (C4A); 83.88, 83.53 (C4); 75.78, 74.78, 74.72, 74.60 (C2, C3, C5); 73.02 (C5A); 63.01 (C6); 51.97 (C6A); 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). \( \delta_{\text{C}}(\text{D}_2\text{O}/\text{HCl}, \text{pH} \sim 1) \)
104.49, 103.82 (C1); 85.83 (C4A); 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 (C5A); 63.48, 63.14 (C6); 51.14 (C6A); 47.96, 47.45, 47.34 (dipnC1, dipnC3, dipnC4); 39.32 (dipnC6); 26.44, 25.18 (dipnC2, dipnC5).

6\textsuperscript{A}-(2-(2-(2-aminoethyl)aminoethyl)aminoethyl)amino-6\textsuperscript{A}-deoxy-\textbeta-cyclodextrin 27

A mixture of 2-(2-(2-aminoethyl)aminoethyl)aminoethylamine \( \cdot \text{HCl} \) (0.855 g, 2.92 × 10\textsuperscript{-3} mol) and sodium hydroxide (0.474 mg, 11.85 × 10\textsuperscript{-3} mol in ethanol (30 cm\textsuperscript{3}) was
stirred at room temperature for 15 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm³). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm³) 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%). Rc 0.28. Electrospray-ms m/z 1263 (M⁺). (Found C, 44.83; H, 6.89; N, 4.42. Calculated for C₄₈H₄₈N₄O₃₅ C, 44.99; H, 6.92; N, 4.37%). δ_H(D₂O/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, H4A); 2.93 (d, J = 12 Hz, 1H, H6A); 2.5 - 2.7 (m, 13H, H6A', trienH). δ_H(D₂O, 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, H4A); 3.06 (d, J = 11.6 Hz, 1H, H6A); 2.4 - 2.9 (m, 13H, H6A', trienH). δ_H(D₂O/HCl, pH ~ 1) 4.95 (bs, 7H + solvent, H1), 4.08 (t, J = 9.0 Hz, 1H, H5A); 3.6-3.9 (m, 25H, H3, H5, H6); 3.2-3.6 (m, 28H). δ_C(D₂O/NaOH, pH ~ 14) 106.7, 106.3, 106.2, 105.8 (C1); 87.9 (C4A); 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 (C5A); 63.6 (C6); 53.9 (trienC1?); 52.2 (C6A); 50.6, 50.2 (trienC2-5), 43.1 (trienC6). δ_C(D₂O, pH ~ 9) 104.55, 104.19 (C1); 86.31 (C4A); 83.88, 83.63 (C4); 75.81, 75.74, 74.75, 74.56 (C2, C3, C5); 73.08 (C5A); 63.01 (C6); 52.69 (C6A); 52.01, (51.32), 50.51, 50.30, 50.21, (49.95), (43.02), 42.35. δ_C(D₂O/HCl, pH ~ 1) 104.48, 103.62 (C1); 85.71 (C4A); 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 (C5A); 63.57, 63.03 (C6); 51.59 (C6A); 47.31, 46.19, 45.98, 45.88, 37.94.

6A-(1,4,7-triazacyclonon-1-yl)-6A-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 cm³) was stirred at room temperature for 15 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm³). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm³) 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 cm³) and the cyclodextrins were then precipitated by addition of ethanol (100 cm³). 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 29.3H₂O (C₄₈H₅₀N₅O₇) C, 44.34; H, 6.90; N, 3.23%). δ_H(D₂O/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, H4ᴬ, H6ᴬ); 2.3-2.7 (m, 7H, H6ᴬ, tacnH). δ_H(D₂O, pH ~ 9) 5.0 (s, 7H, H1); 3.7-4.0 (m, 26H, H3, H5, H6); 2.7-3.7 (m, 23H, H4ᴬ, H6ᴬ, tacnH). δ_H(D₂O/HCl, pH ~ 1) 3.96 (bt, 1H, H₅ᴬ); 3.5-3.8 (m, 25H, H₃, H₅, H₆); 3.0-3.4 (m, 23H', H4, H6', tacnH). δ_C(D₂O/NaOH, pH ~ 14) 107.0, 106.6, 106.3, 106.0, 104.8 (C₁); 87.8 (C₄ᴬ); 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 (C₅ᴬ); 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(D₂O, pH ~ 9) 104.7, 104.6, 104.0 (C₁); 86.3 (C₄ᴬ); 83.9, 83.3, 83.1 (C₄); 75.9, 75.6, 74.7 (C₂, C₃, C₅); 72.9 (C₅ᴬ); 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(D₂O/HCl, pH ~ 1) 104.4, 103.8 (C₁); 86.5 (C₄ᴬ); 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 (C₅ᴬ); 63.4, 63.0 (C₆); 56.7 (C₆ᴬ); 50.0 (tacnCl); 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.

6⁴(1,5,9-triazacyclododecan-1-yl)-6⁴-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 cm³) was stirred room temperature for 90 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm³). The combined filtrates were evaporated under reduced pressure to give the free amine as a yellow oil. This was dissolved in NMP (5 cm³) and the tosylate 32 (2.081 g,
1.61 \times 10^{-3} \text{ mol}) and KI (0.030 \text{ 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 \text{ g}, 34\%). R_c 0.75 Electrospray-ms \text{ m/z} 1288 (M^+). (Found C, 45.28; H, 7.34; N, 3.08\%.)

\(\delta_H(D_2O/NaOH, \text{ pH } = 14)\) 4.9 (bs, 7H+solvent, H1); 4.14 (t, \(J = 6.0 \text{ Hz}, 1H, H^5A\)); 3.7-4.0 (m, 25H, H3, H5, H6); 3.17 (t, \(J = 6.0 \text{ Hz}, 1H, H^4A\)); 2.88 (d, \(J = 15 \text{ Hz}, 1H, H^6A\)); 2.64 (m, 13H, H6A, tacdoH1, tacdoH3, tacdoH4); 1.66 (m, 6H, tacdoH2, tacdoH5).

\(\delta_H(D_2O/\sim \text{ 1 eq HCl, pH } \sim 8.5)\) 5.09 (s, 7H+solvent, H1); 4.26 (t, \(J = 9.0 \text{ Hz}, 1H, H^5A\)); 3.8-4.2 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.39 (t, \(J = 9.0 \text{ Hz}, 1H, H^4A\)); 2.7-3.3 (m, 14H, H6A, tacdoH1, tacdoH3, tacdoH4); 1.6 - 2.0 (m, 6H, tacdoH2, tacdoH5).

\(\delta_H(D_2O/\sim \text{ 2 eq HCl, pH } \sim 6.0)\) 5.07 (bs, 7H, H1); 4.25 (t, \(J = 9.0 \text{ Hz}, 1H, H^5A\)); 3.8-4.1 (m, 25H, H3, H5, H6); 3.5-3.7 (m, 13H, H2, H4); 3.43 (t, \(J = 9.0 \text{ Hz}, 1H, H^4A\)); 2.5-3.2 (m, 14H, H6A, tacdoH1, tacdoH3, tacdoH4); 1.7-2.2 (m, 6H, tacdoH2, tacdoH5).

\(\delta_H(D_2O/HCl, \text{ pH } \sim 1)\) 5.0 (bs, 7H+solvent, H1); 4.33 (bt, 1H, H5A); 3.7-4.0 (m 25H, H3, H5, H6); 3.2-3.6 (m, 27H, H2, H4, H6A, tacdoH1, tacdoH3, tacdoH4); 2.2 (broad, 6H, tacdoH2, tacdoH5).

\(\delta_C(D_2O/NaOH, \text{ pH } \sim 14)\) 106.9, 106.6, 106.4, 106.3, 105.8, 105.7, 104.3 (C1); 87.7 (C4A); 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 (C5A); 63.4, 63.1 (C6); 55.9 (C6A); 54.6 (tacdoC1); 48.7, 48.6 (tacdoC3, tacdoC4); 28.0, 26.5 (tacdoC2, tacdoC5).

\(\delta_C(D_2O/\sim \text{ 1 eq HCl, pH } \sim 8.5)\) 104.8, 104.5, 103.4, 103.0 (C1); 86.4 (C4A); 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 (C5A); 63.3, 63.2 (C6); 54.3 (C6A); 51.9, 49.0 (tacdoC1, tacdoC3, tacdoC4); 26.6, 25.4 (tacdoC2, tacdoC5).

\(\delta_C(D_2O/\sim \text{ 2 eq HCl, pH } \sim 6.0)\) 104.8, 104.7, 103.7 (C1); 86.5 (C4A); 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 (C5A); 63.4, 63.2 (C6); 53.8, (53.1), 47.5 (broad), (45.7) (C6A, tacdoC1, tacdoC3, tacdoC4); 25.1 (broad), 23.9 (broad) (tacdoC2, tacdoC5). \(\delta_C(D_2O/HCl, \text{ pH } \sim 1)\) 104.9. 104.7, 104.6, 103.7 (C1); 86.3 (C4A); 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 (C5A); 63.8, 63.3 (C6); 58.6, 50.9 (broad), 47.5, 45.2, 43.9 (C6A, tacdoC1, tacdoC3, tacdoC4); (25.4), 23.7, 19.9 (tacdoC2, tacdoC5).
Experimental

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\textsuperscript{A}-deoxy-6\textsuperscript{A}-(1,4,7,10-tetraazacyclododecan-1-yl)-\textbeta-cyclodextrin 31

A mixture of 1,4,7,10-tetraazacyclododecane sulfate (1.845 g, 5.0 \times 10^{-3} \text{ mol}) and sodium hydroxide (0.784 g, 19.6 \times 10^{-3} \text{ mol}) was stirred in ethanol (40 cm\textsuperscript{3}) at room temperature for 90 minutes. The mixture was filtered and the collected solid was washed with ethanol (10 cm\textsuperscript{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\textsuperscript{3}) and the tosylate 32 (2.177 g, 1.69 \times 10^{-3} \text{ 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 $m/\varepsilon$ 1289 (M\textsuperscript{+}). (Found C, 44.16; H, 7.10; N, 4.36. Calculated for 31.3\text{H}_{2}\text{O} (C_{50}H_{94}N_{4}O_{37}) C, 44.71；H, 7.05；N, 4.17%).

δ\textsubscript{H}(D_{2}O/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\textsubscript{A}); 2.2-2.8 (m, 18H, H6\textsubscript{A}, cyclenH). δ\textsubscript{H}(D_{2}O, 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\textsubscript{A}); 2.4 - 2.9 (broad, 18H, H6\textsubscript{A}, cyclenH). δ\textsubscript{H}(D_{2}O/HCl, pH ~ 1) 4.9 (s, 7H + solvent, H1); 4.07 (t, $J = 9.4$ Hz, 1H, H5\textsubscript{A}); 3.4-3.9 (m, 25H, H3, H5, H6); 2.9-3.5 (m, 32H, H6\textsubscript{A}, cyclenH). δ\textsubscript{C}(D_{2}O/NaOH, pH ~ 14) 106.9, 106.8, 106.6, 106.4, 105.7, 105.3, 104.4 (C1); 86.35 (C4\textsubscript{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\textsubscript{A}); 63.1, 62.9, 62.8 (C6); 59.0 (C6\textsubscript{A}); 55.0, (50.2), 48.0, 47.1, 46.1 (cyclenC). δ\textsubscript{C}(D_{2}O, pH ~ 10) 104.9, 104.6, 104.3, 104.2, 103.8(C1); 85.6 (C4\textsubscript{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\textsubscript{A}); 63.1, 63.0, 62.8 (C6); 58.3 (C6\textsubscript{A}); 54.3, (50.2), 48.2, 46.9, 46.2 (cyclenC). δ\textsubscript{C}(D_{2}O/HCl, pH ~ 1) 104.5, 104.4. 104.3, 103.6 (C1); 86.4 (C4\textsubscript{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
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-$\beta$-cyclodextrin 24

1D proton spectrum data: $\delta_H$ 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, H4A); 2.93 (d, $J = 12.4$ Hz, 1H, H6A); 2.70 (m, 1H, H6A'); 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: $\delta_H$ 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 (H5A ?); 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-$\beta$-cyclodextrin 26

1D proton spectrum data: $\delta_H$ 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, H4A); 2.98 (d, $J = 12.6$ Hz, 1H, H6A); 2.65 (dd, $J = 9.0$, 12.6 Hz, 1H, H6A'); 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: $\delta_H$ 1.5 (dipnH2, dipnH5) shows cross-peaks with 2.6-2.4 (dipnH1, dipnH3, dipnH4, dipnH6), 2.65 (H6A), 3.7-3.9 (H3, H5); 2.48 (dipnH1, dipnH3, dipnH4) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.65 (H6A'), 2.98 (H6A), 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 (H6A') shows cross-peaks with 2.48 (dipnH1, dipnH3, dipnH4), 2.98 (H6A), 3.27
Experimental

(H4A), 3.9 (H5A); 2.98 (H6A) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.48 (dipnH1, dipnH3, dipnH4), 2.65 (H6A), 3.7-3.9 (H3, H5); 3.27 (H4A) shows cross-peaks with 2.65 (H6A'); 3.7-3.9 (H3, H5) shows cross-peaks with 1.5 (dipnH2, dipnH5), 2.48 (dipnH1, dipnH3, dipnH4), 2.58 (dipnH6), 2.98 (H6A).

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

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

1D proton spectrum data: δH 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, H4A); 2.99 (d, J = 13.2 Hz, 1H, H6A); 2.71 (m, 1H, H6A'); 2.64 (t, J = 7.2 Hz, 2H, hhH6); 2.45 (t, J = 7.2 Hz, 2H, hhH1); 2.41 (s, 3H, Me); 1.4-1.5 (m, 4H, hhH2, hhH5); 1.2-1.3 (m, 4H, hhH3, hhH4).

2D ROESY cross-peaks: δH 1.2-1.3 (hhH3,4) shows cross-peaks with 1.4-1.5 (hhH2,5), 2.45 (hhH1), 2.64 (hhH6), 3.8 (H5), 3.9 (H3); 1.4-1.5 (hhH2,5) shows cross-peaks with 1.2-1.3 (hhH3,4), 2.45 (hhH1), 2.64 (hhH6), 3.8 (H5), 3.9 (H3); 2.41 (Me) shows a cross-peak with 7.29 (ArH3); 2.45 (hhH1) shows cross-peaks with 1.2-1.3 (hhH3,4), 1.4-1.5 (hhH2,5), 3.8 (H5), 3.9 (H3); 2.64 (hhH6) shows cross-peaks with 1.2-1.3 (hhH3,4), 1.4-1.5 (hhH2,5), 3.8 (H5), 3.9 (H3); 2.71 (H6A') shows a cross-peak with 3.26 (H4A); 3.26 (H4A) shows a cross-peak with 2.71 (H6A'); 3.8 (H5) shows cross-peaks with 1.2-1.3 (hhH3,4), 1.4-1.5 (hhH2,5), 7.29 (ArH3), 7.80 (ArH2); 3.9 (H3) shows cross-peaks with 1.2-1.3 (hhH3,4), 1.4-1.5 (hhH2,5), 2.45 (hhH1), 2.64 (hhH6), 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 6A-(6-aminohexyl)amino-6A-deoxy-β-cyclodextrin 24

1D proton spectrum data: δH 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, H4A); 2.93 (d, J = 12.6 Hz, 1H, H6A);
2.67 (dd, J = 12.6, 9.5 Hz, 1H, H6A'); 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: δH 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 (H5A); 2.62 (hnH6) shows cross-peaks with 1.25 (hnH3,
hnH4), 1.39 (hnH2, hnH5), 3.7(H5), 3.75 (H3); 2.67 (H6A') shows cross-peaks with 2.93
(H6A), 3.19 (H4A); 2.93 (H6A) shows cross-peaks with 2.67 (H6A'), 3.7 (H5), 3.8 (H5A);
3.19 (H4A) shows cross-peaks with 2.67 (H6A'), 3.75 (H3), 3.8 (H5A); 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 (H5A) shows cross-peaks with 2.45 (hnH1), 2.93 (H6A), 3.19
(H4A); 7.30 (ArH2,2', ArH3,3') shows cross-peaks with 3.58 (αCH), 3.75 (H3).

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

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

1D proton spectrum data: δH 7.68 (d, J = 7.8 Hz, 2H, H6); 7.18 (d, J = 7.8 Hz, 2H,
Hm); 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, H4A); 2.83 (d, J =13.2 Hz, 1H, H6A); 2.4-2.6 (m, 9H, H6A', dienH2-4);
2.31 (s, 3H, Me).

2D ROESY cross-peaks: δH 2.31 (Me) shows cross-peaks with 3.5-3.8 (H3, H5),
7.18 (Hm); 2.6 (H6A) shows cross-peaks with 2.83 (H6A), 3.14 (H4A); 2.83 (H6A) shows
cross-peaks with 2.6 (H6A), 3.65 (H5); 3.14 (H4A) shows cross-peaks with 2.6 (H6A); 3.65
Experimental

(H5) shows cross-peaks with 2.31 (Me), 2.83 (H6A), 7.18 (Hm), 7.68 (Ho); 3.8 (H3) shows cross-peaks with 2.31 (Me), 7.18 (Hm), 7.68 (Ho); 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 6A-(3-(3-aminopropyl)aminopropyl)amino-6A-deoxy-β-cyclodextrin 26

1D proton spectrum data: $\delta_H$ 7.71 (d, $J = 7.8$ Hz, 2H, H0); 7.20 (d, $J = 7.8$ Hz, 2H, Hm); 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, H4A); 2.89 (d, $J = 12.0$ Hz, 1H, H6A); 2.62 (dd, $J = 9.0$, 12.0 Hz, 1H, H6A); 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: $\delta_H$ 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 (Hm); 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 (C6A') shows cross-peaks with 2.89 (C6A), 3.18 (C4A); 2.89 (C6A) shows cross-peaks with 2.62 (C6A'), 3.7 (H5A); 3.18 (C4A) shows cross-peaks with 2.62 (C6A'); 3.6-3.9 (H3, H5) shows cross-peaks with 2.34 (Me), 7.20 (Hm), 7.71 (Ho); 7.20 (Hm) shows cross-peaks with 2.34 (Me), 3.6-3.9 (H3, H5); 7.71 (Ho) 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\(^{-3}\))\(^a\)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
<th>y</th>
<th>pH</th>
<th>x</th>
<th>y</th>
</tr>
</thead>
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<td>7.6</td>
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<td>32.0</td>
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<td>8.69</td>
<td>8.0</td>
<td>36.9</td>
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<td>8.2</td>
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<td>5.91</td>
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<td>7.3</td>
<td>18.0</td>
<td>8.20</td>
<td>8.4</td>
<td>43.8</td>
<td>5.62</td>
</tr>
</tbody>
</table>

\(^a\) 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\(^{-3}\))\(^a\)

<table>
<thead>
<tr>
<th>pH</th>
<th>x</th>
<th>pH</th>
<th>x</th>
</tr>
</thead>
<tbody>
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<td>3.9</td>
<td>9.1</td>
<td>23.6</td>
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<tr>
<td>8.1</td>
<td>4.9</td>
<td>9.5</td>
<td>34.6</td>
</tr>
</tbody>
</table>

\(^a\) Stock boric acid solution prepared by dissolving 6.184 g H\(_3\)BO\(_3\) 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\(_2\)O\(_5\) 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...
Experimental

of the cyclodextrin in buffer to give stock solutions of $1.03 \times 10^{-3}$ 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 $1.0 \times 10^{-3}$ 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 $1.0 \times 10^{-5}$ mol dm$^{-3}$.

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

The variation of molar absorbance $\varepsilon_{400}$ 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 0.337-8.41 $\times 10^{-5}$ 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 $\varepsilon_{400}$ 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 $1.0 \times 10^{-3}$ 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

<table>
<thead>
<tr>
<th>10^5[49] /mol dm^-3</th>
<th>A_400</th>
<th>pH 6.9</th>
<th>pH 7.1</th>
<th>pH 7.3</th>
<th>pH 7.6</th>
<th>pH 7.8</th>
<th>pH 8.0</th>
<th>pH 8.5</th>
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<tbody>
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<td>0.033</td>
<td>0.037</td>
<td>0.039</td>
<td>0.048</td>
<td>0.052</td>
<td>0.054</td>
<td>0.059</td>
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<tr>
<td>0.520</td>
<td>0.047</td>
<td>0.056</td>
<td>0.066</td>
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<td>0.075</td>
<td>0.079</td>
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<tr>
<td>0.841</td>
<td>0.085</td>
<td>0.090</td>
<td>0.108</td>
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<td>0.125</td>
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<td>0.174</td>
<td>0.176</td>
<td>0.196</td>
<td>0.202</td>
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<td>0.349</td>
<td>0.359</td>
<td>0.400</td>
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<tr>
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<td>0.862</td>
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<tr>
<td>\varepsilon_400^b</td>
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<td>11300</td>
<td>12500</td>
<td>13500</td>
<td>14500</td>
<td>15300</td>
<td>16500</td>
<td></td>
</tr>
</tbody>
</table>

\^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.¹

<table>
<thead>
<tr>
<th>pH</th>
<th>( \varepsilon_{400} )</th>
<th>( 10^4 \text{slope}^b )/AU s⁻¹</th>
<th>( 10^4 \text{average} )/AU s⁻¹</th>
<th>( 10^8 k_0^c )/mol dm⁻³ s⁻¹</th>
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</thead>
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<td>0.772</td>
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</table>

¹ 0.05 mol dm⁻³ HEPES. ² Determined from the least squares fit of A₄₀₀ against time for the first 2% of reaction. ³ Rate of formation of the phenol 49 calculated by dividing the average slope by the value of \( \varepsilon_{400} \). ⁴ From interpolation of a plot of \( \varepsilon_{400} \) 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.¹

<table>
<thead>
<tr>
<th>pH</th>
<th>ε₄₀₀</th>
<th>10⁴slope b /AU s⁻¹</th>
<th>10⁴average /AU s⁻¹</th>
<th>10⁸kₒbs c /mol dm⁻³ s⁻¹</th>
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</table>

¹ 0.05 mol dm⁻³ HEPES.  b Determined from the least squares fit of A₄₀₀ 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 ε₄₀₀.  d From interpolation of a plot of ε₄₀₀ against pH.

The molar absorbance ε₄₀₀ of 4-nitrophenol 49 is affected by the presence of cyclodextrins. Therefore it was necessary to determine the value of ε₄₀₀ for each solution containing a cyclodextrin. The molar absorbance ε₄₀₀ of 4-nitrophenol 49 in solutions containing cyclodextrins was calculated from the observed absorbance at 400 nm measured by adding 0.05 cm³ 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 ε₄₀₀ 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 ε₄₀₀, the reactions of the ester 48 in buffer alone were repeated, and the initial rate values, kₒ, were calculated using a value of ε₄₀₀ 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.

<table>
<thead>
<tr>
<th>pH</th>
<th>A₄₀₀ᵃ</th>
<th>ε₄₀₀</th>
<th>10⁴slopeᵇ</th>
<th>10⁴average</th>
<th>10⁸kₒᶜ</th>
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</thead>
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<td>0.173</td>
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<td>20700</td>
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<td>39.1</td>
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</tbody>
</table>

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 ε₄₀₀.ᵇ Determined from the least squares fit of A₄₀₀ 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 ε₄₀₀.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.

<table>
<thead>
<tr>
<th>pH</th>
<th>A₄₀₀ᵃ</th>
<th>ε₄₀₀</th>
<th>10⁴slopeᵇ</th>
<th>10⁴average</th>
<th>10⁸kₐₒₛᵉᶜ⁹</th>
<th>mol dm⁻³ s⁻¹</th>
</tr>
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<tr>
<td>6.6ᵈ</td>
<td>0.325</td>
<td>8900</td>
<td>0.634</td>
<td>0.629</td>
<td>0.707</td>
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<tr>
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<td></td>
<td>0.639</td>
<td>0.614</td>
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</tr>
<tr>
<td>7.2ᵈ</td>
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<td>3.34</td>
<td>3.28</td>
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<tr>
<td></td>
<td>(0.481)ᵍ</td>
<td>(13200)ᵍ</td>
<td>3.23</td>
<td>(3.19)ᵍ</td>
<td>(2.42)ᵍ</td>
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</tr>
<tr>
<td></td>
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<td>3.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3.16, 3.22)ᵍ</td>
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</tr>
<tr>
<td>7.8ᵈ</td>
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<td>32.0</td>
<td>34.1</td>
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</tr>
<tr>
<td>8.1ᵉ</td>
<td>0.776</td>
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<td>48.8</td>
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<tr>
<td></td>
<td>(0.691)ᵍ</td>
<td>(18900)ᵍ</td>
<td>269</td>
<td>(322)ᵍ</td>
<td>(170)ᵍ</td>
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<td></td>
<td></td>
<td></td>
<td>(321, 322)ᵍ</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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 ε₄₀₀. ᵇ Determined from the least squares fit of A₄₀₀ 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 ε₄₀₀. 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(ClO₄)₂.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.2[^d]</td>
<td>0.468</td>
<td>12800</td>
<td>2.69</td>
<td>2.05</td>
</tr>
<tr>
<td>1.0</td>
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<td>3.00</td>
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<tr>
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<tr>
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<td>0.685</td>
<td>18800</td>
<td>141</td>
<td>74.5</td>
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</tbody>
</table>

[^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 ε₄₀₀.  
[^b]: Determined from the least squares fit of A₄₀₀ 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 ε₄₀₀.  
[^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 cm³ of a stock solution (1.03 × 10⁻³ mol dm⁻³) of the aminoalkylamino-β-cyclodextrin 21, 22 or 24 (or the corresponding free amine NH₂(CH₂)₃NH₂) 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 cm³ 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 $1.0 \times 10^{-5}$ mol dm$^{-3}$ and the amine at a concentration of $1.0 \times 10^{-3}$ 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.$^a$

<table>
<thead>
<tr>
<th>Added reactant$^b$</th>
<th>$10^3$ observed rate/s$^{-1}$$^c$</th>
<th>$10^3$ average rate/s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>nil</td>
<td>0.190, 0.208</td>
<td>0.200</td>
</tr>
<tr>
<td>$\text{NH}_2(\text{CH}_2)_2\text{NH}_2$</td>
<td>0.893, 0.877, 0.900</td>
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<tr>
<td>$\text{NH}_2(\text{CH}_2)_3\text{NH}_2$</td>
<td>1.33, 1.35, 1.37</td>
<td>1.35</td>
</tr>
<tr>
<td>$\text{NH}_2(\text{CH}_2)_6\text{NH}_2$</td>
<td>1.27, 1.30, 1.26</td>
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<tr>
<td>2</td>
<td>0.339, 0.341, 0.351</td>
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<td>21</td>
<td>7.17, 7.46</td>
<td>7.32</td>
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<tr>
<td>22</td>
<td>3.79, 3.81, 3.89</td>
<td>3.83</td>
</tr>
<tr>
<td>24</td>
<td>1.13, 1.13</td>
<td>1.13</td>
</tr>
</tbody>
</table>

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

The first order rate constants were calculated by fitting the collected data to a first order rate equation ($A_{\text{inf}}-A_t = A_{\text{inf}}e^{-kt}$). The amount of 4-nitrophenyl acetate 48 remaining in the reaction mixture at any time, $t$, is related to the absorbance at infinite time, $A_{\text{inf}}$, minus the absorbance at that time, $A_t$. Plots of $\ln(A_{\text{inf}}-A_t)$ 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

<table>
<thead>
<tr>
<th>pH</th>
<th>10^3k_0/s-¹</th>
<th>10^3k_0/s-¹ average</th>
<th>10^3k_0/s-¹</th>
<th>10^3k_0/s-¹ average</th>
<th>10^3 (k_obs/k_0)</th>
<th>10^4[24] non-protonated</th>
<th>10^4[24] mono-protonated</th>
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<td>1.87</td>
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<td>0.200</td>
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<td>1.13</td>
<td></td>
<td>1.13</td>
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<td></td>
</tr>
</tbody>
</table>

⁠^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 pKₐs = 10.47 and 8.72.
Experimental

E.3.5. Preparation of N-acetyl-aminoalkyl amino $\beta$-cyclodextrins

$6^A$-(2-acetamidoethylamino-$6^A$-deoxy-$\beta$-cyclodextrin 53

A mixture of the cyclodextrin 21 (0.100 g, $0.085 \times 10^{-3}$ mol) and 4-nitrophenyl acetate 48 (0.015 g, $0.082 \times 10^{-3}$ mol) in NMP (2 cm$^3$) was stirred at room temperature for 18 hours. TLC analysis showed the presence of $\beta$-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 ($5 \times 20$ 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 ($\text{NH}_4^+$ 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$_2$O (C$_{46}$H$_{91}$ClN$_2$O$_{41}$) C, 40.52; H, 6.72; N, 2.05%). $\delta_H$ (D$_2$O) 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$_2$NAc); 3.06 (d, $J = 11.7$ Hz, 1H, H6$^A$); 2.77 (m, 3H, H6$^A$, CH$_2$NH); 1.99 (s, 3H, Me). $\delta_C$-(D$_2$O) 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 (enCl); 41.26 (enC2); 24.63 (Me).

$6^A$-(3-acetamidopropyl)amino-$6^A$-deoxy-$\beta$-cyclodextrin 54

A mixture of the cyclodextrin 22 (0.096 g, $0.086 \times 10^{-3}$ mol) and 4-nitrophenyl acetate 48 (0.016 g, $0.088 \times 10^{-3}$ mol) in NMP (2 cm$^3$) was stirred at room temperature for 18 hours. TLC analysis showed the presence of $\beta$-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 ($5 \times 10$ 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 ($\text{NH}_4^+$ 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%). \( R_c 1.1 \). Electrospray-ms 1233 (M⁺). (Found C, 41.63; H, 6.81; N, 2.23. Calculated for \( \text{C}_4\text{H}_9\text{ClN}_2\text{O}_4 \) C, 41.52; H, 6.74; N, 2.06%). \( \delta_H (\text{D}_2\text{O}, 25 ^\circ\text{C}) 5.06 (s, 7\text{H}, \text{H}1); 3.8-4.0 (m, 26\text{H}, \text{H}3, \text{H}5, \text{H}6); 3.5-3.7 (m, 14\text{H}, \text{H}2, \text{H}4); 3.0-3.5 (m, 5\text{H}); 2.80 (m, 0.3\text{H}); 2.58 (t, \( J = 6.9 \) Hz, 0.6\text{H}); 2.00, 1.98 (s, 3\text{H}, \text{Me} ratio 2:1); 1.68 (m, 2\text{H}, \text{pnH}2). \( \delta_C (\text{D}_2\text{O}) 176.69, 167.46 (\text{C}=	ext{O}); 104.82, 104.33 (\text{C}1); 86.57 (\text{C}4\text{A}); 83.92, 83.52 (\text{C}4); 75.94, 74.95, 74.67 (\text{C}2, \text{C}3, \text{C}5); 72.67 (\text{C}5\text{A}); 65.38, 63.03 (\text{C}6); 51.86 (\text{C}6\text{A}); 48.52 (\text{pnC}1); 39.95 (\text{pnC}3); 30.93 (\text{pnC}2); 24.64 (\text{Me}). \( \delta_H (\text{D}_2\text{O}, 50 ^\circ\text{C}) 5.35 (s, 7\text{H}, \text{H}1); 4.0-4.3 (m, 26\text{H}, \text{H}3, \text{H}5, \text{H}6); 3.8-4.0 (m, 13\text{H}, \text{H}2, \text{H}4); 3.68 (t, \( J = 8.9 \) Hz, 1\text{H}, \text{H}4\text{A}); 3.47 (t, \( J = 6.9 \) Hz, 2\text{H}, \text{CH}_2\text{NAc}); 3.30 (d, \( J = 11.9 \) Hz, 1\text{H}, \text{H}6\text{A}); 3.04 (dd, \( J = 11.9, 7.1 \) Hz, 1\text{H}, \text{H}6\text{A}'); 2.87 (t, \( J = 6.9 \) Hz, 2\text{H}, \text{CH}_2\text{NH}); 2.26 (s, 3\text{H}, \text{Me}); 1.94 (bs, 2\text{H}, \text{pnH}2).

\( \underline{6^\text{A}}\)-\( (6\text{-acetamidohexylamino-}6^\text{A}-\text{deoxy-} \beta\text{-cyclodextrin} \) \( \underline{55} \)

A mixture of the cyclodextrin \( \underline{24} \) (0.101 g, 0.082 × 10⁻³ mol) and 4-nitrophenyl acetate \( \underline{48} \) (0.014 g, 0.078 × 10⁻³ mol) in NMP (2 cm³) was stirred at room temperature for 18 hours. TLC analysis showed the presence of \( \beta\)-cyclodextrin \( \underline{2} \) and a new spot (\( R_c 1.2 \)). The yellow solution was diluted with 1 mol dm⁻³ HCl (30 cm³) and washed with dichloromethane (5 × 20 cm³). 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 cm³) and loaded onto a column of BioRex 70 (NH₄⁺ form). The column was washed with water and fractions (8 cm³) 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 \( \underline{55}\)HCl.5H₂O (\( \text{C}_{5\text{g}}\text{H}_{9\text{g}}\text{ClN}_2\text{O}_{\text{g}} \)) C, 42.84; H, 6.97; N, 2.00%). \( \delta_H (\text{D}_2\text{O}) 5.07 (s, 7\text{H}, \text{H}1); 3.5-4.0 (m, 39\text{H}, \text{H}2, \text{H}3, \text{H}4, \text{H}5, \text{H}6); 3.40 (t, \( J = 9.0 \) Hz, 1\text{H}, \text{H}4\text{A}); 3.16 (t, \( J = 7.2 \) Hz, 2\text{H}, \text{CH}_2\text{NAc}); 3.05 (d, \( J = 12.6 \) Hz, 1\text{H}, \text{H}6\text{A}); 2.76 (m, 1\text{H}, \text{H}6\text{A}'); 2.58 (t, \( J = 7.2 \) Hz, 2\text{H}, \text{CH}_2\text{NH}); 1.99 (s, 3\text{H}, \text{Me}); 1.2-1.6 (m, 8\text{H}, \text{hnH}2, \text{hnH}3, \text{hnH}4, \text{hnH}5). \( \delta_C(\text{D}_2\text{O})-176.51 (\text{C}=	ext{O}); 104.81, 104.73, 104.61, 104.43, 103.36 (\text{C}1); 85.72 (\text{C}4\text{A}); 84.03, 83.89, 83.75, 82.83 (\text{C}4); 76.48, 76.04, 75.85, 75.65, 74.79, 74.45 (\text{C}2, \text{C}3, \text{C}5); 71.24

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(C5\(^\alpha\)); 63.04, 62.90 (C6); 50.47 (C6\(^\alpha\)); 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 \(\times\) 10\(^{-3}\) mol) in dry acetonitrile (0.1 cm\(^3\)) was added to a stirred solution of the cyclodextrin 24 (15 mg, 0.012 \(\times\) 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\(_2\)O (0.8 cm\(^3\)). The 300 MHz \(^1\)H NMR spectrum of this solution showed the presence of a complex mixture of products. Signals due to acyl methyl groups appeared at \(\delta\) 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 \(\geq\) 12 by the addition of sodium hydroxide and left to stand at room temperature for 2 hours. The 300 MHz \(^1\)H NMR spectrum of this solution showed that the signal at \(\delta\) 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 \(\times\) 10\(^{-3}\) mol) in place of 4-nitrophenyl acetate 48. The 300 MHz \(^1\)H NMR spectrum of the solution after work-up showed signals at \(\delta\) 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 \(\times\) 10\(^{-4}\) mol dm\(^{-3}\) in ester 48 and either 2.0 \(\times\) 10\(^{-2}\) mol dm\(^{-3}\) or 8.0 \(\times\) 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 cm³ 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

<table>
<thead>
<tr>
<th>cyclodextrin</th>
<th>10³k–obs/s⁻¹</th>
<th>10³average k–obs/s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>4.24, 4.49, 4.59</td>
<td>4.44</td>
</tr>
<tr>
<td>22</td>
<td>2.17, 2.19 (0.704, 0.606, 0.678)ᵇ</td>
<td>2.18 (0.663)ᵇ</td>
</tr>
<tr>
<td>24</td>
<td>0.943, 0.916, 0.889</td>
<td>0.916</td>
</tr>
</tbody>
</table>

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 6⁴-(6-aminohexyl)amino-6⁴-deoxy-β-cyclodextrin 24**

1D proton spectrum data: δ_H 4.65 (m, 7H, H1); 3.81 (t, J = 9.6 Hz, 1H, H5₄); 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, H4₄); 2.92 (d, J = 14.0 Hz, 1H, H6₄); 2.58 (dd, J = 14.0, 9.6 Hz, 1H, H6₄); 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 (H5₇), 3.7 (H₃?); 1.69 (AdH4) shows cross-peaks with 1.45 (AdH₄'), 1.99 (AdH2), 3.53 (H5₇), 3.7 (H₃?); 1.76 (AdH2) shows cross-peaks with 1.99 (AdH2), 3.53 (H5₇), 3.7 (H₃?); 1.99 (AdH3) shows cross-peaks with 1.45 (AdH₄'), 1.69 (AdH4), 1.76 (AdH2), 3.53 (H5₇), 3.7 (H₃?); 2.23 (hnH1) shows cross-peaks with 2.42 (hnH6), 2.92 (H6₄), 3.81 (H₅₇); 2.42 (hnH6) shows cross-peaks with 2.23 (hnH1), 3.81 (H₅₇); 2.58 (H₆₄') shows cross-peaks with 2.92 (H₆₄), 3.06 (H₄₄); 2.92 (H₆₄) shows cross-peaks
with 2.58 (H6A), 3.81 (H5A); 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 (H5A) shows cross-peaks with 2.23 (hnH1), 2.42 (hnH6), 2.92 (H6A).

E.4. Experimental for Chapter 4

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

6A-(12-aminododecyl)amino-6A-deoxy-β-cyclodextrin 60

A solution of the tosylate 32 (1.256 g, 0.974 x 10^{-3} mol) and 1,12-diaminododecane (0.620 g, 3.10 x 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 x 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_{4}OH. 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_{2}O_{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_{2}O (C_{54}H_{102}N_{2}O_{37}) C, 47.29; H, 7.49; N, 2.04%). \delta_{H}(D_{2}O, p\text{H} \sim 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, H4A); 3.09 (m, 1H, H6A); 2.3-2.9 (m, 5H, H6A', ddnH1, ddnH12); 1.0-1.7 (m, 20H, ddnH). \delta_{H}(D_{2}O, p\text{H} \sim 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, H4A); 2.87 (m, 1H, H6A); 2.58 (m, 1H, H6A'); 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). δC(D2O, pH ~ 10, 50 MHz) 104.9 (C1); 87.0 (C4A); 83.7 (C4); 76.2, 74.8 (C2, C3, C5); 70.0 (C5A); 62.6 (C6); 51.2, 49.4, 47.3 (C6A, ddnC1); 42.9 (ddnC12); 32.3, 31.9, 30.9, 29.7, 28.6, 27.5, 26.6 (ddnC). δC(D2O, pH ~ 14, 75 MHz) 106.3, 106.2, 106.1, 105.8 (C1); 87.9 (C4A); 84.6, 84.4, 84.3 (C4); 77.2, 77.0 (C2); 76.4 (C3); 75.3, 75.1 (C5); 71.2 (C5A); 63.1 (C6); 51.9, 50.2 (ddnC1, C6A); 43.7 (ddnC12); 34.9, 31.3, 31.1, 29.4, 28.8 (ddnC).

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

1D proton spectrum data: δH 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, H4A); 2.87 (m, 1H, H6A); 2.58 (m, 1H, H6A'); 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: δH 1.1-1.4 (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 (H5A?); 2.52 (ddnH12) shows cross-peaks with 1.1-1.4 (ddnH2-ddnH11), 3.63 (H3?); 2.58 (H6A') shows cross-peaks with 2.87 (H6A), 3.03 (H4A); 2.87 (H6A) shows cross-peaks with 2.58 (H6A'), 3.5 (H5?), 3.7 (H5A?); 3.03 (H4A) shows cross-peaks with 2.58 (H6A'), 3.63 (H3?), 3.7 (H5A?); 3.5-3.8 (H3, H5) shows cross-peaks with 1.1-1.4 (ddnH2-ddnH11), 2.42 (ddnH1), 2.52 (ddnH12), 2.87 (H6A), 3.03 (H4A).

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

1D proton spectrum data: δH 4.76 (m, 7H, H1); 3.2-3.8 (m, 39H, H2-H6); 3.05 (t, J = 9.0 Hz 1H, H4A); 2.81 (d, J = 13.2 Hz, 1H, H6A); 2.58 (dd, J = 9.0, 13.2 Hz, 1H, H6A'), 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: δH 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), 1.38 (AdH4'), 1.61 (AdH4), 1.89 (AdH3); 3.55 (H6?) shows cross-peaks with 1.0-1.2 (ddnH3-ddnH10), 1.38 (AdH4'), 1.61 (AdH4), 1.89 (AdH3); 3.44 (H5?) 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 cm³) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite which was washed with dichloromethane (3 x 5 cm³) and the combined filtrate was washed successively with 5% sodium bicarbonate solution (3 x 25 cm³) and brine (25 cm³) and dried over sodium sulfate. The filtered solution was evaporated under reduced pressure and the residue was dissolved in dichloromethane (2 cm³) and subjected to flash chromatography (column 1.5 cm i.d., eluted with dichloromethane). 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 δH (CDCl₃) 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). δC (CDCl₃) 171.6, 168.7, 155.3, 145.2, 125.1, 122.3, 55.7, 55.4, 51.5, 47.1, 47.0. I.R. (CDCl₃) 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.

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

A mixture of 4-nitrophenol 49 (0.142 g, 1.02 x 10⁻³ mol), 1-methoxycarbonyl-2,3-dimethyl-cubane-4-carboxylic acid 63 (0.245 g, 1.05 x 10⁻³ mol) and dicyclohexylcarbodiimide (0.220 g, 1.07 x 10⁻³ mol) in dry dichloromethane (5 cm³) was stirred at room temperature for 20 hours. The reaction mixture was filtered through a pad of Celite which was washed with dichloromethane (3 x 5 cm³) and the combined filtrate was evaporated under reduced pressure. The residue was dissolved in chloroform (2 cm³) and subjected to flash chromatography (column 1.5 cm i.d., eluted with chloroform). 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 (CDCl₃) 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 (CDCl₃) 171.0, 168.3, 155.4, 145.3, 125.1, 122.4, 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 x 10⁻³ mol), adamantane-1-carboxylic acid 59 (0.182 g, 1.0 x 10⁻³ mol) and dicyclohexylcarbodiimide (0.218 g, 1.1 x 10⁻³ mol) in dichloromethane (5 cm³) 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 cm³). The filtrate was loaded onto a squat column (30 g, 4.5 cm i.d.) and eluted with dichloromethane (100 cm³). 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
Experimental

des. (CDCl₃) 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). δC (CDCl₃) 175.1, 156.0, 145.1, 124.9, 122.3, 41.0, 38.4, 38.1, 27.5.

IR. (nujol) 1747 (s) (C=O); 1614 (m), 1589 (m), 1438 (m) (Ar); 1519 (s), 1342 (s) (NO₂); 1199 (s), 1049 (s) (C-O).

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

A solution of the cyclodextrin 24 (0.128 g, 1.0 x 10⁻⁴ mol) and 1-methoxycarbonyl-4-(4-nitrophenoxycarbonyl)cubane 64 (0.036 g, 1.1 x 10⁻⁴ mol) in dry DMF (2 cm³) was stirred at room temperature for 20 hours. The resultant yellow solution was added dropwise with stirring to 1:1 ether:ethanol (50 cm³) 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 cm³) and the Celite was removed by filtration. The filtrate was acidified with dilute hydrochloric acid and extracted with dichloromethane (3 x 20 cm³). 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%). RC = 1.11. Electrospray-ms m/z 1421 (M⁺). (Found C, 45.71; H, 6.79; N, 1.75. Calculated for C₅₉H₁₀₃ClN₂O₄₂ C, 45.77; H, 6.70; N, 1.80%.) δH (D₂O) 4.76 (bs, 7H, H1); 4.2 (m, 6H, cubaneH); 3.4-4.0 (m, 42H, CDH, MeO); 3.0-3.4 (m, 4H, C₄A, C₆A, hnH6); 2.8 (m, 1H, C₆A'); 2.55 (m, 1H, hnH1); 2.40 (m, 1H, hnH1'); 1.1-1.5 (m, 8H, hnH2-5). δC (D₂O) 176.5, 175.6 (C=O); 105.0 (Cl); 87.3 (C₄A); 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 (C₆A, cubaneC, hnC1, hnC6, MeO); 25-31 (broad lump, hnC2-5).
Experimental cyclodextrin 68

A solution of the cyclodextrin 24 (0.123 g, 1.0 \times 10^{-4} \text{ mol}) and 1-methoxycarbonyl-4-(4-nitrophenoxycarbonyl)-2,3-dimethylcubane 65 (0.058 g, 1.6 \times 10^{-4} \text{ mol}) in dry DMF (2 cm³) was stirred at room temperature for 20 hours. This solution was diluted with ether (50 cm³) and the resultant precipitate was collected on a pad of Celite. The pad was washed with ether (3 \times 10 \text{ cm³}) and then suspended in water (25 cm³) to dissolve cyclodextrins. The Celite was filtered off and the filtrate was acidified with dilute hydrochloric acid and extracted with dichloromethane (3 \times 20 \text{ cm³}). 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 \( 68.\text{HCl}_4\text{H}_2\text{O} \ (C_{61}\text{H}_{105}\text{ClN}_{10}\text{O}_{41}) \) C, 47.03; H, 6.79; N, 1.80%.) \( \delta_H (\text{D}_2\text{O}) \ 5.04 \) (bs, 7H, H1); 3.5-4.4 (m, 46H, CDH, MeO); 3.0-3.4 (m, 4H, C4A, C6A, hH6); 2.6 (m, 3H, C6A', hH1) 1.1-1.5 (m, 14H, cubaneMe, hH2-5). \( \delta_C (\text{D}_2\text{O}) \ 175.8, 175.5 \) (C=O); 105.1 (C1); 86.7 (C4A); 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 (C6A, cubaneC, hH1, hH6, MeO); 28.5-31.4 (broad lump, hH2-5); 15.4, 14.5, 14.2 (cubaneMe).

6\text{A}-\text{deoxy}-6\text{A}-(6-\text{N-(adamantan-1-oyl)aminohexyl)amino-β-cyclodextrin 69}

A solution of the cyclodextrin 24 (0.130 g, 1.05 \times 10^{-4} \text{ mol}) and 1-(4-nitrophenylcarboxy)-adamantane 66 (0.037 g, 1.22 \times 10^{-4} \text{ mol}) in dry DMF (4 cm³) 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 cm³) and the resultant precipitate was collected by vacuum filtration and washed with ether (3 \times 10 \text{ cm³}). The crude product was dissolved in water (20 cm³) and the solution was acidified with dilute hydrochloric acid and extracted with dichloromethane (3 \times 15 \text{ cm³}). 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 \( m/z \ 1395 (M^+) \). \( \delta_H (\text{D}_2\text{O}) \ 5.03 \) (bs, 7H, H1); 2.5-4.1 (m, 65H, CDH,
hnH1, hnH6); 1.1-2.4 (m, 31H, AdH, hnH2-5). $\delta_C$ (D$_2$O) 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, 75.1, 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, (C6A, 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 6A-deoxy-6A-(6-N-(4-methoxycarbonyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin 67

1D proton spectrum data: $\delta_H$ 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, C4A, hnH6); 3.06 (m, 1H, H6A); 2.87 (m, 1H, C6A); 2.55 (m, 1H, hnH1); 2.40 (m, 1H, hnH1'); 1.1-1.5 (m, 8H, hnH2-5).

2D-ROESY spectrum data: $\delta_H$ 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 6A-deoxy-6A-(6-N-(4-methoxycarbonyl-cuban-1-oyl)aminohexyl)amino-β-cyclodextrin 67

1D proton spectrum data: $\delta_H$ 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, H4A); 3.17 (m, 3H, H6A, hnH6); 2.87 (m, 1H, H6A); 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: $\delta_H$ 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).
Experimental

Self-inclusion of the dimethylcubanyl group in \(6^A\)-deoxy-\(6^A\)-(6-N-(4-methoxycarbonyl-2,3-dimethyl-cuban-1-oyl)aminoheptyl)amino-\(\beta\)-cyclodextrin 68

1D proton spectrum data: \(\delta_H\) 5.04 (bs, 7H, H1); 3.5-4.4 (m, 46H, cubaneH, CDH, MeO); 3.42, (t, \(J = 9.0\) Hz, 1H, H4A); 3.19 (m, 3H, H6A, hnH6); 2.92 (m, 1H, H6A'); 2.64 (m, 2H, hnH1); 1.1-1.5 (m, 14H, hnH2-5, Me).

2D-ROESY spectrum data: \(\delta_H\) 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)aminoheptyl)amino-\(\beta\)-cyclodextrin 68

1D proton spectrum data: \(\delta_H\) 5.05 (bs, 7H, H1); 3.5-4.2 (m, 46H, cubaneH, CDH, MeO); 3.36 (t, \(J = 9.0\) Hz, 1H, H4A); 3.10 (m, 3H, H6A, hnH6); 2.81 (dd, \(J = 7.2, 14.3\) Hz, 1H, H6A'); 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: \(\delta_H\) 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)aminoaoyl)amino-\(\beta\)-cyclodextrin 69

1D proton spectrum data: \(\delta_H\) 5.1 (bs, 7H, H1); 3.5-4.0 (m, 39H, CDH); 3.32 (t, \(J = 9.0\) Hz, 1H, H4A); 3.25 (m, 1H, hnH6); 3.0 (m, 2H, hnH6', H6A); 2.79 (dd, \(J = 9.0, 12.0\) Hz, 1H, H6A'); 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: \(\delta_H\) 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\(^{\text{A}}\)-deoxy-6\(^{\text{A}}\)-(6-N-(adamantan-1-oyl)aminohexyl)amino-\(\beta\)-cyclodextrin 69**

1D proton spectrum data: \(\delta_H\) 5.1 (bs, 7H, H1); 3.5-4.0 (m, 39H, CDH); 3.07 (d, \(J = 12.0\) Hz, 1H, H6\(^{\text{A}}\)); 2.80 (dd, \(J = 9.0, 12.0\) Hz, 1H, H6\(^{\text{A}}\)); 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: \(\delta_H\) 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-nitrophenoxy carbonyl)-cubane 71

A mixture of 4-nitrophenol 49 (0.282 g, 2.00 \(\times 10^{-3}\) mol), cubane-1,4-dicarboxylic acid 70 (0.193 g, 1.00 \(\times 10^{-3}\) mol) and dicyclohexylcarbodiimide (0.411 g, 2.0 \(\times 10^{-3}\) 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 \(\times 20\) 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. \(\delta_H\) (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). \(\delta_H\) (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
Experimental

1,4-bis((6-N-(6-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 cm³) was stirred at room temperature for 20 hours. The reaction mixture was diluted with ether (40 cm³) and the resultant precipitate was collected by vacuum filtration and washed with ether (3 × 10 cm³). The crude product was dissolved in water (20 cm³) and the solution was acidified with dilute hydrochloric acid and extracted with dichloromethane (3 × 15 cm³). 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 dm³) and loaded onto a column of Sephadex G-10 (600 × 20 mm i.d.). The column was eluted with 10% aqueous ethanol and 10 cm³ 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<sub>c</sub> = 0.71. Electrospray-ms m/z 2622 (M⁺). δ<sub>H</sub> (D<sub>2</sub>O) 5.00 (bs, 14H, H1); 2.5-4.4 (m, 142H, cubaneH, CDH, hhH1, hhH6); 1.1-1.8 (m, 16H, hhH2-5). δ<sub>C</sub> (D<sub>2</sub>O) 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<sub>A</sub>, cubaneC, hhCl, hhC6); 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, hhC2-5).

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

600 MHz 1D proton spectrum data: δ<sub>H</sub> 5.06 (s, 14H, H1); 4.16 (s, 6H, cubaneH); 4.01 (t, J = 9.2 Hz, 2H, H5<sup>A</sup>); 3.9-3.5 (m, 76H, H2-H6), 3.40 (t, J = 9.2 Hz, 2H, H4<sup>A</sup>); 3.30 (d, J = 13.2 Hz, 2H, H6<sup>A</sup>); 3.18 (m, 4H, hhH6); 3.01 (m, 2H, H6<sup>A</sup>); 2.80 (m, 2H, hhH1); 2.68 (m, 2H, hhH1'); 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: \( \delta_H \) 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).
References

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References


Appendix 1: Publications arising from this thesis


Preparation and characterization of $\beta^6$-polyamine-mono-substituted $\beta$-cyclodextrins

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* Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

General syntheses for eleven $\beta$-cyclodextrins (cyclomaltoheptaosides) mono-substituted at the C6 position by a polyamine are described. The basis of the synthesis is the reaction of $\beta^6$-O-4(4-methylphenylsulfonyl)-$\beta$-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 $pK_a$ of the protonated amine groups and the $^{13}$C NMR spectra of the modified $\beta$-cyclodextrins with pH are reported.

Introduction

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

We require a range of $\beta$-CDXs which can be produced in reasonable yield and high purity for our host-guest complex and metallo-cyclodextrin studies. Some of these $\beta$-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 $\beta$-CDXs: For the liquid polyamines, heating either $\beta$-cyclodextrin ($\beta$-CD), $\beta^6$-O-4(4-methylphenylsulfonyl)-$\beta$-cyclodextrin ($\beta$-CD@), or $\beta^6$-deoxy-$\beta$-cyclodextrin ($\beta$-CDII) in excess polyamine in a sealed tube yields $\beta$-CDX which requires purification by lengthy chromatographic separation. For either the more expensive liquid or solid polyamines, reaction of $\beta$-CD@ with the polyamine in DMF under similar conditions yields $\beta$-CDX, but we found it difficult to avoid some formulation of the $\beta$-CDX which necessitated tedious separations using this method. We now report a simple general procedure for the synthesis of some reported $\beta$-CDXs where X is either the 2-aminoethylamino, 2-aminoethylpropylamino, 2-aminoethylaminoethylamino, 2-[6-(2-aminoethylamino)ethylamino]ethylamino, 2-[6-(2-aminoethyl)ethylenedioxy-1-y] $\beta$-CD@, 6-aminoethylaminoethylamino, 2-aminoethylaminoethylamino, 2-[6-(2-aminoethylamino)ethylamino]-1-y] $\beta$-CD@, or 1,4,7,10-tetraazacyclodecan-1-y] $\beta$-CD@ and some new $\beta$-CDXs that yield clean products under mild conditions.

The $\beta$-CDX's protonated amine groups exhibit a wide range of $pK_a$ 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 $\beta$-CDX with pH to gain an insight into the factors influencing these characteristics.

Results and discussion

Preparative aspects

The synthesis of $\beta^6$-[2-[6-(2-aminoethylamino)ethylamino]-6-deoxy-$\beta$-cyclodextrin ($\beta$-CDTren)] serves to illustrate pre-


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

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<th>Reaction time (h)</th>
<th>Yield (%)</th>
<th>Elemental analyses (%)</th>
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<tr>
<td>β-CDOn-H2O</td>
<td>42.70</td>
<td>C: 67.2 ± 0.39</td>
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<tr>
<td>β-CDnp-H2O</td>
<td>43.67</td>
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<td>β-CDFe-3H2O</td>
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<td>C: 69.2 ± 0.42</td>
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<td>41.76</td>
<td>C: 69.2 ± 0.43</td>
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<td>β-CDppc-3H2O</td>
<td>42.62</td>
<td>C: 67.9 ± 0.39</td>
</tr>
<tr>
<td>β-CDcm-3H2O</td>
<td>43.42</td>
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<td>C: 71.0 ± 0.46</td>
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<td>42.51</td>
<td>C: 70.5 ± 0.17</td>
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Table 2 pKα values for some protonated aliphatic polyamine-substituted β-cycloextrins and the corresponding free polyamines in aqueous NaClO4 (0.1 M) at 298.2 K

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<th>pKα</th>
<th>Species</th>
<th>pKα</th>
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</table>

* Errors represent one standard deviation. 1 Ref. 7, 2 Ref. 10, 3 Ref. 28, 4 Ref. 29, 5 Ref. 30.

NMP is a dipolar aprotic solvent that has been shown to be superior to DMF for nucleophilic substitution of toluene-2-sulfonyl derivatives but is more stable than DMF under acidic or basic conditions. When β-CDx was heated at 70°C for 4 h with 3.3 equiv. of tritylchloride in DMF, a yield of 67% 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 tritylchloride and water. The formation of β-CDx in the reaction was shown by TLC of the reaction mixture during the course of the reaction. A series of β-CDx, having either linear, branched or cyclic polyamine substituents, was prepared under the same conditions (Table 1). All of the β-CDx prepared by this procedure were shown to be pure by TLC and 1H and 13C NMR spectroscopy and mass spectrometry. (A referee has pointed out that the cyclic solvent is the diamine increases in size. The increase in pKα magnitude coincides with increases in hydrophobicity as the aliphatic chain lengths and indicates a decrease in the ability of surrounding water to solvate a proton from the protonated amine as overall hydration decreases. The two pKα of β-CDx are less than those of the analogous free diamine.

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 solution 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 five structures is seen in the 13C 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-CDtla and β-CDpnp in Figs. 2 and 3.) A similar interpretation has been presented for β-CDtla (where pKα magnitude increases in the sequence -NH4+ ? NH2+ ? CDβ-NH2+ ? CDβ-NH2+ and that is identified by 13C NMR spectroscopy) which together with its β-CDtla homologue shows similar trends (Table 2) to those discussed above. Generally, similar trends in pKα magnitudes are observed for the polypeptide β-CDx as for their diamine analogues and their origins are probably similar.

The substrates X on the β-CDx C6 carbon of the A glucose-pyranose unit renders it and the other six glucopyranose (often labelled B-G) inequivalent, and as a result they each exhibit six 13C unique resonances to give a total of 42 resonances when the magnetic inequivalence is sufficiently large.
Appendix

Experimental

Materials and Instrumental methods

The polyanilines 1,2-diaminobenzene (ben), 1,3-diaminopropane (prop), 1,4-diaminobutane (but), 1,6-diaminohexane (hex), 2,4-diaminoethylenelamintetrahydroxymethane (dim), 3,3'-diaminopropylamine (propamine) (dipn), 3,3'-diaminoethylaminotetrahydroxymethane (dipn), 3,3'-diaminopropylamine (dipn), and 3,3'-diaminoethylaminotetrahydroxymethane (dipn) (NMP, Aldrich) were purified by two recrystallizations from ethanol-water. 

The 1H NMR spectra of the polyanilines were recorded on a Microsystem 900 spectrometer at 25°C, using CDCl3 as solvent, and the chemical shifts were referred to TMS as internal standard. The 13C NMR spectra of the polyanilines were recorded on a Bruker AC 300 spectrometer at 75.47 MHz (13C) for all β-CDXs except for 6'-deoxy-6'-deoxy-6'-deoxy-6'-deoxy-cyclodextrin (β-CDX) where a Varian Gemini 200 spectrometer operating at 200 (1H) and 50.29 MHz (19F) was used.

All solutions were prepared with water purified to give a specific resistance of >15 MΩ cm. The pH of the solutions was adjusted to the appropriate values using 0.1 M HCl or NaOH.

The CD spectra were recorded on a Jasco J-815 spectropolarimeter using a 0.1 cm pathlength cell containing an 1.0 mg/mL solution of the polyaniline in DMSO-d6. The CD spectra were corrected for the solvent contribution.

The 1H NMR spectra of the polyanilines 1-4 were obtained at 25°C, using either DMSO-d6 or CDCl3 as solvents, and the chemical shifts were referred to TMS as internal standard. The 13C NMR spectra of 1-4 were recorded on a Varian Gemini 200 spectrometer operating at 75.47 MHz (13C) and 125 MHz (1H) for all β-CDXs except for 6'-deoxy-6'-deoxy-6'-deoxy-6'-deoxy-cyclodextrin (β-CDX) where a Varian Gemini 200 spectrometer operating at 200 (1H) and 50.29 MHz (19F) was used.

The CD spectra were recorded on a Jasco J-815 spectropolarimeter using a 0.1 cm pathlength cell containing an 1.0 mg/mL solution of the polyaniline in DMSO-d6. The CD spectra were corrected for the solvent contribution.

General procedure for preparation of amino-substituted β-cyclodextrins

A solution of β-CDX in 0.00 g, 1.55 × 10⁻¹ mol, K10, 0.25 g, 0.15 × 10⁻¹ mol in D2O. NMR is

Appendix

6'-4-[2-[(2-aminoethyl)amino]ethylamino]-4'-deoxy-β-cycloDEXTRIN (β-CDrere)

A mixture of β-CD (2.048 g, 1.59 x 10^(-2) mol), tri-2-aminoethylamine (0.74 g, 5.07 x 10^(-2) mol) and KCl (0.024 g) in NMP (5 cm^3) was treated according to the general procedure to give β-CDrere as a white powder (1.192 g, 59%). Rf: 0.31.

Electrophoresis under the condition of the gel (123 M, *M*), C: 43.84 H: 7.28 N: 4.40. Calc.: for β-CDrere, H_2O (C_6H_11NO_5), C: 43.76; H: 7.04; N: 4.25. β-CD: C_6D_10O_16H_22, pH 7.0 (0.01M NaCl)

1.5-3.8 (m, 6H), 3.8-7.0 (m, 12H), 4.3-5.6 (m, 4H), 6.4-7.6 (m, 6H).

Acknowledgements

We are grateful for the award of an Australian Postgraduate Award to S. D. K. and to the Australian Research Commission for supporting this research and to Nihon Shokuhin Kako Co for a gift of β-cycloextrin.

References


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Host-guest complexation of aromatic carboxylic acids and their conjugate bases by 6-(o-aminoalkylamino)-6'-deoxy-β-cyclo-
dextrins† in aqueous solution

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A potentiometric titration study of the complexation of the guest benzoic acid, 4-methoxybenzoic acid and (AR)-2-
phenylpropanoic acid (Fig. 1) with the 6-(o-aminoalkylamino)-6'-deoxy-β-cyclodextrins, where

\[
\text{Host-guest complexation of aromatic carboxylic acids and their conjugate bases by 6-(o-aminoalkylamino)-6'-deoxy-β-cyclo-
dextrins† in aqueous solution}
\]

Appendix

PERKIN

INTRODUCTION

The range of cyclodextrins (CDs), their modified forms and the host-guest complexes formed by them is extensive. In most

†E-Cyclodextrin = cycloheptanotetrasiloxane.

action of all solutions. A Metrohm Dosimat E565 titrator, an Orion 5A T50 potentiometer and an Orion 1172 Ross Surface

balance were used. The titrations were performed at 298.2 ± 0.1 K in a water-jacketed 20 cm³ titration vessel which

was stirred at 300 rpm to reach thermal equilibrium. Sodium hydroxide solution (0.10 mol dm⁻³) was used in all titrations. During
each titration a small stream of nitrogen bubble was passed through the solution which was continuously stirred and thermostated at
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the solution which was continuously stirred and thermostated at 298.2 ± 0.1 K in a water-jacketed 20 cm³ titration vessel.
The 'H NMR (1D and 2D ROESY (mixing time of 0.35 s)) experiments were run on a Varian Inova 600 spectrometer. Either [CD$_3$]CH$_2$NH$_2$ or equimolar amounts of the guest species and [CD$_3$]CH$_2$NH$_2$ were dissolved in D$_2$O to give total concentrations of 0.05 mol dm$^{-3}$ of each species, and the pH was adjusted to 7.01. The resultant assignments are: 1D 'H spectrum; $\delta$ 6.10 (m, 4H, 1H$_{a}$, 1H$_{b}$, 1H$_{c}$, 1H$_{d}$); 2.60 (m, 2H, 1H$_{e}$, 1H$_{f}$); 1.40 (s, 4H, 1H$_{g}$, 1H$_{h}$), 1.70 (br, 4H, 2H, 2H). The 2D ROESY spectrum shows the cross-peaks of $\delta$ 1.25 (H$_{a}$) shows cross-peaks with 1.39 (H$_{b}$), 2.45 (H$_{c}$), 3.22 (H$_{d}$), 3.73 (H$_{e}$), 4.67 (H$_{f}$) shows cross-peaks with 2.93 (H$_{g}$), 3.39 (H$_{h}$), 2.91 (H$_{i}$) shows cross-peaks with 2.87 (H$_{j}$), 3.17 (H$_{k}$), 3.8 (H$_{l}$) shows cross-peaks with 3.35 (H$_{m}$), 3.19 (H$_{n}$), 3.71 (H$_{o}$), 3.67 (H$_{p}$) shows cross-peaks with 2.93 (H$_{q}$), 3.39 (H$_{r}$), 2.91 (H$_{s}$) shows cross-peaks with 2.87 (H$_{t}$), 3.17 (H$_{u}$), 3.8 (H$_{v}$) shows cross-peaks with 3.35 (H$_{w}$), 3.19 (H$_{x}$), 3.71 (H$_{y}$), 3.67 (H$_{z}$) shows cross-peaks with 2.93 (H$_{A}$), 3.39 (H$_{B}$), 2.91 (H$_{C}$) shows cross-peaks with 2.87 (H$_{D}$), 3.17 (H$_{E}$), 3.8 (H$_{F}$) shows cross-peaks with 3.35 (H$_{G}$), 3.19 (H$_{H}$), 3.71 (H$_{I}$), 3.67 (H$_{J}$) shows cross-peaks with 2.93 (H$_{K}$), 3.39 (H$_{L}$), 2.91 (H$_{M}$) shows cross-peaks with 2.87 (H$_{N}$), 3.17 (H$_{O}$), 3.8 (H$_{P}$) shows cross-peaks with 3.35 (H$_{Q}$), 3.19 (H$_{R}$), 3.71 (H$_{S}$), 3.67 (H$_{T}$) shows cross-peaks with 2.93 (H$_{U}$), 3.39 (H$_{V}$), 2.91 (H$_{W}$) shows cross-peaks with 2.87 (H$_{X}$), 3.17 (H$_{Y}$), 3.8 (H$_{Z}$) shows cross-peaks with 3.35 (H$_{a}$), 3.19 (H$_{b}$), 3.71 (H$_{c}$), 3.67 (H$_{d}$) shows cross-peaks with 2.93 (H$_{e}$), 3.39 (H$_{f}$), 2.91 (H$_{g}$) shows cross-peaks with 2.87 (H$_{h}$), 3.17 (H$_{i}$), 3.8 (H$_{j}$) shows cross-peaks with 3.35 (H$_{k}$), 3.19 (H$_{l}$), 3.71 (H$_{m}$), 3.67 (H$_{n}$) shows cross-peaks with 2.93 (H$_{o}$), 3.39 (H$_{p}$), 2.91 (H$_{q}$) shows cross-peaks with 2.87 (H$_{r}$), 3.17 (H$_{s}$), 3.8 (H$_{t}$) shows cross-peaks with 3.35 (H$_{u}$), 3.19 (H$_{v}$), 3.71 (H$_{w}$), 3.67 (H$_{x}$) shows cross-peaks with 2.93 (H$_{y}$), 3.39 (H$_{z}$), 2.91 (H$_{A}$) shows cross-peaks with 2.87 (H$_{B}$), 3.17 (H$_{C}$), 3.8 (H$_{D}$) shows cross-peaks with 3.35 (H$_{E}$), 3.19 (H$_{F}$), 3.71 (H$_{G}$), 3.67 (H$_{H}$) shows cross-peaks with 2.93 (H$_{I}$), 3.39 (H$_{J}$), 2.91 (H$_{K}$) shows cross-peaks with 2.87 (H$_{L}$), 3.17 (H$_{M}$), 3.8 (H$_{N}$) shows cross-peaks with 3.35 (H$_{O}$), 3.19 (H$_{P}$), 3.71 (H$_{Q}$), 3.67 (H$_{R}$) shows cross-peaks with 2.93 (H$_{S}$), 3.39 (H$_{T}$), 2.91 (H$_{U}$) shows cross-peaks with 2.87 (H$_{V}$), 3.17 (H$_{W}$), 3.8 (H$_{X}$) shows cross-peaks with 3.35 (H$_{Y}$), 3.19 (H$_{Z}$), 3.71 (H$_{a}$), 3.67 (H$_{b}$) shows cross-peaks with 2.93 (H$_{c}$), 3.39 (H$_{d}$), 2.91 (H$_{e}$) shows cross-peaks with 2.87 (H$_{f}$), 3.17 (H$_{g}$), 3.8 (H$_{h}$) shows cross-peaks with 3.35 (H$_{i}$), 3.19 (H$_{j}$), 3.71 (H$_{k}$), 3.67 (H$_{l}$) shows cross-peaks with 2.93 (H$_{m}$), 3.39 (H$_{n}$), 2.91 (H$_{o}$) shows cross-peaks with 2.87 (H$_{p}$), 3.17 (H$_{q}$), 3.8 (H$_{r}$) shows cross-peaks with 3.35 (H$_{s}$), 3.19 (H$_{t}$), 3.71 (H$_{u}$), 3.67 (H$_{v}$) shows cross-peaks with 2.93 (H$_{w}$), 3.39 (H$_{x}$), 2.91 (H$_{y}$) shows cross-peaks with 2.87 (H$_{z}$), 3.17 (H$_{A}$), 3.8 (H$_{B}$) shows cross-peaks with 3.35 (H$_{C}$), 3.19 (H$_{D}$), 3.71 (H$_{E}$), 3.67 (H$_{F}$) shows cross-peaks with 2.93 (H$_{G}$), 3.39 (H$_{H}$), 2.91 (H$_{I}$) shows cross-peaks with 2.87 (H$_{J}$), 3.17 (H$_{K}$), 3.8 (H$_{L}$) shows cross-peaks with 3.35 (H$_{M}$), 3.19 (H$_{N}$), 3.71 (H$_{O}$), 3.67 (H$_{P}$) shows cross-peaks with 2.93 (H$_{Q}$), 3.39 (H$_{R}$), 2.91 (H$_{S}$) shows cross-peaks with 2.87 (H$_{T}$), 3.17 (H$_{U}$), 3.8 (H$_{V}$) shows cross-peaks with 3.35 (H$_{W}$), 3.19 (H$_{X}$), 3.71 (H$_{Y}$), 3.67 (H$_{Z}$) shows cross-peaks with 2.93 (H$_{a}$), 3.39 (H$_{b}$), 2.91 (H$_{c}$) shows cross-peaks with 2.87 (H$_{d}$), 3.17 (H$_{e}$), 3.8 (H$_{f}$) shows cross-peaks with 3.35 (H$_{g}$), 3.19 (H$_{h}$), 3.71 (H$_{i}$), 3.67 (H$_{j}$).
Table 1

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>$\Delta G_{m}^{\ddagger}$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) $\text{CDNH}<em>{3}^+ + \text{benzoic acid} \rightleftharpoons [\text{CDNH}</em>{3}^+\text{benzoic acid}]^+$</td>
<td>820 ± 170</td>
</tr>
<tr>
<td>(2) $\text{CDNH}<em>{3}^+ + \text{benzoic acid} \rightleftharpoons [\text{CDNH}</em>{3}^+\text{benzoic acid}]^+$</td>
<td>870 ± 70</td>
</tr>
<tr>
<td>(3) $\text{CDNH}<em>{3}^+ + \text{benzoic acid} \rightleftharpoons [\text{CDNH}</em>{3}^+\text{benzoic acid}]^+$</td>
<td>915 ± 60</td>
</tr>
</tbody>
</table>

The $\Delta G_{m}^{\ddagger}$ values for the reaction of $\text{CDNH}_{3}^+$ with benzoic acid were determined in aqueous solution at $\ell = 0.10$ mol dm$^{-3}$ (NaClO$_4$) and 298.2 K.

Trhid. Equilibria and $pK_a$ values determined in aqueous solution at $\ell = 0.10$ mol dm$^{-3}$ (NaClO$_4$) and 298.2 K.

The $pK_a$ of $\text{CDNH}_{3}^+$ is 4.00 and 4.20 and 4.43, respectively. From reference 2.

A schematic representation of the variation of complex stability and the factors underlying it is.

Fig. 3 A schematic representation of the variation of complex stability and the factors underlying it.
The titration curves for 0.001 mol dm⁻³ solution of BCD-N\(\text{CH}_2\text{CN}=\text{H}_2\) and 0.001 mol dm⁻³ solutions of BCD-N\(\text{CH}_2\text{CN}=\text{H}\) and 4-methylbenzenediazonium tetrafluoroborate respectively at 0.020 mol dm⁻³ in CH\(_2\text{CN}=\text{H}_2\) at 25°C in the presence of 0.00001 mol dm⁻³ of Na\(\text{H}_2\text{PO}_4\).

The ¹H NMR (CD\(_3\)CN) and 2D ROESY (mixing time of 0.35 s) experiments were run on a Varian Inova 600 spectrometer. Either BCD-N\(\text{CH}_2\text{CN}=\text{H}_2\) alone or equivalent amounts of the guest species and BCD-N\(\text{CH}_2\text{CN}=\text{H}\) were dissolved in D\(_2\)O to give total concentrations of 0.05 mol dm⁻³ of each species, and the pH was adjusted to 7.1. The resultant solutions were filtered and degassed by freeze-drying before the spectra were recorded. The spectral assignments below are according to the glucopyranose numbering system H1-16 (where a superscript A specifies the glucopyranose unit bearing the 1-6-Sambucus substructure) in Figure 1. All the proton signals are labelled H\(_n\) as it becomes appears from the secondary amine group, and aromatic guest protons are labelled as H\(_n\) and H\(_n\) where the former is adjacent to the carbohydrate group.

The experimental assignments for BCD-N\(\text{CH}_2\text{CN}=\text{H}_2\) alone at pH 7.15 are: 1D ¹H spectra: \(\delta_0.40\) (H\(_9\) in solvent, H\(_1\)); 1.5-3.5 ppm (H\(_2\), H\(_3\), H\(_4\), H\(_5\), H\(_6\), H\(_7\), H\(_8\), H\(_9\), H\(_10\), H\(_11\), H\(_12\), H\(_13\), H\(_14\), H\(_15\), H\(_16\); 5.3-6.0 ppm (H\(_1\) and H\(_2\)); 3.1-3.3 ppm (H\(_3\) and H\(_4\)); 2.9 ppm (H\(_5\) and H\(_6\)); 1.2-1.5 ppm (H\(_7\) and H\(_8\)); 0.8-1.0 ppm (H\(_9\) and H\(_10\)); 0.3 ppm (H\(_11\) and H\(_12\)); 0.2 ppm (H\(_13\) and H\(_14\)); 0.1 ppm (H\(_15\) and H\(_16\)).

The 2D ROESY spectrum shows the following cross-peaks: 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)); 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)).

The ROESY spectrum shows the following cross-peaks: 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)); 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)).

The stability constants of the complex was calculated using the ROESY spectrum and the following cross-peaks: 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)); 1.36 (H\(_9\)-H\(_9\)) shows an interaction with 2.30 (H\(_9\)-H\(_9\)).

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guest and complex hydration superimposing on the stabilizing effect of the hydrophobic phenyl group of the guest into the hydrophobic centre of the SCD annulus.

The SCD annulus and the two amine groups represent consis
tent structural features in the four SCDNH(CH$_2$)$_3$NH$_2$ hosts. Hence, variations in effects (3-4) generated by the CD hosts arise predominantly from SCD changes in the length of the +NH(CH$_2$)$_n$NH$^-$ substituents indicated by n. The phenyl ring and the carboxylic acid group are invariant in all four guest carboxylic acids, and therefore differences in their complexation characteristics arise from differences in hydrophilicity and stereoechemistry engendered by the CH$_2$ and CH$_2$CH mole
cles in 4-methylbenzoic acid and (A) or (B-2-phenylpropionic acid), respectively, when compared with those of benzoic acid.

Spectroscopic details in Table 3 are equivalent to increasing solubility pH so that the host's charge decreases with decreasing protonation and its hydrophilicity decreases. On the horizontal axis, host hydrophobicity increases and the charge separation in the diprotonated host increases as n in SCDNH(CH$_2$)$_n$NH$_2$ increases. Thus, in the upper left hand corner of Fig. 3, the host has a dispersive 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 stabilizes the complex. The intensity of this interaction probably diminishes as it increases and the changes in SCDNH(CH$_2$)$_n$NH$_2$ Moderately move further apart as that the stability 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 SCDNH(CH$_2$)$_n$NH$_2$ charge as its protonation changes as gauged 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 compound formed between SCD and SCDNH$_2$ and the same carboxylates as those studied here are characterized by stability constants in the range 10$^{-13}$-10$^{-19}$ M$^{-1}$ while the complex constants by equilibrium (3) provide SCDNH$_2$, with the analogous acids are characterized by stability constants in the range 10$^{-13}$-10$^{-17}$ M$^{-1}$. However, as hydrophilicity increases with n as Fig. 3 is traversed from left to right, complexation of the carboxylic acid guest structures 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 stabilization, it also appears (from the NMR and molecular modelling studies discussed below) that the NH$^-$

-CH$_2$NH$^-$ substituent enters the annulus in basic solution, both in SCD and SCDNH$_2$, and in its carboxylate complex.

As the SCDNH(CH$_2$)$_n$NH$_2$ carboxylate complexes are of the more stable species, it seems that this latter effect stabilizes both host-guest complexes while the short -NH$_2$-CH$_2$-NH$_2$ substituent is least effective in this role. Similar self-complications are observed for the pendant naphthyl groups and pendant dimyristoyl group of 4',6'-N-(4-chloromethyl-1-

-phenylpyridinium)dimyristoyl-2'-deoxy-beta-cyclodextrin and 3',5'-N-(4-naphthylpropyl)-2',5'deoxy-beta-cyclodextrin, respectively. The charged -NH$_2$-CH$_2$-NH$_2$, substituent does not appear to enter the SCD annulus as discussed below.

Superimposed on these effects is that of hydration. The hydration of (CD)NH$_2$CH$_2$NH$_2$ and its protonated analogues probably has two main components: water occupying the annulus but interesting weakly with the methyl, methine and ether oxygen atoms defining the hydrophobic centre of the annulus, and water hydrogen bonding with the hydroxyl groups at either end of the annulus. From 6-6.5 H$_2$O have been observed in the SCD annulus in solid state neutron and X-ray diffraction studies.

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. Depending on whether it is SCDNH(CH$_2$)$_n$NH$_2$ 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 hydration by that of the guest is likely to produce a decrease in the overall hydration of the host 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 (3) and (3), (6) and (7), (10) and (11), and (14) and (13) in Table 1. Hydration effects on stability are likely to be less for equilibria (5), (5), (7), (7), (11) and (16) where no change in overall charge occurs on complexation. Thus, a degree of complex stabilization may be achieved through minimizing 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 its hydrophilic moieties.

There is no apparent systematic stereosteric effect of the variation of n in SCDNH(CH$_2$)$_n$NH$_2$ and its conjugate acids and of the stereocchemistry of the carboxylic acid and the conju
gate base other than those subsumed into the discussion of factors (3-4) above. However, there is a small chiral discrimination in equilibria (9) and (12) when n = 2 and 3, where SCDNH$_2$CH$_2$NH$_2$ (B-2-phenylpropionic acid) is more stable than its (D)-analogues when n = 3 and vice versa when n = 2. Although small, these differences are consistent with o of the NH$_2$-CH$_2$-NH$_2$ substituents influencing guest orientation as it also the ease for the NH$_2$-CD$_2$ substituent where n = 2 and 6 in equilibria (12) and (16).

NMR structural studies

The detailed assignment of the H NMR spectrum of SCD-

-CH$_2$NH$_2$ at pH 11.5 (Fig. 4) is presented in the experi
cental section, and the cross-peaks observed in the ROESY and COSY spectra are shown in Fig. 6 and Table 3. Typical cross-peaks arising from interaction between H-$

O$-His of the -NH$_2$-CH$_2$-NH$_2$ substituent and H and H of the SCD annulus and H$_2$O and H$_2$O are consistent with recombination of -NH$_2$-CH$_2$-NH$_2$ inside the annulus as shown schematically in Fig. 5. These cross-peaks are absent from the ROESY spectrum obtained after saturation of the sample solution to pH 1 with hydrochloric acid consistent with the protonated -NH$_2$-CH$_2$-NH$_2$ substituent being excluded from the annulus as a result of decreased hydrophilicity.

The detailed data discussed above indicate that the SCD

-CH$_2$NH$_2$CH$_2$NH$_2$ (4-methylbenzenesulphonamide) complex illustrates 33% of the total SCDNH$_2$CH$_2$NH$_2$ and (4-methylenethane) at the pH 11.5 of the NMR study. The H chemical shifts and the spectral resolution of the -NH$_2$-CH$_2$-NH$_2$ substituent methyl signals of SCDNH$_2$CH$_2$NH$_2$ and SCDNH$_2$CH$_2$NH$_2$ (Fig. 5 and Table 3) are consistent with the assignment of the -NH$_2$-CH$_2$-NH$_2$ substituent being inside the SCD annulus and parallel to the first of the aromatic rings where they experience an anisotropically field arising from the high $\pi$ electron density of the guest. Cross-peaks between H-$

O$-His of -NH$_2$-CH$_2$-NH$_2$ and H and H and H$_2$O and H of the 4-methylenethane and H$_2$O (Fig. 6 and Table 2) are consistent with the assignment of both entities in the SCD annulus. However, there are no cross-peaks due to interactions between the -NH$_2$-CH$_2$-

NH$_2$ substituent and 4-methylenethane. These data are consist
ent with either a single complex where the carboxylate interacts with the primary or the tertiary face (Fig. 5) of the SCD annulus (respectively delocalized by primary and secondary bonds) as discussed below.

Appendix

The table below shows the 1H NMR cross-peaks observed for BCDNH(CH$_2$)$_2$NH$_2$ and its 4-methylbenzene and (S)-2-phenylpropanoate complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Protons</th>
<th>Cross-peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCDNH(CH$_2$)$_2$NH$_2$</td>
<td>Anular protons</td>
<td>6-Aminothiazole substituent protons</td>
</tr>
<tr>
<td></td>
<td>H$_3$</td>
<td>H$_2$b H$_3$</td>
</tr>
<tr>
<td></td>
<td>H$_3^*$</td>
<td>+ + + + + +</td>
</tr>
</tbody>
</table>

* The intensity of the cross peaks increases from + to ++. The concentrations of BCDNH(CH$_2$)$_2$NH$_2$, and either 4-methylbenzene or (S)-2-phenylpropanoate, were 0.06 and 0.04 mM, respectively.

**Fig. 4.** The 1H 600 MHz ROESY NMR spectrum of BCDNH-(CH$_2$)$_2$NH$_2$. Cross-peaks are formed between H$_3$ and H$_2$, H$_a$-H$_b$, and H$_3$ and H$_3$ and H$_2$ and H$_3$.

**Fig. 5.** Schematic representations of the structures of (a) the intramolecular complex formed by BCDNH(CH$_2$)$_2$NH$_2$, and (b) the intermolecular (BCDNH(CH$_2$)$_2$NH$_2$ +4-methylbenzene) complex.

**Fig. 6.** The 1H 600 MHz ROESY NMR spectrum of the (BCDNH(CH$_2$)$_2$NH$_2$ +4-methylbenzene) complex showing cross-peaks formed between H$_3$ and H$_2$, H$_3$ and H$_2$, H$_a$ and H$_b$ of 4-methylbenzene and H$_3$, and Ha of 4-methylbenzene and H$_3$ and H$_2$.

Gas phase force-field molecular modelling produced the global energy minimum (37.7 kJ mol$^{-1}$) structure of BCDNH-(CH$_2$)$_2$NH$_2$ with the $-$NH(CH$_2$)$_2$NH$_2$ substituent complexed inside the BCD annulus as shown in Fig. 7. Similar modelling showed that the $-$NH(CH$_2$)$_2$NH$_2^+$ substituent of BCDNH-(CH$_2$)$_2$NH$_2^+$ does not enter the BCD annulus. Both of these complexes are shown in Fig. 8.
The NH₂-nicotinoyl complex produced a global energy minimum of 44.4 kJ mol⁻¹ for the structure shown in Fig. 8 where 4-methylnicotinoyl is oriented with its carboxylate group towards the secondary face of the pCD annulus and the NH₂(CH₂)₄NH₂ substituent is also completely included outside the pCD annulus consistent with the NMR data. This orientation of the carboxylate group to the secondary face is also found in adamantan-1-carboxylate complexes of pCD and JCD and the cyclohexanecarboxylate complex of pCD. When the 4-methylnicotinoyl 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 favored orientation. The model structure of the [pCDNH(CH₂)₄NH₂-4-methylnicotinoyl] complex shows the 4-methylnicotinoyl guest to have its carboxylate group in the vicinity of the primary face of the pCD annulus. This reversal of orientation, consistent with that in the [pCDNH(CH₂)₄NH₂-4-methylnicotinoyl] complex, is consistent with the charge of the NH₂(CH₂)₄NH₂ substitutes (where n = 0 or 1 and x = 0 or 1) substantially influencing guest orientation through electrostatic interactions. This has also been found to occur in modelling studies of the 4-methylnicotinoyl complex of protonated heptakis(6-mono-6-deoxy-β-cyclohexodextrin and also its amino acid complexes. While our modelling studies show the probable orienting effects of charge in [pCDNH(CH₂)₄NH₂-4-methylnicotinoyl] and [CH₂]₂NH₂-4-methylnicotinoyl], the latter complex was not discussed in solution as discussed above.

Molecular modelling also shows that both the NH₂(CH₂)₄NH₂ substituent and (S)-phenylpropanoate are completely within the pCD annulus in the [pCDNH(CH₂)₄NH₂-p(phenyl-propanoate)] complex. The carboxylate group is oriented towards the secondary face of the pCD annulus. The [pCDNH(CH₂)₄NH₂-(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 pCD annulus. The globalised energy minima of the [pCDNH(CH₂)₄NH₂-p-phenylpropanoate] complex and its (R)-analogues are 718.5 and 712.3 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-(o-aminoalanyl)-1,4-deoxy-β-cyclodextrin hosts and their protonated forms (where the amine/alanylamine groups are NH₂(CH₂)₄NH₂ and n = 3, 4 and 6) and the guest, benzoic acid, 4-methylnicotinic acid and (S)- or (R)-3-phenylpropanoate acid and their congener 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 1H NMR studies show that the NH₂(CH₂)₄NH₂ substituent of [pCDNH(CH₂)₄NH₂-p(phenylpropanoate)] self-complexes inside the pCD annulus, and that in [pCDNH(CH₂)₄NH₂-p-phenylpropanoate] and its (S)-phenylpropanoate analogue both the guest and the NH₂(CH₂)₄NH₂ substituent are simultaneously complexed within the pCD annulus. Gas phase force-field modelling also shows that when both the pCD and pCDNH(CH₂)₄NH₂ 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 NH₂(CH₂)₄NH₂ substituent into the pCD annulus may significantly affect complex stability, as may also be the case for the NH₂(CH₂)₄NH₂ substituents where n = 3, 4 and 6 if they also enter the pCD annulus. In contrast the fully protonated NH₂(CH₂)₄NH₂ substituents does not enter the pCD annulus according to the 'H NMR and molecular modelling studies.

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Shokuhin

References

Appendix

20 Bioprep7 of San Diego.
21 D. M. Laszlo, A. Tene, A. W. Coleman and C. de Rango, Carbohydr. Res., 1996, 282, 121. X-ray crystallography shows \( \text{SCDNNH}_2\text{CH}_2\text{NH}_3 \), molecules align head to tail in a zig-zag array in the crystal. The \(-\text{NH}(\text{CH}_3)\text{NH}_2\) substituents of each \( \text{SCDNNH}_2\text{CH}_2\text{NH}_3 \) enter the annulus of an adjacent \( \text{SCDNNH}_2\text{CH}_2\text{NH}_3 \) from the secondary face so that \( \text{CH}_2\text{N} \) chain is largely within the hydrophobic annulus region with the \(-\text{NH}_2\) group protruding from the primary face. This solid state intermolecular arrangement contrasts with the intramolecular compression proposed in the present solution study in which no exocone for other than monomeric \( \text{SCDNNH}_2\text{CH}_2\text{NH}_3 \) was found.

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Appendix 2: Publications in this area from prior work


