



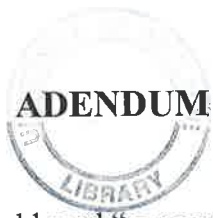
MODIFIED CYCLODEXTRINS AND THEIR COMPLEXES

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Doctor of Philosophy
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ADENDUM

Page ix, paragraph 3, line 6:

“appears to governed” should read “appears to be governed”

Page 23, last line:

“ β CD-NH⁺” should read “ β CD-NH₃⁺”

Page 30, paragraph 1, line 4:

“so to does” should read “so too does”

Page 55, paragraph 1, line 9:

“so to did” should read “so too did”

Page 71, line 12:

“phenylpropanooic acid” should read “phenylpropanoic acid”

Page 93, paragraph 2, line 2:

“The of β CD-X” should read “The pK_as of β CD-X”

Page 94, paragraph 1, line 3:

“methos,” should read “methods,”

Page 94, paragraph 1, line 5:

“cane” should read “can be”

Page 96, paragraph 2, line 3:

“an chiral” should read “a chiral”

Page 109, Figure 4.6, label of the ‘y’ axis:

“speices” should read “Species”

Page 112, paragraph 1, line 5:

“Thus,,” should read “Thus,”

Page 112, paragraph 2, last line:

“absorbtion” should read “absorption”

Page 112, paragraph 3, line 1:

“exhibit a” should read “exhibit”

Page 127, paragraph 2, line 3:

“This lead” should read “This led”

Page 130, paragraph 1, line 5:

“enhaced” should read “enhanced”

Page 152, line 3 (main text):

“verses” should read “versus”

Page 156, Reference 1d:

“R. Delgado,” should read “R. Delgado,”

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DECLARATION

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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ABBREVIATIONS

en	1,2-diaminoethane
pn	1,3-diaminopropane
bn	1,4-diaminobutane
hn	1,6-diaminohexane
dien	2-(2-aminoethyl)aminoethylamine
dipn	3-(3-aminopropyl)aminopropylamine
tacn	1,4,7-triazacyclononane
tacdo	1,5,9-triazacyclododecane
trien	2-(2-(2-aminoethyl)aminoethyl)aminoethylamine
tren	tris-(2-aminoethyl)amine
cyclen	1,4,7,10-tetraazacyclododecane
DO3A	1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane
β CD-en	6 ^A -deoxy-6 ^A -(2-aminoethylamino)- β -cyclodextrin
β CD-pn	6 ^A -deoxy-6 ^A -(3-aminopropylamino)- β -cyclodextrin
β CD-bn	6 ^A -deoxy-6 ^A -(4-aminobutylamino)- β -cyclodextrin
β CD-hn	6 ^A -deoxy-6 ^A -(6-aminoethylamino)- β -cyclodextrin
β CD-dien	6 ^A -deoxy-6 ^A -(2-(2-aminoethylamino)ethylamino)- β -cyclodextrin
β CD-dipn	6 ^A -deoxy-6 ^A -(3-(3-aminopropyl amino)propylamino)- β -cyclodextrin
β CD-tacn	6 ^A -deoxy-6 ^A -(1,4,7-triazacyclononanyl) - β -cyclodextrin
β CD-tacdo	6 ^A -deoxy-6 ^A -(1,5,9-triazacyclododecanyl)- β -cyclodextrin
β CD-trien	6 ^A -deoxy-6 ^A -(2-(2-(2-aminoethylamino) ethylamino)ethylamino)- β -cyclodextrin
β CD-tren	6 ^A -deoxy-6 ^A -(2-(bis(2-aminoethyl)amino)ethylamino)- β -cyclodextrin
β CD-cyclen	6 ^A -deoxy-6 ^A -(1,4,7,10-tetraazacyclododecanyl)- β -cyclodextrin
β CD-DO3A	6 ^A -deoxy-6 ^A -(1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane)- β -cyclodextrin

α CD	α -cyclodextrin
β CD	β -cyclodextrin
γ CD	γ -cyclodextrin
trp ⁻	tryptophanoate
trpH	tryptophan
DNA	deoxyribose nucleic acid
RNA	ribose nucleic acid
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
ROESY	Rotating frame nuclear Overhauser Effect Spectroscopy
CPK	Corey-Pauling-Koltun

ABSTRACT

The cyclodextrins are cyclic oligosaccharides capable of forming inclusion complexes in which a guest molecule resides within the cyclodextrin cavity, held in place by non-covalent forces alone. The focus of this study is to gain a greater insight into the factors that govern the stability of cyclodextrin inclusion complexes with a range of systematically modified cyclodextrins.

Apparent stability constants for the inclusion of carboxylic acids and carboxylates by di- tri- and tetraamino mono-substituted β -cyclodextrins are determined by potentiometric titrations. Host and guest orientations are further investigated by two-dimensional ^1H NMR ROESY spectroscopy and force-field molecular modelling. The inclusion is affected by such factors as the size, hydrophobicity, charge and charge separation of the substituent on the cyclodextrin. Chiral recognition of the carboxylic acids and carboxylates studied is quite low. An apparent stability constant of for the complex of with 6^A-deoxy-6^A-(3-(3-aminopropyl amino)propylamino)- β -cyclodextrin is one of the largest stability constants reported for modified cyclodextrin host with the guest carboxylic acid and carboxylates studied.

The formation of metallocyclodextrins, in which a metal ion is coordinated to a tetraamino mono-substituted β -cyclodextrin, is monitored by potentiometric titrations. The presence of Ni^{2+} , Cu^{2+} and Zn^{2+} is found to increase the stability of the cyclodextrin complexes of tryptophan and tryptophanoate, relative to the stability of their tetraamino mono-substituted β -cyclodextrin complexes. The stability of the metallocyclodextrin inclusion complexes appears to governed primarily by the interaction of the tetraamino mono-substituted β -cyclodextrin with the guests. Chiral recognition of tryptophan and tryptophanoate is quite low.

A modified cyclodextrin capable of binding a lanthanide ion is synthesised. The Europium (III) complex of the modified cyclodextrin is investigated for its ability to act as a chiral NMR shift reagent, its AETE properties and its ability to act as an RNA phosphatase mimic. Complete chiral resolution is observed for the proton signals of (*R/S*)-histidinoate in the presence of the Europium (III) modified cyclodextrin complex by ^1H NMR spectroscopy. Complete chiral resolution is observed for the proton signals of (*R/S*)-tyrosinoate by ^1H NMR

spectroscopy, in the presence of the modified cyclodextrin only. The Europium (III) complex of the modified cyclodextrin proved to be an effective catalyst of bis (2,4-dinitrophenyl)phosphate (an RNA mimic).



CHAPTER ONE

INTRODUCTION

1.1. The Physical and Chemical Properties of Cyclodextrins.

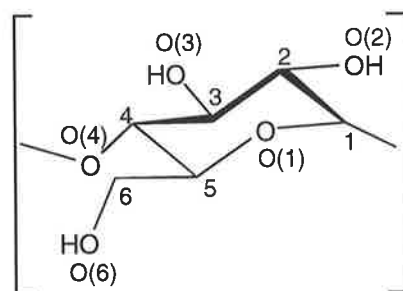
First isolated by Villiers in 1891,¹ cyclodextrins are naturally occurring cyclic oligosaccharides made up of 6 or more α -1,4 linked (α)-D-glucopyranose units, as shown in Figure 1.1. They are produced by the degradation of starch by the enzyme cyclodextrin transglycosylase^{2,3} found in such bacteria as *Bacillus macerans*.

The most common cyclodextrins are α -, β -, and γ -cyclodextrin (α CD, β CD, γ CD) each containing 6, 7, and 8 glucopyranose units respectively. Of all the cyclodextrins, these have been the most extensively studied and their physical properties and dimensions the most well defined, a few of which are listed in Table 1.1. Cyclodextrins containing less than 6 (α)-D-glucopyranose units are not known possibly due to excessive strain in the ring.⁴ Cyclodextrins containing as many as 13 glucopyranose units have been reported³ and successfully purified⁵ although they display poor complexing abilities.

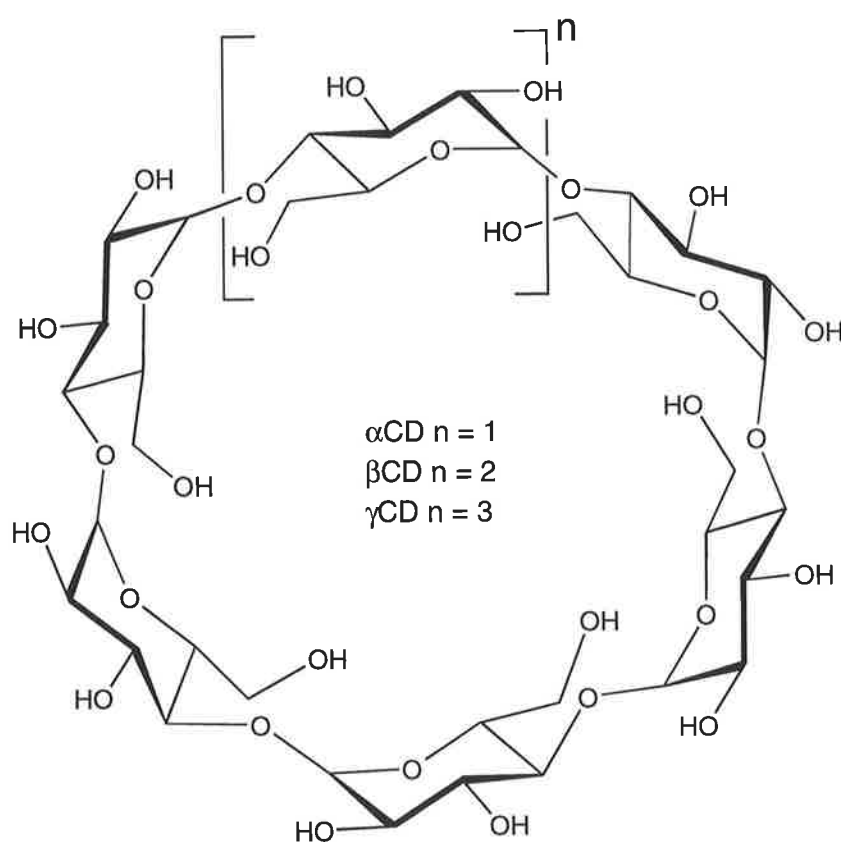
Table 1.1. Some dimensions and physical properties of cyclodextrins.³

	α CD	β CD	γ CD
No of glucose units	6	7	8
No of chiral centres	30	35	40
Molecular Mass	973	1135	1297
External diameter (pm) ^a	1370-1460	1530-1540	1690-1750
Internal diameter (pm) ^a	470-520	600-650	750-850
Cavity volume (nm ³)	0.176	0.346	0.510
Length of cavity (pm)	790-800	790-800	790-800
Solubility in water (g/100cm ³ at 25°C)	14.50	1.85	23.20
Melting point (K)	551	572	540

^aThe smaller and larger values represent the primary and secondary rims of the cavity.



a)



b)

Figure 1.1. a) a single (α)-D-glucopyranose unit and the numbering of atoms within it. b) The structural formula of the cyclodextrins.

Cyclodextrins are often drawn as truncated cones, as shown by Figure 1.2, the inside of which represents the cyclodextrin cavity or annulus. This is a consequence of the α -D-glucopyranose units existing in a ${}^4C_1$ chair conformation and there being a lack of rotation about the glycosidic bonds.² The narrow primary end of the cone is defined by the primary hydroxyl groups whereas the wider secondary end is defined by the secondary hydroxyl groups. Additional rigidity is provided by hydrogen bonding between the secondary hydroxyl groups,³ thus there is an increase in rigidity with an increase in the size of the cyclodextrin. Similarly the symmetry of the cyclodextrins increases in the order $\alpha\text{CD} < \beta\text{CD} < \gamma\text{CD}$. By a similar argument the flexibility of the cyclodextrins decreases in the same order. Crystallographic studies revealed αCD had a much lower symmetry relative to βCD and γCD , which were quite symmetrical, due to the partial rotation of one of its glucopyranose units.² Traditionally this has been the accepted model for cyclodextrins in both their solid state and in solution but recent studies involving both molecular modelling and analytical techniques have given rise to the current view that, in solution, the cyclodextrins are fairly flexible and exist in a range of conformations, some of which may depart considerably from the highly symmetrical conformation of their solid form.⁶

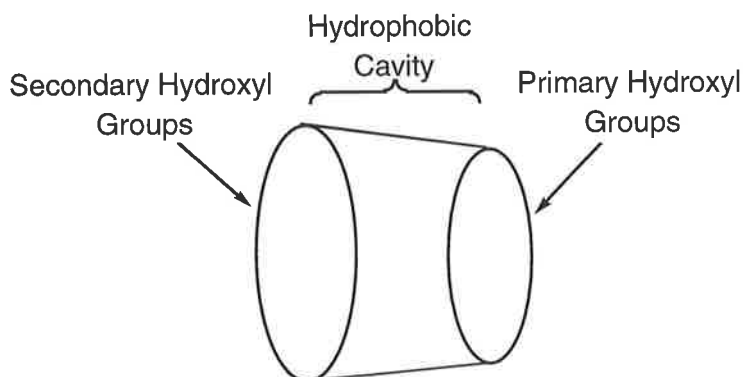


Figure 1.2. The truncated cone representation of a cyclodextrin showing the position of the primary and secondary hydroxyl groups.

The static arrangement of the glucopyranose units gives rise to areas within the cyclodextrin that are hydrophilic or hydrophobic in nature.⁴ The rims of the cyclodextrin cavity are lined by hydroxyl groups, which are highly hydrophilic and are free to hydrogen bond with water, allowing cyclodextrins to be soluble in aqueous solution. Alternatively, the interior of the cyclodextrin cavity is lined with C(3)-H and C(5)-H hydrogens and by the ether like O(4) oxygens giving rise to a very hydrophobic environment.

Another property arising from the static conformation of the (α)-*D*-glucopyranose units is that of an inherent dipole. It has previously been held, that since each glucopyranose unit has a dipole moment (the dipole moment of *D*-glucose itself is quite large with values of 8 - 14 Debye (1 Debye = 3.33564×10^{-30} Cm) having been reported⁶) and as each unit is held in the same conformation within a cyclodextrin, the individual dipoles add together to give an overall dipole moment. Thus, as the number of glucopyranose units within the cyclodextrin increases, the dipole moment was expected to increase ie. $\alpha\text{CD} < \beta\text{CD} < \gamma\text{CD}$. Molecular orbital calculations based on X-ray structures of various inclusion complexes determined that cyclodextrins possess a dipole moment directed from the secondary hydroxyl groups to the primary hydroxyl groups, as depicted in Figure 1.3, running approximately through the centre of the cavity with values of around 10 - 20 Debye.⁷ Alternate calculations using the AM1 method gave dipole moments of 7.06, 2.03 and 2.96 Debye for αCD , βCD and γCD respectively.⁸ A more recent report gave the dipole of βCD being calculated as 2.9 Debye when the primary hydroxyl groups are perpendicular to the axis of the cavity and 14.9 when they are parallel.⁹ In solution, the orientation of the primary hydroxyl groups will be influenced by interactions with the solvent so an intermediate value may be expected.

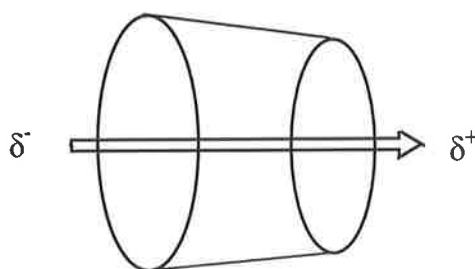


Figure 1.3. A cyclodextrin showing its dipole moment directed from the secondary to the primary hydroxyl groups.

One of the most important properties arising from the static conformation of the glucopyranose groups within the cyclodextrin is that of the formation of an annulus (mentioned earlier) and the subsequent ability of the cyclodextrins to form inclusion complexes.

1.2 Inclusion Complexes of Cyclodextrins.

Inclusion complexes can be defined as chemical species consisting of two or more associated molecules which interact non-covalently. Cyclodextrins possess a cavity thus can act as host molecules to admit guest molecules which might result in a stable association without the formation of covalent bonds.¹⁰ An intricate example of an inclusion complex is that of the highly specific enzyme-substrate interactions that occur in biological systems. It is through the study of inclusion complexes that a greater understanding of these systems can be achieved.

Cyclodextrins form inclusion complexes with a wide variety of guest molecules with the only condition seeming to be that the guests fit entirely or at least partially into the cyclodextrin cavity,¹¹ a possible exception being the complex formed between bilirubin and β CD where the guest is believed to be hydrogen bonded to the secondary hydroxyl groups only.¹² The range of guest molecules studied is extensive and include inorganic salts, gases (including noble gases), various aliphatic species and even fullerenes.¹³ In general it is held that hydrophobic guests are bound within the cyclodextrin cavity more strongly than hydrophilic guests.¹⁴ The guests used throughout this study all contain an aromatic moiety.

1.2.1. Forces Governing The Formation of Cyclodextrin Inclusion Complexes.

The stabilities of cyclodextrin inclusion complexes are governed by several forces such as hydrogen bonding, hydrophobic interactions, dipole-dipole interactions, dispersive interactions/Van der Waals forces, and the release of high energy cavity water (change in solvation).^{2,7,15} The extent to which each of these factors contribute is still debateable but it is generally viewed that the size of the guest is one of the most important factors determining the stability of a cyclodextrin complex. A good matching of the guest size to that of the cyclodextrin cavity is required for there to be efficient intermolecular contacts (due to Van der Waals and dispersion forces¹¹) leading to an increased stability of the inclusion complex.¹⁵ If the guest molecule is small then interactions between it and the surrounding cyclodextrin are generally weak.

Arguably the dominating driving forces for cyclodextrin complex formation in aqueous solution with a hydrophobic guest, are hydrophobic interactions.¹⁶ The name belies the origin of this interaction, as the complexation of a hydrophobic guest by a hydrophobic host is not due to mutual attraction but rather to the intrinsic cohesion of the water. The role of water in complex formation is crucial since hydration of the cyclodextrin inclusion complex is energetically favoured when compared with the separate hydration of the components.¹⁷

The extrusion of 'high energy water' from the cyclodextrin cavity is an important driving force in the formation of inclusion complexes with organic/hydrophobic guests.¹⁵ As stated previously, cyclodextrin cavities are hydrophobic in nature thus water within the cavity will be in an unfavourable environment, unable to satiate their tetrahedral hydrogen bonding capacity as can those in the bulk solvent.^{11,17} Energetically unfavourable polar-apolar interactions will be occurring between the hydrophobic cavity and the included water¹⁵ and between water and the hydrophobic guest. Complex formation is favoured by a gain in entropy on expulsion of the included water, and favourable apolar-apolar interactions occurring between the hydrophobic guest and the hydrophobic cyclodextrin cavity.

The contribution of hydrogen bonding interactions are somewhat trivialised in aqueous solution. Intermolecular hydrogen bonds are hardly formed in aqueous solutions because of strong hydration of hydrogen bonding sites of both the host and guest.¹⁸ Hydrogen bonds may still be of importance in aqueous solution when the hydrogen bonding sites are located in a microscopically hydrophobic environment and/or are situated very closely together.¹⁸

1.2.2. Types of Inclusion Complexes.

Binary, or 1:1 host:guest complexes are the most common type of inclusion complex. Ternary complexes may also exist for cyclodextrin systems depending on the size of the host and guest molecules involved. If the cyclodextrin cavity is small compared to the size of the guest, it is possible that the formation of a binary complex will see the guest only partially encapsulated by the cyclodextrin and a second cyclodextrin may bind to the guest by that part protruding from the original host, to form a 2:1 host:guest complex. This type of complexation is known to happen for α CD due to its small size.¹⁹ If the guest size is small compared to the size of the cyclodextrin cavity it is possible for a 1:2 host:guest ternary complex to form. This may occur by two guests being held within the cyclodextrin cavity at the same time as has been reported for Tropaeolin 000 No2 with β CD.²⁰

Ternary cyclodextrin complexes consisting of three components have also been described. These complexes usually consist of a cyclodextrin, a guest, and a third molecule in a 1:1:1 ratio.⁶ The guest and the third molecule are believed to reside in the cyclodextrin annulus simultaneously with the third molecule said to be acting as a space filler or regulator. The presence of molecules that act as space regulators has been known to enhance the stability of the complex formed as was the case for toluene in the complexation of pyrene by γ CD.²¹

1.2.3. The Orientation of Guest Molecules within the Cyclodextrin Cavity.

The orientation of a guest molecule within the cyclodextrin cavity has traditionally been believed to be governed by dipole-dipole interactions. For native cyclodextrins the dipole moment of the guest and host will be aligned so that they are antiparallel to each other. This is illustrated in Figure 1.4 which depicts the formation of a cyclodextrin inclusion complex with 4-methylbenzoic acid as an example guest. The carbonyl group of the guest is situated at the primary end of the cyclodextrin and the aromatic ring within the cavity. X-ray crystallographic analysis studies performed on α CD and β CD with benzoic acid as the included guest supports this orientation.⁷

It has been argued that the structure of cyclodextrin inclusion complexes derived from X-ray crystallographic data are governed predominantly by crystal packing forces, and the question asked as to how relevant to the dilute aqueous solution these complex structures are.⁶ The recent, huge, advancements in two dimensional NMR spectroscopic and computational study capabilities are now allowing cyclodextrin chemists to gain further insight into the structure of cyclodextrin inclusion complexes in solution.^{22,23} Both of these methods were employed in this study. There is generally good agreement between the structures determined using these methods and those of the crystalline cyclodextrin complexes²² with dipole-dipole interactions still believed to be important.

Dipole-dipole interactions are unable to predict the structure of all cyclodextrin inclusion complex structures. The binding of adamantane-1-carboxylate by β CD gives an orientation of host and guest with parallel alignment of dipoles.²⁴ This structure is consistent with an alternate model for predicting the structure of cyclodextrin inclusion complexes proposed by Lichtenthaler and Immel²⁵ whereby optimisation of the complementarity of hydrophobic and hydrophilic sites on the host and guest governs complex structure.

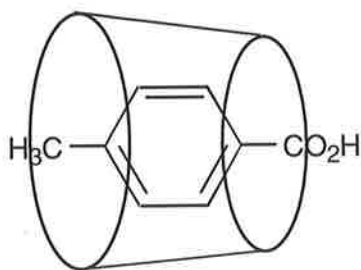
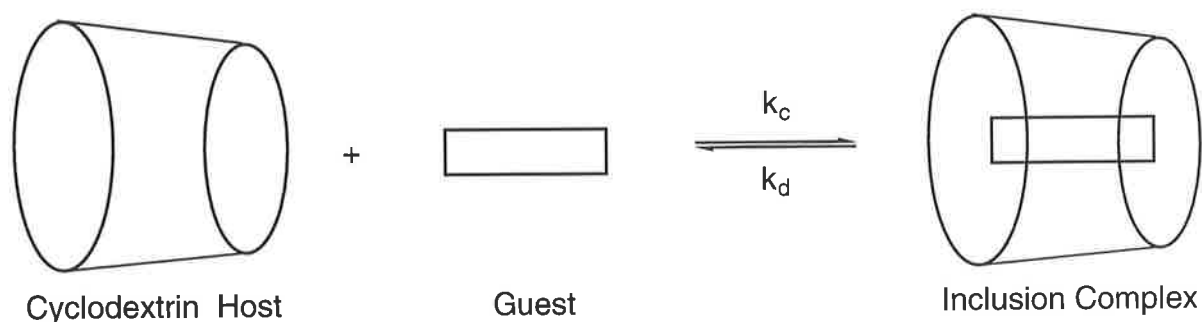


Figure 1.4 A schematic representation of an inclusion complex between a cyclodextrin, the host, and 4-methylbenzoic acid, the guest.

1.2.4. Determination of the Stabilities of Cyclodextrin Inclusion Complexes.

Upon complexation, a guest molecule experiences a change in its chemical environment as it moves from the bulk solution to the cavity of the cyclodextrin.² When in the bulk solution, the guest molecule is surrounded by solvent molecules whereas, upon complexation, it becomes whole or partially encapsulated by the hydrophobic cavity of the cyclodextrin. This results in a change in the physical and chemical properties of the included guest. Such changes that can be measured by an accurate method, may be used to determine the apparent stability (K) of the inclusion complex formed. Inspection of Scheme 1.1 reveals that K is simply $[\text{inclusion complex}]/([\text{cyclodextrin host}][\text{guest}]$). Alternately $K = k_c/k_d$ where k_c and k_d are the rates of complexation and decomplexation of the host:guest complex, respectively. The simplest interpretation of an increase or decrease in complex stability is that the rate of decomplexation, relative to the rate of complexation, has slowed or quickened, respectively.

Scheme 1.1.



Inclusion in a cyclodextrin cavity may protect a guest from collisional quenching of an excited state by oxygen or the solvent, and may result in enhancement of the fluorescence spectrum of the guest. Perturbation of the electronic energy levels of a guest, either by a direct interaction with the cyclodextrin or by the exclusion of solvating water molecules, often causes changes in the electronic spectrum of the guest. Changes in the environment of nuclei of the guest and host due to such factors as anisotropic shielding, conformational and solvation changes, or direct contact between the host and guest, often cause changes in the NMR spectrum of both the cyclodextrin and the guest. The circular dichroism spectrum of a chiral guest may be changed by interaction with the chiral cyclodextrin, and an achiral guest may exhibit an induced circular dichroism spectrum due to inclusion within the chiral cyclodextrin cavity. Complexation may also alter the pK_a values of acidic and basic guests, allowing complex formation to be monitored by potentiometric titrations.

The cyclodextrin host may also undergo a change in its chemical and physical properties upon including a guest molecule. The chemical modification of cyclodextrins may impart new properties to the host which can also be utilised in the determination of complex formation.

1.3. The Modification of Cyclodextrins.

The chemical modification of cyclodextrins has been extensively studied in order to improve their chemical and physical properties.²⁶ The aim of most modifications has been to improve the complexing, enantioselective and catalytic properties of cyclodextrins in order to develop "artificial enzymes".^{2,27} Native (unmodified) cyclodextrins are relatively poor enzyme models as enzymes usually depend upon several catalytic groups working together. By modifying the cyclodextrin the number of molecular recognition sites is increased thus improving its enzyme like properties.²⁸

Modifications of cyclodextrins have included linking cyclodextrins together,²⁹⁻³³ which may result in enhanced guest complexation as a consequence of cooperativity between the two cyclodextrin moieties. In addition, linked cyclodextrins may be tailored to optimise the cyclodextrin moiety distance and the position of a catalytic group on the link to produce selectivity in the catalysis of a guest. The main modification made to cyclodextrins is the introduction of various functional groups.

The enantioselectivity of guest complexation is often greater with a modified cyclodextrin where, through the modification, the degree of asymmetry of the cyclodextrin

may be increased, and there are additional interactions between chiral centres of the cyclodextrin and those of the guest. The stability constants for the complexes of (*R*)- and (*S*)-2-phenylpropanoate with β CD are 63 and 52 dm³ mol⁻¹, respectively, while those of the analogous complexes of protonated 6^A-amino-6^A-deoxy- β -cyclodextrin, are 36 and 13 dm³ mol⁻¹.³⁴ This indicates that only a small modification can bring about a large degree of selectivity, also that unfavourable interactions between substituents of the modified cyclodextrin host and the racemic guests may destabilise complexes, but lead to greater enantioselectivity. The enantioselectivity displayed by modified cyclodextrins is discussed in greater detail in Section 1.4.

Reactions of the native cyclodextrins as models for enzyme catalysts are limited because they can only involve the cyclodextrin hydroxyl groups. This restriction has been overcome with modified cyclodextrins through the introduction of a variety of reactive functional groups.³⁵⁻³⁷

The binding of a guest to a modified cyclodextrin is often stronger where, through the modification, an additional recognition or binding site is added. The stability constant for the complex of (*S*)-tryptophanoate with β CD is characterised by $\log (K/\text{dm}^3 \text{ mol}^{-1}) = 2.33$, while that of the analogous complex of 6^A-deoxy-6^A-(3-aminopropylamino)- β -cyclodextrin, is 3.40.³⁸ Favourable interactions between the functional group used to modify the cyclodextrin, and the guest, has lead to stronger binding. Alternatively, the introduced functional group may have induced changes in the cyclodextrin which have improved its complexing ability. The stability constant for the complex of (*S*)-tryptophanoate with the binary Zn²⁺ complex of 6^A-deoxy-6^A-(3-aminopropylamino)- β -cyclodextrin is characterised by $\log (K/\text{dm}^3 \text{ mol}^{-1}) = 5.90$. The additional binding site, provided by the metal centre, has lead to increased complexation of the guest.³⁸ The binding of metal ions to cyclodextrins has lead to different class of modified cyclodextrins called metallocyclodextrins.

1.3.1. Metallocyclodextrins.

Metallocyclodextrins are cyclodextrins with a metal ion coordinated either directly to the cyclodextrin hydroxyl groups or to a functional group substituted on a modified cyclodextrin. The direct coordination of metal ions to a cyclodextrin tends to be weak and involves the formation of hydroxo species.³⁹⁻⁴¹ The latter case is the one more commonly referred to in the discussion of metallocyclodextrins and they are often called binary metallocyclodextrins. The subsequent inclusion of a guest in the annulus of a binary

metallocyclodextrin leads to the formation of ternary metalocyclodextrins. Metallocyclodextrins have shown high degrees of enantioselectivity and increased rates of catalysis.

The Ni^{2+} metalocyclodextrin of 6^A-deoxy-6^A-(3-aminopropylamino)- β -cyclodextrin is enantioselective for the (*S*)-tryptophan anion in a ratio of 10:1 over the (*R*)-tryptophan anion in forming the ternary metalocyclodextrin complex.⁴¹ A possible structure for this complex is shown in Figure 1.5. The stability of the ternary metalocyclodextrin complex appears to be governed by three major factors: 1) the hydrophobic interaction between the guest and the cyclodextrin annulus, 2) the coordination of the guest to the metal centre and 3) the interaction of the guests chiral centre with the chirality of the cyclodextrin. When the contribution of the last factor differs for the diastereomeric ternary metalocyclodextrin thermodynamic enantioselectivity occurs. This is illustrated by the absence of enantioselectivity in the ternary complexes of 6^A-deoxy-6^A-(2-(*N,N*-bis(2-aminoethyl)amino)ethylamino)- β -cyclodextrin with (*R/S*)-tryptophan and M^{2+} (where *M* = Ni, Cu, Zn), where factors 1) and 2) appear to dominate despite a considerable increase in stability over the 6^A-deoxy-6^A-(3-aminopropylamino)- β -cyclodextrin ternary metalocyclodextrin complexes.⁴³

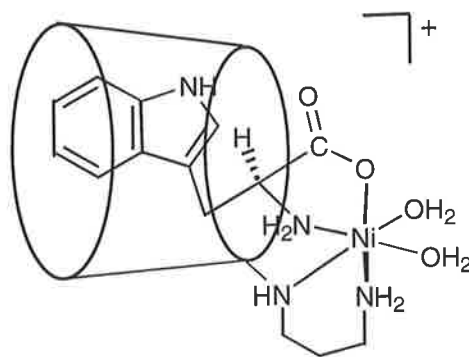


Figure 1.5 The proposed structure for the ternary Ni^{2+} , tryptophanoate, 6^A-deoxy-6^A-(3-aminopropylamino)- β -cyclodextrin host-guest complex

The 1000-fold rate acceleration of the deacylation of *p*-nitrophenyl acetate caused by 6^A-deoxy-6^A-(1,4,7,10-tetraazadodecyl)- β -cyclodextrincobalt(III) and the smaller acceleration caused by 3^A-deoxy-3^A-(1,4,7,10-tetraazadodecyl)- β -cyclodextrincobalt(III) indicate the importance of the relative orientations of the metal centre and guest in ternary metallocyclodextrins.^{44,45} The probability of the nucleophilic attack on the included *p*-nitrophenyl acetate by a hydroxo ligand bound to the Co³⁺ metal centre appears to be decreased though steric hindrance in the case of the latter. The [Co(cyclen)(OH)(H₂O)]²⁺ complex has no catalytic effect but 6^A-deoxy-6^A-(1,4,7,10-tetraazadodecyl)- β -cyclodextrin causes an 8.6 fold deacylation rate acceleration under the same conditions. The influence of the metal centre on catalysis is illustrated by the Ni²⁺, Cu²⁺ and Zn²⁺ binary metallocyclodextrins of 6^A-deoxy-6^A-(1,4,7,10-tetraazadodecyl)- β -cyclodextrin which cause only 16, 14, and 12-fold accelerations of the deacylation of *p*-nitrophenyl acetate.⁴⁶

1.4. Enantioselectivity by Cyclodextrins.

Cyclodextrins are themselves chiral, existing only as the *D* enantiomer. Upon complexation with a racemic mixture of an optically active guest species there is a possibility of forming two diastereomers: (+)-guest.*D*(+)-cyclodextrin and (-)-guest.*D*(+)-cyclodextrin. It is possible that the cyclodextrin may preferentially bind one enantiomer of the racemate and produce different physical and chemical properties in the two diastereomeric complexes. If this thermodynamic discrimination is large enough, then it may allow the measurement of different stability constants for the enantiomer containing complexes. It is also possible that in the case where thermodynamic discrimination is occurring but not to an accurately measurable extent, spectroscopic methods may still detect small energy differences between the diastereomeric complexes. The enantioselectivity of cyclodextrins is exhibited in both the solid and the liquid state.⁴⁷

The first example of enantioselectivity by β CD was reported by Cramer and Dietsche, who studied the partial optical rotation of mandelic acid derivatives.⁴⁸ Native cyclodextrins show a relatively low enantioselectivity for racemic guests but have since been extensively studied, the work of Armstrong *et al.* culminating in the development of cyclodextrin based chromatographic systems^{49,50} whose efficiency is a result of repeated interactions between the racemates and the cyclodextrin. The low enantioselectivity of the cyclodextrins has been attributed to their inherent symmetry.

The 'three point rule' is the general mechanism for chiral recognition in host-guest chemistry.¹⁸ Point interactions include hydrogen bonding, formation of coordinative bonds, electrostatic interaction and dipole-dipole interactions. An alternate model for chiral recognition might be the 'lock-and-key mechanism' originally proposed by Emil Fischer.¹⁸ In general, an inclusion complex must form, facilitated by there being a tight fit between the enantiomer and the cyclodextrin cavity. The C(2) and C(3) hydroxyl groups are unidirectional and believed to be particularly important in chiral recognition thus, the chiral centre or a substituent of the chiral centre must be near to and interact with the mouth of the cyclodextrin cavity, the mouth being the larger end of the cyclodextrin annulus.⁵⁰

Symmetrically substituted cyclodextrins generally show no greater enantioselectivity than the analogous native cyclodextrin even though the modification may result in more favourable interactions between the racemic guest and cyclodextrin host, which is reflected by their stability constants.⁵¹ The complexation of (*R*)- and (*S*)-2-phenylpropanoic acids by termethylated α - and β CD led to enantioselectivity that was not much different to that observed for the analogous native cyclodextrin.¹⁹

The chemical modification of a cyclodextrin to impart a degree of asymmetry, can lead to increased enantioselectivity. The asymmetric modification of β CD to give mono and di substituted amino cyclodextrins lead to an increased enantioselectivity for the complexation of nucleotides compared with the fully substituted amino cyclodextrin.⁵² An example of where a modification may have a detrimental affect on the stability of the complexes formed, but lead to an increase in enantioselectivity, has been mentioned previously (Section 1.3).

The enantioselectivity displayed by cyclodextrins and their ability to hydrolyse esters has lead to an interest in their use as enzyme mimics. It is generally held that the enantioselectivity observed is primarily due to the differences in reactivity of the diastereomeric complexes as opposed to their stability. This was shown to be the case for the hydrolysis of the *meta* nitrophenol esters of (*R/S*)-2-phenylpropanoic acid by β CD. An overall discrimination of 19 in favour of the (*R*)- enantiomer was determined.⁵³ While both the complexation of the enantiomers, and the reactivities of the resulting diastereomeric complexes were in favour of the (*R*)- enantiomer, the ratio of *R:S* for both the association constants, and the rate constants, gave values of 1.2 and 15.5, respectively. This indicated that the rate of hydrolysis, or reactivity, was the main contributing factor to the overall discrimination observed.

The enzymatic hydrolysis of esters is essentially a two step process, the cleavage of the ester linkage to liberate the alcohol followed by hydrolysis at the reactive centre to regenerate the enzyme and release the acylated product. Recent interest has been given to determining the selectivity of each of these steps. The hydrolysis of the diastereomeric esters derived from the reaction of Ibuprofen acid chloride and β CD displayed a 10-fold diastereoselectivity in favour of the ester formed from (*R*)-Ibuprofen.⁵⁴ The initial formation of the esters afforded a 5:1 ratio of the diastereomers in favour of the one derived from (*R*)-Ibuprofen. Thus, the overall enantioselectivity for the two step reaction for the hydrolysis of Ibuprofen acid chloride by β CD was about 50:1 in favour of the (*R*)- enantiomer.

An example of a metallocyclodextrin displaying a significant degree of enantioselectivity has been mentioned earlier (Section 1.3.1). Only a limited number of studies of enantioselectivity by metallocyclodextrins have been reported. It is believed that the binding of both a cyclodextrin and a guest to a metal, which increases the extent of interaction between the cyclodextrin and the guest, will generally increase the enantioselectivity.⁵¹

1.5. The Use of Cyclodextrins in Industry.

Cyclodextrins are non-toxic, biodegradable carbohydrates.⁵⁵ Their environmental friendliness and relatively low cost make cyclodextrins very attractive industrial agents. The practical application of cyclodextrins, in both their native and modified forms, continues to increase rapidly, with their finding employment in areas as diverse as detoxification of polluted soil to use as an oral deodoriser. The primary application of cyclodextrins by industry, is derived from their ability to include or 'micro-encapsulate' other molecules.⁵⁶ This can lead to advantageous changes in the chemical and physical properties of the guest molecule. Micro-encapsulation by cyclodextrins has four main advantages which are being utilised in industry today:¹¹

- 1) the stabilisation of light or air sensitive substances,
 - 2) the modification of the chemical activity of the guest molecule,
 - 3) the fixation of volatile substances
- and 4) the modification of the physico-chemical properties of guest molecules.

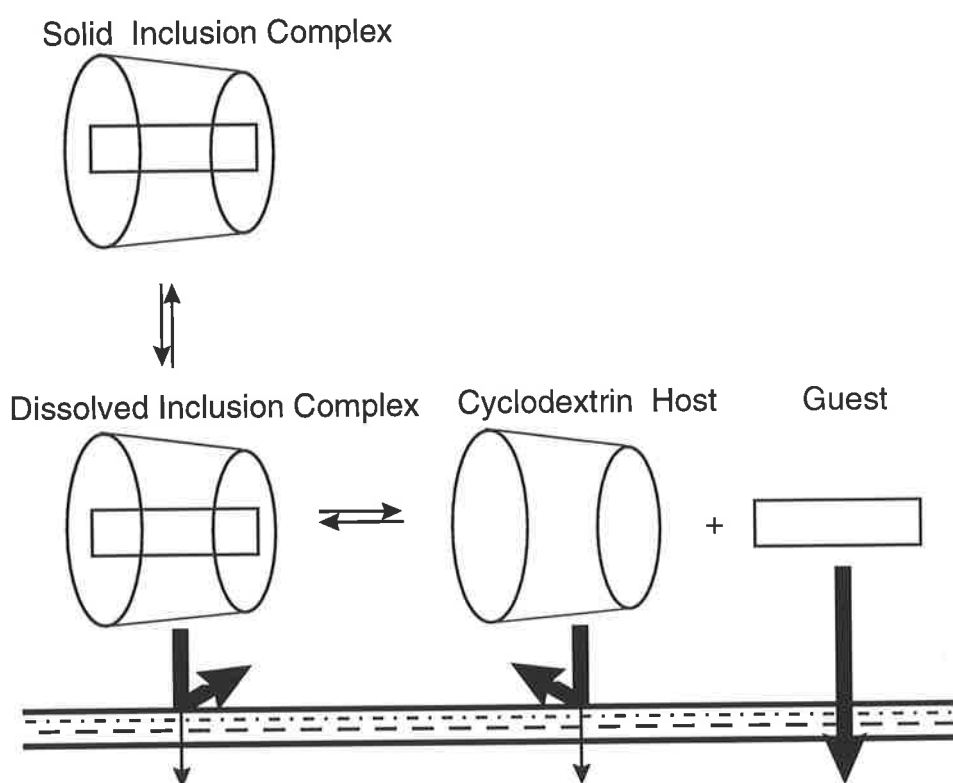
Cyclodextrins have found a niche in the ongoing search for more effective drug delivery systems. After oral ingestion, native cyclodextrins are hydrolysed in the colon by α -1,4-glucanohydase.⁵⁷ The hydrolysis of cyclodextrins is not as rapid as that of the linear

dextrins and they are not hydrolysed by enzymes that can attack terminal groups.¹¹ However, modified cyclodextrins, due to their various substitutions, are not recognised as natural sugars and are not metabolised. Long term ingestion may lead to weight gain due the modified cyclodextrins complexing normal molecules within the gastrointestinal tract.⁵⁸ In addition, the intramuscular and intravenous administration of the native cyclodextrins results in ulcerations, and nephrotoxicity and haemolytic effects, respectively. Depending on the modification, the haemolytic effect of modified cyclodextrins may be increased or decreased.^{58,59} Cyclodextrins themselves cannot easily traverse a cell membrane, but they can effectively deliver a drug to a biological membrane. The mechanism by which the drug is delivered to, then absorbed through the membrane, is given in Scheme 1.2. They have been used to prepare aqueous formulations of poorly water soluble drugs and to modify the rate of release of the drug, be it immediate, prolonged or delayed.⁶⁰ Cyclodextrins have proven successful in optimising the delivery of drugs through oral, transdermal and transmucosal administration. The inclusion of a drug molecule by the cyclodextrin may decrease undesirable side effects,⁶¹ reduce the irritating taste, such in the case for Ibuprofen,⁵⁶ and diminish any unpleasant odour. Inclusion of a drug within a cyclodextrin may increase its bioavailability,⁶¹⁻⁶³ though for this to occur it is necessary to have good water solubility of the inclusion complex but with a low stability constant.⁵⁶

The main functions of cyclodextrins in the food industry are to maintain the appeal/appearance of the product, to help preserve the product and/or to aid in packaging. The protection of active ingredients from reactions that may cause an undesired colour change, the reduction or removal of undesired tastes and odours, the reduction of hygroscopicity and the increase in solubility of poorly soluble components, are all ways that cyclodextrins maintain the appeal of the food through encapsulation of the active ingredients. Food is preserved by the prevention of the decomposition, and loss by volatility, of active ingredients through encapsulation. Cyclodextrins may also be used to remove contaminants. Packaging of food is aided by the formation of stable and homogenous composites.⁶⁴

Cyclodextrins are used as stabilising agents and emulsifiers in the cosmetic industry and are effective deodorisers.^{56,65} Cyclodextrins are used to stabilise volatile fragrances in household and toiletry products.⁶⁵ They can include detergent molecules thus act as defoaming agents.^{56,65} Their addition to the rinsing water in laundries can lead to the removal of the last traces of detergent thus reduce water consumption.⁵⁶

Scheme 1.2. The delivery to and absorption through a biological membrane of an active ingredient complexed within a cyclodextrin.



Cyclodextrins have wide applications in the farming industry. These include the stabilisation of volatile substances, such as ethylene, the intracellular production of which ethylene is connected to the ripening process of fruits and the abscission of leaves, and the protection of vitamins, dispersed in the feed of animals, from decomposition.⁵⁶ Cyclodextrins may be used extensively with pesticides. Their role includes the delivery of volatile substances with the aim of increasing the bioavailability of the pesticide, imparting homogeneity to the final product and reducing environmental pollution due to overdosing.⁵⁵ The contact toxicity of some pesticides is decreased, though, because the inclusion complex cannot be absorbed directly into the body of the pest.⁵⁶

Scientific applications of cyclodextrins have been found in the area of biotechnology in the cultivation of cells and enhancement of bioconversions.⁶⁶ Commercially available cyclodextrin-based chromatography columns illustrate their use as an analytical tool.⁶⁷

1.6. Aims of This Research.

As mentioned in Section 1.2, it is through the study of inclusion complexes that a greater understanding of the highly specific enzyme-substrate interactions that occur in biological systems can be obtained. Thus, the first two sections of this thesis are concerned with a study into the factors governing the formation of complexes with benzoic, 4-methylbenzoic, and (*R/S*)-2-phenylpropanoic acids and their conjugate bases with a range of 6^A-polyamine-mono-substituted- β -cyclodextrins. The amine groups used to functionalise the cyclodextrin were varied systematically, providing hosts of varying size, charge and hydrophobicity. High guest selectivity with respect to enantiomers is common in biological systems, thus, both the (*R*)- and (*S*)- enantiomers of 2-phenylpropanoic acid were used in the hope of observing enantioselective complexation.

Section 1.4 outlined the extent of enantioselectivity displayed by modified cyclodextrins. Only a limited number of studies of enantioselectivity by metallocyclodextrins have been reported. The third section of this thesis is concerned with the Ni²⁺, Cu²⁺ and Zn²⁺ metallocyclodextrins of 6^A-deoxy-6^A-(1,4,7,10-tetraazadodecyl)- β -cyclodextrin and 6^A-deoxy-6^A-(2-(2-(2-aminoethylamino)ethylamino)amino)- β -cyclodextrin and their complexation of tryptophan. To determine the effect of the metal chelating environment on both metal and guest selectivity a comparison was made with the complexation of tryptophan by the Ni²⁺, Cu²⁺ and Zn²⁺ metallocyclodextrins of 6^A-deoxy-6^A-(2(bis(2-aminoethyl)amino)ethylamino)- β -cyclodextrin, previously reported.⁴³

Metalloenzyme activity is very metal centre specific and this may be partly due to the influence of the metal centre on the metalloenzyme and its ability to complex substrates. The influence of the metal centre on the ability of the metallocyclodextrin to complex tryptophan is discussed. High enantioselectivity is common in biological systems, thus, both the (*R*)- and (*S*)- enantiomers of tryptophan were used in the hope of observing enantioselective complexation. An understanding of the factors influencing the stabilities and enantioselectivities of metallocyclodextrins and their complexes, should be furthered by this investigation of the effects that variation in the metal chelating environment and nature of the metal have on complexation.

The final area with which this thesis is concerned is with a new range of metallocyclodextrin which involve the use of lanthanides. This relatively new area of metallocyclodextrin chemistry is gaining more interest, with lanthanide bound cyclodextrins finding uses as chiral NMR shift reagents,⁶⁸ fluorescent probes⁶⁹⁻⁷¹ and as possible catalysts for the hydrolysis of DNA/RNA.⁷²

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CHAPTER TWO

**THE COMPLEXATION OF AROMATIC CARBOXYLIC ACIDS BY
6^A-DEOXY-6^A-(ω -AMINOALKYL)AMINO- β -CYCLODEXTRINS****2.1. Introduction**

The cyclodextrins and their modified forms form an extensive range of host-guest complexes. In most cases, host guest complexation involves the entry of a hydrophobic moiety of the guest into the hydrophobic region of the host cyclodextrin annulus. The cyclodextrin may discriminate between guests on the basis of their size, hydrophobicity, charge and chirality. This study is particularly concerned with the determination of the effect of varying charge and hydrophobicity on the host cyclodextrin and the guest on complexation.

The 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins are cyclodextrin hosts that exhibit two protonations and self complexation of the (ω -aminoalkyl)amino substituent. They facilitate the systematic study of charge variation on host-guest complex stabilisation. By varying the aliphatic chain length of the (ω -aminoalkyl)amino substituent, the hydrophobicity of the modified cyclodextrin may also be varied. In addition, by increasing the aliphatic chain length, a steric factor is introduced which may affect the complexation of guests. The guests selected for this study, benzoic, 4-methylbenzoic and (*R/S*)-2-phenylpropanoic acids and their conjugate bases, also exhibit differences in hydrophobicity and charge. The use of these aromatic carboxylic acids as guests in this, and previous studies, is due to their propensity to form complexes with β CD and related hosts that have measurable apparent stability constants, due to their size and aromaticity. The use of the potentiometric method to determine stability constants for these types of systems is quite common.¹⁻³ The structures of the aromatic carboxylic acids, (HA), and the four 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins, (β CD-X), used in this study are shown in Figure 2.1.

A potentiometric titration study of the complexation of β CD and 6^A-deoxy-6^A-amino- β -cyclodextrin (β CD-NH₂) with benzoic, 4-methylbenzoic and (*R/S*)-2-phenylpropanoic acids has been reported.¹ A similar study involving the complexation of termethylated α - and β -cyclodextrins has also appeared in the literature.² As hosts, each of these cyclodextrins are quite simple, existing only as a single species, with the exception being β CD-NH₂ which can also exist as β CD-NH⁺, the protonated species. The trends seen for the variation of the

complex stabilities with the selected guest acids were rationalised in terms of guest and host size, electrostatic interactions, the extent of hydrophobic interactions between the guest and the cyclodextrin annulus and in terms of hydration. Subsequently, it is expected that all of these factors will play an important role in the determination of any complexes formed with the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins and the same guests.

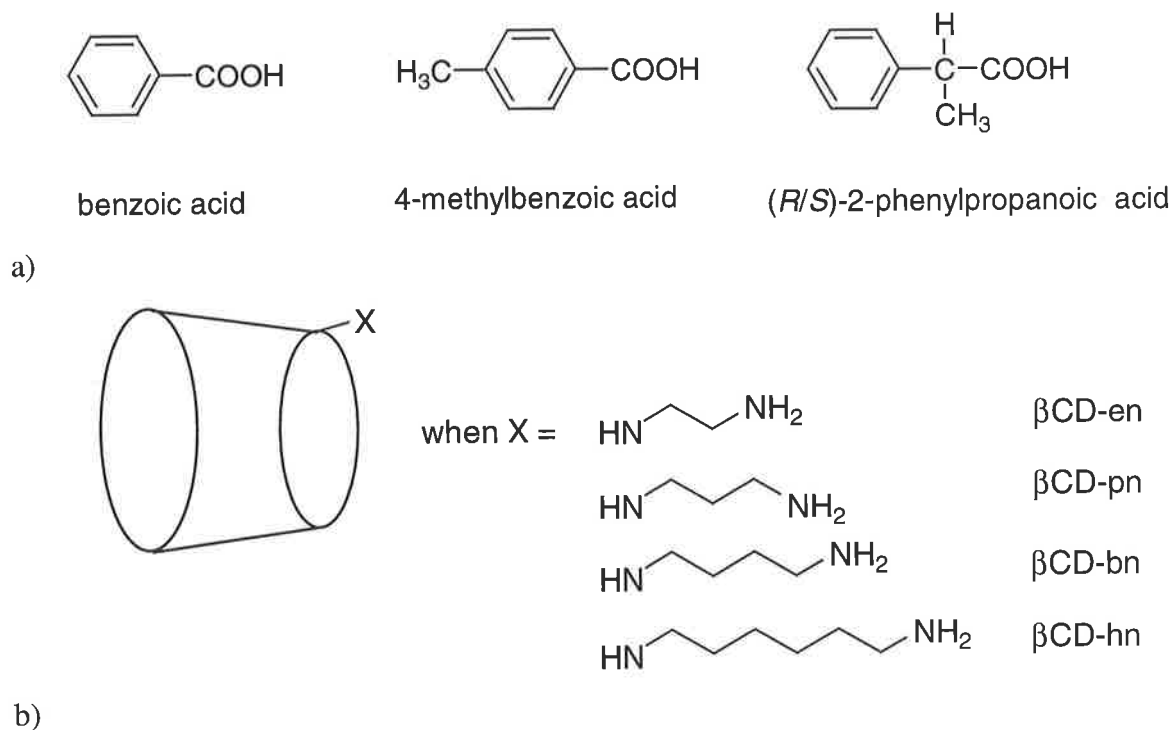
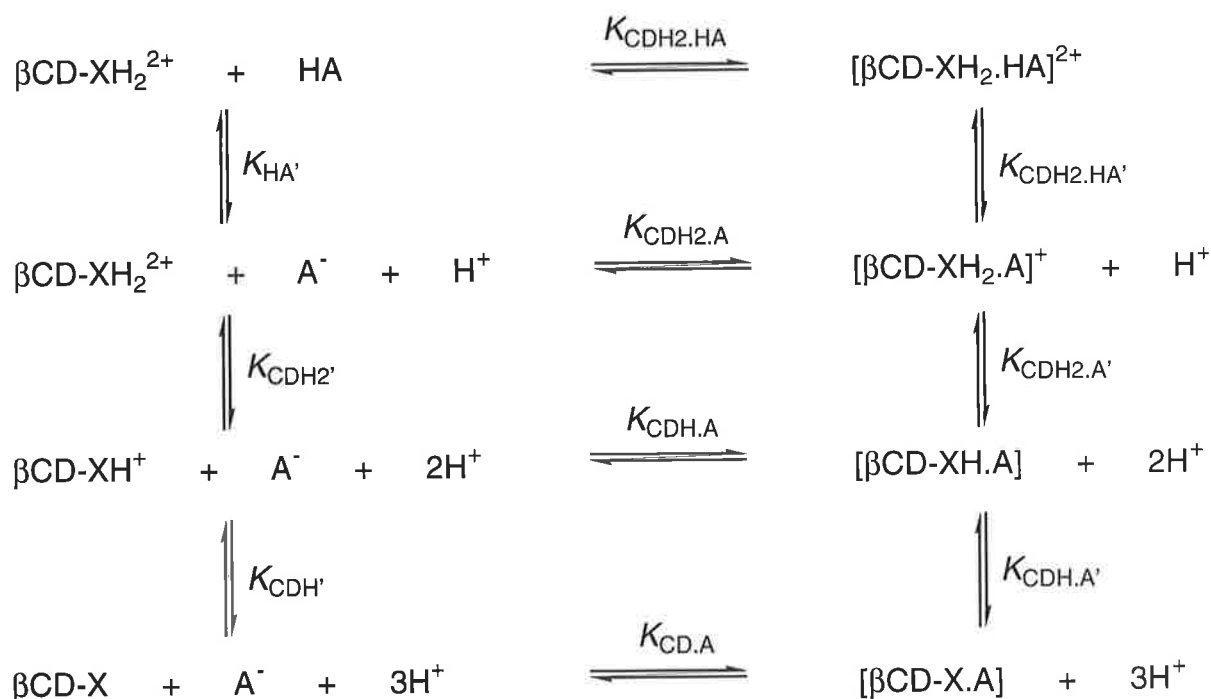


Figure 2.1. a) the structures of the aromatic carboxylic acid guests studied and b) the structure of the four 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins studied.

By virtue of the acidic and basic natures of the guests and hosts, respectively, a range of complexes may be formed, in principle, as shown in Scheme 2.1. However, not all of those shown will necessarily be detected as considerable variations in stability are anticipated.

Scheme 2.1.

The subscripts used to define the apparent stability constants, K , are CD, for $\beta\text{CD-X}$, and A for the carboxylate guest, with protonation as indicated. The acid dissociation constants of both the complexed and free species use the same nomenclature but are distinguishable by a prime symbol ('). For example the stability constant for the complexation of A by $\beta\text{CD-XH}$ is $K_{\text{CDH}.\text{A}'}$ whereas $K_{\text{CDH}.\text{A}}$ is the protonation constant of the complex $[\beta\text{CD-XH}.\text{A}]^+$. $K_{\text{HA}'}$ is the protonation constant of the free carboxylic acid whereas $K_{\text{CDH}_2.\text{HA}'}$ is the protonation constant of HA in the $[\beta\text{CD-XH}_2.\text{HA}]^{2+}$ complex. $K_{\text{CDH}_2.\text{HA}}$ and $K_{\text{CDH}_2.\text{A}}$ are the apparent stability constants for the complexation of HA and A^- by $\beta\text{CD-XH}_2^{2+}$ respectively.

Complexation of HA can only occur at low pH. Hence, the species $[\beta\text{CD-XH}.\text{HA}]^+$ and $[\beta\text{CD-X}.\text{HA}]$ are unlikely to form because of pK_a differences of the hosts and guests, thus, only complexes with A^- need be considered for $\beta\text{CD-XH}$ and $\beta\text{CD-X}$. $K_{\text{CDH}_2.\text{A}'}$ is the protonation constant of $\beta\text{CD-XH}_2^{2+}$ in the complex $[\beta\text{CD-XH}_2.\text{A}]^+$. Provided the protonation constants of the free guests and hosts are different from those of the complexed species, the acid-base titration of an acidified solution containing both HA and $\beta\text{CD-X}$ should allow the determination of the above apparent stability constants.

2.2. Results and Discussion.

A series of 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins, (β CD-X), were prepared and the pK_as of their protonated forms determined by potentiometric titration ($I = 0.10 \text{ mol dm}^{-3}$ (NaClO₄), 298.2 K).⁴ The pK_as of the free protonated diamines were determined to allow comparison with those of β CD-X (Figure 2.2, Table 2.1). A potentiometric titration study in aqueous solution ($I = 0.10 \text{ mol dm}^{-3}$ NaClO₄, 298.2 K) of the complexation of benzoic, 4-methylbenzoic, and (*R/S*)-2-phenylpropanoic acids with 6^A-deoxy-6^A-(aminoethylamino)- β -cyclodextrin (β CD-en), 6^A-deoxy-6^A-(aminopropylamino)- β -cyclodextrin (β CD-pn), 6^A-deoxy-6^A-(aminobutylamino)- β -cyclodextrin (β CD-bn), and 6^A-deoxy-6^A-(aminohexylamino)- β -cyclodextrin (β CD-hn) was undertaken.⁵ The apparent stability constants derived from the best fit of the equilibrium algorithm (represented by Scheme 2.) to the titrimetric data are shown in Table 2.2. Complexes whose maximum concentrations were $\leq 5\%$ of the total β CD-XH_nⁿ⁺ concentration were considered insignificant.

Table 2.1. The pK_a values for the protonated 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins (β CD-XH_nⁿ⁺ where $n = 0, 1$ or 2) and the protonated free diamines at $I = 0.10 \text{ mol dm}^{-3}$ (NaClO₄) and 298.2 K and the corresponding literature values for the protonated free diamines.

X		β CD-XH ₂ ²⁺	free XH ₂ ²⁺	literature XH ₂ ²⁺
en	pK _{a1}	9.42 $\pm$ 0.01	9.97 $\pm$ 0.03	9.98 $\pm$ 0.01 ^a
	pK _{a2}	5.70 $\pm$ 0.02	7.16 $\pm$ 0.02	7.28 $\pm$ 0.28 ^a
pn	pK _{a1}	9.94 $\pm$ 0.02	10.56 $\pm$ 0.02	10.55 $\pm$ 0.01 ^a
	pK _{a2}	7.44 $\pm$ 0.01	8.79 $\pm$ 0.01	8.88 $\pm$ 0.02 ^a
bn	pK _{a1}	10.26 $\pm$ 0.02	10.91 $\pm$ 0.02	10.80 $\pm$ 0.002 ^b
	pK _{a2}	8.06 $\pm$ 0.01	9.49 $\pm$ 0.01	9.63 $\pm$ 0.003 ^b
hn	pK _{a1}	10.27 $\pm$ 0.03	11.01 $\pm$ 0.06	11.02 $\pm$ 0.004 ^b
	pK _{a2}	8.72 $\pm$ 0.01	10.04 $\pm$ 0.03	10.24 $\pm$ 0.008 ^b

^a Reference 6. ^b Reference 7.

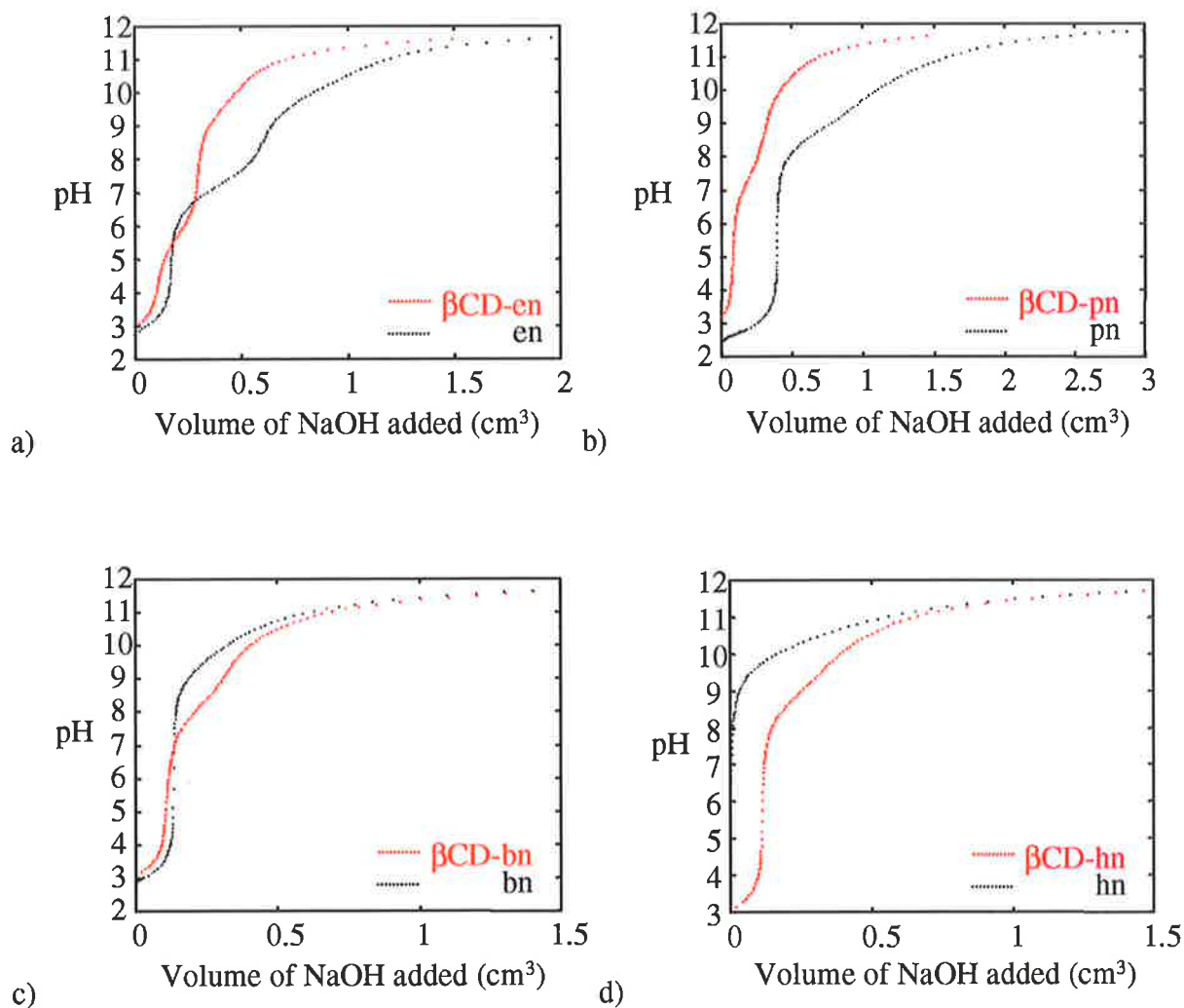


Figure 2.2. The titration profiles of each of the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins, (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) shown with that of the corresponding free diamine (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. The curves represent raw data from titrations of a) β CD-en and en, b) β CD-pn and pn, c) β CD-bn and bn and d) β CD-hn and hn, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K. The initial volume of the titration solution was 10.00 cm^3 .

Table 2.2. The apparent stability constants for the complexes of $\beta\text{CD-enH}_n^{n+}$, $\beta\text{CD-pnH}_n^{n+}$, $\beta\text{CD-bnH}_n^{n+}$ and $\beta\text{CD-hnH}_n^{n+}$ (where $n = 0, 1$, or 2) with benzoic, 4-methylbenzoic and (*R*)- and (*S*)-2-phenylpropanoic acid and their conjugate bases at $I = 0.10$ (NaClO_4) and 298.2 K

Guest	K $\text{dm}^3 \text{ mol}^{-1}$	$\beta\text{CD-enH}_n^{n+}$	$\beta\text{CD-pnH}_n^{n+}$	$\beta\text{CD-bnH}_n^{n+}$	$\beta\text{CD-hnH}_n^{n+}$
Benzoic acid/ benzoate ⁻	$K_{\text{CDH2.HA}}$	820 ± 170	350 ± 80	740 ± 100	545 ± 140
	$K_{\text{CDH2.A}}$	870 ± 70			
	$K_{\text{CDH.A}}$	180 ± 15		305 ± 35	275 ± 30
	$K_{\text{CD.A}}$		915 ± 60	950 ± 120	1000 ± 120
4-methylbenzoic acid/ 4-methylbenzoate ⁻	$K_{\text{CDH2.HA}}$		420 ± 60	415 ± 65	
	$K_{\text{CDH2.A}}$	1000 ± 170			
	$K_{\text{CDH.A}}$	140 ± 35			180 ± 20
	$K_{\text{CD.A}}$		885 ± 65	535 ± 50	750 ± 20
(<i>R</i>)-2-phenylpropanoic acid/ (<i>R</i>)-2-phenylpropanoate ⁻	$K_{\text{CDH2.HA}}$	850 ± 170	395 ± 50	420 ± 65	
	$K_{\text{CDH2.A}}$	790 ± 80			
	$K_{\text{CDH.A}}$				250 ± 35
	$K_{\text{CD.A}}$		760 ± 75	630 ± 30	1150 ± 295
(<i>S</i>)-2-phenylpropanoic acid/ (<i>S</i>)-2-phenylpropanoate ⁻	$K_{\text{CDH2.HA}}$	345 ± 40	690 ± 70	570 ± 80	
	$K_{\text{CDH2.A}}$	630 ± 30			
	$K_{\text{CDH.A}}$				285 ± 80
	$K_{\text{CD.A}}$		465 ± 60	630 ± 105	1760 ± 150

2.2.1. Determination of the pK_a s of the Cyclodextrin Bound and Free Protonated Diamines

A comparison of the pK_a s of the protonated free diamines determined via this study and those in the literature,^{6,7} show good agreement, with any differences appearing to be systematic. The values for β CD-pnH₂²⁺ reported in the literature,³ given as 7.39 ± 0.04 and 9.9 ± 0.1 for pK_{a1} and pK_{a2} respectively, agree very well with those determined here. These values were determined using the same laboratory equipment and method and indicate the high reproducibility of results between different operators. Values for the first and second pK_a s of β CD-enH₂²⁺ were determined as being 5.6 and 9.2 respectively, by Matsui *et al*, under unreported conditions.⁸ Bonomo *et al* reported values of 5.56 and 8.92 at $I = 0.10 \text{ mol dm}^{-3}$ KNO₃ and 298.2 K.⁹ While these values are in general agreement with the ones reported in this study. The differences between them may be attributable to different experimental conditions and operators. A more likely origin of the differences may be the purity of the modified cyclodextrin. A study into the production of modified cyclodextrins of high purity and reasonable yield has been undertaken within our laboratory and the resultant synthetic protocol published.⁴

Cucinotta *et al*, performed a ¹³C NMR titration study of a diethylenetriamine mono-substituted β -cyclodextrin and were able to assign the protonation order of the three nitrogen atoms.¹⁰ A ¹H NMR titration study on β CD-en by Bonomo *et al* also allowed assignation of a protonation order.⁹ In both cases it was determined that the first site of protonation was the primary amine, followed by the secondary amine adjacent the cyclodextrin moiety and finally, in the case of the diethylenetriamine substituent, at the secondary amine separating the two ethylene carbon chains. Even with reports such as these, there still appears to be some confusion in the literature as to the order of protonation with Yoshida *et al* depicting the first protonation of β CD-en at the secondary amine.¹¹

Based on the above studies we expect that the first protonation step of β CD-XH_nⁿ⁺, involves the primary amine of the diamine substituent, while the second involves the secondary amine adjacent to the cyclodextrin. Due to the effects of hydrogen bonding, this assignment may not so straight forward for β CD-enH₂²⁺. A study by Vacca and Arenare into the effects of a methyl substituent on the protonation thermodynamics of aliphatic diamines lead them to suggest that monoprotonated *N*-methylethylenediamine exists in two tautomeric forms, one protonated on the primary amine, the other on the secondary amine.⁶ Bonomo *et al* gave a structure consistent with these findings for β CD-enH⁺.⁹

2.2.2. Variations of the pK_a s of the Cyclodextrin Bound and Free Protonated Diamines

As the length of the protonated diamine bound to the cyclodextrin increases, there is a systematic increase in the pK_a s. This mirrors the trend set by the analogous free diamines. This increase in magnitude of pK_a s can be attributed to the increase in the aliphatic chain length. As the aliphatic chain length increases so to does the charge separation of the protonated amine groups, resulting in a decrease in electrostatic repulsion in the diprotonated species. In addition, the hydrophobicity of the system, hence the hydration of the diamine decreases. This results in the ability of the surrounding water to accept a proton from the protonated amine to decrease. As the length of the amine chain bound to the cyclodextrin increases, there is also a decrease in the difference between the two pK_a s, this too mirrors the trend set by the analogous free diamines and is also attributable to a decrease in electrostatic repulsion in the diprotonated species through increased charge separation.

The two pK_a s of $\beta\text{CD-XH}_2^{2+}$ are lower when compared with those of the corresponding protonated free diamine. This is due to being bound to a cyclodextrin and not just a result of a simple substitution at an amine nitrogen of the diamine. Substitution of ethylenediamine by a methyl, ethyl or propyl group at one nitrogen results in only minor changes in the pK_a s of the system (Table 2.3). The size and polarity of the βCD substituent may induce changes in the basicity of the amino nitrogens of the diamine, through steric and electronic effects. It may also induce a difference in solvation experienced by the protonation sites in $\beta\text{CD-X}$ compared to those of the free diamine. In addition, the diamine moiety in $\beta\text{CD-X}$ is bound adjacent the ring of six primary hydroxyl groups which delineate the narrow end of the cyclodextrin cavity. Hydrogen bonding between the protonated amine nitrogens and the primary hydroxyl groups may increase the basicity of the former. A similar explanation was given for the particularly low pK_a observed for $\beta\text{CD-NH}_3^+$.¹

Table 2.3. The pK_a values of ethylenediamine and *N*-methyl-, *N*-ethyl-, and *N*-propylethylenediamine.

	pK_{a1}	pK_{a2}
ethylenediamine, $\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}_2$	9.97 ^a	7.16 ^a
<i>N</i> -methylethylenediamine, $\text{CH}_3\text{NH}(\text{CH}_2)_2\text{NH}_2$	10.11 ^b	7.04 ^b
<i>N</i> -ethylethylenediamine, $\text{CH}_3\text{CH}_2\text{NH}(\text{CH}_2)_2\text{NH}_2$	10.16 ^b	7.10 ^b
<i>N</i> -propylethylenediamine, $\text{CH}_3(\text{CH}_2)_2\text{NH}(\text{CH}_2)_2\text{NH}_2$	10.19 ^b	7.39 ^b

^a Results of this study. ^b Values from reference 12 determined at $I = 0.10 \text{ mol dm}^{-3}$ and 298 K

2.2.3. Formation of Complexes with the Aromatic Carboxylic Acids.

Stability constants were determined from a comparison of the variation in pH of a titration involving the cyclodextrin, $\beta\text{CD-XH}_n^{n+}$, and guest species of interest, to that of a titration involving just $\beta\text{CD-XH}_n^{n+}$ (Figure 2.3 and Appendix A). The magnitude of the stability constants encompassed by the range of complexes detected varied from 140 and 1760 $\text{dm}^3 \text{ mol}^{-1}$ for $[\beta\text{CD-enH.4-methylbenzote}]$ and $[\beta\text{CD-hn.(S)-2-phenylpropanoate}]$ respectively. Scheme 2.1 assumes complexation of all species in solution, hence for the four guests and four hosts studied there is a probability of forming 64 possible complexes in total. Of these 64 complexes only 35 were detected. Each possible type of complex was detected for each guest species, but each type of complex was not detected for each $\beta\text{CD-XH}_n^{n+}$ host. Inspection of Table 2.2 reveals systematic absences about the diagonal for $\beta\text{CD-enH}_n^{n+}$ through to $\beta\text{CD-hnH}_n^{n+}$ or as the length of the carbon chain increases. The $[\beta\text{CD-X.A}]$, (negatively charged), complex was not detected for $\beta\text{CD-en}$. The $[\beta\text{CD-XH.A}]$, (neutral), and $[\beta\text{CD-XH}_2.\text{A}]^+$, (mono positively charged), complexes were not detected for $\beta\text{CD-pnH}_n^{n+}$ or $\beta\text{CD-bnH}_n^{n+}$ (where $n = 1$ or 2 , except in the case of $\beta\text{CD-bnH}^+$ when the guest was benzoate for the neutral complex). For $\beta\text{CD-hnH}_n^{n+}$, the only positively charged complex formed $[\beta\text{CD-hnH}_2.\text{benzoic acid}]^{2+}$.

Various weak secondary bonding interactions between the $\beta\text{CD-XH}_n^{n+}$ host and the guest aromatic carboxylic acids/carboxylates determine the relative stability of any complex formed. The complexation of the guest aromatic carboxylic acids/carboxylates by $\beta\text{CD-XH}_n^{n+}$ appears to involve some conformational changes in and dehydration of both the host and guest to an extent dependant on the nature of each. Variations in the stabilities of the complexes formed by $\beta\text{CD-XH}_n^{n+}$ with the guest aromatic carboxylic acids/carboxylates may arise from a range of causes. The simplest interpretation of an increase in complex stability is that the rate of decomplexation has slowed relative to the rate of complexation.

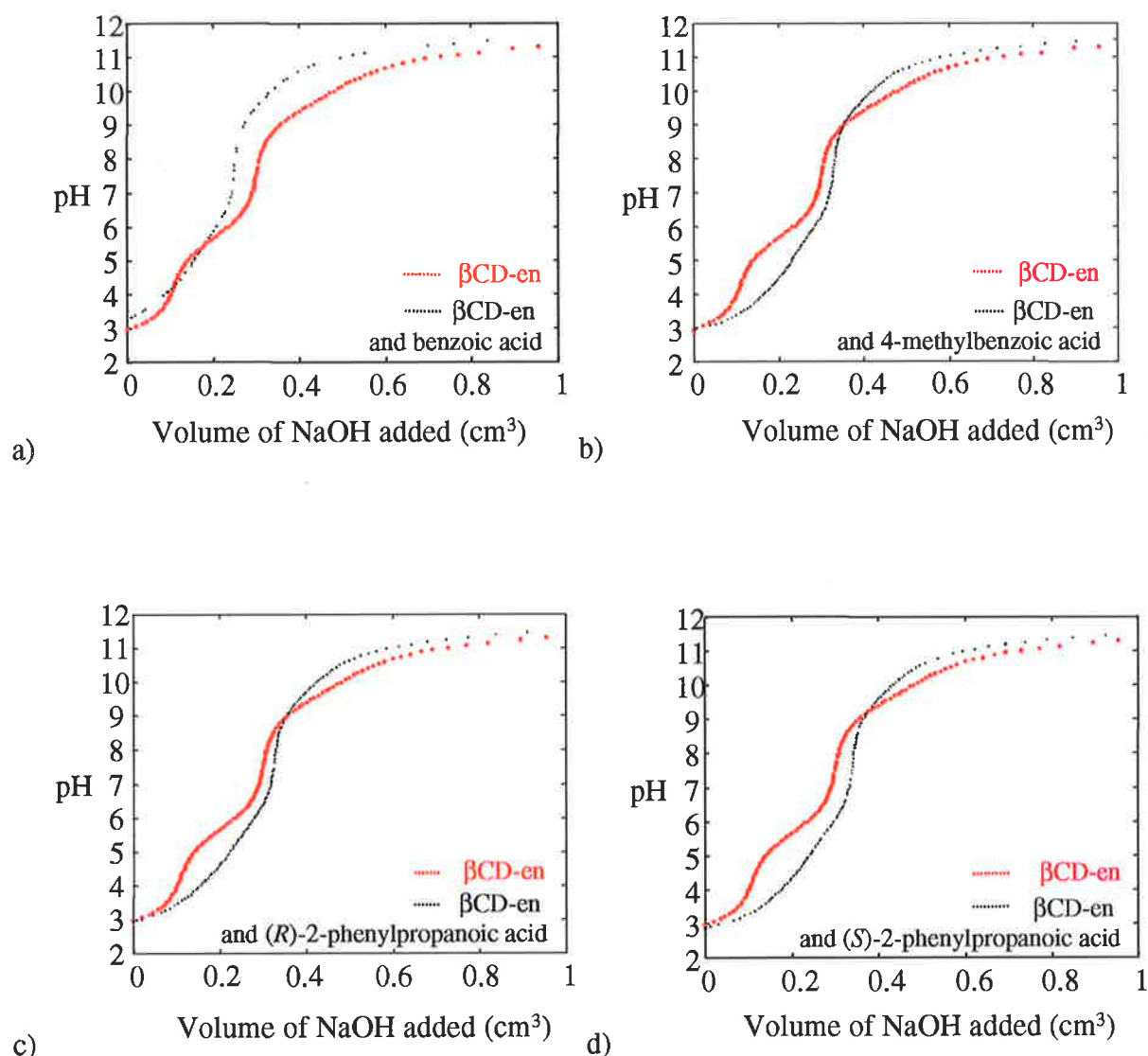


Figure 2.3. The titration profiles of 6^A-deoxy-6^A-(aminoethyl)amino- β - in the presence cyclodextrin (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest acids (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) used in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. A comparison of titration profiles allowed determination of the complex formation and the derivation of stability constants. The curves represent raw data from titrations of β CD-en in the presence and absence of benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K. The initial volume of the titration solution was 10.00 cm^3 .

The pattern that emerges for the variation in stability constants for a single carboxylic acid and its carboxylate being complexed by $\beta\text{CD-XH}_n^{n+}$ is represented by Figure 2.4. The most stable complexes occur in the top left and bottom right corners of the rectangle (with the exception of $[\beta\text{CD-enH}_2\text{.4-methylbenzoic acid}]^{2+}$ which was not detected). There is an absence of complexes about the diagonal running from bottom right to top left of the rectangle. In aqueous media the main factors affecting the variation in stabilities of complexes with cyclodextrins are i) hydrophobicity and ii) charge, which both impact upon iii) hydration, and finally iv) steric interactions between the guest and host. These factors superimpose on the apparent stabilising effect of the hydrophobic portion of the guest. The systematic absences in Table 2.2 may be explained by considering these factors (i – iv) and their interplay in the systems studied.

The cyclodextrin annulus and the two amine groups of the hosts are constant features. Hence variations in the factors affecting complex stabilisation and attributable to the cyclodextrins, will arise predominantly from the variation in the carbon chain length of the amine substituent. The aromatic moiety and carboxylic acid group of the four guests remain constant. Thus, differences in their complexation characteristics arise from differences in their hydrophobicity and stereochemistry imparted by the addition of alkyl moieties in 4-methylbenzoic acid and (*R/S*)-2-phenylpropanoic acid when compared with those of benzoic acid.

Descending the vertical axis of Figure 2.4 is the same as increasing the pH of the solution. Correspondingly, the charge on $\beta\text{CD-XH}_n^{n+}$ decreases with decreasing protonation and its hydrophobicity subsequently increases. Simultaneously, the guest becomes negatively charged which may decrease its hydrophobicity and in turn increasing its hydration. On the horizontal axis, $\beta\text{CD-XH}_n^{n+}$ hydrophobicity increases and charge separation in $\beta\text{CD-XH}_2^{2+}$ increases from right to left of Figure 2.4 (ie. from $\beta\text{CD-enH}_n^{n+}$ to $\beta\text{CD-hnH}_n^{n+}$) corresponding to an increase in aliphatic chain length. Thus, in the upper left hand corner of Figure 2.4, the host has a dipositive charge and the guest has either no charge or a negative charge, and it appears that either a charge-dipole or a charge-charge interaction stabilises complex formation. As the charge separation on $\beta\text{CD-XH}_2^{2+}$ increases, the intensity of this interaction appears to diminish with a corresponding decrease in complex stabilities in the upper right hand corner of Figure 2.4. These results give no information as to the orientation of the guest within the annulus and any changes that occur with variation in host protonation. This was investigated by molecular modelling studies and the results are discussed in Section 2.2.4.

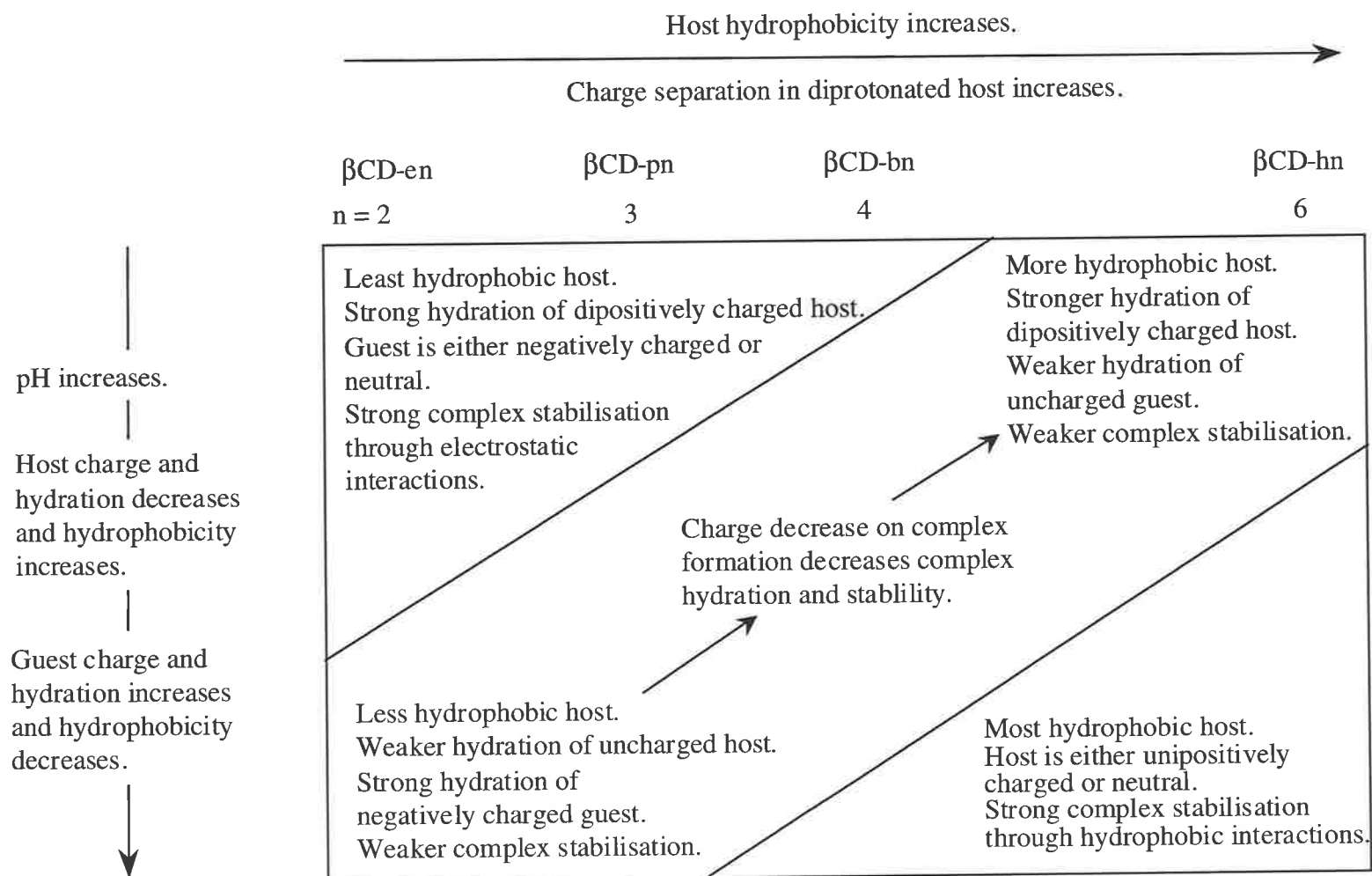


Figure 2.4. A schematic representation of the variation of stabilities observed in Table 2.2 and the factors contributing to them where n is the number of carbon atoms in the aliphatic chain of the diamine substituent.

At the lower left-hand corner of Figure 2.4, the host is uncharged, the guest is negatively charged and no complexes are observed. Proceeding from left to right of Figure 2.4, the hydrophobicity of the β CD-X increases and complexation of the carboxylate guest strengthens to give more stable complexes in the lower right hand corner. These results indicate that an increase in β CD-X hydrophobicity is the main source of complex stabilisation.

Coinciding with changes in hydrophobicity and charge are changes in hydration. The hydration of β CD-XH_nⁿ⁺ probably has two main components: water interacting weakly within the annulus and water hydrogen bonding to the hydroxyl groups delineating the annulus ends. A major change in hydration is experienced upon the displacement of water from the annulus by the hydrophobic moiety of the guest during complexation and probably makes a significant contribution to the free energy of complexation. Depending on the protonation of both β CD-X and guest, the extent of hydration may increase or decrease on formation of the complex. The complete or partial cancellation of the charge on the host by that of the guest is likely to produce a decrease in overall hydration of the complex, when compared with that of the charged components, lessening the complex stability. This occurs in the centre of Figure 2.4 and is exemplified by the complexation of β CD-XH₂²⁺ and β CD-XH⁺ with the guest carboxylates. Hydration effects on stability are likely to be less for the complexes of β CD-X and the carboxylates or β CD-XH₂²⁺ and the carboxylic acids, as there is no overall change in the charge on the host. Additional stability may be imparted to a complex through the hydrophobic aromatic guest being only partially encapsulated by the hydrophobic β CD-XH_nⁿ⁺ annulus thus allowing their hydrophilic moieties to retain hydration. This was also investigated by molecular modelling studies (Section 2.2.4)

There is no apparent systematic trend in steric interaction with variation in aliphatic carbon chain length for β CD-XH_nⁿ⁺ other than those mentioned in the discussion of factors i) to iv) previously. This is also true for the guests. There is, however, a small chiral discrimination for (*R*)-2-phenylpropanoic acid over (*S*)-2-phenylpropanoic acid by β CD-enH₂²⁺ and *vice versa* for β CD-pnH₂²⁺. Although small, this discrimination is consistent with the length of the aliphatic carbon chain influencing the guest orientation. This is also the case for β CD-hn, which is slightly selective for (*S*)-2-phenylpropanoate over the (*R*)- enantiomer, and β CD-pn, for which the opposite trend is seen (Figure 2.5).

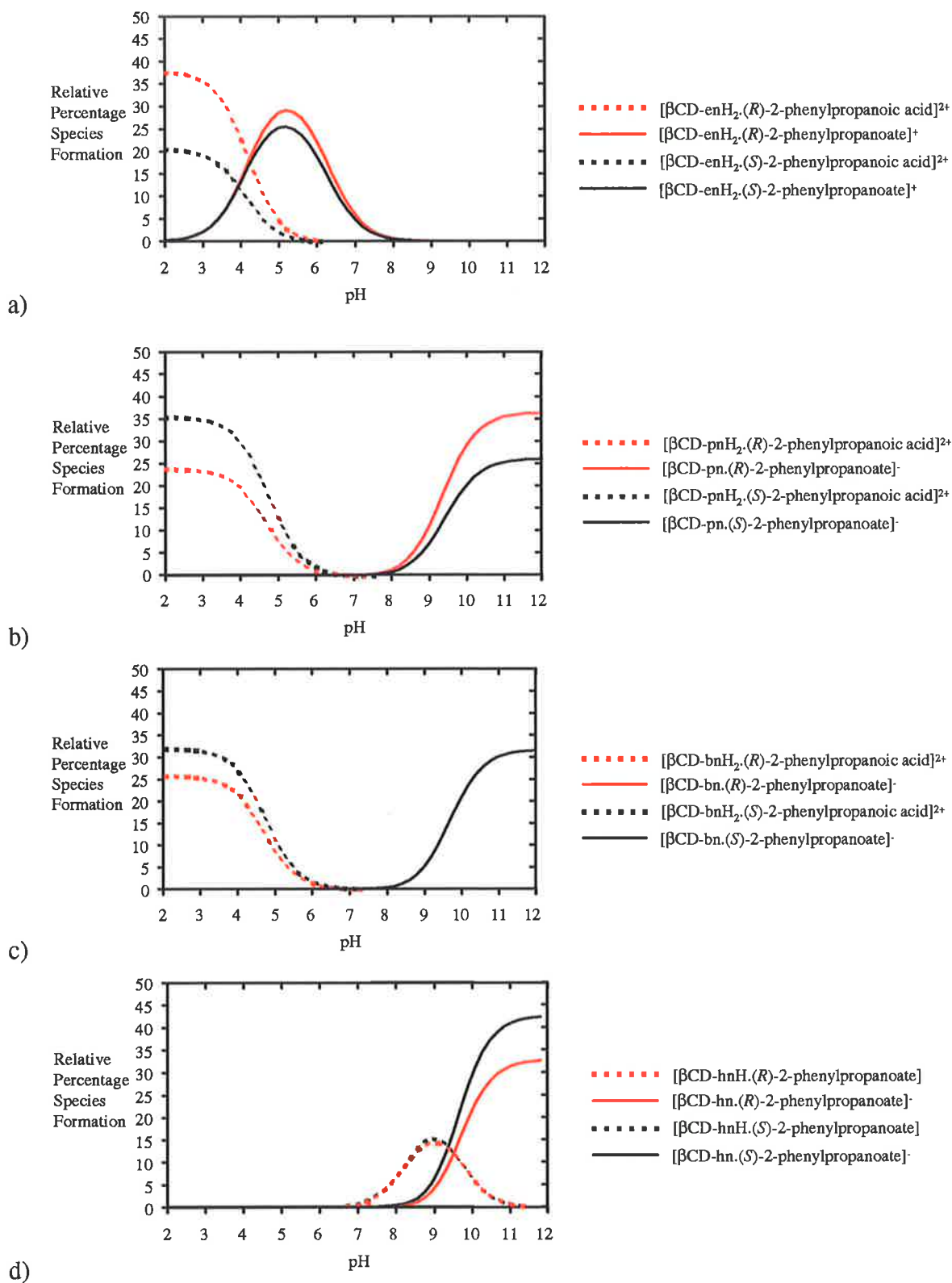


Figure 2.5. The percentage of complexes containing (*R*)- or (*S*)-2-phenylpropanoic acid in solutions $2 \times 10^{-3} \text{ mol dm}^{-3}$ of each $[\text{guest}]_{\text{total}}$ and $[\beta\text{CD-X}]_{\text{total}}$. Plots were calculated from the data in Table 2.2 and plotted relative to $[\beta\text{CD-X}]_{\text{total}} = 100\%$. The plots represent the complexes containing (*R*)- and (*S*)-2-phenylpropanoic acid and its conjugate base with a) $\beta\text{CD-enH}_n^{n+}$, b) $\beta\text{CD-pn}$, c) $\beta\text{CD-bn}$ and d) $\beta\text{CD-hn}$.

2.2.4. Molecular Modelling of Complexes with the 6^A-Deoxy-6^A-(ω -aminoalkyl)amino- β -Cyclodextrins.

Gas phase molecular modelling using a CVFF force field, (Section 6.2.3), was employed to investigate the stereochemistry of selected complexes of the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins encompassed by Table 2.2. In addition, the complexes of 4-nitrophenylacetate and 1-adamantane carboxylate with β CD-hn were investigated.

The global energy minimised (877.1 kJ mol⁻¹) structure of β CD-hn, (Figure 2.6), clearly shows the aminoalkyl chain residing within the cyclodextrin annulus and the secondary amine group hydrogen bonding to a secondary hydroxyl group (bond distance of 2.14 Å). This structure is consistent with NMR data.⁵ A ¹H ROESY NMR spectrum of β CD-hn at pH $\geq$ 11.5 shows NOE cross-peaks between hnH1 – hnH6 of the hn substituent and the H3 and H5 protons of the cyclodextrin annulus (Figure 2.7). Together with the cross-peaks between hnH1 – hnH3 and H5^A, these results give a strong indication of self complexation of the hn substituent by β CD-hn. The ¹H ROESY NMR spectrum of β CD-hnH₂²⁺ gives no cross-peaks. This is consistent with the global energy minimised (1162.7 kJ mol⁻¹) structure of β CD-hnH₂²⁺ which revealed the hnH₂²⁺ substituent to point up and away from the cyclodextrin moiety. A summary of the one dimensional ¹H NMR and two dimensional ¹H ROESY NMR assignments for β CD-hn are given in Table 2.3.

Gas phase modelling of the [β CD-hn.4-methylbenzoate]⁻ complex produced a global energy minimum of 797.4 kJ mol⁻¹. The orientation of the 4-methylbenzoate guest was such that the charged carboxylate group hydrogen bonded to the secondary end of the annulus (bond distances of 1.70 and 1.55 Å) and the hydrophobic 4-methylbenzoate moiety was complexed inside the hydrophobic annulus of the β CD-hn. In addition, the hn substituent is still complexed inside the annulus (Figure 2.8). The complexation of two components within a cyclodextrin annulus has been known to enhance the stability of a complex.¹³ Toluene was found to enhance the binding of pyrene to γ -cyclodextrin by the formation of a ternary complex. The toluene is considered to act as a space filler or regulator, and the hn substituent appears to fill a similar role in the complexes studied here. The observed orientation of the guest within the cyclodextrin (Figure 2.8) goes against the orientation anticipated from complimentary alignment of the dipoles of the guest and cyclodextrin host,^{1,2} but has also

been found in adamantane-1-carboxylate complexes of α CD and β CD¹⁴ and the cyclohexanecarboxylate complex of β CD.¹⁵ When the orientation of 4-methylbenzoate is reversed so that the carboxylate group is orientated towards the primary end of the cyclodextrin, the complex energy is 870.4 kJ mol⁻¹. This is consistent with the former orientation being the more favoured in the gas phase. However, the effect of hydration is not taken into account in these modelling studies. Such hydration could modify the relative stabilities of the complexes.

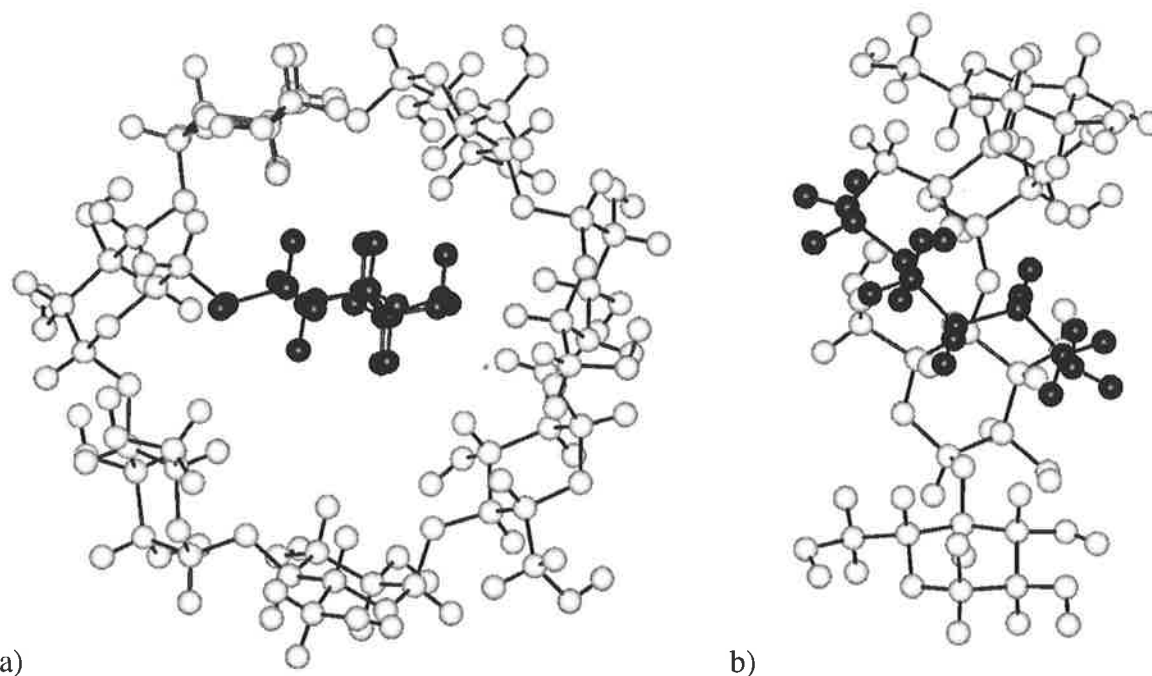


Figure 2.6. The global energy minimised structure of β CD-hn viewed from a) the primary face of the annulus and b) from the side with three glucose units cut away. The hn substituent is shown in dark shading.

Table 2.3. The spectral assignments for β CD-hn alone at $\text{pH} \geq 11.5$. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the hn substituent) as in Figure 1.1. The hexyl protons are labelled hnH1 – hnH6 as distance increases from the secondary amine.

1D ^1H NMR spectrum		
δH	Appearance, Integration	Assignment
4.80	singlet, 7H + solvent	H1
3.5-3.8	multiplet, 26H	H3, H5, H6
3.2-3.4	multiplet, 13H	H2, H4
3.11	triplet, $J = 9.3$ Hz, 1H	H4 ^A
2.93	doublet, $J = 12.4$ Hz, 1H	H6 ^A
2.70	multiplet, 1H	H6 ^{A'}
2.65	multiplet, 2H	hnH6
2.46	multiplet, 2H	hnH1
1.40	broad singlet, 4H	hnH2, hnH5
1.26	broad singlet, 4H	hnH3, hnH4

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
1.26	hnH3, hnH4	1.40	hnH2, hnH5
		2.46	hnH1
		2.65	hnH6
		3.7	H5
		3.8	H3
1.40	hnH2, hnH5	1.26	hnH3, hnH4
		2.46	hnH1
		2.65	hnH6
		3.7	H5
		3.8	H3
2.46	hnH1	1.26	hnH3, hnH4
		1.40	hnH2, hnH5
		3.9	H5 ^A
2.65	hnH6	1.40	hnH2, hnH5
		3.7	H5
		3.8	H3

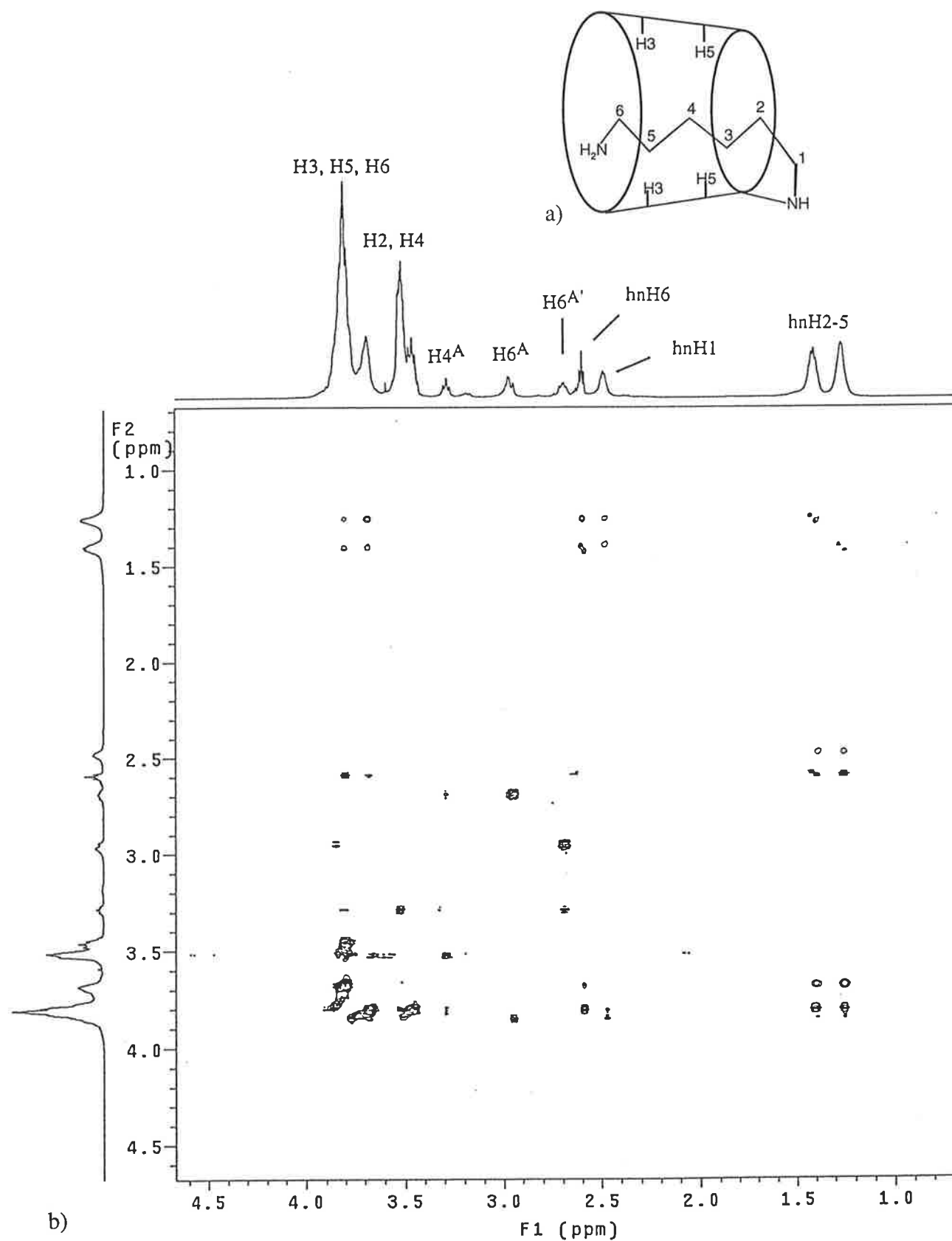


Figure 2.7. a) A representation of β CD-hn showing the hydrogen numbering scheme. b) The ^1H 600 MHz ROESY NMR spectrum of β CD-hn. Cross-peaks are formed between $\text{hnH2} - \text{hnH6}$ and H3 of β CD-hn, $\text{hnH1} - \text{hnH6}$ and H5 of β CD and $\text{hnH1} - \text{hnH3}$ and H5^A of β CDhn. The signals along the diagonal have been removed for clarity

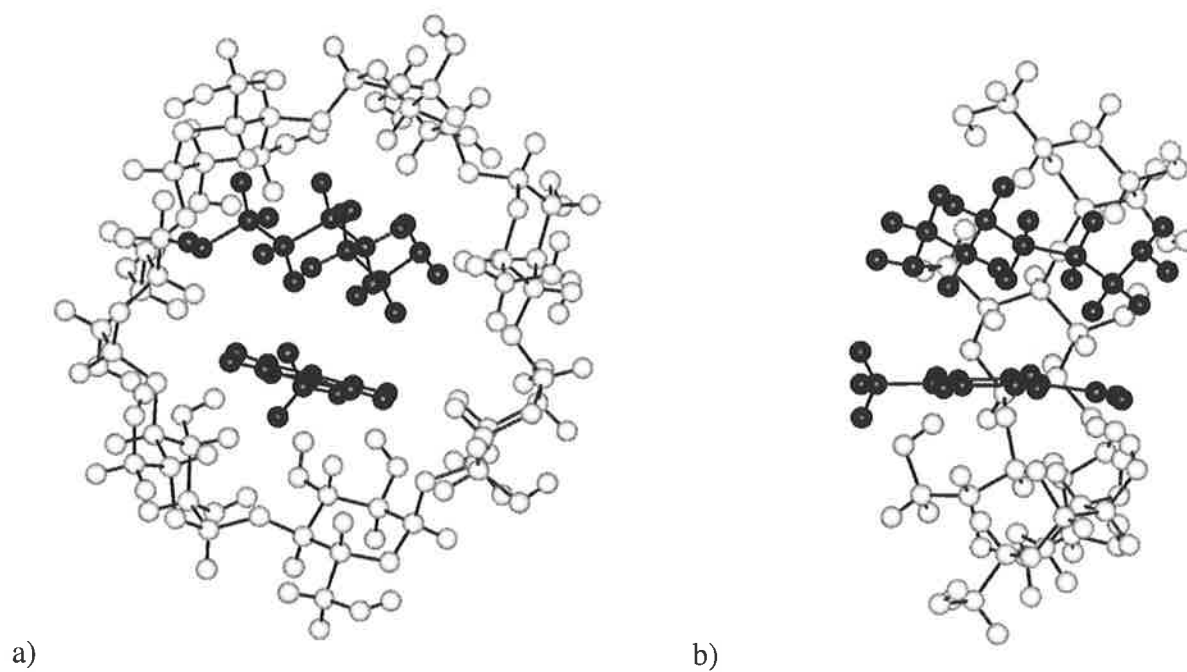
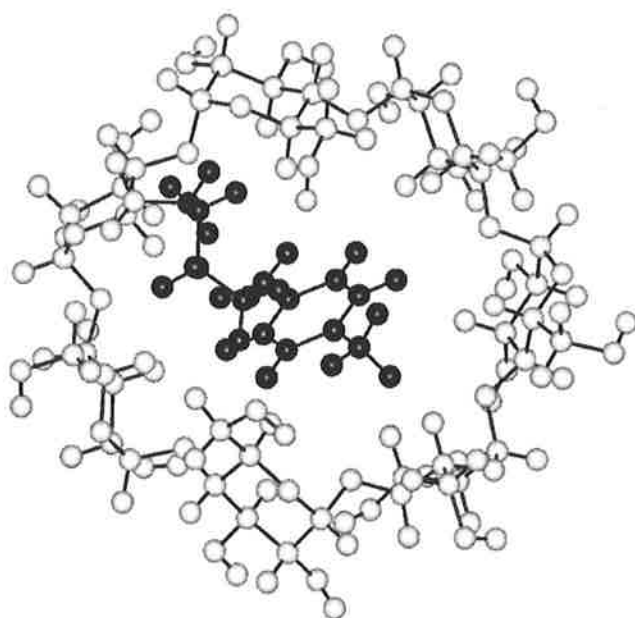
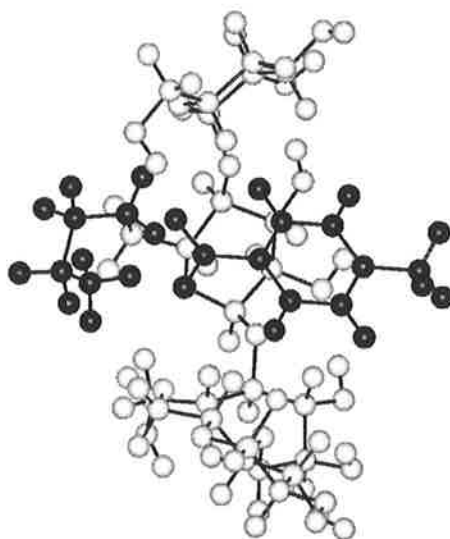


Figure 2.8. The global minimised structure of $[\beta\text{CD-hn.4-methylbenzoate}]^-$ viewed from a) the primary face of the βCD annulus and b) the side with three glucopyranose units cut away. The hn substituent and the 4-methylbenzoate are shown in dark shading.

The global energy minimised ($610.6 \text{ kJ mol}^{-1}$) structure of $[\beta\text{CD-enH}_2\text{,4-methylbenzoate}]^{2+}$, (Figure 2.9), clearly shows the 4-methylbenzoate orientated with the carboxylate towards the primary end of the cyclodextrin. The carboxylate group is within hydrogen bonding distance of both the amine nitrogens and a primary hydroxyl group (Table 2.5). When the orientation of 4-methylbenzoate is reversed so that the carboxylate group is orientated towards the secondary end of the cyclodextrin, the complex energy is $811.9 \text{ kJ mol}^{-1}$. Thus the former orientation is preferred and is consistent with the charge on the diamine substituent substantially influencing guest orientation through electrostatic interactions.



a)

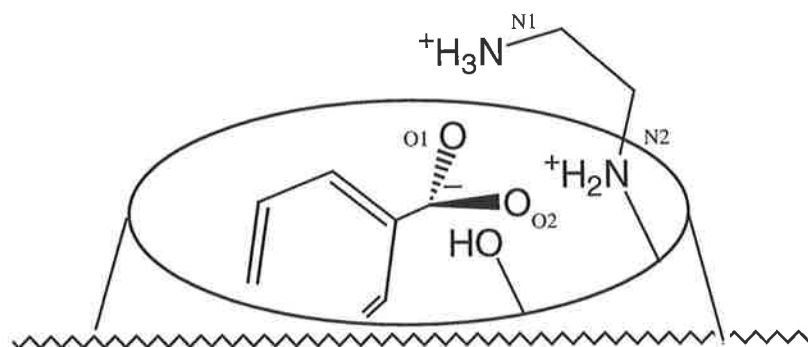


b)

Figure 2.9. The global energy minimised structure of $[\beta\text{CD-enH}_2\text{,4-methylbenzoate}]^{2+}$ viewed from a) the primary face of the βCD -annulus and b) from the side with three glucopyranose units cut away. The enH_2^{2+} substituent and the 4-methylbenzoate are in dark shading.

Table 2.5. The distances between the two carboxylate oxygens (O1, O2) of 4-methylbenzoate and the primary, (N1), and the secondary, (N2), amines and a primary hydroxyl group of β CD-enH₂²⁺. Distances were obtained from gas phase molecular model of the complex $[\beta$ CD-enH₂.4-methylbenzoate]⁺. The atoms of interest are labelled as per the stylised diagram below.

4-methylbenzoate ⁻	β CD-enH ₂ ²⁺	Distance (Angstroms)
O1	N1	1.78
	N2	1.77
	OH	3.50
O2	N1	1.89
	N2	2.13
	OH	1.53



Molecular modelling of both the (*R*)- and (*S*)-2-phenylpropanoate complexes of β CD-hn produced similar structures where the hn substituent and the carboxylate guest are complexed within the β CD-hn annulus simultaneously. The carboxylate groups of the guests are orientated towards the secondary end of the annulus. There are some differences in orientation of the two guests within the β CD-hn annulus (Figure 2.10). The globalised energy minima of $[\beta$ CD-hn.(*R*)-2-phenylpropanoate]⁻ and its (*S*)- analogue are 812.3 and 738.5 kJ mol⁻¹, respectively, showing the (*S*)- diastereomer to be more stable in the gas phase. This is in qualitative agreement with the results from the potentiometric titration study (Table 2.2).

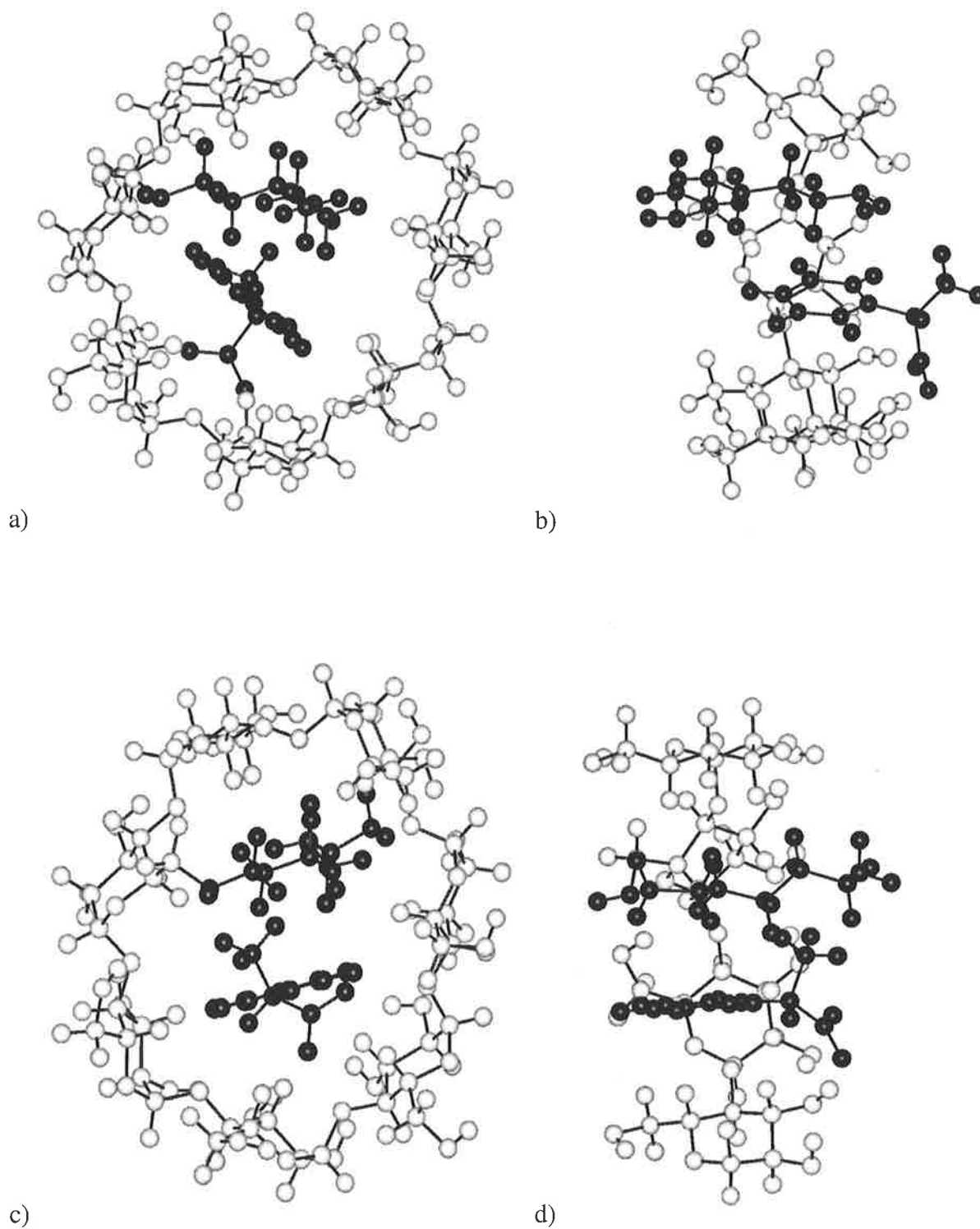


Figure 2.10. The global energy minimised structures of $[\beta\text{CD-hn.}(R)\text{-2-phenylpropanoate}]^-$ (viewed from a) the primary face of the βCD annulus and b) from the side with three glucopyranose units cut away) and $[\beta\text{CD-hn.}(S)\text{-2-phenylpropanoate}]^-$ (viewed from c) the primary face of the βCD annulus and b) from the side with three glucopyranose units cut away). The hn substituents and the (R/S)-2-phenylpropanoates are shown in dark shading.

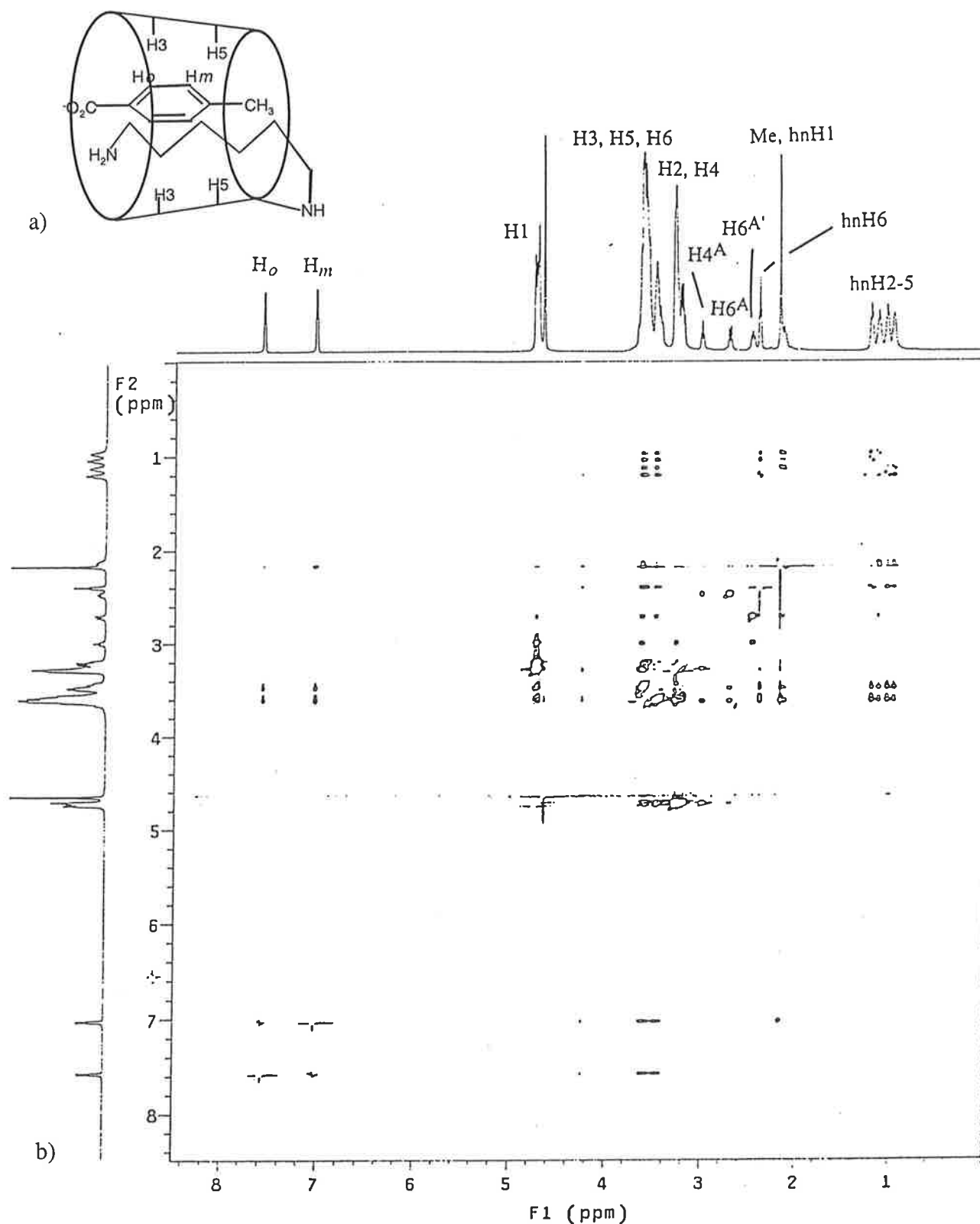
The simultaneous complexation of both the hn substituent and a carboxylate guest by β CD-hn, as depicted by the modelling study, is consistent with results seen from ^1H ROESY NMR studies.⁵ In the ^1H ROESY NMR spectra of $[\beta\text{CD-hn.4-methylbenzoate}]^-$ (Figure 2.11), the hnH1 – hnH6 protons showed cross-peaks with the H3 and H5 protons of β CD-hn. In addition cross-peaks were observed due to interactions between the *m* and *o* protons of 4-methylbenzoate and the H3 and H5 protons of β CD-hn. However, there are no cross-peaks due to interactions between the hn substituent and the carboxylate guest. Similar results were obtained for $[\beta\text{CD-hn.(R)-2-phenylpropanoate}]^-$ and its (*S*)- analogue. A summary of the one dimensional ^1H NMR and two dimensional ^1H ROESY NMR assignments for $[\beta\text{CD-hn(4-methylbenzoate)}]^-$ are given in Tables 2.6 and 2.7 respectively.

Table 2.6. The 1D ^1H NMR spectral assignments for $[\beta\text{CD-hn.4-methylbenzoate}]^-$ at $\text{pH} \geq 11.5$. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the hn substituent) as in Figure 1.1. The hexyl protons are labelled hnH1 – hnH6 as distance increases from the secondary amine. The aromatic guest protons are labelled as Ho and Hm where the former is adjacent to the carboxylate group.

1D ^1H NMR spectrum		
δH	Appearance, Integration	Assignment
7.80	doublet, $J = 7.8$ Hz, 2H	Ho
7.29	doublet, $J = 7.8$ Hz, 2H	Hm
5.00	multiplet, 7H + solvent	H1
3.6-3.9	multiplet, 26H,	H3, H5, H6
3.4-3.6	multiplet, 13H	H2, H4
3.26	triplet, $J = 9.6$ Hz, 1H	H4 ^A
2.99	doublet, $J = 13.2$ Hz, 1H	H6 ^A
2.71	multiplet, 1H	H6 ^{A'}
2.64	triplet, $J = 7.2$ Hz, 2H	hnH6
2.45	triplet, $J = 7.2$ Hz, 2H	hnH1
2.41	singlet, 3H,	Me
1.4-1.5	multiplet, 4H	hnH2, hnH5
1.2-1.3	multiplet, 4H	hnH3, hnH4

Table 2.7. The 2D ^1H ROESY NMR spectral assignments for $[\beta\text{CD-hn.4-methylbenzoate}]^-$ at $\text{pH} \geq 11.5$ with proton numbering as for Table 2.6.

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
1.2-1.3	hnH3, hnH4	1.4-1.5	hnH2, hnH5
		2.45	hnH1
		2.64	hnH6
		3.8	H5
		3.9	H3
1.4-1.5	hnH2, hnH5	1.2-1.3	hnH3, hnH4
		2.45	hnH1
		2.64	hnH6
		3.8	H5
		3.9	H3
2.41	Me	7.29	Hm
2.45	hnH1	1.2-1.3	hnH3, hnH4
		1.4-1.5	hnH2, hnH5
		3.8	H5
		3.9	H3
2.64	hnH6	1.2-1.3	hnH3, hnH4
		1.4-1.5	hnH2, hnH5
		3.8	H5
		3.9	H3
2.71	H6 ^A	3.26	H4 ^A
3.26	H4 ^A	2.71	H6 ^{A'}
3.8	H5	1.2-1.3	hnH3, hnH4
		1.4-1.5	hnH2, hnH5
		7.29	Ho
		7.80	Hm
3.9	H3	1.2-1.3	hnH3, hnH4
		1.4-1.5	hnH2, hnH5
		2.45	hnH1
		2.64	hnH6
		7.29	Ho
		7.80	Hm
7.29	Ho	2.41	Me
		3.8	H5
		3.9	H3
		7.80	Hm
7.80	Hm	3.8	H5
		3.9	H3
		7.29	Ho



Further molecular modelling of β CD-hn looked at the structures of complexes with 4-nitrophenylacetate, (*p*NPA). β CD-hn is known to catalyse the deacylation of *p*NPA and it was envisioned that the modelled structures would represent the ground state Michaelis complexes in solution.¹⁶ As was observed for the carboxylic acids, the hn substituent was found to include simultaneously with the *p*NPA in the β CD-hn annulus. Similar energies were determined for the complexes of [β CD-hn.*p*NPA] when the guest was orientated with its acetate group adjacent to either the secondary face or the primary face of the β CD-hn annulus (Figures 2.12 and 2.13), being 971.6 and 958.9 kJ mol⁻¹ respectively. For the former complex, the distance from the carbonyl carbon of *p*NPA to the primary amine group of the hn substituent is 3.65 Å, for the latter it is 5.13 Å. The small energy difference between the isomeric complexes is consistent with the coexistence of both species in appreciable amounts and with one being the reactive complex (more likely the former due to the proximity of reactive sites) and the other not leading to deacylation. Energies of 1244.8 and 1243 kJ mol⁻¹ were determined for the above complexes respectively with the hn substituent located outside the cyclodextrin annulus. This gives an indication of the stabilising effect of inclusion of the hn substituent.

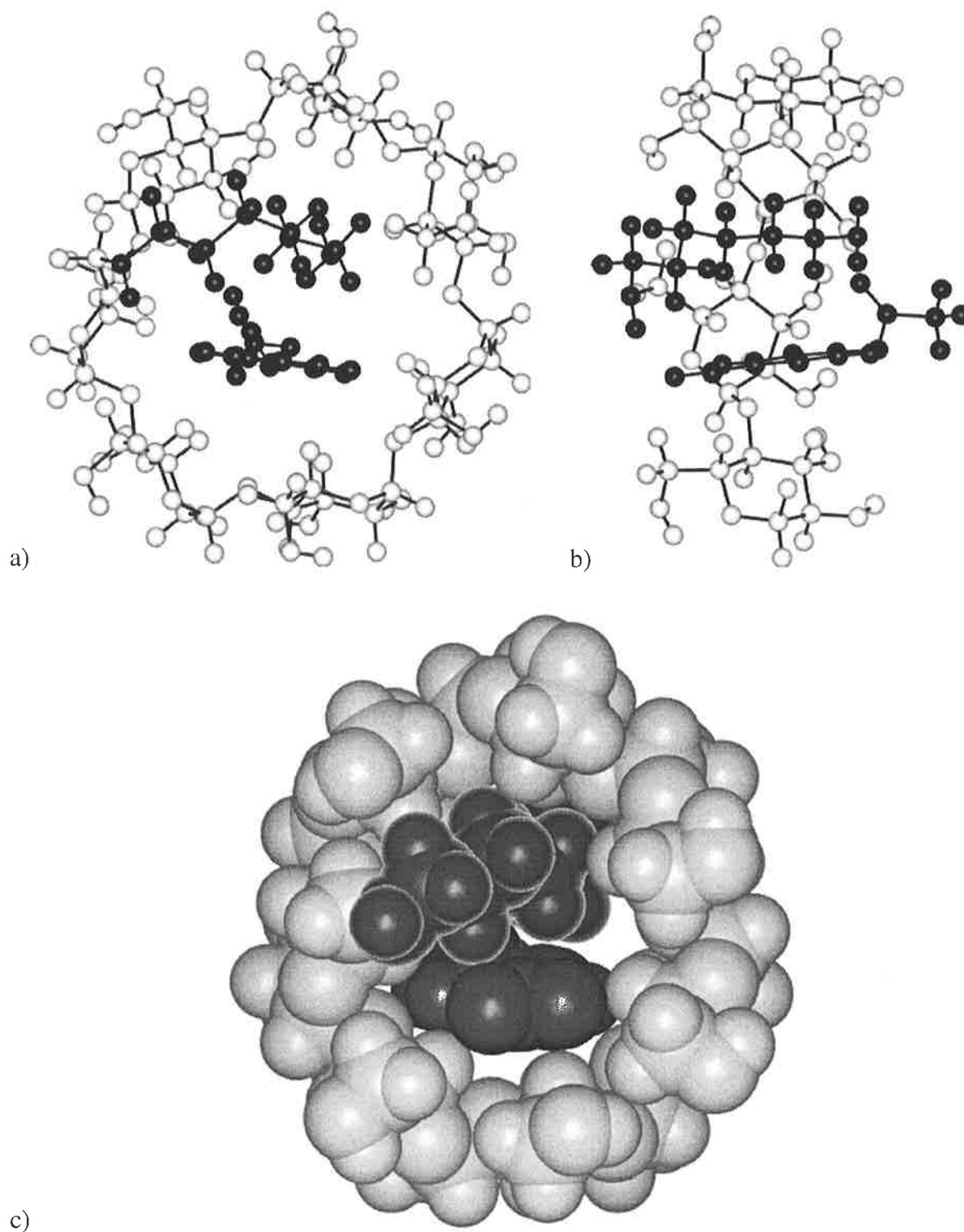


Figure 2.12. The global energy minimised structure of the complex formed between β CD-hn and pNPA (where pNPA is orientated with the acetate group adjacent the secondary end of the β CD annulus viewed from a) the primary face of the β CD annulus and b) from the side with three glucopyranose units cut away. The CPK representation of a) is also shown (c).

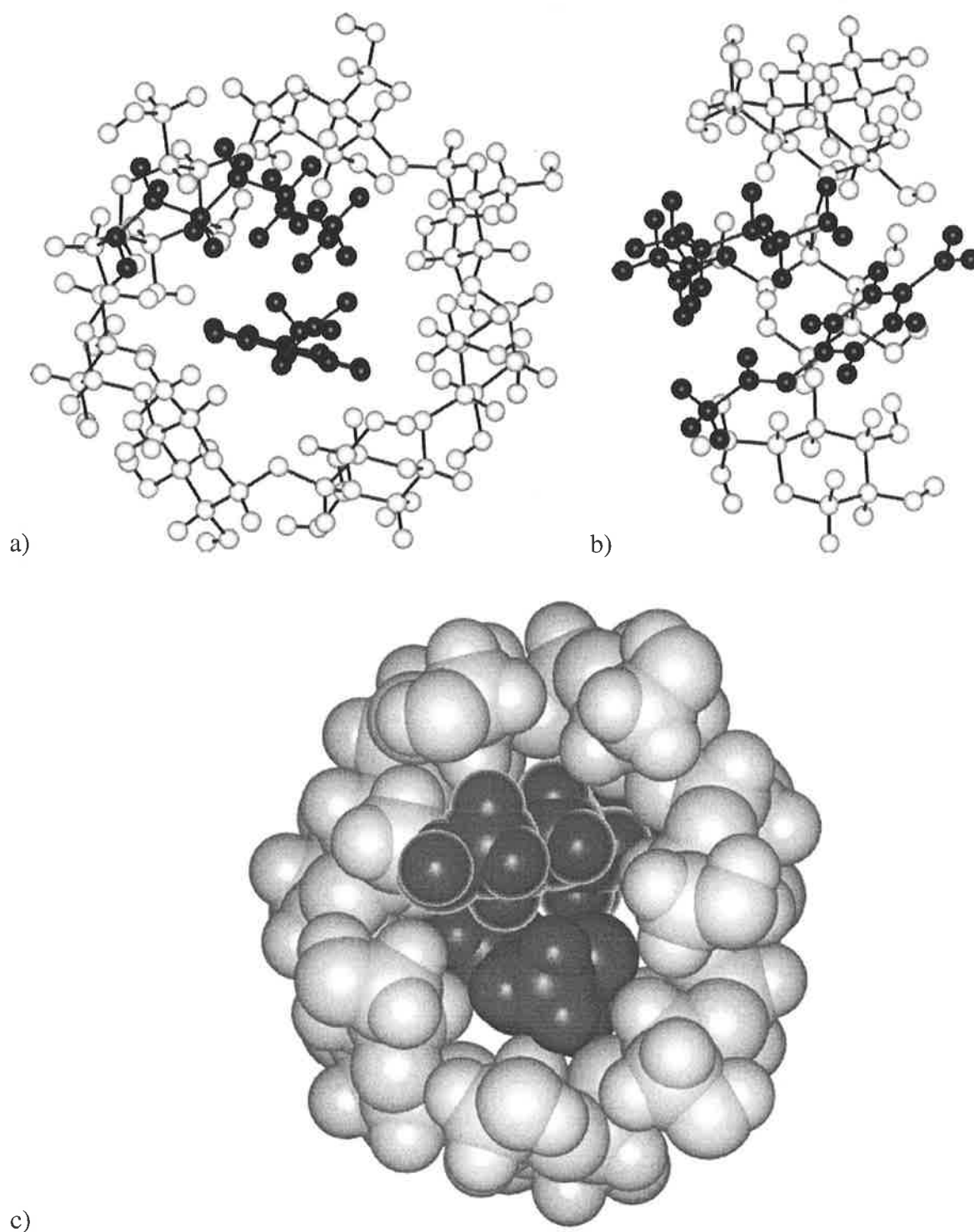


Figure 2.13. The global energy minimised structure of the complex formed between β CD-hn and pNPA (where pNPA is orientated with the acetate group adjacent the primary end of the β CD annulus viewed from a) the primary face of the β CD annulus and b) from the side with three glucopyranose units cut away. The CPK representation of a) is also shown (c).

Exclusion of the hn substituent from the β CD-hn annulus was observed for the gas phase, energy minimised (1025.5 kJ mol⁻¹) structure of its complex with adamantane-1-carboxylate. The carboxylate group is orientated towards the secondary face (Figure 2.14) with the reversed orientation giving an energy of 1111.4 kJ mol⁻¹. This structure is consistent with the ¹H NMR ROESY data where no cross-peaks are observed for the hn substituent with the interior of the cyclodextrin.¹⁶ The ¹H NMR ROESY spectrum (Figure 2.15), however, does show strong cross-peaks between the hnH1 and hnH6 protons of the hn substituent suggesting a coiled configuration. This is unlike the modelled structure where it appears to lie along the primary rim of the cyclodextrin. These differences may reflect the influence of hydration on the gas phase modelled structure in aqueous solution. (Attempts to determine the stability of complexes formed with adamantane-1-carboxylate and the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins using the titrimetric method were unsuccessful due to the low solubility of 1-adamantane carboxylic acid (Section 6.2.4).) A summary of the one dimensional ¹H NMR and two dimensional ¹H ROESY NMR assignments for [β CD-hn.1-adamantane carboxylate] are given in Tables 2.8 and 2.9 respectively.

Table 2.8. The 1D ¹H NMR spectral assignments for [β CD-hn.4-methylbenzoate] at pH $\geq$ 11.5. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the hn substituent) as in Figure 1.1. The hexyl protons are labelled hnH1 – hnH6 as distance increases from the secondary amine. The 1-adamantane carboxylate protons are labelled as in Figure 2.15.

1D ¹ H NMR spectrum		
δ H	Appearance, Integration	Assignment
4.65	multiplet, 7H	H1
3.81	triplet, J = 9.6 Hz, 1H	H5 ^A
3.5-3.8	multiplet, 25H	H3, H5, H6
3.2-3.4	multiplet 13H	H2, H4
3.06	triplet, J = 9.6 Hz, 1H	H4 ^A
2.92	doublet, J = 14.0 Hz, 1H	H6 ^A
2.58	doublet of doublets, J = 14.0, 9.6 Hz, 1H	H6 ^{A'}
2.42	multiplet, 2H	hnH6
2.23	doublet of triplets, J = 5.4, 10.8 Hz, 1H	hnH1
2.14	doublet, J = 5.4 Hz, 1H	hnH1'
1.99	broad singlet, 3H	AdH3
1.76	broad singlet, 6H	AdH2
1.69	broad doublet, J = 10.8 Hz, 3H	AdH4
1.45	broad doublet, J = 10.8 Hz, 3H	AdH4'
1.0-1.4	multiplet, 8H	hnH2 - hnH5

Table 2.9. The 2D ^1H ROESY NMR spectral assignments for $[\beta\text{CD-hn.4-methylbenzoate}]^-$ at $\text{pH} \geq 11.5$ with proton numbering as in Table 2.8.

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
1.45	AdH4'	1.69	AdH4
		1.99	AdH2
		3.53	H5
		3.7	H3
1.69	AdH4	1.45	AdH4'
		1.99	AdH2
		3.53	H5
		3.7	H3
1.76	AdH2	1.99	AdH2
		3.53	H5
		3.7	H3
1.99	AdH2	1.45	AdH4'
		1.69	AdH4
		1.76	AdH2
		3.53	H5
		3.7	H3
2.23	hnH1	2.42	hnH6
		2.92	H6 ^A
		3.81	H5 ^A
2.42	hnH6	2.23	hnH1
		3.81	H5 ^A
2.58	H6 ^A '	2.92	H6 ^A
		3.06	H4 ^A
2.92	H6 ^A	2.58	H6 ^A '
		3.81	H5 ^A
3.53	H5	1.45	AdH4'
		1.69	AdH4
		1.76	AdH2
		1.99	AdH2
		3.7	H3
3.7	H3	1.45	AdH4'
		1.69	AdH4
		1.76	AdH2
		1.99	AdH2
3.81	H5 ^A	2.23	hnH1
		2.42	hnH6
		2.92	H6 ^A

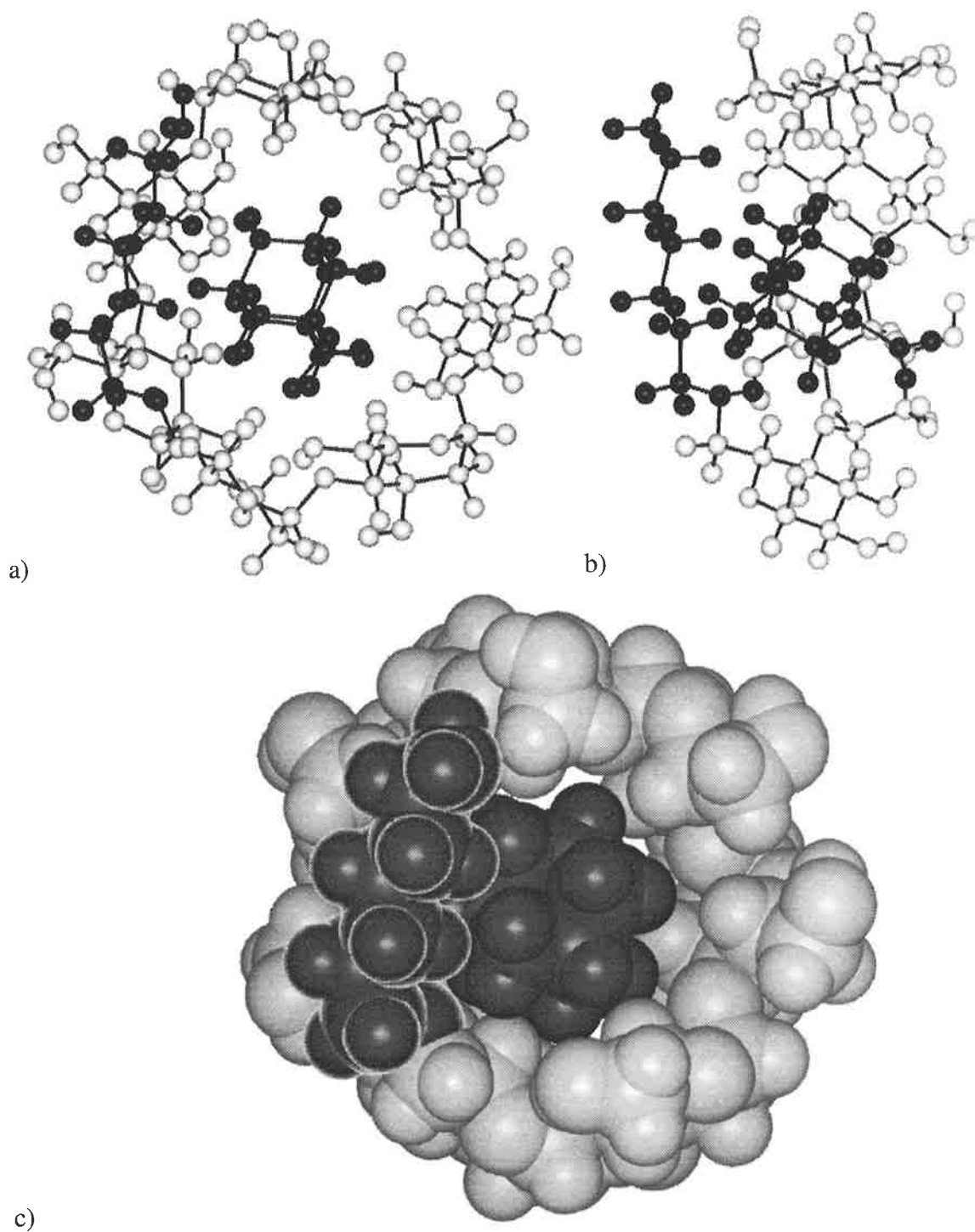


Figure 2.14. The global energy minimised structure of the complex formed between β CD-hn and 1-adamantane carboxylate viewed from a) the primary face of the β CD annulus and b) from the side with three glucopyranose units cut away. The CPK representation of a) is also shown (c).

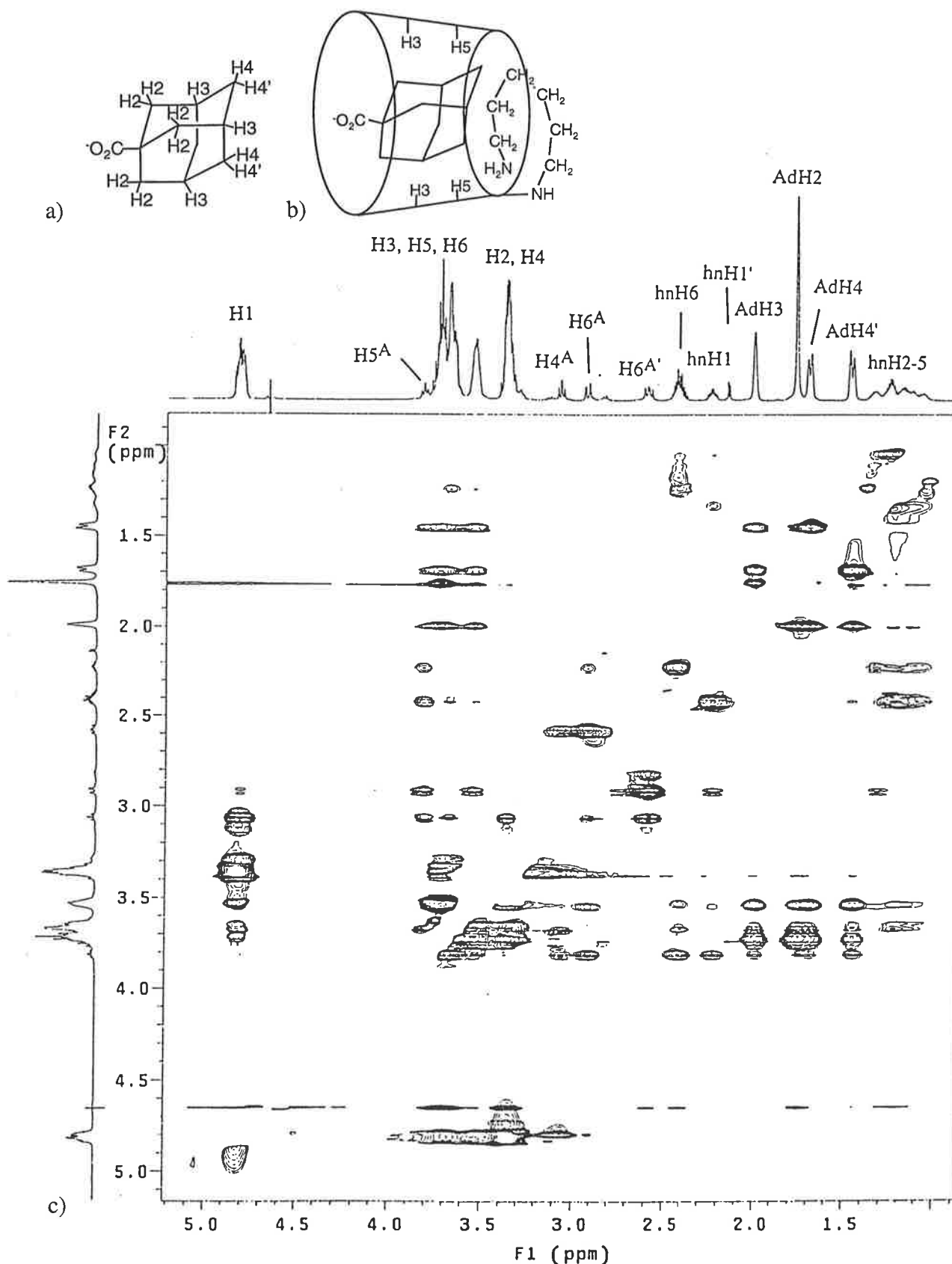


Figure 2.15. a) A representation of 1-adamantane carboxylate, showing the hydrogen numbering scheme. b) The proposed structure of $[\beta\text{CD-hn.1-adamantane carboxylate}]$. c) The ^1H ROESY NMR spectrum of the complex formed by $\beta\text{CD-hn}$ and 1-adamantane carboxylate showing cross-peaks between hnH1 and hnH6 of the hn substituent and H3 and H5 of the cyclodextrin with H2 – H4 of the 1-adamantane carboxylate. The signals along the diagonal have been removed for clarity

2.3. Summary and Conclusions.

The pK_a s of a range of protonated 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrin were determined. The pK_a s of the analogous free protonated diamines were also determined to allow comparison. Based on literature results,^{9,10} a protonation order was assigned to the cyclodextrin bound diamines, with the first site being that of the primary amine and the second being that of the secondary amine. The pK_a s of β CD-XH_nⁿ⁺ were consistently lower compared with those of the free diamines. This was attributed to the size and polarity of the β CD substituent of β CD-XH_nⁿ⁺, imparting steric and electronic effects, which induced changes in the basicity of the amino nitrogens of the diamine. As the length of the aliphatic chain in the X substituent increased so to did the size of the pK_a s determined. This was attributed to an increase in hydrophobicity and a decrease in hydration. Similarly, as the aliphatic carbon chain length increased, there was a decrease in the difference between the two pK_a s of the diamine due to increased charge separation.

A potentiometric titration study into the complexation of benzoic, 4-methylbenzoic, (*R*)- and (*S*)-2-phenylpropanoic acids with the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins, found that of the 64 possible complexes that could exist in solution, only 35 were detected. The pattern of complexation that emerged for the variation in stability constants for a single carboxylic acid and its carboxylate being complexed by β CD-XH_nⁿ⁺, was discussed in terms of hydrophobicity, charge, hydration and steric interactions. Electrostatic interactions and hydrophobic interactions were determined to be the main driving forces for complexation. No trend was observed for steric interactions with increasing aliphatic carbon chain length or variation in guest size, however some stereoselectivity was observed for the enantiomeric guests.

Gas phase molecular modelling of the complexes formed between the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins and the carboxylic acid guests, in conjunction with ¹H NMR ROESY spectroscopy,⁵ gave orientations of the guest species within the cyclodextrin annulus, consistent with hydrophobic and electrostatic interactions being the dominating factors for complexation. Evidence for the simultaneous inclusion of a guest and the (aminohexyl)amino group bound to β CD-hn was obtained.

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CHAPTER THREE

**THE COMPLEXATION OF AROMATIC CARBOXYLIC ACIDS BY
6^A-DEOXY-6^A-TRIAZAALKYL- AND 6^A-DEOXY-6^A-
TETRAAZAALKYL- β -CYCLODEXTRINS**

3.1. Introduction

Chapter Two described a potentiometric titration study of the complexation of four 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins with benzoic, 4-methylbenzoic and (*R/S*)-2-phenylpropanoic acids and their conjugate bases. The trends, seen for the formation of complexes and their stabilities with the selected guest acids, were rationalised in terms of charge, hydrophobicity, hydration and steric interactions (Section 2.2.3). Both ¹H ROESY NMR spectroscopy and force-field molecular modelling were consistent with the findings of the potentiometric study.¹

It was envisioned that a similar study involving the complexation of 6^A-deoxy-6^A-(2-(2-aminoethylamino)ethylamino)- β -cyclodextrin (β CD-dien), 6^A-deoxy-6^A-(3-(3-aminopropyl amino)propylamino)- β -cyclodextrin (β CD-dipn), 6^A-deoxy-6^A-(2-(2-(2-aminoethylamino) ethylamino)ethylamino)- β -cyclodextrin (β CD-trien), 6^A-deoxy-6^A-(1,4,7-triazacyclononanyl) - β -cyclodextrin (β CD-tacn), 6^A-deoxy-6^A-(1,5,9-triazacyclododecanyl)- β -cyclodextrin (β CD-tacdo) and 6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecanyl)- β -cyclodextrin (β CD-cyclen) (Figure 3.1), would give further insight into the interplay of the forces that govern the complexation of benzoic, 4-methylbenzoic and (*R/S*)-2-phenylpropanoic acids and their conjugate bases by polyamino-monosubstituted- β -cyclodextrins. These modified cyclodextrins provide a wide variety of hosts with varying degrees of charge and polyamino substituent stereochemistry.

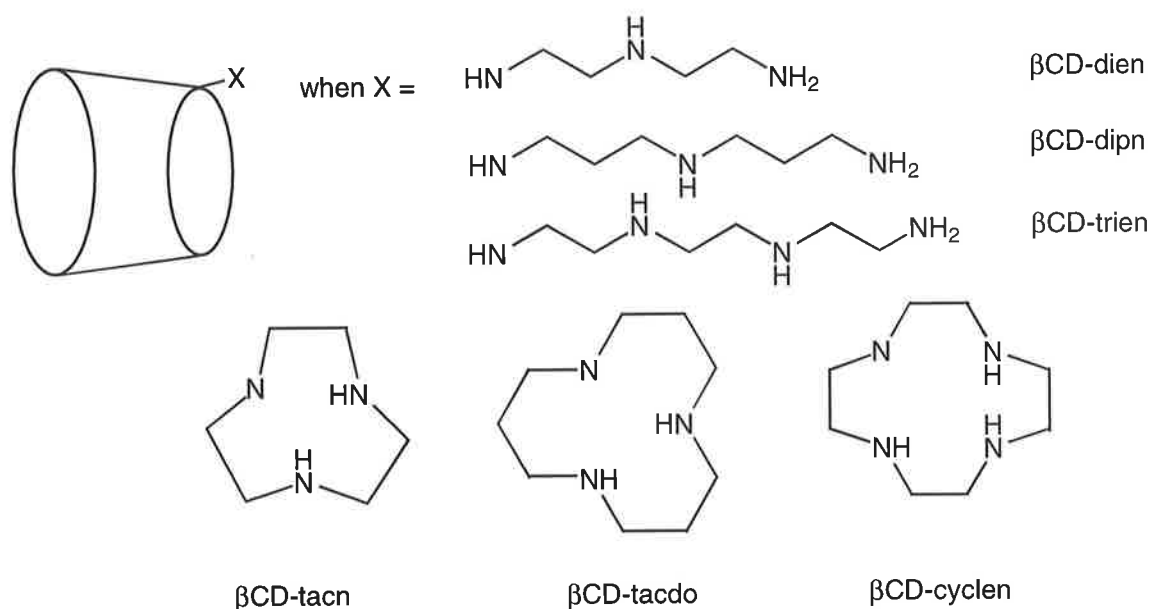


Figure 3.1. The structure of the 6^A-deoxy-6^A-triazalkyl- β -cyclodextrins and the 6^A-deoxy-6^A-tetraazaalkyl- β -cyclodextrins studied. The individual atoms of the triaza or tetraaza substituent are labelled 1, 2, ... n as distance from the cyclodextrin moiety increases.

3.2. Results and Discussion.

A series of 6^A-deoxy-6^A-triazalkyl- and 6^A-deoxy-6^A-tetraazaalkyl- β -cyclodextrins were prepared and the pK_a s of their protonated form, determined by potentiometric titration ($I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4), 298.2 K). The pK_a s of most of the free protonated polyamines corresponding to the $\beta\text{CD-X}$ were determined to allow comparison (Figure 3.2, Table 3.1). A potentiometric titration study in aqueous solution ($I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4), 298.2 K) of the complexation of benzoic, 4-methylbenzoic, and (*R/S*)-2-phenylpropanoic acids and their conjugate bases with the above modified cyclodextrins was also undertaken.

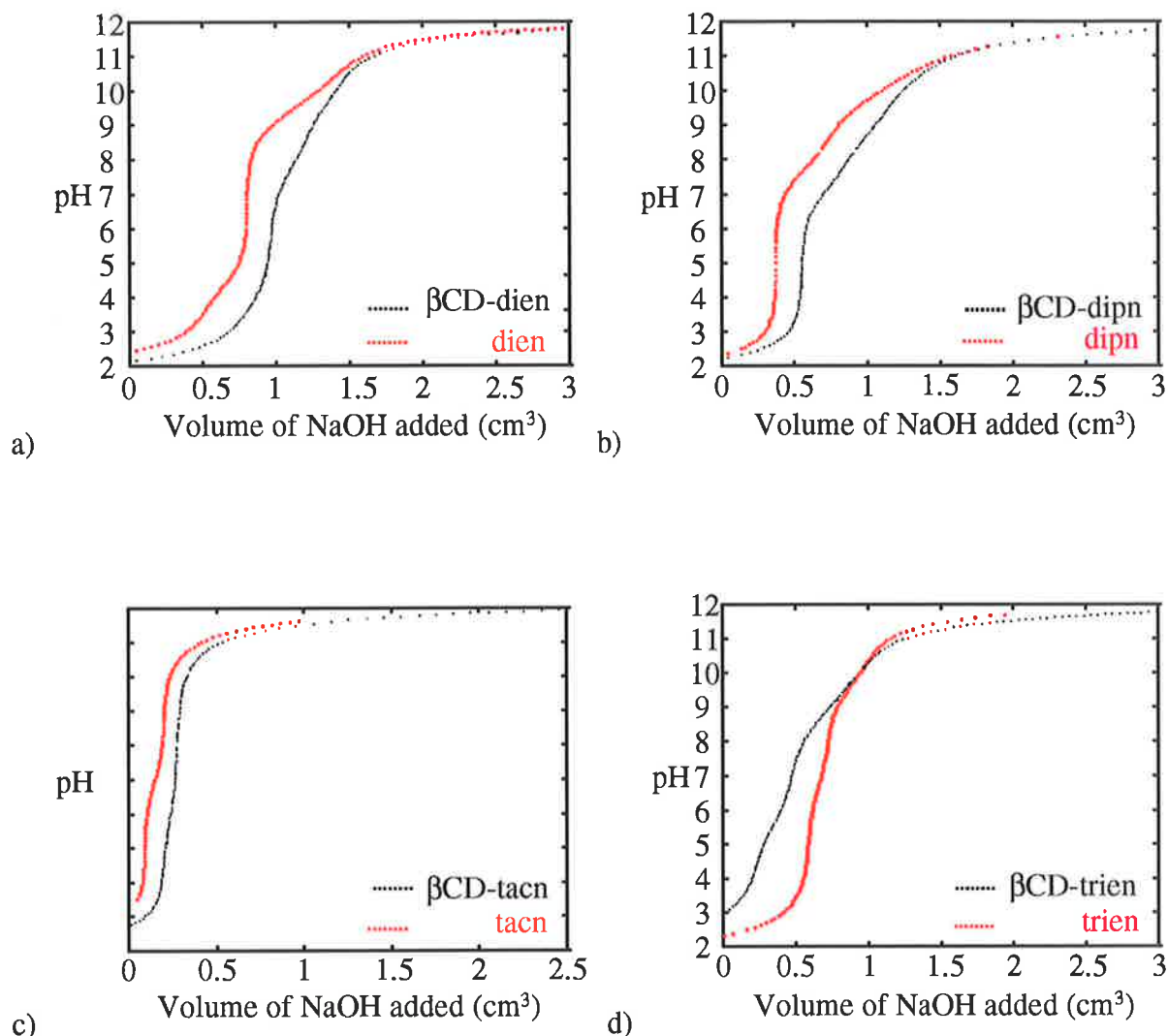


Figure 3.2. The titration profiles of some of the protonated 6^A-deoxy-6^A-triazaalkyl- and 6^A-deoxy-6^A-tetraazaalkyl- β -cyclodextrins (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) shown with that of the corresponding free polyamine group (at $2 \times 10^{-3} \text{ mol dm}^{-3}$), both in the presence of $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$. The initial volume of the titrated solution was 10.00 cm^3 . The curves represent raw data from titrations of a) β CD-dien and dien in the presence of $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$, b) β CD-dipn and dipn in the presence of $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$, c) β CD-tacn and tacn in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$ and d) β CD-trien and trien in the presence of $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K .

Table 3.1. The pK_a values for the protonated 6^A-deoxy-6^A-triazalkyl- and 6^A-deoxy-6^A-tetraazaalkyl- β -cyclodextrins and the corresponding free protonated polyamine groups at $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K and the corresponding literature values for the free polyamine groups.

X		$\beta\text{CD-XH}_n^{n+ \text{ a}}$	free $\text{XH}_n^{n+ \text{ a}}$	literature $\text{XH}_n^{n+ \text{ a}}$
dien	pK_{a1}	9.52 ± 0.02	9.78 ± 0.01	9.84 ± 0.05^b
	pK_{a2}	7.63 ± 0.03	8.99 ± 0.03	9.02 ± 0.06^b
	pK_{a3}	3.88 ± 0.07	4.32 ± 0.03	4.23 ± 0.03^b
dipn	pK_{a1}	10.06 ± 0.02	10.56 ± 0.05	10.65^b
	pK_{a2}	8.44 ± 0.03	9.44 ± 0.06	9.57^b
	pK_{a3}	6.72 ± 0.03	7.54 ± 0.06	7.72^b
tacn	pK_{a1}	10.0 ± 0.1	10.69 ± 0.02	10.42 ± 0.02^c
	pK_{a2}	5.89 ± 0.07	7.01 ± 0.01	6.82 ± 0.02^c
	pK_{a3}	2.35 ± 0.2	-	Strong ^c
tacdo	pK_{a1}	11.24 ± 0.04	-	12.60^d
	pK_{a2}	5.85 ± 0.03	-	7.57^d
	pK_{a3}	2.80 ± 0.1	-	2.41^d
trien	pK_{a1}	9.33 ± 0.02	9.83 ± 0.04	9.74 ± 0.06^b
	pK_{a2}	8.23 ± 0.03	8.93 ± 0.05	9.08 ± 0.02^b
	pK_{a3}	5.61 ± 0.03	6.40 ± 0.05	6.56 ± 0.02^b
	pK_{a4}	3.13 ± 0.07	3.0 ± 0.1	3.25 ± 0.03^b
cyclen	pK_{a1}	10.40 ± 0.01	-	10.6^c
	pK_{a2}	8.62 ± 0.02	-	9.6^c

^a Where n is equal to the number of pK_a s determined. ^b Reference 2. ^c Reference 3.

^d Reference 4. ^e Reference 5.

3.2.1. Determination of the pK_a s of the Protonated Substituent Polyamines and the Corresponding Free Protonated Polyamines.

There is good agreement between the results determined from this study and those in the literature for the pK_a s of the free protonated polyamine groups.²⁻⁵

The pK_a s of β CD-dienH₃³⁺ have been determined elsewhere via a ¹³C NMR titration study.⁶ Values of 9.41, 7.41 and 3.97 for the first, second and third pK_a s respectively are in general agreement with the values determined by this study. Additionally, a protonation order of the amine groups of the dien moiety for β CD-dien was reported.⁶ It is probable that the order of protonation for the amine groups of β CD-dipn is the same as that for β CD-dien, thus the first site of protonation is the primary amine group, followed by the secondary amine group adjacent to the cyclodextrin moiety and lastly the secondary amine group separating the two propylene carbon chains. The assignment of a protonation order to any of the other β CD-XH_nⁿ⁺ studied here was not attempted.

The trends observed for the change in pK_a magnitudes for the β CD-XH_nⁿ⁺ and the corresponding free protonated polyamine analogue are similar to those observed for the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrin systems (Section 2.2.1). A ¹³C NMR spectroscopic investigation of the modified cyclodextrins supported the argument, to some extent, that hydrogen bonding of the protonated amine nitrogens to the primary hydroxyl groups, delineating the rim of the cyclodextrin cavity, leads to an increase in the acidity of the protonated substituent,¹ as is discussed below.

The substitution of a primary hydroxyl group with a polyamine moiety on the A glucopyranose unit of β CD renders all seven glucopyranoses (A-G) inequivalent. If the magnetic inequivalence of the glucopyranoses is sufficiently large, a possible total of forty-two ¹³C resonances may be observed, six from each glucopyranose unit. Generally, the ¹³C chemical shifts differences between the seven glucopyranose units are insufficient for all forty-two ¹³C signals to be observed separately. It was found that the ¹³C spectra observed for β CD-X changed as the polyamine moiety changed its state of protonation. In basic solution more fine structure was observed in the ¹³C NMR spectra of the polyamine modified cyclodextrin than was seen in acidic solution. This is consistent with the deprotonated polyamine moiety hydrogen bonding to the cyclodextrin primary hydroxyl groups more effectively than its protonated analogue, an argument consistent with that presented for the pH dependant ¹³C NMR spectra of β CD-dien.⁶ This phenomenon was strongest in the cases of

β CD-cyclen and β CD-tacdo (Figures 3.3 and 3.4 respectively).

The ^{13}C NMR spectrum of $\beta\text{CD-cyclenH}_4^{4+}$ and $\beta\text{CD-tacdoH}_3^{3+}$ show relatively weak resolution of the carbon resonances. This is consistent with the cyclen/tacdo substituents swinging out from the cyclodextrin and interacting weakly, if at all, with the primary hydroxyl groups. Thus the differentiation of the seven glucopyranose units is small. Conversely in the ^{13}C NMR spectra of $\beta\text{CD-cyclen}$ and $\beta\text{CD-tacdo}$ (the neutral, deprotonated species) all seven C(1) and C(4) resonances are observed. This is consistent with the cyclen/tacdo substituents hydrogen bonding with the primary hydroxyl groups of the cyclodextrin and maximising the magnetic inequivalence of the seven glucopyranose units.

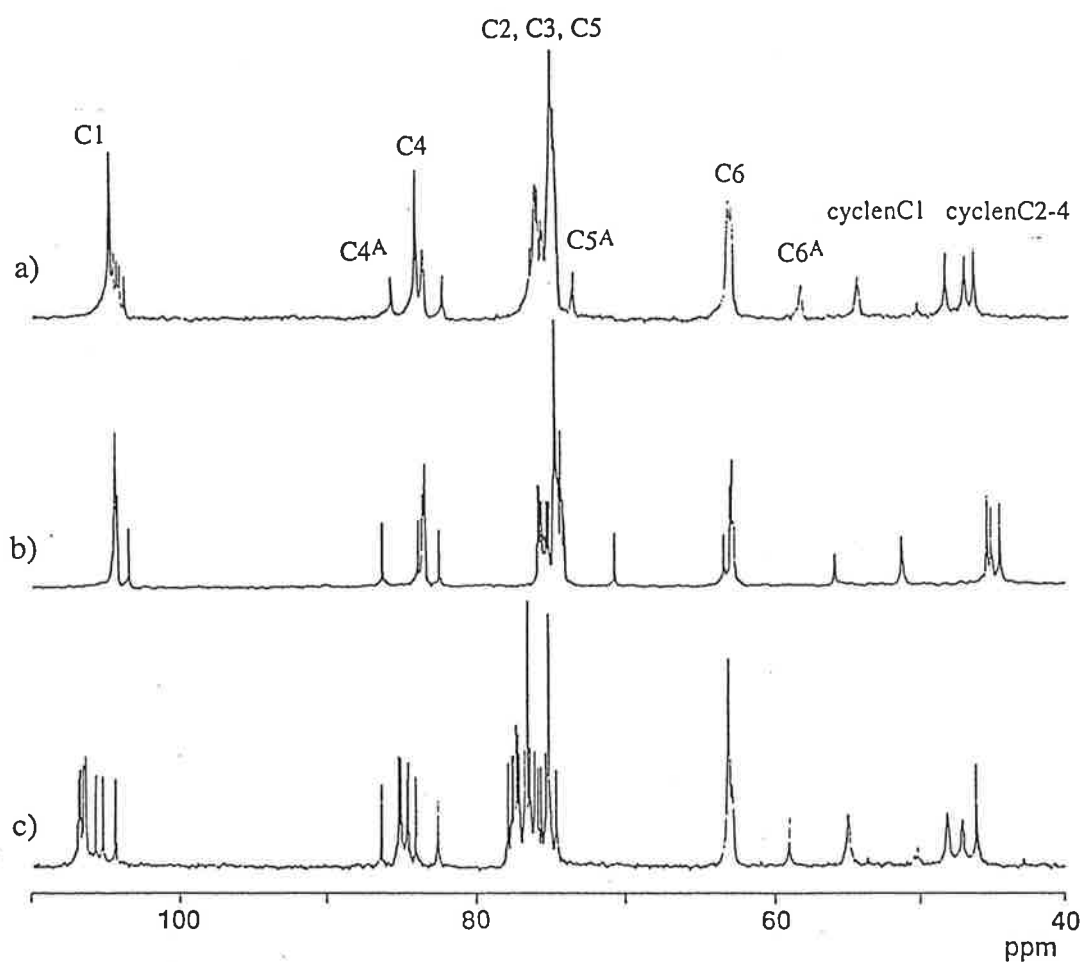


Figure 3.3. ^{13}C 75.47 MHz NMR spectra of βCD -cyclen in D_2O at a concentration of 0.06 mol dm^{-3} at a) pH ~ 2 : δ 104.5, 104.4, 104.3, 103.6 (C1); 86.4 (C4^{A}); 84.0, 83.7, 83.6, 82.6 (C4); 75.9, 75.8, 75.6, 75.3, 74.0, 74.8, 74.7, 74.6, 74.4, 74.3, 74.2 (C2, C3, C5); 70.9 (C5^{A}); 63.6, 63.2, 63.0 (C6); 56.2 (C6^{A}); 51.5 (cyclenC1); 45.7, 45.4, 44.9 (cyclenC2-C4); b) pH ~ 9 : δ 104.9, 104.6, 104.3, 104.2, 103.8 (C1); 85.6 (C4^{A}); 84.1, 83.6, 83.5, 82.2 (C2, C3, C5); 63.1, 63.0, 62.8 (C6); 58.3 (C6^{A}), 54.3, (50.2), 48.2, 46.9, 46.2 (cyclenC1-C4); and c) pH ~ 12 : δ 106.9, 106.8, 106.6, 106.4, 105.7, 105.3, 104.4 (C1); 86.35 (C4^{A}); 85.2, 85.0, 84.7, 84.6, 84.0, 82.6 (C4); 77.9, 77.7, 77.5, 77.4, 77.2, 77.1, 76.8, 76.6, 76.4, 76.1, 76.0, 75.6, 75.2, 75.1, 74.6, (C2, C3, C5); 63.1, 62.9, 62.8, (C6); 59.0 (C6^{A}); 55.0, (50.2), 48.0, 47.1, 46.1 (cyclenC1-C4).

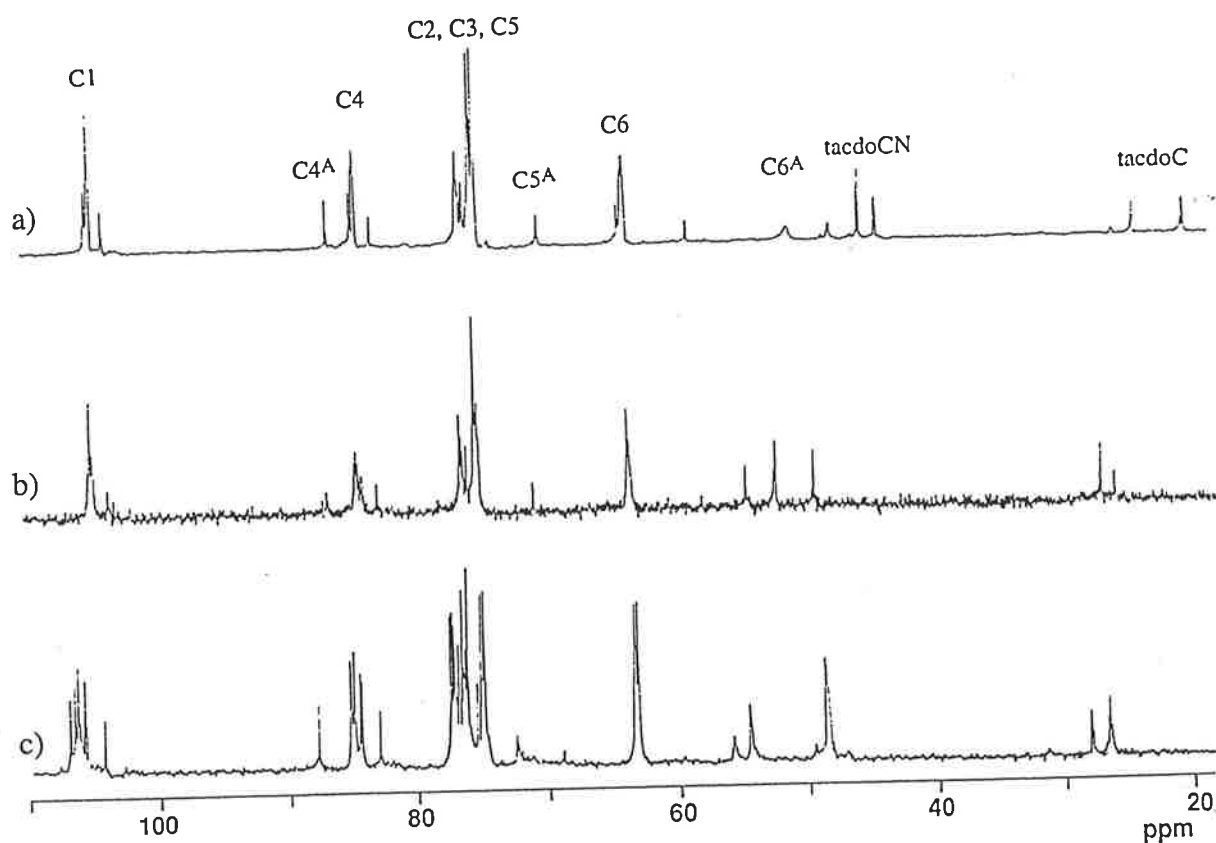


Figure 3.4. ^{13}C 75.47 MHz NMR spectra of $\beta\text{CD-tacdo}$ in D_2O at a concentration of 0.06 mol dm^{-3} at a) pH ~ 2 : δ_{C} 104.9, 104.7, 104.6, 103.7 (C1); 86.3 (C4A); 84.3, 84.0, 82.8 (C4); 76.1, 76.0, 75.9, 75.6, 75.2, 75.0, 74.7, 74.6 (C2, C3, C5); 70.0 (C5A); 63.8, 63.3 (C6); 58.6, 50.9 (broad), 47.5, 45.2, 43.9 (C6A, tacdoC1, tacdoC3, tacdoC4); (25.4), 23.7, 19.9 (tacdoC2, tacdoC5); b) pH ~ 8.5 : δ_{C} 104.8, 104.5, 103.0 (C1); 86.4 (C4A); 84.1, 83.9, 83.7, 82.5 (C4); 76.1, 76.0, 75.9, 75.6, 74.9, 74.7 (C2, C3, C5); 63.3, 63.2 (C6); 54.3 (C6A); 51.9, 49.0 (tacdoC1, tacdoC3, tacdoC4); 26.6, 25.4 (tacdoC2, tacdoC5) and c) pH ~ 12 : δ_{C} 106.9, 106.6, 106.4, 106.3, 105.8, 105.7, 104.3 (C1); 87.7 (C4^A); 85.2, 85.1, 85.0, 84.9, 84.5, 84.3, 82.9 (C4); 77.4, 77.2, 77.1, 77.0, 76.9, 76.8, 76.7, 76.5, 76.3, 76.1, 75.4, 75.1, 74.9 (C2, C3, C5); 72.5 (C5^A); 63.4, 63.1 (C6); 55.9, (C6^A); 54.6 (tacdoC1); 48.7, 48.6 (tacdoC3, tacdoC4); 28.0, 26.5 (tacdoC2, tacdoC5).

3.2.2. Determination of the Stability Constants of the Complexes Formed by the 6^A-Deoxy-6^A-triazalkyl- and 6^A-Deoxy-6^A-tetraazaalkyl- β -cyclodextrins with the Aromatic Carboxylic Acids and their Conjugate Bases.

The determination of the apparent stability constants depends on differences in the pK_a s between the complexed and uncomplexed states of the species involved. The identities of the complexes formed were established from the best fit of sequential equilibria (which encompassed all states of protonation of β CD-X and the guest aromatic carboxylic acids) to the potentiometric data. Depending upon the pK_a s of their constituents and other factors, a wide range of complexes was detected.

The schemes used to fit the data for each system, reflect the probability of complexes forming in solution. This is substantially based on the simultaneous existence of species in solution, determined by inspection of their pK_a s. Figure 3.5 depicts the presence in solution at varying pH of β CD-dipn and 4-methylbenzoate and their protonated analogues, assuming no complexation. Inspection of this plot reveals that the likelihood of complexes forming between β CD-dipn, β CD-dipnH⁺, β CD-dipnH₂²⁺ and 4-methylbenzoic acid is improbable, and no complexation between them was detected.

The pK_{a3} of β CD-dienH₃³⁺, β CD-tacnH₃³⁺ and β CD-tacdoH₃³⁺ are all lower than those of the guest aromatic carboxylic acids studied, hence the formation of the species $[\beta$ CD-XH₂.HA]²⁺ for these modified cyclodextrins is possible. The data for these systems were finally fitted to Scheme 3.1. The pK_a s of β CD-dipnH_nⁿ⁺ are all greater than those of the carboxylic acids studied, hence the formation of $[\beta$ CD-dipnH₂.HA]²⁺ is improbable. The data for this system were finally fitted to Scheme 3.2. The data for β CD-trienH_nⁿ⁺ and β CD-cyclenH_nⁿ⁺ were finally fitted to Schemes 3.3 and 3.4 respectively. The lowest two pK_a s of β CD-cyclenH₄⁴⁺ could not be determined using the potentiometric method. Thus, any complexes formed by these species were undetectable.

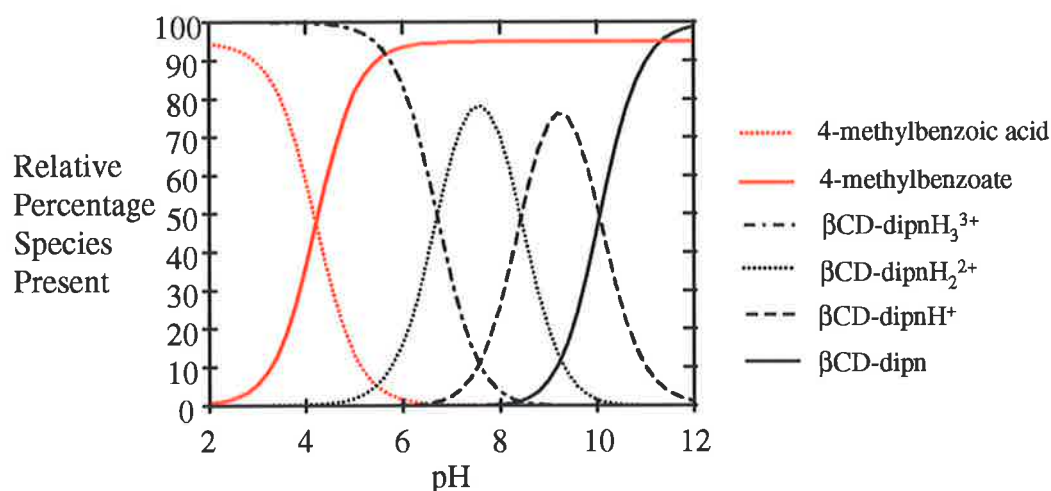
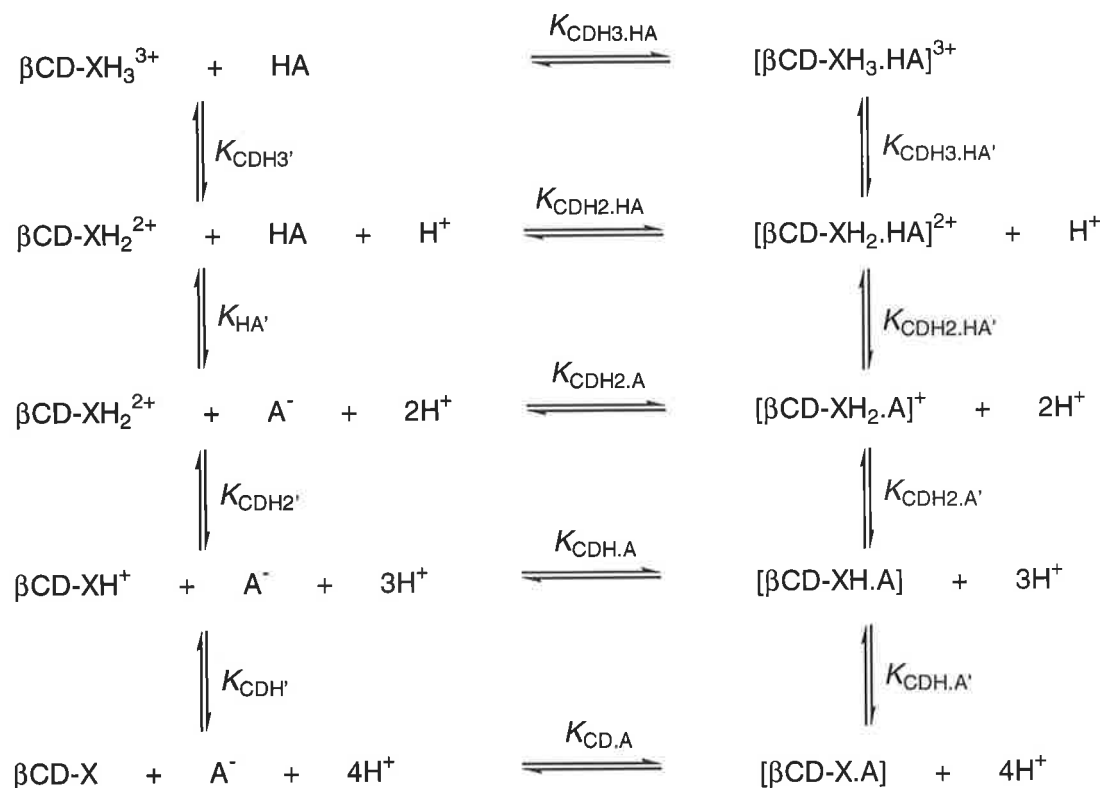
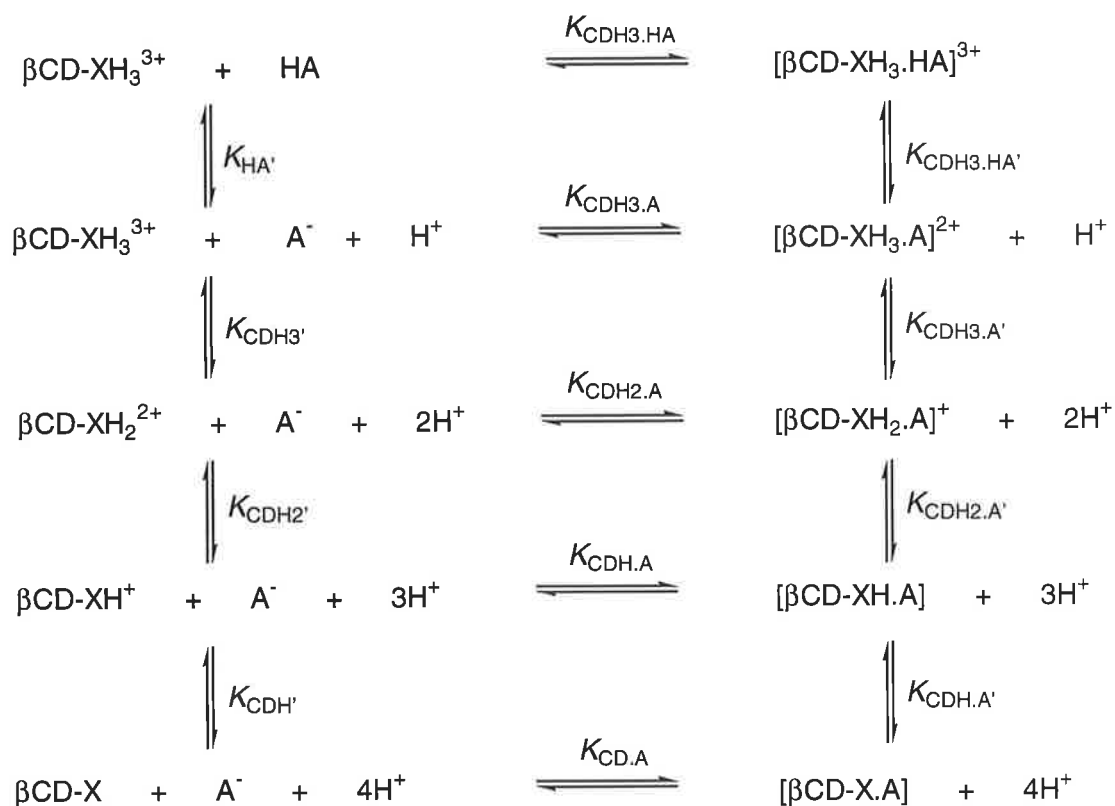


Figure 3.5. A speciation plot depicting the existence of 4-methylbenzoic acid/4-methylbenzoate, (at $0.095 \text{ mol dm}^{-3}$), and β CD-dipnH_nⁿ⁺, (at $0.100 \text{ mol dm}^{-3}$ and 298.2 K), in its various protonation states over the pH range of 2 - 12. No complexation is assumed. The plot was calculated from the data in Table 3.1 and using a pK_a of 4-methylbenzoic acid of 4.24. The plot allows determination of which species will be present simultaneously at a given pH and thus have the potential to form a complex. The definition of percentage of species is referred to $0.100 \text{ mol dm}^{-3}$ β CD-dipnH_nⁿ⁺ as 100%.

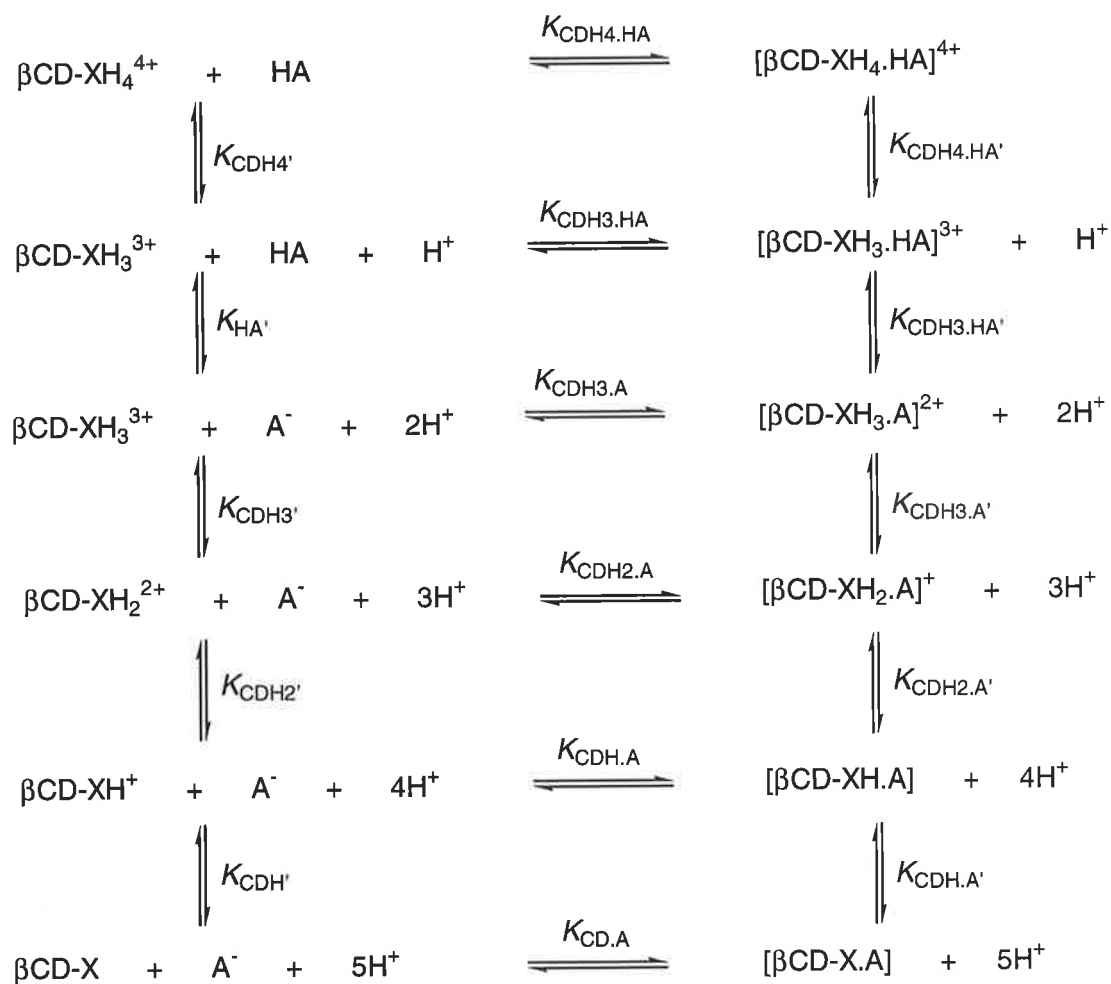
Scheme 3.1.

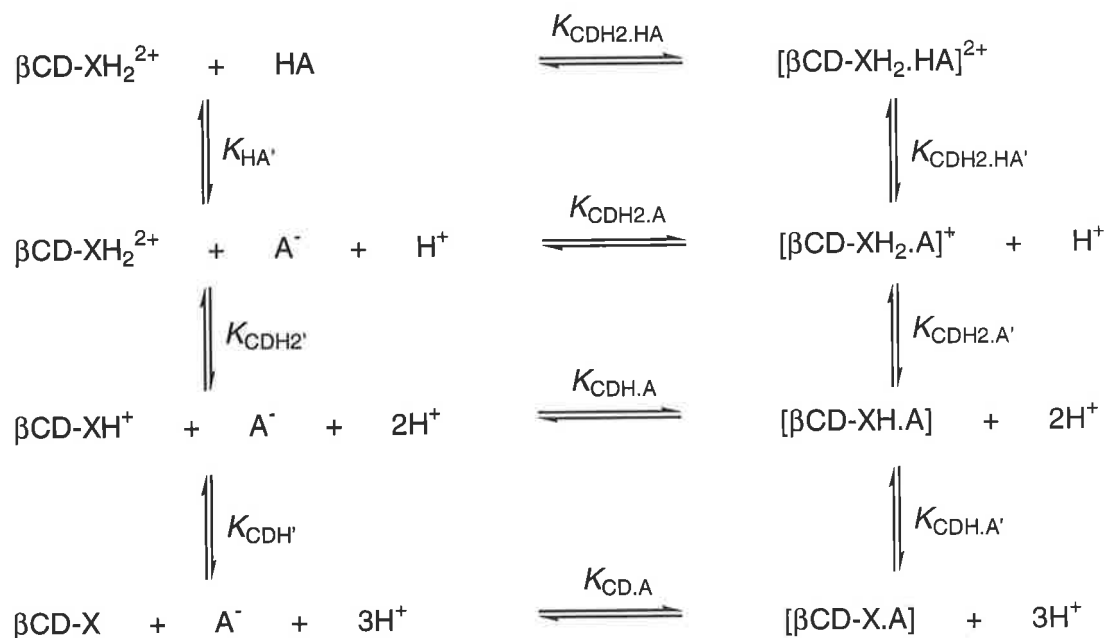


Scheme 3.2.



Scheme 3.3.



Scheme 3.4.

The nomenclature for the apparent stability constants above, are the same as those in Chapter Two (Section 2.1). The subscripts used to define the apparent stability constants K , are CD for $\beta\text{CD-X}$ and A for the carboxylate guest, with protonation as indicated. Provided the $\text{p}K_{\text{a}}$ s of the free guests and host are different to those of the complexed species, the acid-base titration of an acidified solution containing both HA and $\beta\text{CD-X}$ should allow the determination of apparent stability constants as indicated above.

3.2.3. General Stability Variations observed for the Complexation of the Aromatic Carboxylic Acids by the 6^A-Deoxy-6^A-triazalkyl- and 6^A-Deoxy-6^A-tetraazaalkyl- β -Cyclodextrins.

The results for the complexation of the aromatic guest carboxylic acids and carboxylates with $\beta\text{CD-XH}_n^{n+}$ from the best fit of the data, (Figure 3.6 and Appendix B) to Schemes 3.1 - 3.4, are shown in Table 3.2. The magnitude of the stability constants encompassed by the range of complexes detected varied from 170 ± 30 and $48,000 \pm 11,000 \text{ dm}^3 \text{ mol}^{-1}$ for $[\beta\text{CD-trienH}_2 \cdot (R)\text{-2-phenylpropanoate}]^+$ and $[\beta\text{CD-dipnH}_3 \cdot (S)\text{-2-phenylpropanoic acid}]^{3+}$ respectively. The latter value being one of the highest reported for a complex of the selected guests studied, with a modified βCD .⁷⁻¹⁰ The quoted errors represent one standard deviation for the fit of the appropriate equilibrium algorithm to the titrimetric data. The magnitude of the errors for some of the stability constants are quite large and appears to be associated with an increased number of complexes resulting from the multiple protonation states of $\beta\text{CD-XH}_n^{n+}$. This is illustrated by the complexation of (*R*)- and (*S*)-2-phenylpropanoic acid and their conjugates bases by $\beta\text{CD-dipnH}_n^{n+}$ where four complexes are detected for each guest enantiomer. Their similar titration curves yield *K* values for each diastereomeric complex which approach each other within their large standard deviations shown in Table 3.2. Complexes whose maximum concentrations were $\leq 5\%$ of the total $\beta\text{CD-XH}_n^{n+}$ concentration are not reliably detected by the pH titrimetric method used in this study and are listed in Table 3.2 as not detected.

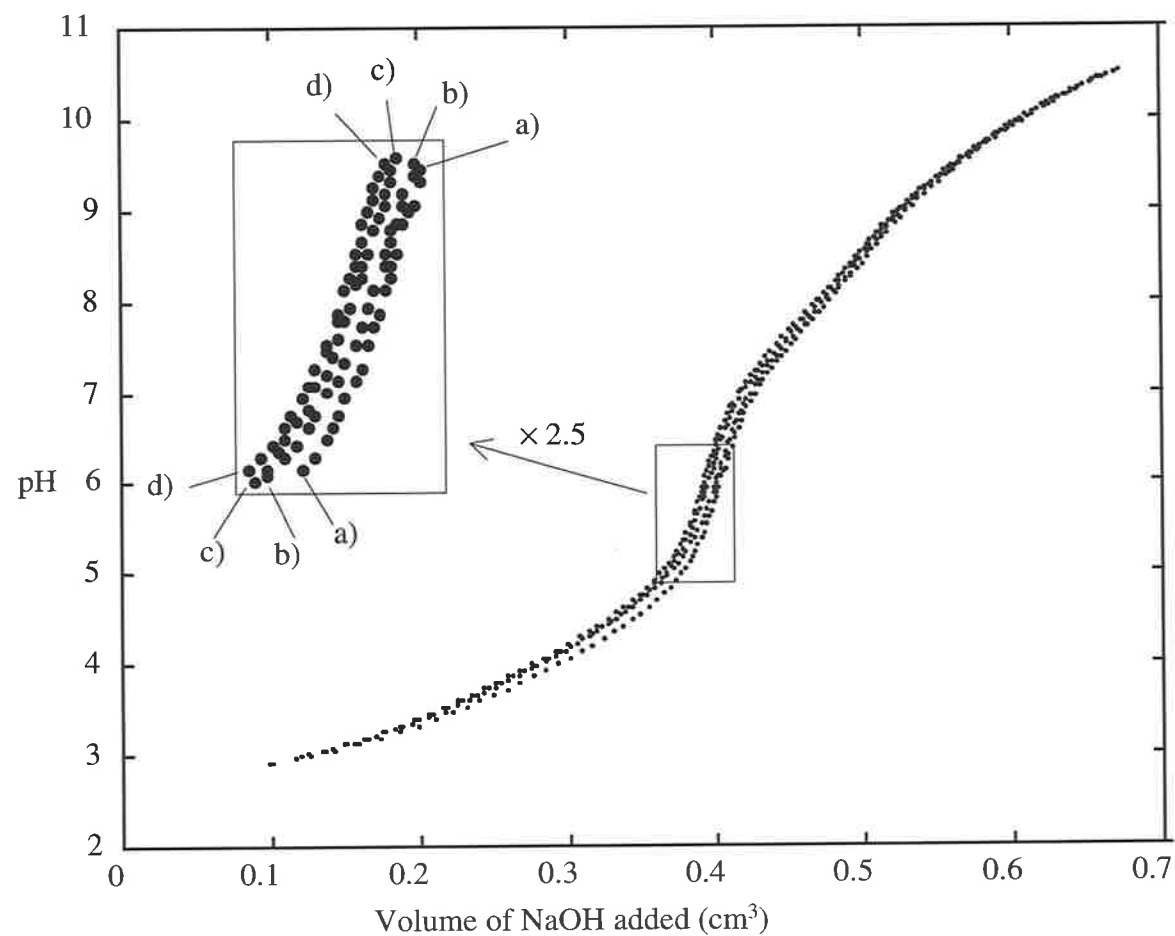


Figure 3.6. The titration profiles of β CD-dien H_n^{n+} in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of β CD-dien H_n^{n+} in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K.

Table 3.2. The apparent stability constants for the complexes of $\beta\text{CD-XH}_n^{n+}$ with benzoic, 4-methylbenzoic and (R)- and (S)-2-phenylpropanoic acid and their conjugate bases at $I = 0.10$ (NaClO_4) and 298.2 K. The shaded areas indicate the complexes which cannot form either because the pK_a s of the potential constituents preclude their coexistence or the protonation state of the $\beta\text{CD-XH}_n^{n+}$ does not exist.

Guest	K $\text{dm}^3 \text{mol}^{-1}$	$\beta\text{CD-}$ dienH_n^{n+}	$\beta\text{CD-}$ dipnH_n^{n+}	$\beta\text{CD-}$ tacnH_n^{n+}	$\beta\text{CD-}$ tacdoH_n^{n+}	$\beta\text{CD-}$ trienH_n^{n+}	$\beta\text{CD-}$ cyclenH_n^{n+}
Benzoic acid/ benzoate	$K_{\text{CDH4,HA}}$						
	$K_{\text{CDH3,HA}}$	1200 ± 300	2000 ± 500			705 ± 180	
	$K_{\text{CDH3,A}}$		1900 ± 250			725 ± 130	
	$K_{\text{CDH2,HA}}$	2400 ± 500		6500 ± 1000	10000 ± 1800		790 ± 130
	$K_{\text{CDH2,A}}$	220 ± 50	750 ± 110	5200 ± 600	4000 ± 400	225 ± 60	
	$K_{\text{CDH,A}}$			2500 ± 300			15000 ± 1300
	$K_{\text{CD,A}}$				24000 ± 800		
4-methylbenzoic acid/ 4-methylbenzoate ⁻	$K_{\text{CDH4,HA}}$						
	$K_{\text{CDH3,HA}}$	1300 ± 360	12000 ± 2800			450 ± 110	
	$K_{\text{CDH3,A}}$		12000 ± 2600			840 ± 140	
	$K_{\text{CDH2,HA}}$	2200 ± 420		2800 ± 400	32000 ± 6000		800 ± 200
	$K_{\text{CDH2,A}}$	530 ± 70	4100 ± 250	2500 ± 300	8700 ± 1000	235 ± 60	
	$K_{\text{CDH,A}}$		1050 ± 200	1200 ± 190			1200 ± 70
	$K_{\text{CD,A}}$				12000 ± 1000		6000 ± 750
(R)-2-phenylpropanoic acid/ (R)-2-phenylpropanoate	$K_{\text{CDH4,HA}}$						
	$K_{\text{CDH3,HA}}$		26000 ± 6000				
	$K_{\text{CDH3,A}}$		22000 ± 5000			480 ± 80	
	$K_{\text{CDH2,HA}}$	1500 ± 270		7200 ± 1100	15000 ± 1800		1000 ± 330
	$K_{\text{CDH2,A}}$	600 ± 100	7200 ± 1200	5300 ± 700	6000 ± 500	170 ± 30	
	$K_{\text{CDH,A}}$		1900 ± 200	2700 ± 300			3200 ± 170
	$K_{\text{CD,A}}$				13000 ± 1300		41000 ± 2800
(S)-2-phenylpropanoic acid/ (S)-2-phenylpropanoate	$K_{\text{CDH4,HA}}$						
	$K_{\text{CDH3,HA}}$		48000 ± 11000				
	$K_{\text{CDH3,A}}$		37000 ± 5000			655 ± 110	
	$K_{\text{CDH2,HA}}$	1400 ± 250		1200 ± 300	32000 ± 3500		3000 ± 580
	$K_{\text{CDH2,A}}$	600 ± 100	12800 ± 3800	1500 ± 300	8700 ± 800	180 ± 50	
	$K_{\text{CDH,A}}$		3400 ± 300	1400 ± 200			2700 ± 170
	$K_{\text{CD,A}}$				9700 ± 500		44000 ± 4500

Of the 168 complexes represented in Table 3.2, 16 cannot form due to the lack of a 4+ protonation state on the $\beta\text{CD-XH}_n^{n+}$ represented. Another 12 are not possible due to the 3+ and 4+ protonation states of $\beta\text{CD-cyclenH}_n^{n+}$ being undetectable by the titrimetric method. Hence, any complexes formed by these species were also undetected. A further 20 complexes are not established because the coexistence of the prospective hosts and guests in the same pH range is excluded by their pK_a s. Of the remaining 120 complexes represented, only 67 were of sufficient stability to be detected by the titrimetric method as shown in Table 3.2.

Most of the apparent stability constants for the complexes of $\beta\text{CD-XH}_n^{n+}$ with the aromatic guests are substantially greater than those determined for the complexes with the diamino-monosubstituted- β -cyclodextrins in their un- mono- and diprotonated states with the same guests (Section 2.2, Table 2.2).⁷

Various weak secondary bonding interactions between the $\beta\text{CD-XH}_n^{n+}$ host and the guest aromatic carboxylic acids/carboxylates determine the relative stability of any complex formed. The complexation of the guest aromatic carboxylic acids/carboxylates by $\beta\text{CD-XH}_n^{n+}$ appears to involve some conformational changes in the $\beta\text{CD-XH}_n^{n+}$ host and the aromatic guest, in addition to dehydration of both substituents to an extent dependant on the nature of each. Variations in the stabilities of the complexes formed by $\beta\text{CD-XH}_n^{n+}$ with the guest aromatic carboxylic acids/carboxylates may arise from a range of causes. The simplest interpretation of an increase in complex stability is that the rate of decomplexation has slowed relative to the rate of complexation.

The variation in the stabilities of the complexes, determined in Table 3.2, are attributable to the effects of changes in the charge, hydrophobicity and the stereochemistry of both the $\beta\text{CD-XH}_n^{n+}$ host and the guest aromatic carboxylic acids/carboxylates. The changes in complex stability with change in protonation may be partially attributable to the interaction between the $\beta\text{CD-XH}_n^{n+}$ dipole moment and that of the guests, believed to play an important role in determining the orientation of the guest within the cyclodextrin annulus (Section 1.2.3). Changes in host, guest and complex hydration also contribute to complex stability. These effects superimpose on the stabilising effect of entry of the hydrophobic phenyl moieties of the aromatic guests into the hydrophobic annuli of the six $\beta\text{CD-XH}_n^{n+}$ studied here.

Of the 120 complexes represented in Table 3.2, systematic trends in complex stability are seen. The cyclodextrin annulus is a constant feature of the $\beta\text{CD-XH}_n^{n+}$ hosts studied. Thus, the variations in the stabilities of a complex formed with a particular guest must be due to the differences in the nature of the polyamino substituent. For $\beta\text{CD-dienH}_n^{n+}$, $\beta\text{CD-dipnH}_n^{n+}$, $\beta\text{CD-tacnH}_n^{n+}$ and $\beta\text{CD-tacdoH}_n^{n+}$, the polyamino substituents incorporate three protonatable amino groups. They differ, in that the dien and dipn substituents are linear and tacn and tacdo substituents are cyclic. In addition the number of methylene groups increases from four, for dien, to six, for dipn and tacn, to nine, for tacdo, with a corresponding increase in bulk and hydrophobicity. For $\beta\text{CD-trienH}_n^{n+}$ and $\beta\text{CD-cyclenH}_n^{n+}$, the polyamino substituents incorporate four protonatable amino groups. They differ in that trien is linear and contains 6 methylene groups, whereas cyclen is cyclic and contains 8 methylene groups. Hence, the cyclen substituent has more bulk and is of greater hydrophobicity than trien. The four guests all have a phenyl moiety and either a carboxylic acid or carboxylate group in common. The differences in stability of a complex formed with a particular host is attributable to the addition of alkyl moieties in 4-methylbenzoic acid and (*R/S*)-2-phenylpropanoic acids and their conjugate bases when compared with those of benzoic acid/benzoate. The additional alkyl moieties increase the bulk and hydrophobicity of the guest. The trends in complex stability are now interpreted in the context of these observations.

3.2.4. The Complexation of the Aromatic Carboxylic Acids by the 6^A-Deoxy-6^A-triazalkyl- and 6^A-Deoxy-6^A-tetraazaalkyl- β -Cyclodextrins.

No complexes were detected with β CD-trienH₄⁴⁺. This is consistent with the high degree of protonation and small charge separation imparted by the 6 methylene groups of the trienH₄⁴⁺ substituent, lowering the hydrophobicity of β CD-trienH₄⁴⁺.

For the complexes formed with β CD-XH₃³⁺, β CD-dipnH₃³⁺ formed complexes with each of the guest aromatic carboxylic acids and carboxylates studied, the stabilities of which increased as guest size and hydrophobicity of the guest increased. β CD-dienH₃³⁺ only forms complexes with benzoic acid and 4-methylbenzoic acid. Similarly, β CD-trienH₃³⁺ forms complexes with the guest aromatic carboxylates and with benzoic and 4-methylbenzoic acids only, the stabilities of which are significantly lower than the analogous complexes formed by β CD-dipnH₃³⁺. This is consistent with the 6 methylene groups and the greater charge separation of the dipnH₃³⁺ substituent lessening the effect of protonation on the hydrophobicity of β CD-dipnH₃³⁺ and therefore on its complexing ability, by comparison with that of both β CD-dienH₃³⁺ and β CD-trienH₃³⁺. In contrast, neither β CD-tacnH₃³⁺ nor β CD-tacdoH₃³⁺ form detectable complexes with the carboxylic acid guests. This is attributable to the charges of the two triprotonated cyclic substituents being held close to the β CD annulus lowering its hydrophobicity. In addition, any steric component imparted by the bulkier cyclic substituents is lost, due the protonated tacn and tacdo moieties swinging away from the primary face of the cyclodextrin, as indicated by ¹³C NMR studies (Section 3.2.1).

For the β CD-XH₂²⁺ complexes with the guest aromatic carboxylic acids only, β CD-dienH₂²⁺ forms the least stable complexes of the triamino substituted cyclodextrins studied here. This is consistent with β CD-dienH₂²⁺ being the least hydrophobic of these hosts. The stabilities of these complexes with the triamino substituted cyclodextrins increase as the hydrophobicity of X increases in the order dienH₂²⁺ < tacnH₂²⁺ < tacdoH₂²⁺. The hydrophobicity of X is assessed on the ratio of methylene groups to amine groups. Thus the above order corresponds to an increase in the methylene:amine group ratio for dien, tacn and tacdo, of 4:3, 6:3 and 9:3, respectively.

For the β CD-XH₂²⁺ complexes detected with the guest aromatic carboxylates only, β CD-trienH₂²⁺ and β CD-dienH₂²⁺ are the least stable. This is consistent with these being the

least hydrophobic of the cyclodextrin hosts on the assessment of methylene:amine groups ratios of the X substituent.

The complexes of the diprotonated hosts, $\beta\text{CD-dienH}_2^{2+}$, $\beta\text{CD-tacnH}_2^{2+}$, $\beta\text{CD-tacdoH}_2^{2+}$ and $\beta\text{CD-cyclenH}_2^{2+}$, with the guest aromatic carboxylic acids/carboxylates, increase in stability as the hydrophobicity of the guest increases. This is exemplified by $\beta\text{CD-cyclenH}_2^{2+}$ forming complexes with the hydrophobic guest aromatic carboxylic acids only. ($\beta\text{CD-dipnH}_2^{2+}$ and $\beta\text{CD-trienH}_2^{2+}$ are unable to form complexes with the guest aromatic carboxylic acids due to differences in their pK_{a} s.)

Only 11 of the possible 24 complexes formed between the guest aromatic carboxylates and $\beta\text{CD-XH}^+$ were detected. These were of lowered stability compared with other complexes formed for the same $\beta\text{CD-X}$ host. This is consistent with hydration changes upon complexation having a destabilising effect. Complexation of the guest aromatic carboxylates by $\beta\text{CD-XH}^+$, leads to charge cancellation. It is expected that this leads to a loss of solvation energy by comparison with that of the separate hosts and guests, thus lowering the stability of the complexes. A lesser effect is expected to apply where a charge reduction occurs upon complexation.

Only $\beta\text{CD-tacdo}$ and $\beta\text{CD-cyclen}$ form complexes with the guest aromatic carboxylates in their deprotonated state. The cyclen substituent is of similar hydrophobicity to that of tacn and dipn, each possessing a 2:1 ratio of methylene to amine groups, thus hydrophobicity of the host is not the sole reason for the formation of these complexes. It appears as though the cyclen and tacdo substituents are of an appropriate size to hydrogen bond to adjacent primary hydroxyl groups of the βCD moiety (both substituents contain 12 atoms in the backbone of their rings). Evidence for this was obtained from their ^{13}C NMR spectra (Figures 3.3 and 3.4, Section 3.2.1). In addition, $\beta\text{CD-tacdo}$ is highly hydrophobic in its deprotonated state, qualitative proof of which, is the difficulty encountered in dissolving $\beta\text{CD-tacdo}$ in aqueous basic solutions. Further evidence arises from TLC analyses of the modified cyclodextrins. The linear amino-substituted cyclodextrins had R_f values of ~ 0.6 with respect to βCD , $\beta\text{CD-tacn}$ and $\beta\text{CD-cyclen}$ gave values of ~ 0.3 and $\beta\text{CD-tacdo}$ gave a value of 0.75 (TLC solvent used was 7:7:5:4 ethylacetate:isopropanol:ammonia:water). It is envisioned that when the tacdo substituent caps the rim of the cyclodextrin, the propylene groups then form a hydrophobic rim of their own as depicted in Figure 3.7, rendering the cyclodextrin highly hydrophobic. Further evidence arises from the ^1H ROESY NMR

spectrum of a solution β CD-tacdo and 4-methylbenzoate where cross peaks are not only observed between the annular protons of the cyclodextrin and the aromatic protons of the guest but between the methyl protons of the guests and the tacdo protons of β CD-tacdo (Section 3.2.5). Both the hydrophobic nature of the deprotonated β CD-tacdo and the capping of the cyclodextrin cavity by the cyclic triamine leads to strong complex stabilisation.

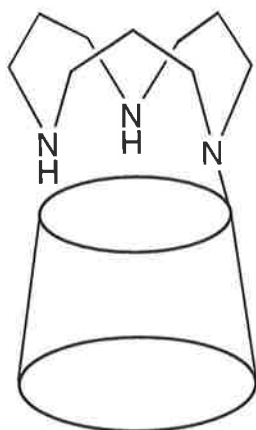


Figure 3.7. A schematic representation of β CD-tacdo showing the capping of the primary end of the cyclodextrin cavity by the tacdo substituent and the propylene groups forming a hydrophobic rim.

Small, but significant, chiral discrimination is observed for β CD-dipnH_nⁿ⁺, β CD-tacnH_nⁿ⁺, β CD-tacdoH_nⁿ⁺ and β CD-cyclenH_nⁿ⁺ with the (*R*)- and (*S*)-2-phenylpropanoic acids/phenylpropanoates (Figure 3.8). This is exemplified by a six-fold discrimination for (*R*)-2-phenylpropanoic acid over the (*S*)- enantiomer by β CD-tacnH₂²⁺ and a two fold discrimination for (*S*)-2-phenylpropanoic acid over the (*R*)-enantiomer by β CD-tacdoH₂²⁺.

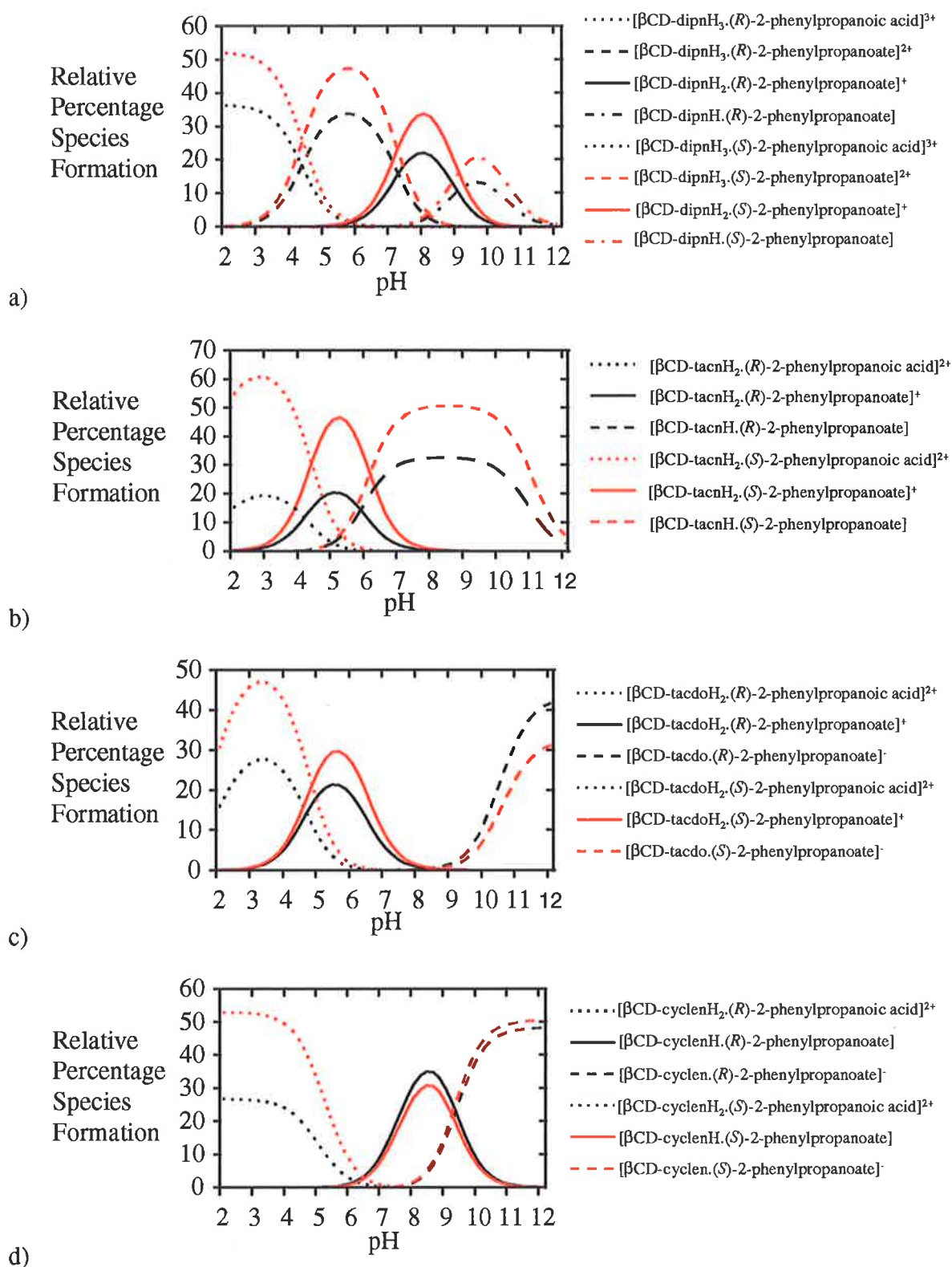


Figure 3.8. The percentage of complexes containing (R)- or (S)-2-phenylpropanoic acid in solutions $2 \times 10^{-3} \text{ mol dm}^{-3}$ of each $[\text{guest}]_{\text{total}}$ and $[\beta\text{CD-X}]_{\text{total}}$. Plots were calculated from the data in Table 3.2 and plotted relative to $[\beta\text{CD-X}]_{\text{total}} = 100\%$. The plots represent the complexes containing (R)- and (S)-2-phenylpropanoic acid and its conjugate base with a) $\beta\text{CD-dipnH}_n^{n+}$, b) $\beta\text{CD-tacnH}_n^{n+}$, c) $\beta\text{CD-tacdoH}_n^{n+}$ and d) $\beta\text{CD-cyclenH}_n^{n+}$.

3.2.5. Guest Orientation within the Cyclodextrin Cavity.

Depending on the nature of $\beta\text{CD-XH}_n^{n+}$ and the aromatic guest, the complex formed may exist in two isomeric forms. The guest may be orientated with the carboxylic acid or carboxylate group towards either the primary end of the cyclodextrin annulus or the secondary end. The overall stability constant is the weighted average of the stability constants of the two isomeric complexes. While direct evidence of this isomerism is not available, indirect evidence is given by hydrolysis studies in the presence of 6^A-deoxy-6^A-amino- β -cyclodextrin where inclusion of *para*-nitrophenyl acetate, (*p*NPA), was found to occur such that the carboxyl group of the ester was located in either the vicinity of the secondary hydroxyl groups or in the previously undetected vicinity of the primary hydroxyl groups.¹¹ Some evidence for the existence of similar isomerism was found in hydrolysis studies of *p*NPA by $\beta\text{CD-X}$ where X is an aliphatic diamine.¹²

Section 1.2.3 discussed the factors determining orientation of a guest within a cyclodextrin cavity. The dipole moments of the host and guest appear to greatly influence host-guest complex orientation. While the extent to which the polyamine substituent modifies the βCD dipole moment is unknown, it is anticipated that protonation of the amine groups will reinforce it and thereby tend to orientate the carboxylic acid or carboxylate group towards the primary and of the cyclodextrin annulus.

The data obtained from the potentiometric titrations gave no direct evidence for the orientation of the guest aromatic acids included in the cyclodextrin cavity. A two dimensional ¹H ROESY NMR study was performed on selected $\beta\text{CD-X}$ with 4-methylbenzoate to obtain structural information about the complexes formed.

The two dimensional ¹H ROESY NMR spectrum of $\beta\text{CD-dipn}$ alone (Figure 3.10), shows strong cross-peaks between H3 and H5 of the cyclodextrin annulus and the protons on the dipn substituent (dipnH1-6). A similar spectrum of $\beta\text{CD-dien}$ shows no such interaction. These results are consistent with the dien substituent being less hydrophobic than the dipn substituent. The hydrophobicity of the dipn substituent favours the self complexation of $\beta\text{CD-dipn}$ (Figure 3.9). This is a result of the decreased ability of water to solvate the dipn substituent, relative to the increase in the hydrophobic interactions with the cyclodextrin annulus. A summary of the one dimensional ¹H NMR and two dimensional ¹H ROESY NMR assignments for $\beta\text{CD-dipn}$ are given in Table 3.3.

The cyclic substituents were not expected to include in the cyclodextrin annulus due to their size. The two dimensional spectra of β CD-tacdo and β CD-tacn at $\text{pH} \geq 12$ show no evidence of NOE interactions between the protons on the cyclic substituent and the H3 and H5 protons of the cyclodextrin annulus.

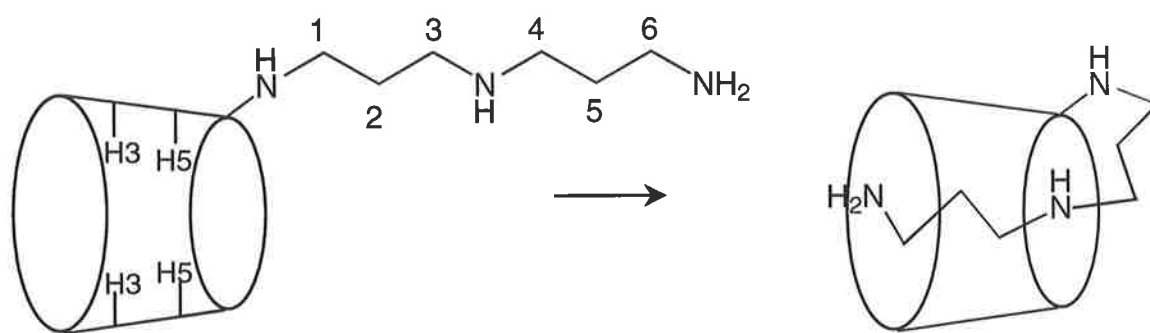


Figure 3.9. Schematic representation of the self complexation of β CD-dipn (showing the numbering of protons) in aqueous solution at $\text{pH} \geq 12$.

Table 3.3. The 1D ^1H NMR and 2D ^1H ROESY NMR spectral assignments for $\beta\text{CD-dipn}$ at $\text{pH} = 12.0$. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the dipn substituent) as in Fig.1.1. The dipn protons are labelled dipnH1 – dipnH6 as distance increases from the βCD moiety.

1D ^1H NMR spectrum			
δH	Appearance, Integration	Assignment	
4.87	broad singlet, 7H + solvent	H1	
3.7-3.9	multiplet, 26H	H3,H5,H6	
3.4-3.6	multiplet, 13H	H2,H4	
3.27	triplet, $J = 9.6$ Hz, 1H	H4^{A}	
2.98	doublet, $J = 12.6$ Hz, 1H	H6^{A}	
2.65	doublet of doublets, $J = 9.0, 12.6$ Hz, 1H	$\text{H6}^{\text{A'}}$	
2.58	triplet, $J = 6.6$ Hz, 2H	dipnH6	
2.48	multiplet, 6H	dipnH1,dipnH3,dipnH4	
1.5	multiplet, 4H	dipnH2,dipnH5	

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
1.5	dipnH2,dipnH5	2.6-2.4	dipnH1,dipnH3,dipnH4,dipnH6
		2.65	$\text{H6}^{\text{A'}}$
		3.7-3.9	H3,H5
2.48	dipnH1,dipnH3,dipnH4	1.5	dipnH2,dipnH5
		2.65	$\text{H6}^{\text{A'}}$
		2.98	H6^{A}
		3.7-3.9	H3,H5
2.58	dipnH6	1.5	dipnH2,dipnH5
		3.7-3.9	H3,H5
2.65	$\text{H6}^{\text{A'}}$	2.48	dipnH1,dipnH3,dipnH4
		2.98	H6^{A}
		3.27	H4^{A}
		3.9	H5^{A}
2.98	H6^{A}	1.5	dipnH2,dipnH5
		2.48	dipnH1,dipnH3,dipnH4
		2.65	$\text{H6}^{\text{A'}}$
		3.7-3.9	H3,H5
3.27	H4^{A}	2.65	$\text{H6}^{\text{A'}}$
3.7-3.9	H3,H5	1.5	dipnH2,dipnH5
		2.48	dipnH1,dipnH3,dipnH4
		2.58	dipnH6
		2.98	H6^{A}

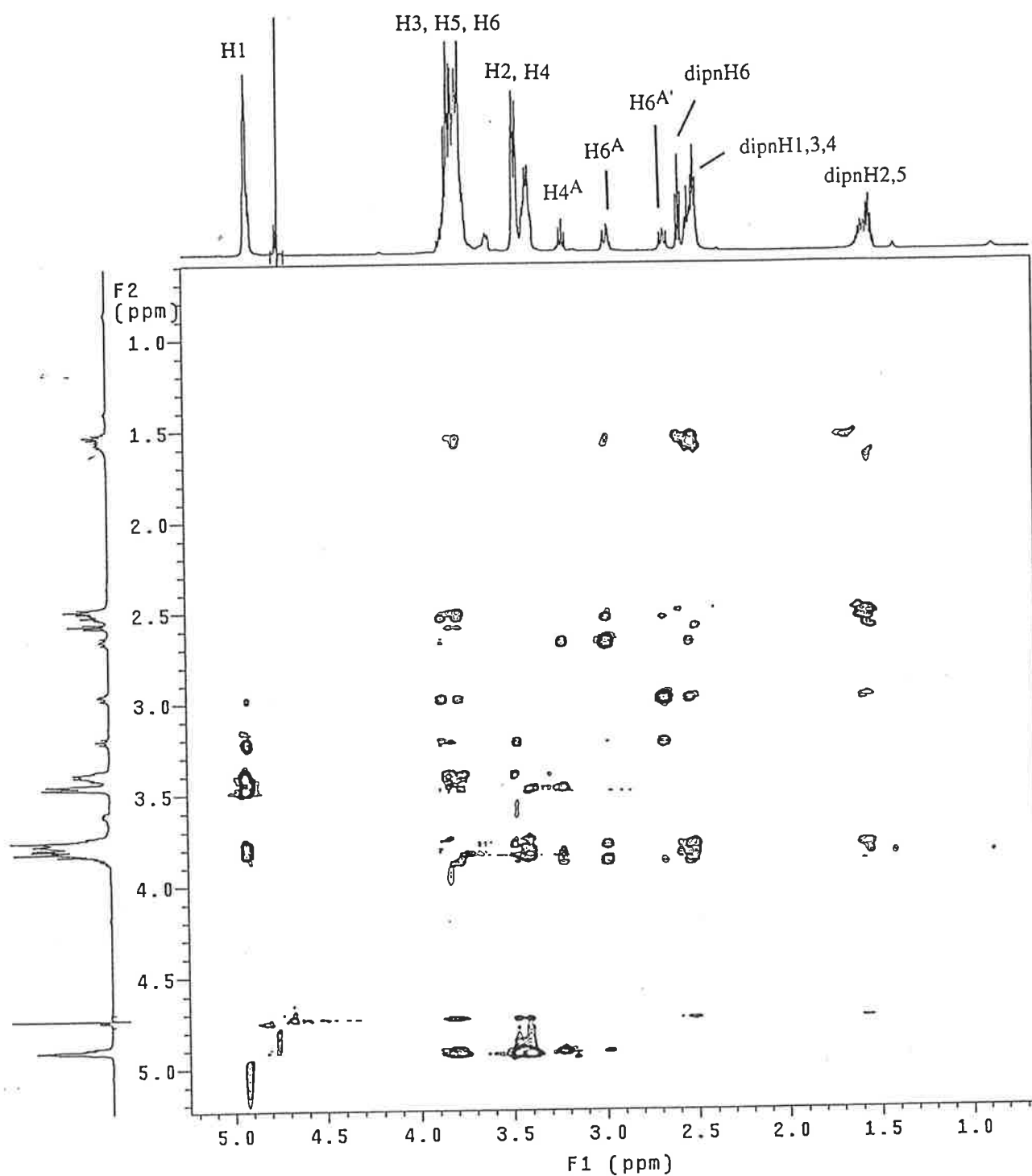


Figure 3.10. The ^1H 600 MHz ROESY NMR spectrum of βCD -dipn at 0.06 mol dm^{-3} . Cross-peaks are formed between the H3 and H5 of the cyclodextrin and dipnH(1-6). The protons are labelled as shown in Figure 3.9. Signals along the diagonal have been removed for clarity.

While it was not possible to detect the formation of a complex between 4-methylbenzoate and β CD-dipn or β CD-dien under the conditions used for potentiometric titration, a two dimensional ^1H ROESY NMR study revealed the presence of complex formation under the conditions used. The concentrations of the hosts and guests were 0.001 and 0.06 mol dm³ in the initial titration and ^1H ROESY NMR solutions, respectively. Thus, a complex characterised by a stability constant of $\approx 100 \text{ dm}^3 \text{ mol}^{-1}$ forms to only a small extent in the titrimetric experiment and is unlikely to be detected in the presence of other more stable complexes. The NMR solutions are 60 times stronger in concentration than the titration solutions, hence, the formation of, and ability to detect any complexes is increased.

There are strong NOE interactions between the *Ho* and *Hm* protons of 4-methylbenzoate with the annular protons (H3 and H5) of β CD-dien (Figure 3.12). In addition the methyl group also shows NOE interactions with the annular protons. The relative intensities of the cross-peaks due to these interactions suggest that one orientation of the 4-methylbenzoate within the annulus be with the carboxylate group pointing towards the primary end of the cyclodextrin (Figure 3.11). A summary of the one dimensional ^1H NMR and two dimensional ^1H ROESY NMR assignments for [β CD-dien.4-methylbenzoate] are given in Table 3.4.

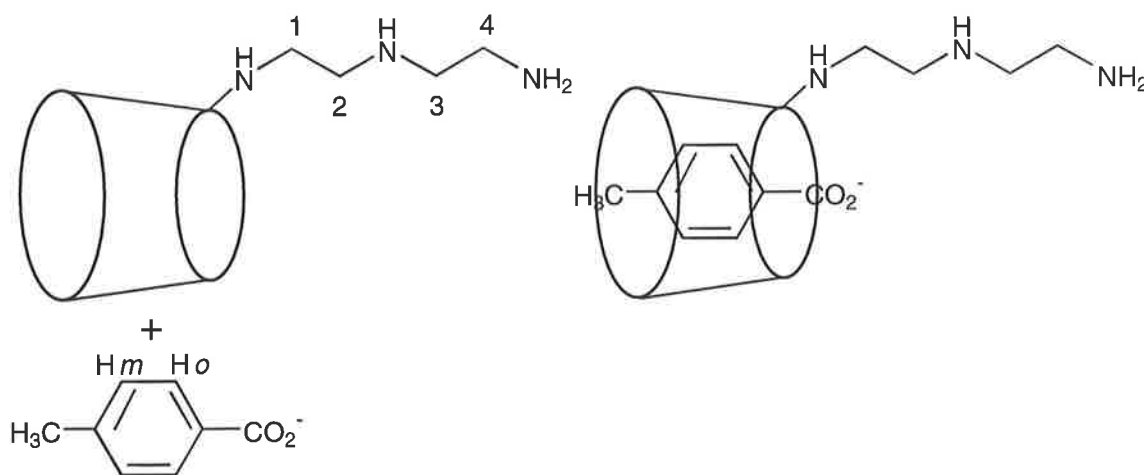


Figure 3.11. The schematic representation of β CD-dien and 4-methylbenzoate showing the numbering of protons and the complex formed by them (detected in solution by ^1H ROESY NMR) showing one orientation of the 4-methylbenzoate within the β CD annulus.

Table 3.4. The 1D ^1H NMR and 2D ^1H ROESY NMR spectral assignments for $[\beta\text{CD-dien.4-methylbenzoate}]^-$ at $\text{pH} = 12.0$. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the dien substituent) as in Fig.1.1. The dien protons are labelled dienH1 – dienH4 as distance increases from the βCD moiety. The aromatic guest protons are labelled as Ho and Hm where the former is adjacent to the carboxylate group.

1D ^1H NMR spectrum			
δH	Appearance, Integration	Assignment	
7.68	doublet, $J = 7.8$ Hz, 2H,	Ho	
7.18	doublet, $J = 7.8$ Hz, 2H	Hm	
4.83	multiplet, 7H	H1	
3.5-3.8	multiplet, 26H	H3, H5, H6	
3.3-3.5	multiplet, 13H	H2, H4	
3.14	triplet, $J = 9.6$ Hz, 1H	H4^{A}	
2.83	doublet, $J = 13.2$ Hz, 1H	H6^{A}	
2.4-2.6	multiplet, 9H	$\text{H6}^{\text{A'}}$, dienH2-4	
2.31	singlet, 3H	Me	

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
2.31	Me	3.5-3.8	H3, H5
		7.18	Hm
2.6	$\text{H6}^{\text{A'}}$	2.83	H6^{A}
		3.14	H4^{A}
2.83	H6^{A}	2.6	$\text{H6}^{\text{A'}}$
		3.65	H5
3.14	H4^{A}	2.6	$\text{H6}^{\text{A'}}$
3.65	H5	2.31	Me
		2.83	H6^{A}
		7.18	Hm
		7.68	Ho
3.8	H3	2.31	Me
		7.18	Hm
		7.68	Ho
7.18	Hm	2.31	Me
		3.6-3.8	H3, H5
7.68	Ho	3.6-3.8	H3, H5

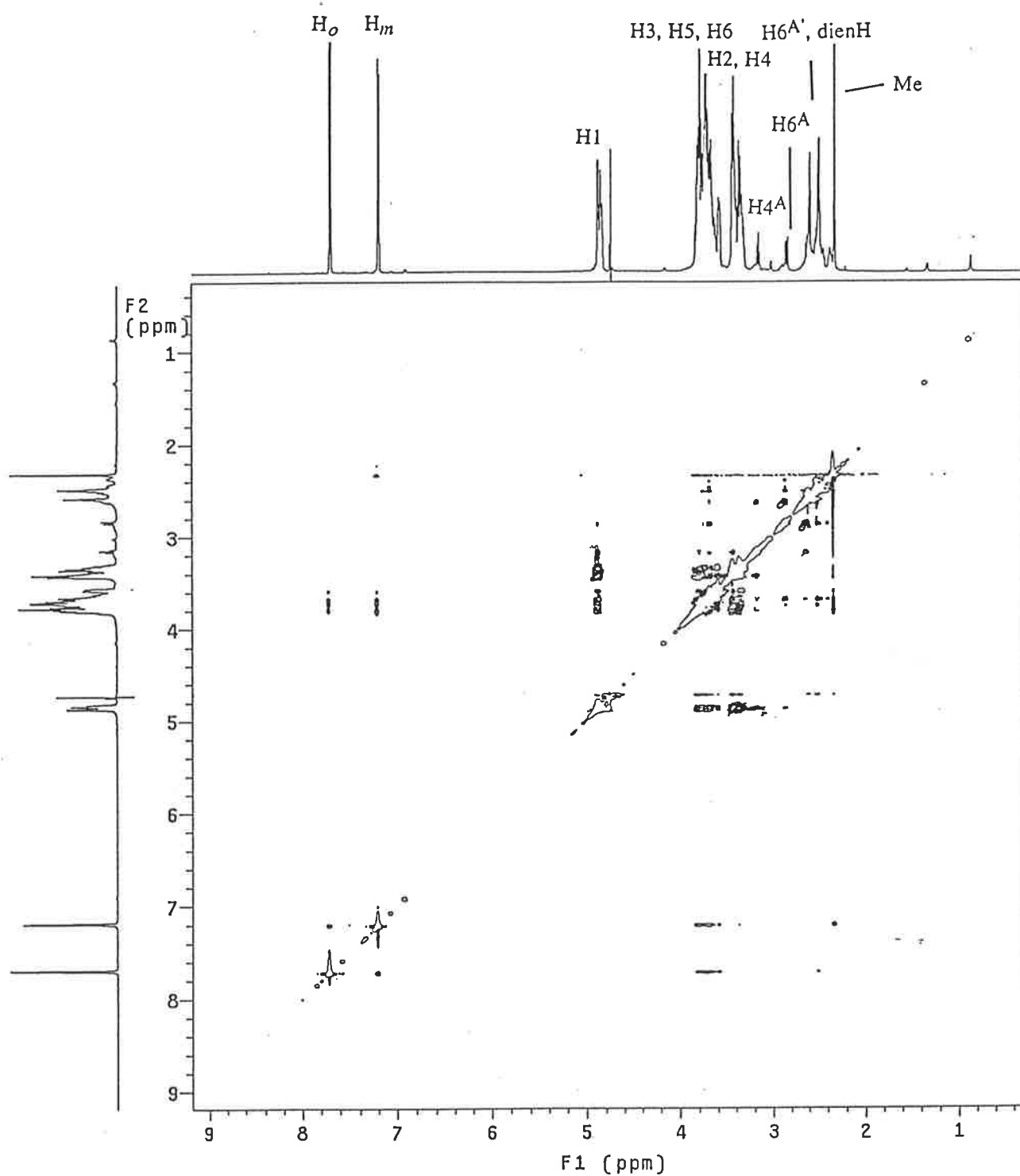


Figure 3.12. The ^1H 600 MHz ROESY NMR spectrum of the complex formed (under the conditions of this study) between 4-methylbenzoate and $\beta\text{CD-dien}$ at 0.06 mol dm^{-3} showing cross-peaks between H_o and H_m and H_3 and H_5 of $\beta\text{CD-dien}$ and between Me and H_3 and H_5 of $\beta\text{CD-dien}$. The protons are labelled as shown in Figure 3.11.

The two dimensional ^1H ROESY NMR spectrum of βCD -dipn and 4-methylbenzoate reveals NOE interactions between *Ho* and *Hm* of the guest and H3 and H5 of the cyclodextrin annulus, (Figure 3.13) the relative intensities of which suggest an orientation of the guest consistent with that for $[\beta\text{CD-dien.4-methylbenzoate}]^-$ mentioned above. There are no observable NOE interactions between the cyclodextrin annular protons and the dipn substituent. It appears as though the inclusion of the 4-methylbenzoate pushes the dipn substituent out of the annulus. Cross-peaks are observed between the H3 and H6, and H4 protons of the dipn substituent suggesting that the linear substituent is held in a conformation that brings these methylene protons near to each other. A summary of the one dimensional ^1H NMR and two dimensional ^1H ROESY NMR assignments for $[\beta\text{CD-dipn.4-methylbenzoate}]^-$ are given in Tables 3.5 and 3.6, respectively.

Table 3.5. The 1D ^1H NMR spectral assignments for $[\beta\text{CD-dipn.4-methylbenzoate}]^-$ at pH = 12.0. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the dipn substituent) as in Fig.1.1. The dipn protons are labelled dipnH1 – dipnH6 as distance increases from the βCD moiety. The aromatic guest protons are labelled as *Ho* and *Hm* where the former is adjacent to the carboxylate group.

1D ^1H NMR spectrum		
δH	Appearance, Integration	Assignment
7.71	doublet, $J = 7.8$ Hz, 2H	<i>Ho</i>
7.20	doublet, $J = 7.8$ Hz, 2H	<i>Hm</i>
4.87	multiplet, 7H	H1
3.6-3.9	multiplet, 26H	H3, H5, H6
3.2-3.5	multiplet, 13H	H2, H4
3.18	triplet, $J = 9.0$ Hz, 1H	H4 ^A
2.89	doublet, $J = 12.0$ Hz, 1H	H6 ^A
2.62	doublet of doublets, $J = 9.0, 12.0$ Hz, 1H	H6 ^{A'}
2.55	triplet, $J = 6.6$ Hz, 2H	dipnH6
2.46	triplet, $J = 7.8$ Hz, 2H	dipnH4
2.40	triplet, $J = 7.8$ Hz, 2H	dipnH3
2.34	multiplet, 5H	dipnH1, Me
1.52	multiplet, 4H	dipnH2, dipnH5

Table 3.6. The 2D ^1H ROESY NMR spectral assignments for $[\beta\text{CD-dipn.4-methylbenzoate}]^-$ at pH = 12.0. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the dipn substituent) as in Fig.1.1. The dipn protons are labelled dipnH1 – dipnH6 as distance increases from the βCD moiety. The aromatic guest protons are labelled as Ho and Hm where the former is adjacent to the carboxylate group.

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
1.52	dipnH2, dipnH5	2.34	dipnH1
		2.40	dipnH3
		2.46	dipnH4
		2.55	dipnH6
2.34	Me	3.6-3.9	H3, H5
		7.20	Hm
2.40	dipnH3	1.52	dipnH2, dipnH5
		2.46	dipnH4
2.46	dipnH4	1.52	dipnH2, dipnH5
		2.40	dipnH3
		2.55	dipnH6
2.55	dipnH6	1.52	dipnH2, dipnH5
		2.46	dipnH4
2.62	C6 ^A	2.89	C6 ^A
		3.18	C4 ^A
2.89	C6 ^A	2.62	C6 ^A
		3.7	H5 ^A
3.18	C4 ^A	2.62	C6 ^A
3.6-3.9	H3, H5	2.34	Me
		7.20	Hm
		7.71	Ho
7.20	Hm	2.34	Me
		3.6-3.9	H3, H5
7.71	Ho	3.6-3.9	H3, H5

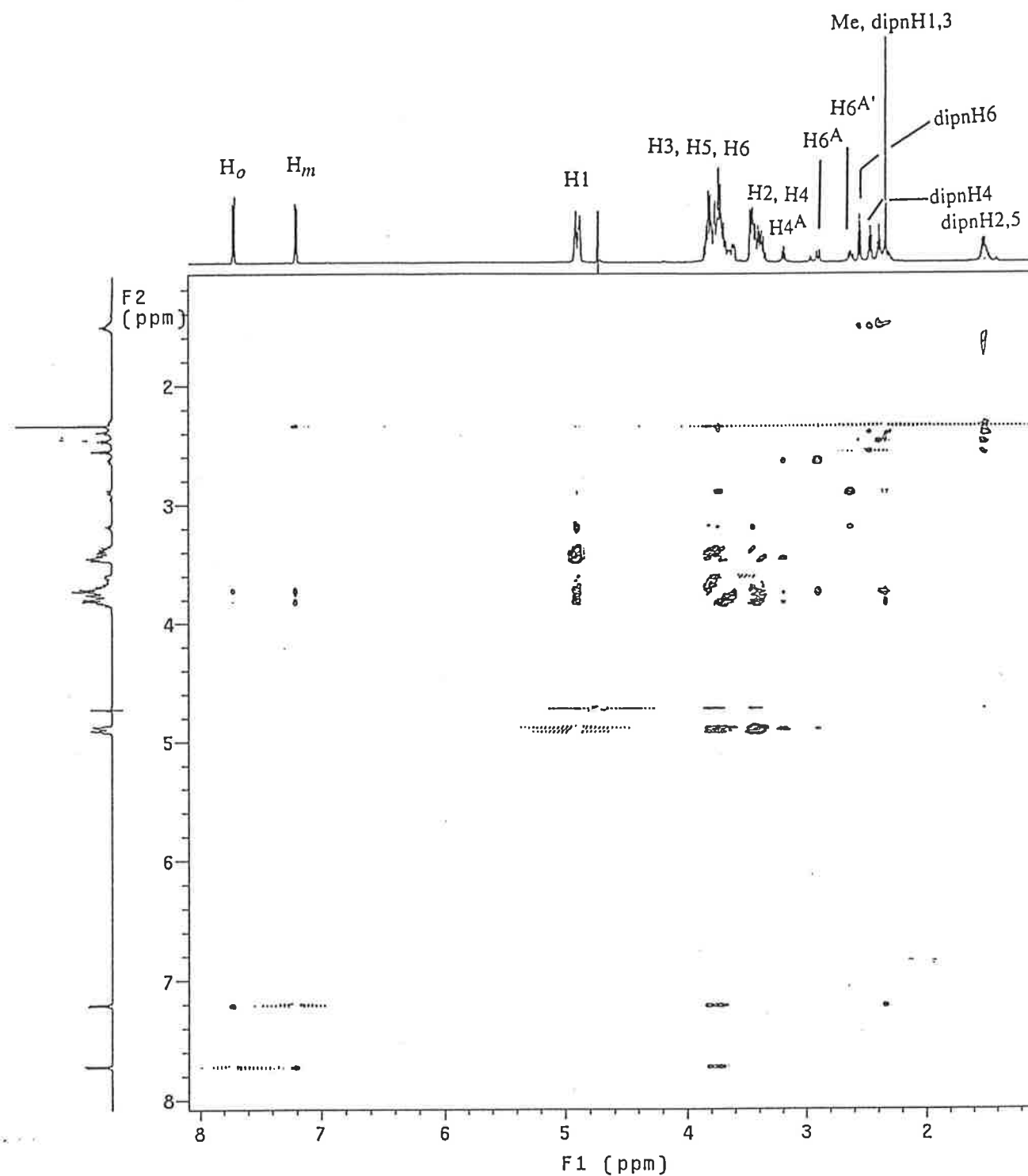


Figure 3.13. The ¹H 600 MHz ROESY NMR spectrum of the complex formed (under the conditions of this study) between 4-methylbenzoate and βCD-dipn at 0.06 mol dm⁻³ showing cross-peaks between *H_o* and *H_m* and *H₃* and *H₅* of βCD-dipn. The protons are labelled as shown in Figures 3.9 and 3.11.

An alternate orientation of the guest within the cyclodextrin cavity is suggested by the two dimensional ^1H ROESY NMR spectrum of βCD -tacdo and 4-methylbenzoate (Figure 3.15). Cross-peaks indicate that the H_o and H_m protons of the guest are situated in the vicinity of the annular protons H3 and H5, with the strongest NOE interaction being between the H_m protons and the annular H5 protons. Further cross-peaks indicate that the methyl group of 4-methylbenzoate is located adjacent to the H5 protons of the cyclodextrin moiety and has NOE interactions with one or more of the methylene groups of the tacdo substituent. These results suggest that one orientation of 4-methylbenzoate in the βCD -tacdo cavity is with the carboxylate group towards the secondary end of the annulus and the guest deeply inserted such that the methyl group penetrates the hydrophobic ring of the tacdo substituent (Figure 3.14). While complexation of 4-methylbenzoate by βCD -tacn is evidenced by cross peaks between the methyl protons and H_o and H_m of the carboxylate guest with the H3 and H5 protons of the annulus of βCD -tacn, no differentiation in NOE signal intensities allows a specific orientation of the guest within the cyclodextrin annulus, to be assumed. A summary of the one dimensional ^1H NMR and two dimensional ^1H ROESY NMR assignments for $[\beta\text{CD-tacdo.4-methylbenzoate}]^-$ is given in Table 3.7.

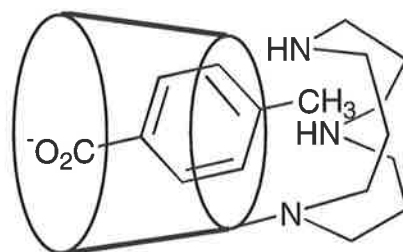


Figure 3.14. The schematic representation of $[\beta\text{CD-tacdo.4-methylbenzoate}]^-$ showing the preferred orientation of the guest as evidenced by 2D ^1H ROESY NMR spectroscopy.

Table 3.7. The 1D ^1H NMR and 2D ^1H ROESY NMR spectral assignments for $[\beta\text{CD-tacdo.4-methylbenzoate}]^-$ at $\text{pH} = 12.0$. The spectral assignments below are according to the glucopyranose numbering system H1-H6 (where a superscript A specifies the glucopyranose unit bearing the dien substituent) as in Fig.1.1. The tacdo protons are labelled tacdoH1 – tacdoH4 as distance increases from the βCD moiety. The aromatic guest protons are labelled as Ho and Hm where the former is adjacent to the carboxylate group.

1D ^1H NMR spectrum			
δH	Appearance, Integration	Assignment	
7.75	doublet, $J = 7.8$ Hz, 2H	Ho	
7.25	doublet, $J = 7.8$ Hz, 2H	Hm	
4.9	multiplet, 7H	H1	
3.7-4.0	multiplet, 26H	H3, H5, H6	
3.4-3.6	multiplet, 13H	H2, H4	
3.17	triplet $J = 6.0$ Hz, 1H	H4 ^A	
2.6-2.8	multiplet, 14H	H6 ^A , tacdoH1, tacdoH3, tacdoH4	
2.34	singlet, 3H	Me	
1.66	multiplet, 6H	tacdoH2, tacdoH5	

2D ^1H ROESY NMR spectrum			
Cross-peaks observed FOR		Cross-peaks observed WITH	
δH	Assignment	δH	Assignment
2.34	Me	2.6-2.7	tacdoH1, tacdoH3, tacdoH4
		3.8-4.0	H3, H5
		7.25	Hm
2.6-2.7	tacdoH1, tacdo H3, tacdoH4	2.34	Me
3.8-4.0	H3, H5	2.34	Me
		2.6-2.7	tacdoH1, tacdoH3, tacdoH4
		7.25	Hm
		7.75	Ho
7.25	Hm	2.34	Me
		3.8-4.0	H3, H5
7.75	Ho	3.8-4.0	H3, H5

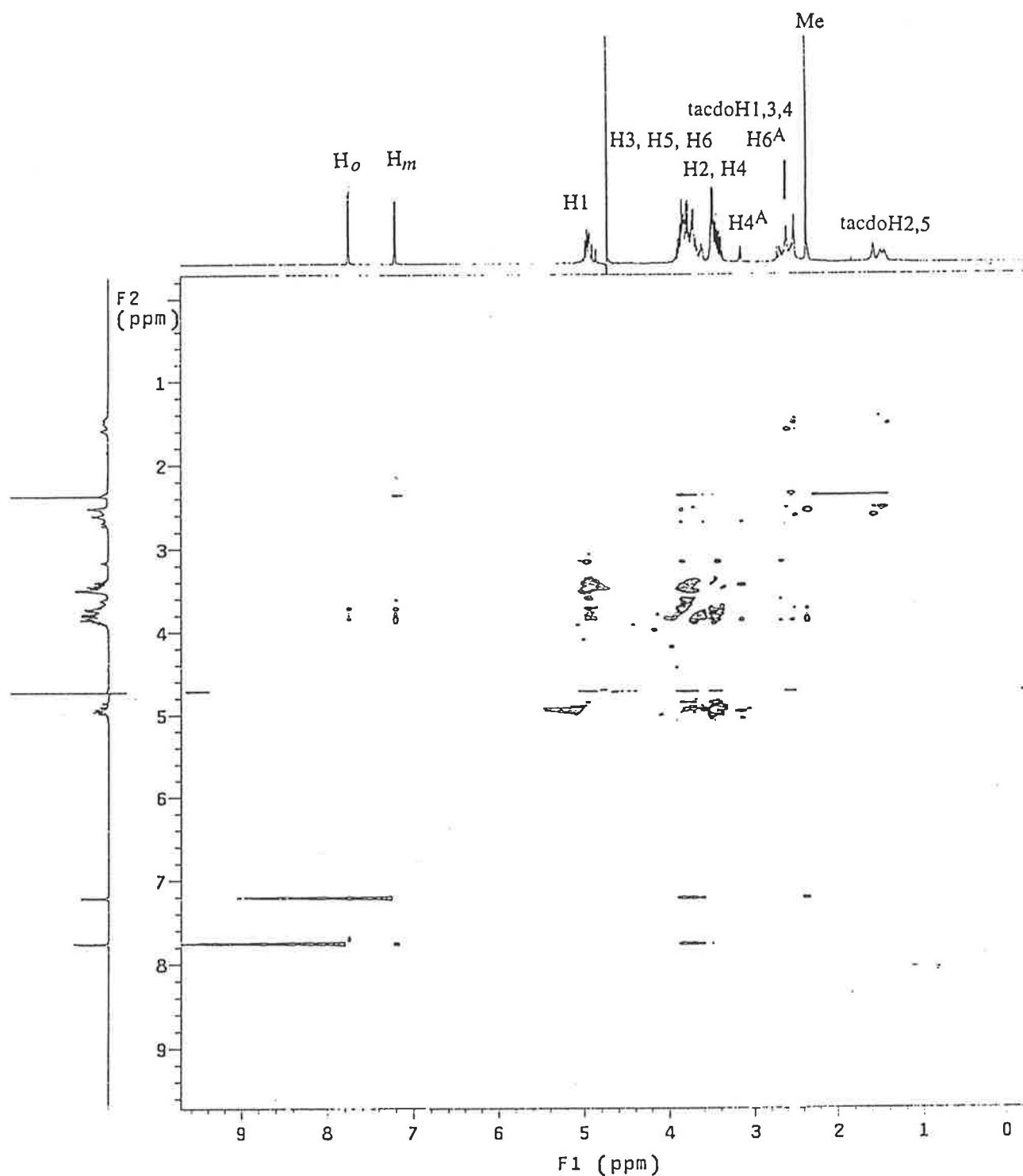


Figure 3.15. The ^1H 600 MHz ROESY NMR spectrum of $[\beta\text{CD-tacdo.4-methylbenzoate}]^-$ at 0.06 mol dm^{-3} showing cross-peaks between H_O and H_m and H_3 and H_5 of $\beta\text{CD-tacdo}$, Me and H_5 of $\beta\text{CD-tacdo}$ and between Me and the methylene protons of the tacdo substituent. The signals along the diagonal have been removed for clarity.

3.3. Summary and Conclusions.

A range of 6^A-deoxy-6^A-triazalkyl- and 6^A-deoxy-6^A-tetraazaalkyl- β -cyclodextrins provided an opportunity to further investigate the factors governing the complexation of benzoic, 4-methylbenzoic and (*R/S*)-2-phenylpropanoic acids and their conjugate bases in aqueous solution with polyamino modified cyclodextrins.

The pK_a s of the cyclodextrin hosts were determined and a comparison made with those of the free polyamine groups. The pK_a s of β CD-X were consistently lower than those of the free polyamine due to the induced changes in the basicity of the amine groups imparted by the steric and electronic effects of the β CD moiety. A ¹³C NMR study revealed that the unprotonated polyamine substituents hydrogen bonded to adjacent primary hydroxyl groups, but subsequent protonation lessened this interaction. The strongest interactions were observed for β CD-tacdo and β CD-cyclen.

The interplay of secondary bonding interactions on the formation of complexes by β CD-XH_nⁿ⁺ with the guest aromatic carboxylic acids and carboxylates, is both additive and subtractive with the consequence that only a generalised explanation of their influence on complex stability can be obtained. The β CD-XH_nⁿ⁺ studied here tend to produce complexes of greater stability than those formed by the diamino-monosubstituted- β -cyclodextrins studied in Chapter 2. A more basic comparison can be made with the complexes of the native β CD. The complexes formed by β CD with benzoic, 4-methylbenzoic and (*R*)- and (*S*)-2-phenylpropanoic acids are characterised by stability constants of 590, 1680, 1090 and 1010 dm³ mol⁻¹, respectively.⁸ Because of the pK_a s of β CD-XH_nⁿ⁺ there are no neutral β CD-X complexes formed with the carboxylic acid guests with which comparison may be made. It is interesting to note that the stabilities of the β CD complexes approach those of the less stable β CD-XH_nⁿ⁺ complexes of the same. The carboxylic acid complexes formed by β CD-tacdoH₂²⁺ and β CD-dipnH₃³⁺ (excluding the complex with benzoic acid for the latter) are at least an order of magnitude more stable than those formed with β CD.

The β CD complexes formed with benzoate, 4-methylbenzoate and (*R*)- and (*S*)-2-phenylpropanoate are characterised by stability constants of 60, 110, 63 and 52 dm³ mol⁻¹, respectively. These stabilities are orders of magnitude lower than those for the analogous complexes formed by neutral β CD-tacdo and β CD-cyclen. However, it is apparent from ¹H

ROESY NMR studies that the $[\beta\text{CD-X-4-methylbenzoate}]^-$ complexes, (where X = dien, dipn and tacn), are of similar low stability to those formed by βCD . Similarly, for the other complexes of $\beta\text{CD-XH}_n^{n+}$ undetected by the titrimetric method, their stabilities may be of similar magnitude to those characterising the βCD carboxylate complexes. While substantial increases in complex stability can be effected by appropriate substitution on βCD , clearly the extent of such increases is very dependant on the natures of both the substituent and the guest.

A two dimensional ^1H ROESY NMR spectroscopic study not only revealed the formation of complexes undetected by the potentiometric method, but gave structural information as to the preferred orientation of the included guest within the cyclodextrin moiety for the complexes studied. No clear trend was observed for the orientation of the guest in these complexes providing further evidence that factors governing complex formation are intrinsic to each individual system studied. Self inclusion of the dipn substituent by βCD -dipn was observed, further evidencing the hydrophobicity of this cyclodextrin host.

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CHAPTER FOUR

THE FORMATION OF METALLO-TETRAAZAALKYL-MONOSUBSTITUTED- β -CYCLODEXTRINS AND THEIR COMPLEXATION OF TRYPTOPHAN AND TRYPTOPHANOATE IN AQUEOUS SOLUTION.**4.1. Introduction.**

The coordination of a metal ion by a modified cyclodextrin to form a binary metallocyclodextrin and the subsequent complexation of a substrate to form a ternary metallocyclodextrin, allows the study of metal centre and cyclodextrin interactions on metallocyclodextrin stability and substrate binding.¹ A number of metallocyclodextrins have been reported in the literature and have been investigated for their complexing, catalytic and enantioselective properties as well as being used as metalloenzyme mimics (Section 1.3.1).

The formation of binary and ternary metallocyclodextrins enable studies into the factors affecting metallocyclodextrin stability and guest binding in some detail. In the case of an chiral guest, the factors affecting enantioselectivity may also be examined. While the chirality of the cyclodextrin is important for enantioselection, the importance of the metal is just as great. Such is the case for 6^A-deoxy-6^A-(aminopropylamino)- β -cyclodextrin and its Ni²⁺ complex.² The former shows no enantioselectivity for tryptophan whereas the latter shows a 10-fold enantioselectivity in favour of the (*S*)-enantiomer.

The formation of the metallocyclodextrins of 6^A-deoxy-6^A-(2-(2-(2-aminoethylamino)ethylamino)ethylamino)- β -cyclodextrin (β CD-trien) and 6^A-deoxy-6^A-(1,4,7,10-tetraaza cyclododecanyl)- β -cyclodextrin (β CD-cyclen) (Figure 4.1), with Zn²⁺, Cu²⁺ and Ni²⁺ and the subsequent complexation of (*R*)- and (*S*)-tryptophan (trpH) and (*R*)- and (*S*)-tryptophanoate (trp⁻) will enable the determination of the effect of the chelating environment of the metal ion on the formation of binary and ternary metallocyclodextrins. The 2-(2-(2-aminoethylamino)ethylamino)ethylamino and 1,4,7,10-tetraazacyclododecanyl substituents are structurally the least and most constrained tetraamine ligands with ethylene linkages for binding metal ions, respectively and it is anticipated that this will be reflected in their metallocyclodextrins. The complexation of a metal ion by a cyclic polyamine ligand has been known to produce striking changes in the properties of the metal ion complexes when compared to the equivalent complexes of linear ligands.³ Typical properties of macrocyclic

complexes include 1) a marked kinetic inertness towards formation from an aqueous metal ion and ligand and towards decomposition; 2) strong metal-nitrogen interactions; 3) possible stabilisation of high oxidation states of the metal centre and 4) high thermodynamic stability which is reflected by the stability constants of macrocycle-metal complexes being several orders of magnitude greater than for the corresponding complex of the linear ligand.³ Intermediate between these substituents is the tripodal substituent of 6^A-deoxy-6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)- β -cyclodextrin, (β CD-tren), whose complexing characteristics provide informative comparisons.¹ The previous metalocyclodextrin studies, undertaken at this institution, detected a subtle interplay between the effects of guest complexation in the β CD-X annulus and the M^{2+} coordination of the guest determining the stability of the ternary metalocyclodextrins.^{1,2} Further understanding of these interactions is sought in this study.

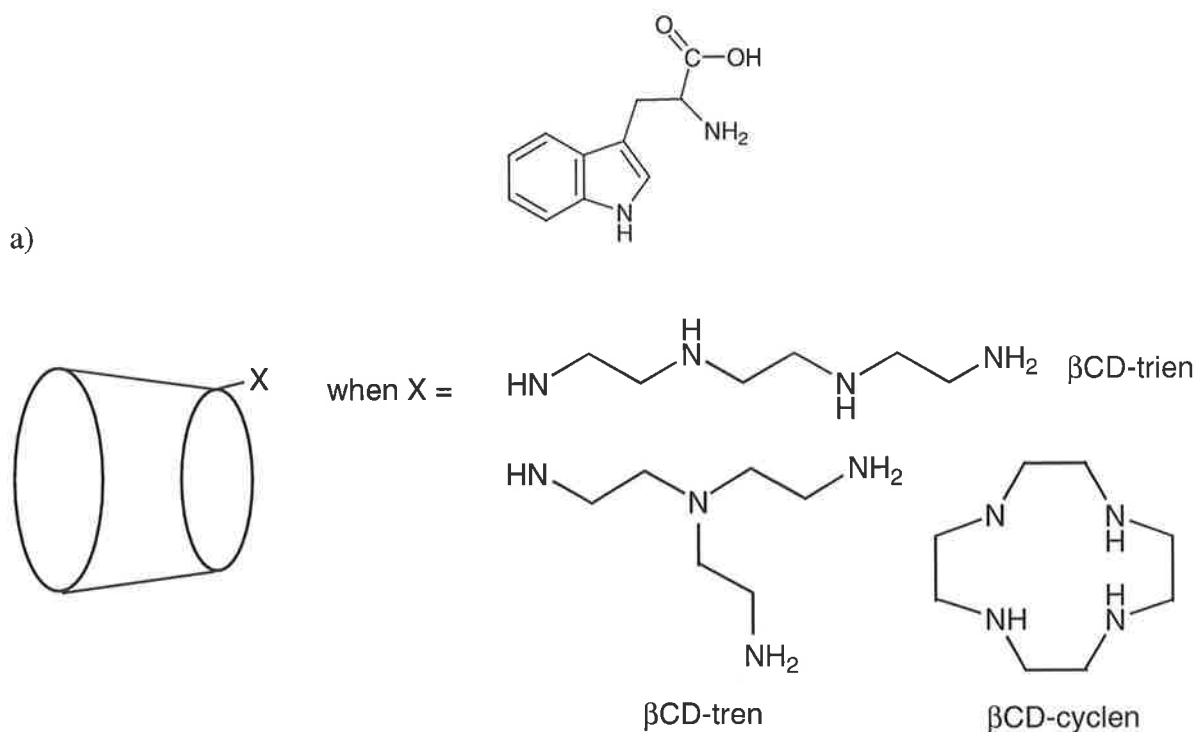


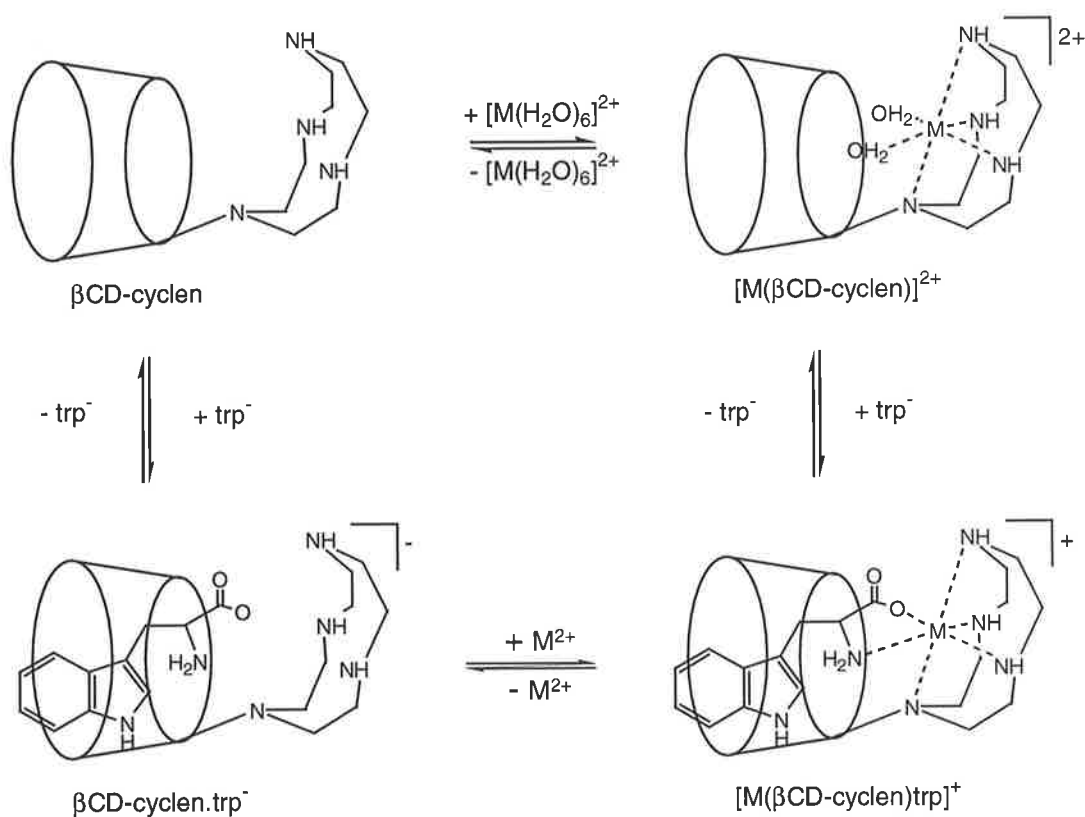
Figure 4.1. The structures of a) (R/S)-tryptophan and b) of the three tetraazaalkyl-monosubstituted- β -cyclodextrins mentioned.

Zn^{2+} , Cu^{2+} and Ni^{2+} are known to form stable complexes with both triethylenetetramine (trien),⁴ and 1,4,7,10-tetraazacyclododecane (cyclen),⁵⁻⁷ hence strong complexation by the cyclodextrin bound analogues is expected. As in previous studies the three M^{2+} were chosen because Zn^{2+} frequently acts as a metal centre in metalloenzymes.⁸⁻¹¹ Ni^{2+} and Cu^{2+} are closely related to Zn^{2+} in size and electronic structure, though themselves are found less often in biological systems. The formation of μ -peroxo species when Co^{2+} is coordinated by four amine groups, precluded studies with this metal ion.^{1,12} Besides being a test for enantioselectivity, tryptophan was chosen for its known ability to form stable complexes with cyclodextrins,¹³ this has been attributed in the past, to its possession of an aromatic moiety of an appropriate size to fit into the cyclodextrin cavity. Complexation studies with (*S*)-tryptophan proved the cavity of βCD to be of the best size to include the amino acid.¹³

4.2. Results and Discussion.

β CD-cyclen and β CD-trien were synthesised and a potentiometric titration study undertaken to investigate their complexation of Zn^{2+} , Ni^{2+} , and Cu^{2+} and of (*R*)- and (*S*)-tryptophan/tryptophanoate as shown in Scheme 4.1 (where $\beta\text{CD-cyclenH}_n^{n+}$ is depicted as the cyclodextrin of interest). The differences between the pH profiles arising from titration of acidified solutions containing various combinations of the complexing species, against NaOH, allows the stabilities of the complexes formed to be calculated (Section 6.3).

Scheme 4.1.



4.2.1. The Formation of 1:1 Complexes with (R)- and (S)-Tryptophan/Tryptophanoate.

The results obtained for the complexation of (R)- and (S)-tryptophan/tryptophanoate with β CD-cyclenH_nⁿ⁺ and β CD-trienH_nⁿ⁺ from the best fit of the data, (Figure 4.2) are given in Table 4.1 alongside the results published for β CD-trenH_nⁿ⁺.¹ The pK_as of protonated tryptophan, trpH₂⁺, and tryptophan, TrpH, are 2.40 and 9.28 respectively.² The pK_as of the cyclodextrin hosts are given in Chapter 3, Table 3.1. The large variation in the types of complexes formed by the three cyclodextrins reflect the varied nature and effect of the tetraamine substituents on complex stabilisation.

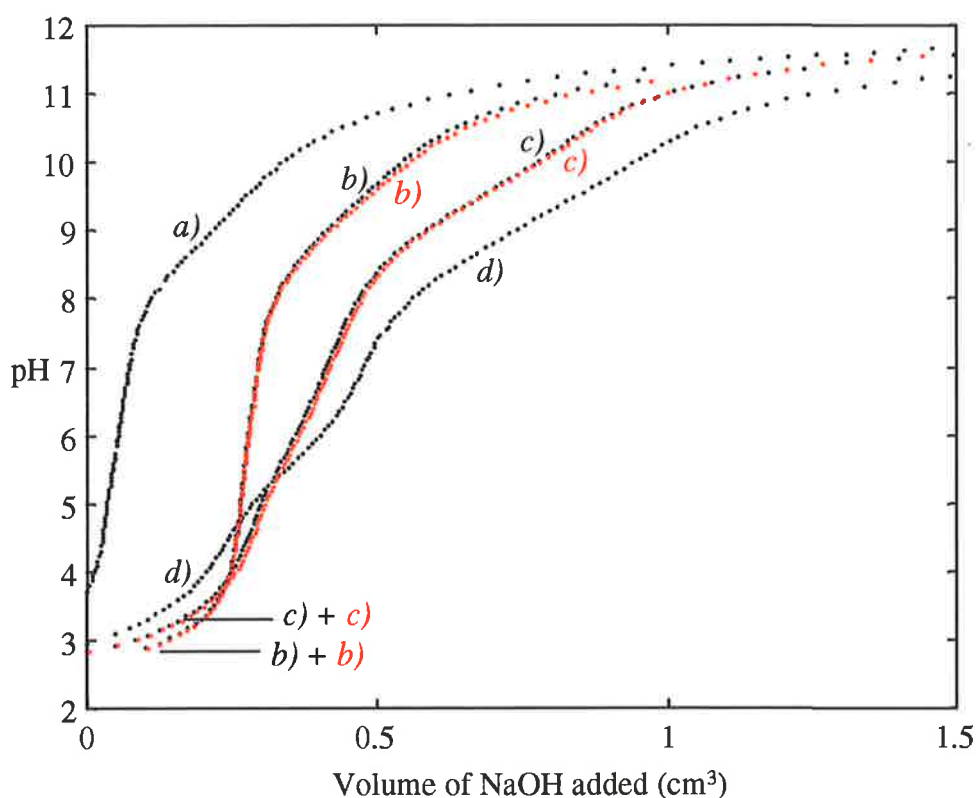


Figure 4.2. The pH profiles of a) β CD-cyclenH_nⁿ⁺ at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$, b) β CD-cyclenH_nⁿ⁺ and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$, b) β CD-cyclenH_nⁿ⁺ and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$, c) β CD-trienH_nⁿ⁺ and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$, c) β CD-trienH_nⁿ⁺ and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ and d) β CD-trienH_nⁿ⁺ at $2 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

Table 4.1. The stability constants determined for the 1:1 binary complexes of β CD-trienH_nⁿ⁺, β CD-cyclenH_nⁿ⁺ and β CD-trenH_nⁿ⁺ with (R)- and (S)-tryptophan/tryptophanoate and their acid dissociation constants (pK_as) at I = 0.10 (NaClO₄) and 298.2 K. Data were fitted over the pH ranges 3 - 11 and 5.5 - 11 for the β CD-trienH_nⁿ⁺ and β CD-cyclenH_nⁿ⁺ systems respectively.

Complex formed	Guest	log (K/mol dm ⁻³)		
		β CD-trienH _n ⁿ⁺	β CD-cyclenH _n ⁿ⁺	β CD-trenH _n ⁿ⁺ ^a
[β CD-X.trp] ⁺	(R)-trp ⁻	-	3.69 ± 0.04	6.36 ± 0.01
	(S)-trp ⁻	-	3.95 ± 0.03	6.5 ± 0.1
[β CD-XH.trp]	(R)-trp ⁻	-	2.82 ± 0.06	5.85 ± 0.03
	(S)-trp ⁻	-	2.87 ± 0.07	5.9 ± 0.1
[β CD-XH.trpH] ⁺	(R)-trpH	3.51 ± 0.04	2.99 ± 0.03	5.59 ± 0.05
	(S)-trpH	3.57 ± 0.03	3.12 ± 0.05	5.61 ± 0.08
[β CD-XH ₂ .trpH] ²⁺	(R)-trpH	4.01 ± 0.05	-	-
	(S)-trpH	4.14 ± 0.04	-	-
[β CD-XH ₃ .trpH] ³⁺	(R)-trpH	4.4 ± 0.1	-	-
	(S)-trpH	4.50 ± 0.06	-	-

	pK _a		
	β CD-trienH _n ⁿ⁺	β CD-cyclenH _n ⁿ⁺	β CD-trenH _n ⁿ⁺
pK _{a1}	9.33 ^b	10.40 ^b	9.85 ^a
pK _{a2}	8.22 ^b	8.62 ^b	8.99 ^a
pK _{a3}	5.61 ^b	small	6.89 ^a
pK _{a4}	3.13 ^b	small	2.6 ^a
[β CD-XH.(R)-trp] = [β CD-X.(R)-trp] ⁻ + H ⁺		9.53 ± 0.05	9.34
[β CD-XH.(S)-trp] = [β CD-X.(S)-trp] ⁻ + H ⁺		9.50 ± 0.09	9.3
[β CD-XH.(R)-trpH] ⁺ = [β CD-XH.(R)-trp] + H ⁺		9.41 ± 0.07	8.99
[β CD-XH.(S)-trpH] ⁺ = [β CD-XH.(S)-trp] + H ⁺		9.31 ± 0.09	8.8
[β CD-XH ₂ .(R)-trpH] ²⁺ = [β CD-XH.(R)-trpH] ⁺ + H ⁺	8.72 ± 0.05		
[β CD-XH ₂ .(S)-trpH] ²⁺ = [β CD-XH.(S)-trpH] ⁺ + H ⁺	8.79 ± 0.02		
[β CD-XH ₃ .(R)-trpH] ³⁺ = [β CD-XH.(R)-trpH] ²⁺ + H ⁺	5.97 ± 0.05		
[β CD-XH ₃ .(S)-trpH] ³⁺ = [β CD-XH.(S)-trpH] ²⁺ + H ⁺	5.96 ± 0.03		

^a Reference 1. ^b Reference 14

As discussed in Chapters Two and Three, the stability of a cyclodextrin complex arises from a summation of weak secondary forces between the cyclodextrin host and the guest. Their formation usually proceeds through entry of a hydrophobic portion of the guest in to the flexible cyclodextrin annulus^{15,16} which is accompanied by some conformational change in the cyclodextrin and the guest, and dehydration of both to an extent dependant on the nature of each.¹⁷⁻¹⁹ Variations in cyclodextrin complex stability may arise from a range of causes, however, in general terms, an increase in complex stability reflects an increase in the rate of complexation compared with the rate of decomplexation. This is reflected in the effects of cyclodextrin substituents on complex stability. Complexes may exist in two major isomeric forms. For the systems studied here, either trp^- or trpH is orientated with the amino acid group towards the primary end of the cyclodextrin annulus in one isomer, with the reverse orientation for the other isomer.

The stability constants derived for the complexes of (*R/S*)- trp^-/trpH are greater than those for the guest aromatic carboxylic acids/carboxylates used in Chapters Two and Three, with the same cyclodextrin host (Tables 4.1 and 3.2). Stability is imparted to the complexation of aromatic guests substantially through the interaction of their phenyl moieties with the hydrophobic cavity of the cyclodextrin. The aromatic portion of (*R/S*)- trp^-/trpH is greater than that of the guest aromatic carboxylic acids/carboxylates studied in Chapters Two and Three. Thus, the interaction of tryptophan with the cyclodextrin annulus is greater, imparting greater complex stability.

The apparent stability constants for the complexes formed by βCD and $\beta\text{CD-pn}$ with (*R/S*)- trp^- are given in Table 4.2.² When $X = \text{cyclen}$, the complexes of $[\beta\text{CD-X.trp}]^-$ (Table 4.1), are 3 times and 30 times more stable than those of $[\beta\text{CD-pn.trp}]^-$ and $[\beta\text{CD.trp}]^-$, respectively. When $X = \text{tren}$, the complexes of $[\beta\text{CD-X.trp}]^-$, (Table 4.1), are approximately 10^3 times and 10^4 times more stable than those of $[\beta\text{CD-pn.trp}]^-$ and $[\beta\text{CD.trp}]^-$, respectively. Analogous complexes were not detected for $\beta\text{CD-trien}$. The increase in stability of the trp^- complexes with change in nature of the cyclodextrin is in the sequence $\beta\text{CD} < \beta\text{CD-pn} < \beta\text{CD-cyclen} < \beta\text{CD-tren}$. Theoretical calculations show βCD to have a dipole moment approximately coincident with the axis of the annulus, with the negative and positive poles adjacent to the secondary and primary faces of the annulus, respectively.¹⁸⁻²⁰ It is held that this dipole moment influences the orientation of dipolar guests complexed in the cyclodextrin annulus (Section 1.2.3). Although the magnitude of the dipole moment varies with the nature of the calculation and the conformations adopted by the glucopyranose units, and the dipole orientation can change substantially on complexation of a guest,^{18,21} its probable existence

has clear implications for the complexes formed by $\beta\text{CD-trienH}_n^{n+}$, $\beta\text{CD-cyclenH}_n^{n+}$ and $\beta\text{CD-trenH}_n^{n+}$. While the effect of the tetraamine substituents of $\beta\text{CD-trien}$, $\beta\text{CD-cyclen}$ and $\beta\text{CD-tren}$ on the magnitude of the dipole moment of βCD is unknown, it is probable that those of the protonated analogues increase in magnitude with the positive pole adjacent to the primary face. Thus, it is anticipated that the dominant isomers of the $\beta\text{CD-XH}_n^{n+}$ (where $n = 0-4$) complexes with trp^- and trpH will be those with the amino acid or aminocarboxylate group adjacent to the primary faces of their annuli.

Table 4.2. The stability constants determined for the 1:1 binary complexes of βCD and $\beta\text{CD-pn}$ with (*R*)- and (*S*)-tryptophan at $I = 0.10$ (NaClO_4) and 298.2 K.

Complex Formed	(<i>R</i>)-tryptophan ^a log ($K/\text{mol dm}^{-3}$)	(<i>S</i>)-tryptophan ^a log ($K/\text{mol dm}^{-3}$)
$[\beta\text{CD.trp}]^-$	2.33 ± 0.06	2.33 ± 0.08
$[\beta\text{CD-pn.trp}]^-$	3.41 ± 0.02	3.40 ± 0.07

^a Reference 2

It is apparent from the differing extent of protonation of the dominant complexes formed by the three $\beta\text{CD-XH}_n^{n+}$ (Table 4.1) that the nature of the substituent plays a primary role in determining complex stability. Hydrogen bonding of the guest with the amine groups of $\beta\text{CD-pn}$, $\beta\text{CD-cyclen}$ and $\beta\text{CD-tren}$, may reinforce the dipolar effect discussed above, consistent with their higher stabilities by comparison with those of βCD . However, the two orders of magnitude difference between the stabilities of the $\beta\text{CD-cyclen}$ and $\beta\text{CD-tren}$ complexes of (*R*)- and (*S*)- trp^- , and the absence of any analogous complexes for $\beta\text{CD-trien}$, is indicative of substituent stereochemistry being a crucial factor affecting complex stability. The extent of substituent stereochemical constraint decreases in the order $\beta\text{CD-cyclen} < \beta\text{CD-tren} < \beta\text{CD-trien}$ from which it is evident that the tripodal 2-(bis(2-aminoethyl)amino)ethyl)amino stereochemistry best stabilises the complexation of trp^- .

The hydrogen bonding characteristics and hydrophobicity of $\beta\text{CD-cyclenH}^+$ and $\beta\text{CD-trenH}^+$ are diminished by comparison with those of $\beta\text{CD-cyclen}$ and $\beta\text{CD-tren}$. Hence the stability of their complexes with (*R*)- and (*S*)- trp^- is decreased by comparison with those of

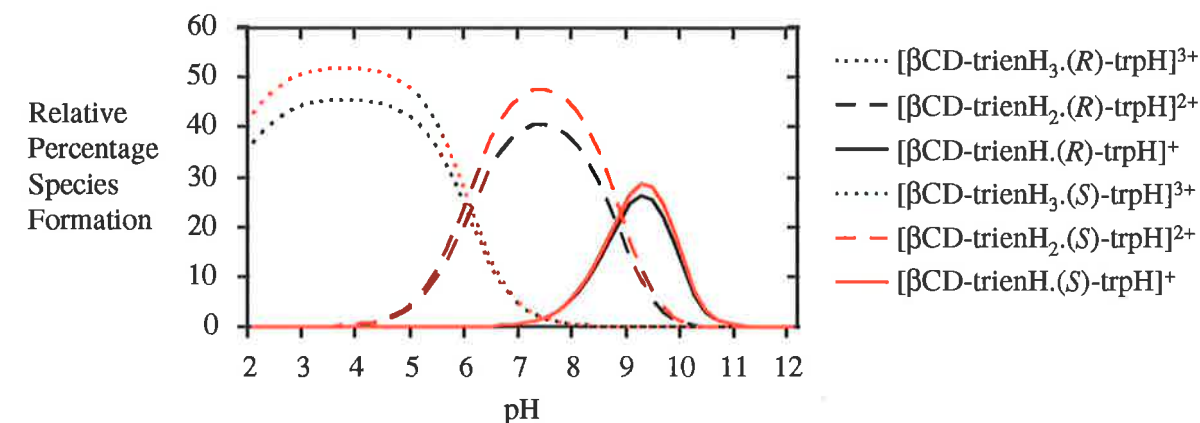
β CD-cyclen and β CD-tren. Superimposing on these factors are changes in hydration. The cancellation of charges on the host and guest upon complexation leads to a loss of solvation energy (compared with the uncomplexed host and guest) which lowers the stability of the complex formed.

The dipolar and hydrogen bonding characteristics of trpH are diminished by comparison with those of trp⁻. Alternately the hydrophobic characteristics of trpH is increased by comparison with that of trp⁻. The subtle interplay of these factors upon complex stabilisation with β CD-XH⁺, in addition to substituent stereochemistry, results in the stability of the $[\beta$ CD-XH.trpH]⁺ complexes increasing with changing X in the order cyclen < trien < tren. This is the only complex formed in common by all three β CD-XH_nⁿ⁺.

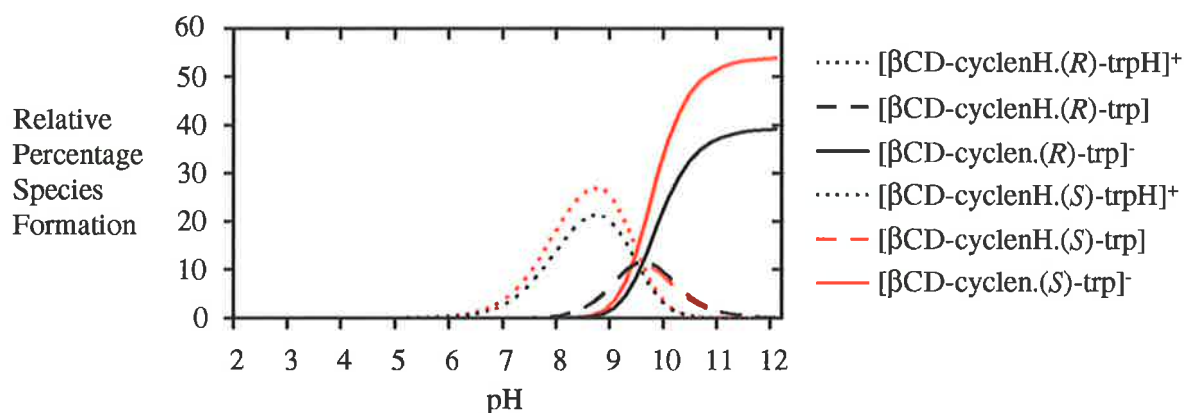
Further protonation is expected to diminish the hydrophobic character of β CD-cyclenH_nⁿ⁺ and β CD-trenH_nⁿ⁺ (n = 2 - 3) so that they are less likely to form complexes with trpH, as is observed. In contrast, β CD-trienH_nⁿ⁺ (n = 2 - 3) does form trpH complexes which again reflects the differences between the substituents of β CD-X and their impact on complex stabilisation. The factors destabilising neutral β CD-trien complexation are diminished by substituent protonation. This infers that the more flexible β CD-trien substituent interacts with the β CD entity to destabilise trp⁻ complexes and that this inhibiting effect is lessened in β CD-trienH_nⁿ⁺ (n = 1 - 3). It is possible that the linear β CD-trien substituent enters the annulus to inhibit complexation, and that protonation of the substituent decreases such interaction and complex inhibition. The more constrained substituents of β CD-cyclen and β CD-tren are less likely to enter the annulus.

The charges of β CD-cyclenH_nⁿ⁺ and β CD-trenH_nⁿ⁺ are constrained closer to the annulus than those of β CD-trienH_nⁿ⁺. While all three β CD-XH⁺ form complexes, perhaps as a result of increases in the strength of their dipoles and their interaction with those of the guest, increased protonation on β CD-cyclenH_nⁿ⁺ and β CD-trenH_nⁿ⁺ causes an absence of detectable complexes. This is coincident with the decreased hydrophobicity of their annuli with increasing charge. The reverse case is observed for β CD-trienH_nⁿ⁺, and the simplest explanation is that the substituent charge may be held at a greater distance by comparison with β CD-cyclenH_nⁿ⁺ and β CD-trenH_nⁿ⁺ thus lessens the effect on annular hydrophobicity.

While this study shows that complexation is very sensitive to both guest and host-substituent protonation, which in turn affects hydrophobicity, in addition to substituent stereochemistry, a more detailed analysis of these effects is not possible from the present available data. It is clear, however, that these effect do not produce significant chiral discrimination between guests despite the homochirality of $\beta\text{CD-XH}_n^{n+}$ (Figure 4.3).



a)



b)

Figure 4.3. The percentage of a) $\beta\text{CD-trienH}_n^{n+}$ complexes and b) $\beta\text{CD-cyclenH}_n^{n+}$ containing (R)- or (S)-tryptophan in solution $2 \times 10^{-3} \text{ mol dm}^{-3}$ of each $[\text{guest}]_{\text{total}}$ and $[\beta\text{CD-XH}_n^{n+}]_{\text{total}}$. The plots were calculated from the data in Table 4.1 and plotted relative to $[\beta\text{CD-XH}_n^{n+}]_{\text{total}}$.

4.2.2. The Formation of Binary Metallocyclodextrins: The Complexation of Ni^{2+} , Cu^{2+} , and Zn^{2+} by the Tetraazaalkyl-Monosubstituted- β -Cyclodextrins.

The stability constants of the binary metallocyclodextrins formed are listed in Table 4.3. The characteristic titration profiles for each metal ion with $\beta\text{CD-trienH}_n^{n+}$ and $\beta\text{CD-cyclenH}_n^{n+}$ are shown in Figures 4.4 and 4.5, respectively. Though not apparent in the smooth nature of the titration curves, precipitates, believed to be of the divalent metal hydroxides, were observed to form above pH 9.5, thus only the data collected below this pH was used to determine stability constants. The bumpy nature of the titration curves for Zn^{2+} and Ni^{2+} with $\beta\text{CD-cyclenH}_n^{n+}$ indicate the slow complexation of these species by the cyclodextrin. As a consequence, it was not possible to derive stability constants for any $[\text{Ni}(\beta\text{CD-cyclenH}_n)]^{(2+n)+}$ complexes. However, stability constants could be derived from the data in the $\text{pH} \geq 5.5$ region for Zn^{2+} (Figure 4.6).

The differing rates of M^{2+} complexation by $\beta\text{CD-cyclenH}_n^{n+}$, reflects the general lability of the metal ion towards ligand substitution and the kinetic restraints placed on it to achieve the final metallocyclodextrin stereochemistries imposed by the cyclic structure and stereochemistry of the cyclen substituent. In contrast, the rapid complexation of M^{2+} by $\beta\text{CD-trienH}_n^{n+}$ (on the pH-titrimetric timescale) was consistent with the trien substituent being less kinetically restricting towards complexation.

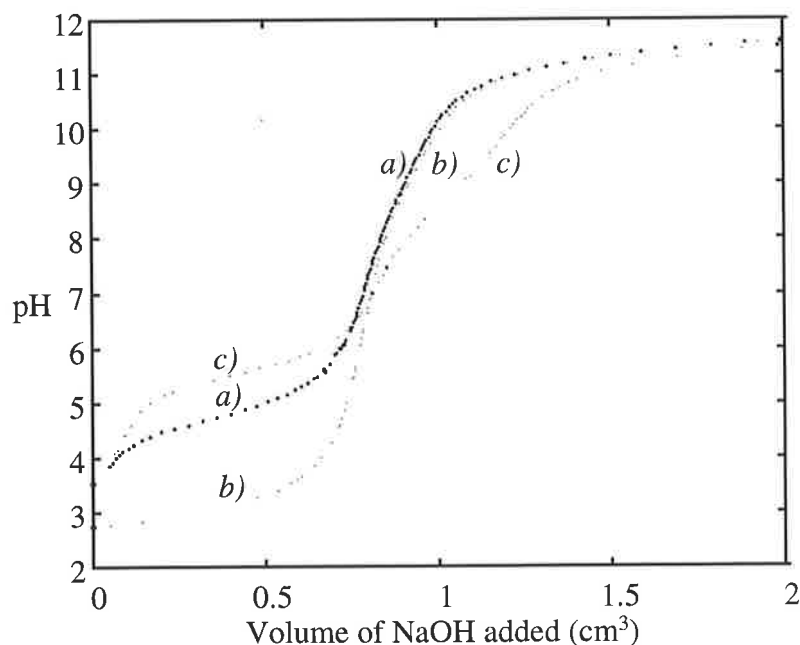


Figure 4.4. The pH profiles for the titrations of $\beta\text{CD-trienH}_n^{n+}$ ($2 \times 10^{-3} \text{ mol dm}^{-3}$) with a) Ni^{2+} b) Cu^{2+} and c) Zn^{2+} (all at $2 \times 10^{-3} \text{ mol dm}^{-3}$) in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial titration solutions were 10 cm^3 in volume.

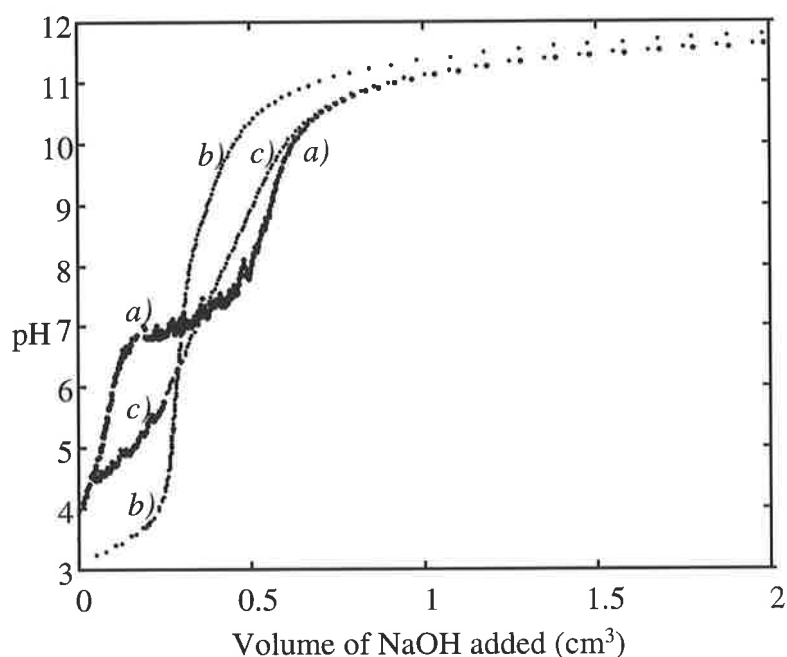


Figure 4.5. The titration profiles of $\beta\text{CD-cyclenH}_n^{n+}$ (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) with a) Ni^{2+} ; b) Cu^{2+} and c) Zn^{2+} (all at $2 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 at $I = 0.10 (\text{NaClO}_4)$ and 298.2 K . The initial volume of the titration solutions was 10.00 cm^3 . The bumpy nature of the titration curves for Zn^{2+} and Ni^{2+} with indicate the slow complexation of these species by $\beta\text{CD-cyclenH}_n^{n+}$. Stability constants for any Ni^{2+} complexes were unable to be derived. Only the data in the $\text{pH} \geq 5.5$ region was used to determine the stability of complexes with Zn^{2+} .

Table 4.3. The stability constants and acid dissociation constants (pK_a s) for the complexes of β CD-trien H_n^{n+} , β CD-cyclen H_n^{n+} and β CD-tren H_n^{n+} with Zn^{2+} , Cu^{2+} and Ni^{2+} at $I = 0.10$ ($NaClO_4$) and 298.2 K. Titrimetric data were fitted over the pH ranges 3.8 - 9.5, 4.4 - 9.5, and 3.5 - 8.5 for β CD-trien H_n^{n+} with Ni^{2+} , Cu^{2+} and Zn^{2+} , respectively. Similarly, titrimetric data were fitted over the pH ranges 4.5 - 8.2 and 5.5 - 9.5 for β CD-cyclen H_n^{n+} with Cu^{2+} and Zn^{2+} , respectively. The stabilities of the analogous complexes of the free tetraamine groups are given in the brackets.

M^{2+}	X	Stability of Species ($\log(K/\text{dm}^3\text{mol}^{-1})$)		pK_a $[M(\beta\text{CD-XH})]^{3+} = [M(\beta\text{CD-X})]^{2+} + H^+$
		$[M(\beta\text{CD-X})]^{2+}$ ($[M(X)]^{2+}$)	$[M(\beta\text{CD-XH})]^{3+}$ ($[M(XH)]^{3+}$)	
Ni^{2+}	trien	11.50 ± 0.02 (13.8) ^b	7.17 ± 0.03 (-)	5.01 ± 0.03
	cyclen	- (16.4) ^c	- (-)	-
	tren ^a	11.65 ± 0.06 (14.6) ^b	8.46 ± 0.06 (-)	6.65
Cu^{2+}	trien	10.70 ± 0.07 (20.1) ^b	8.66 ± 0.05 (3.5) ^{b,f}	7.29 ± 0.07
	cyclen	13.61 ± 0.02 (24.8) ^d	8.38 ± 0.02 (-)	5.18 ± 0.03
	tren ^a	17.29 ± 0.05 (18.5) ^b	11.56 ± 0.02 (-)	4.11
Zn^{2+}	trien	9.40 ± 0.01 (12.0) ^b	5.25 ± 0.04 (-)	5.18 ± 0.04
	cyclen	11.62 ± 0.04 (16.2) ^e	6.98 ± 0.05 (-)	5.77 ± 0.06
	tren ^a	12.25 ± 0.03 (14.5) ^b	7.92 ± 0.02 (-)	5.51

^aReference 1. ^bReference 4. ^cReference 5. ^dReference 6. ^eReference 7. ^f $I=0.1$, 293.2 K.

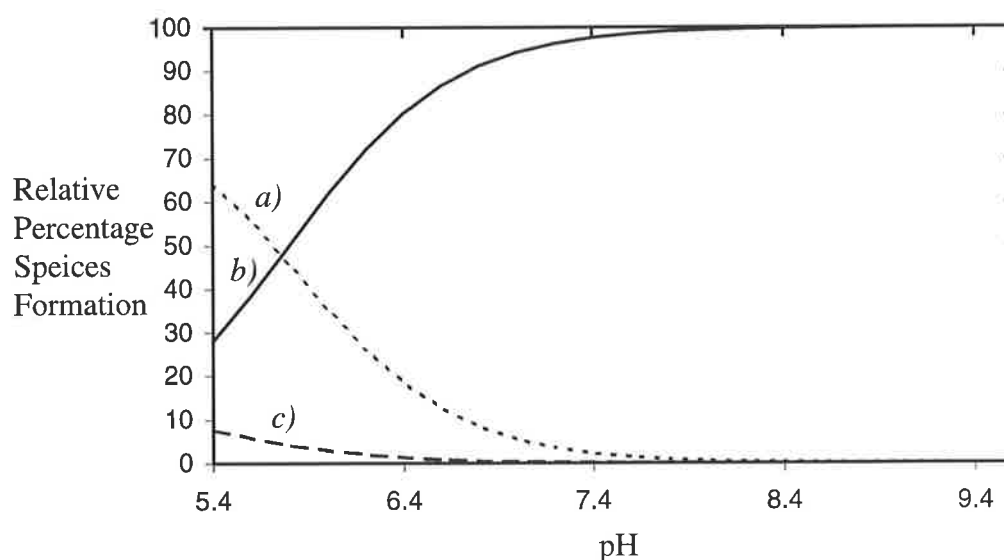


Figure 4.6. A speciation plot of the complexes detected in aqueous solution for $[\beta CD\text{-}cyclenH_n^{n+}]_{total}$ and $[Zn^{2+}]_{total}$ at $2 \times 10^{-3} \text{ mol dm}^{-3}$ over the pH range 5.5 – 9.5. The plot was derived from the data in Table 4.3 and plotted relative to both $[\beta CD\text{-}cyclenH_n^{n+}]_{total}$ and $[Zn^{2+}]_{total}$ being 100%. The curves represent a) $[Zn(\beta CD\text{-}cyclenH)]^{3+}$, b) $[Zn(\beta CD\text{-}cyclen)]^{2+}$ and c) $\beta CD\text{-}cyclenH_2^{2+}$.

The stabilities of the binary metallocyclodextrins, $[M(\beta CD\text{-}X)]^{2+}$, relative to the analogous $[M(X)]^{2+}$ complexes are several orders of magnitude lower (Table 4.3) consistent with the βCD entity hindering the coordination of M^{2+} . This may either arise through increased steric hindrance or a decrease in the electron donating power of the amine group in the tetraamine substituent by comparison with that of the corresponding free tetraamine, being one degree of substitution higher, or a combination of both effects. (Upon substitution by βCD , a primary amine group of both trien and tren becomes secondary, whereas a secondary amine group of cyclen becomes tertiary.) This trend is analogous to that observed for the binding of Cu^{2+} by $\beta CD\text{-dien}$,²² and $\beta CD\text{-en}$,²³ and for the binary metallocyclodextrins reported for $\beta CD\text{-pn}$.² The possibility of the cyclodextrin itself interacting with the metal ion should not be dismissed. The primary hydroxyl group of an adjacent glucose unit to the bound tetraamine substituent may also be involved in the coordination of a metal ion. This has been postulated for the Co^{3+} complex of $\beta CD\text{-cyclen}$.^{24,25} Direct coordination of Ni^{2+} and

Cu^{2+} to natural cyclodextrins has been reported under alkaline conditions,²⁶⁻²⁹ where the hydroxyl groups are deprotonated hence their coordinating ability is increased. However, for most metallocyclodextrins, the cyclodextrin can be considered to act as a second sphere ligand.³⁰

With the exception of the $[\text{M}(\beta\text{CD-trien})]^{2+}$ series, the stabilities of the binary metallocyclodextrins vary with the nature of M^{2+} in the sequence $\text{Ni}^{2+} < \text{Cu}^{2+} > \text{Zn}^{2+}$ observed for the free tetraamine complexes and coincident with the Irving-Williams series³¹ which arises through a combination of varying M^{2+} size and ligand-field effects. However, the metal ion coordinating tetraamine substituents do not have the flexibility inherent in the explanation for this series, and any apparent compliance with it may be coincidental.

The stability of $[\text{M}(\beta\text{CD-XH})]^{3+}$ is less than that of $[\text{M}(\beta\text{CD-X})]^{2+}$. This is to be anticipated from the charge repulsion between M^{2+} and $\beta\text{CD-XH}^+$. In addition the protonation of a nitrogen of the tetraamine moiety restricts the denticity of the substituent to three rather than its maximum of four. The lowered stability of these complexes is most likely due to a combination of these effects.

The stabilities of $[\text{M}(\beta\text{CD-X})]^{2+}$ and $[\text{M(X)}]^{2+}$ do not vary in the same way with the nature of X. For the latter, the series follows the order $\text{trien} < \text{tren} < \text{cyclen}$ in all but the case of Cu^{2+} where the order is $\text{tren} < \text{trien} < \text{cyclen}$. The increased stability of a metal complex containing a macrocyclic tetraamine ligand over that of a similar acyclic tetraamine ligand is called the macrocyclic effect or juxtapositional fixedness.^{3,6} For the metallocyclodextrins the stabilities vary with changing X in the order $\text{trien} < \text{cyclen} < \text{tren}$ regardless of the metal used. The factors destabilising the binding of M^{2+} to $\beta\text{CD-X}$ appear to have the greatest impact when $\text{X} = \text{cyclen}$. The stability imparted by the macrocyclic effect is significantly reduced upon substitution of the macrocycle by βCD . The reduced electron donating power of the tertiary amine of $\beta\text{CD-cyclen}$ and the proximity in which the metal centre will find itself held to the cyclodextrin (likely to be greater than for either $\beta\text{CD-trien}$ or $\beta\text{CD-tren}$ due to the cyclic nature of the cyclen substituent) result in the stabilities of $[\text{M}(\beta\text{CD-cyclen})]^{2+}$ being lower by 6 - 11 orders of magnitude compared with those of $[\text{M(cyclen)}]^{2+}$. The stabilities of $[\text{M}(\beta\text{CD-tren})]^{2+}$ are lower than $[\text{M(tren)}]^{2+}$ by 1 - 3 orders of magnitude. The stabilities of $[\text{M}(\beta\text{CD-trien})]^{2+}$ are lower than $[\text{M(trien)}]^{2+}$ by 2 - 10 orders of magnitude.

4.2.3. The Structure of the Binary and Ternary Metallocyclodextrins.

The transition metal ions Ni^{2+} and Cu^{2+} , exhibit $d-d$ transitions in the visible region of the electromagnetic spectrum, due to their partially filled d orbitals. The geometry and electronic spectra of the $[\text{M}(\text{X})]^{2+}$, where X = trien and cyclen, is well known, thus information on the geometry of $[\text{M}(\beta\text{CD-X})]^{2+}$ (Section 4.2.2) and $[\text{M}(\beta\text{CD-X})\text{trp}]^+$ (Section 4.2.4) complexes may be obtained by a comparison of their spectra. A change in metal coordination number has a significant effect of the electronic spectrum of the complex. Thus any change in coordination number upon complexation of trp^- by the binary metallocyclodextrin, to form the ternary metallocyclodextrin, should be observable in the electronic spectra.

In aqueous solution, trien and cyclen form five-coordinate complexes, $[\text{M}(\text{X})\text{H}_2\text{O}]^{2+}$, with $\text{M}^{2+} = \text{Cu}^{2+}$ and Zn^{2+} , and a six-coordinate complex with Ni^{2+} , $[\text{Ni}(\text{X})(\text{H}_2\text{O})_2]^{2+}$.³²⁻³⁵ Square pyramidal geometries are most probable for the five coordinate complexes of cyclen and trien, whereas octahedral geometries are expected for the six-coordinate complexes, with cyclen adopting a cis-diaquo configuration. Trien may form a meridional and two facial isomers when coordinating Ni^{2+} although they are not separately identified in this study. In all cases, cyclen and trien are believed to act as tetradentate ligands.³³⁻³⁶

Spectra for the complexes formed by Ni^{2+} with cyclen, $\beta\text{CD-cyclen}$ and both $\beta\text{CD-cyclen}$ and trp^- are shown in Figure 4.8. Spectra for the complexes formed with Ni^{2+} with trien, $\beta\text{CD-trien}$ and both $\beta\text{CD-trien}$ and trp^- are shown in Figure 4.9. Over the wavelength range 400 - 900 nm, $[\text{Ni}(\text{cyclen})(\text{H}_2\text{O})_2]^{2+}$ exhibits a major absorbance maximum at 564 nm ($\epsilon = 12.5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$), assigned to the ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ transition,³⁷ in good agreement with the literature (560 nm).³⁴ Over the same wavelength range, $[\text{Ni}(\text{trien})(\text{H}_2\text{O})_2]^{2+}$ exhibits a major absorbance maximum at 562 nm ($\epsilon = 6.30 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The same transition is assigned to this absorbtion

The spectra of $[\text{Ni}(\beta\text{CD-cyclen})(\text{H}_2\text{O})_2]^{2+}$ and $[\text{Ni}(\beta\text{CD-cyclen})\text{trp}]^+$ differ slightly in molar absorbance within this spectral range, exhibiting maxima at 550 nm ($\epsilon = 14.5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) and 564 nm ($\epsilon = 15.2 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$), respectively. Similarly, the spectra of $[\text{Ni}(\beta\text{CD-trien})(\text{H}_2\text{O})_2]^{2+}$ and $[\text{Ni}(\beta\text{CD-trien})\text{trp}]^+$ also differ only slightly in molar absorbance within this spectral range. The spectrum of $[\text{Ni}(\beta\text{CD-trien})(\text{H}_2\text{O})_2]^{2+}$ exhibits a maximum at 572 nm ($\epsilon = 13.5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) and the spectrum of $[\text{Ni}(\beta\text{CD-trien})\text{trp}]^+$

exhibits a maximum at 556 nm ($\epsilon = 15.5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). These metallocyclodextrin spectra closely resemble that of the cyclen and trien complex, consistent with Ni^{2+} also being six-coordinate in these complexes. The space at the centre of the plane formed by the four nitrogens of either cyclen or the βCD -cyclen substituent is too small to accommodate Ni^{2+} . Thus,, both ligands are likely to adopt a folded configuration.²⁵ Similar results and conclusions were obtained for the Ni^{2+} complexes of tren, βCD -tren and both βCD -tren and tryptophan.

Spectra for the complexes formed by Cu^{2+} with cyclen, βCD -cyclen and both βCD -cyclen and trp^- are shown in Figure 4.10. Spectra for the complexes formed with Cu^{2+} with trien, βCD -trien and both βCD -trien and trp^- are shown in Figure 4.11. Over the wavelength range 400 - 900 nm, $[\text{Cu}(\text{cyclen})\text{H}_2\text{O}]^{2+}$ exhibits a major absorbance maximum at 604 nm ($\epsilon = 371 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$), assigned to the ${}^2\text{B}_1 \rightarrow {}^2\text{E}_1$ transition,³⁸ in reasonable agreement with the literature (590 nm).³³ Over the same wavelength range, $[\text{Cu}(\text{trien})(\text{H}_2\text{O})_2]^{2+}$ exhibits a major absorbance maximum at 580 nm ($\epsilon = 230 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The same transition is assigned to this absorbtion.

The spectra of $[\text{Cu}(\beta\text{CD-cyclen})\text{H}_2\text{O}]^{2+}$ and $[\text{Cu}(\beta\text{CD-cyclen})\text{typ}]^+$ each exhibit a maxima at 602 nm ($\epsilon = 318$ and $315 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ for each complex, respectively). The spectra of $[\text{Cu}(\beta\text{CD-trien})\text{H}_2\text{O}]^{2+}$ and $[\text{Cu}(\beta\text{CD-trien})\text{typ}]^+$ exhibit maxima at 588 nm ($\epsilon = 207 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$) and 592 nm ($\epsilon = 188 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$), respectively. The spectral similarity between the metallocyclodextrin complexes and the trien and cyclen complexes of Cu^{2+} is consistent with the metallocyclodextrins also being five-coordinate. Similar conclusions were made for the Cu^{2+} complexes of tren, βCD -tren and both βCD -tren and tryptophan whose spectra exhibited maxima between 840 and 850 nm. Square pyramidal five-coordinate Cu^{2+} complexes have an absorbtion band between 550 - 670 nm whereas trigonal bipyrimidal complexes absorb near 800 - 850 nm.^{39,40} This is consistent with five-coordinate $[\text{Cu}(\text{tren})\text{H}_2\text{O}]^{2+}$ having a trigonal bipyramidal arrangement around the metal centre whereas five-coordinate $[\text{Cu}(\text{cyclen})\text{H}_2\text{O}]^{2+}$ and $[\text{Cu}(\text{trien})\text{H}_2\text{O}]^{2+}$ have a square pyramidal arrangement.

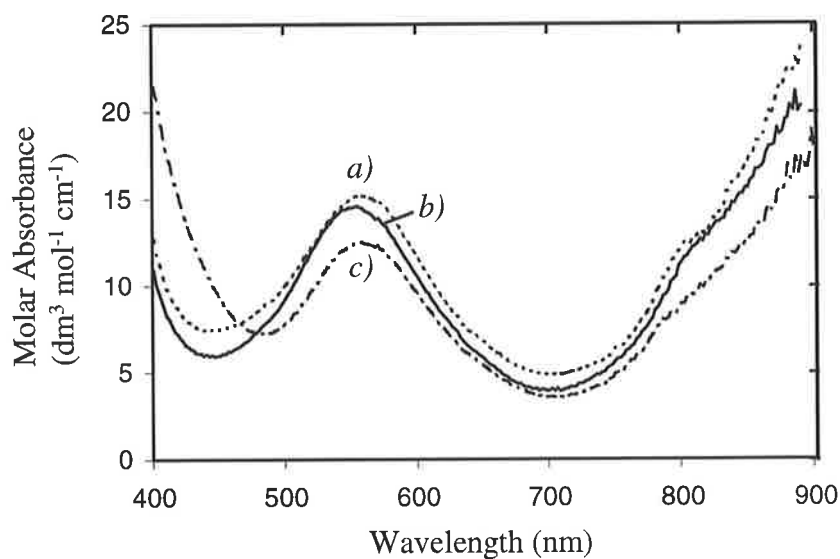


Figure 4.8. Spectra of the complexes formed by Ni^{2+} with a) both βCD -cyclen and tryptophan, b) βCD -cyclen and c) cyclen. The molar absorbances of the complexes were determined at pH 8.0 ($0.025 \text{ mol dm}^{-3}$ NaPIPES buffer) in aqueous solution at 298.2 K and $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4).

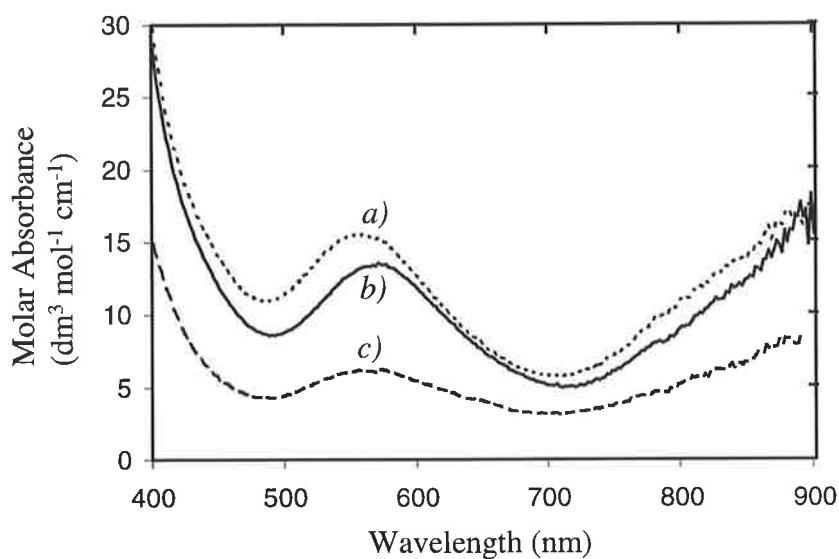


Figure 4.9. Spectra of the complexes formed by Ni^{2+} with a) both βCD -trien and tryptophan, b) βCD -trien and c) trien. The molar absorbances of the complexes were determined at pH 8.0 ($0.025 \text{ mol dm}^{-3}$ NaPIPES buffer) in aqueous solution at 298.2 K and $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4).

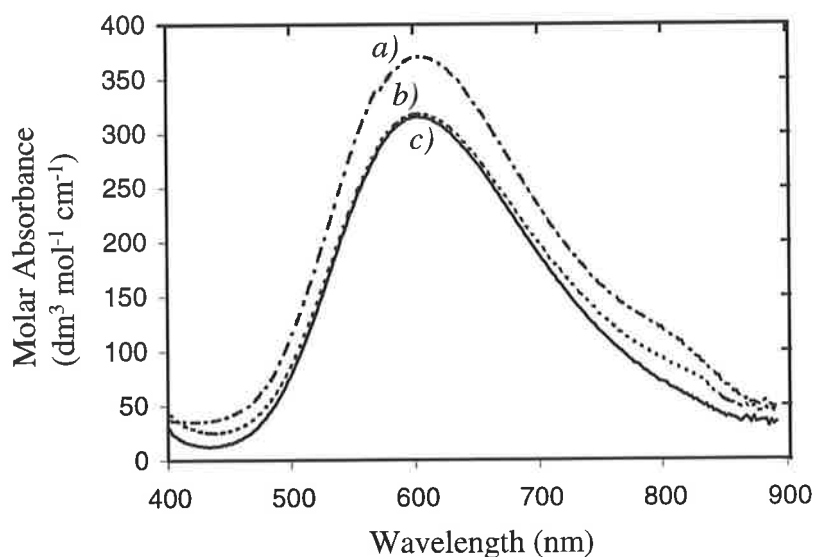


Figure 4.10 Spectra of the complexes formed by Cu^{2+} with a) cyclen, b) βCD -cyclen and c) both βCD -cyclen and tryptophan. The molar absorbances of the complexes were determined at pH 8.0 ($0.025 \text{ mol dm}^{-3}$ NaPIPES buffer) in aqueous solution at 298.2 K and $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4).

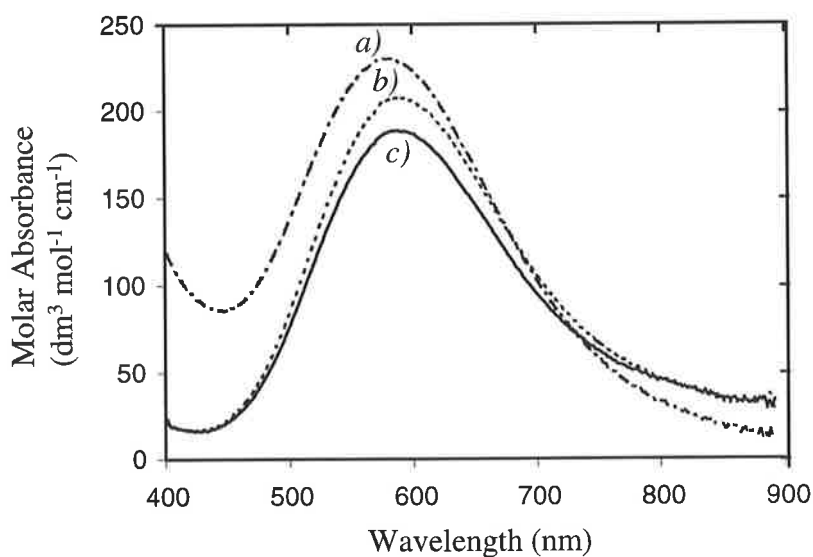


Figure 4.11 Spectra of the complexes formed by Cu^{2+} with a) trien, b) βCD -trien and c) both βCD -trien and tryptophan. The molar absorbances of the complexes were determined at pH 8.0 ($0.025 \text{ mol dm}^{-3}$ NaPIPES buffer) in aqueous solution at 298.2 K and $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4).

Little information about the environment of Zn^{2+} is provided by U/V-Visible spectroscopy because of its d^{10} electronic configuration, which results in no $d-d$ bands. A comparison of the $\log (\text{K}/\text{dm}^3 \text{ mol}^{-1})$ values for $[\text{M}(\beta\text{CD-X})]^{2+}$ with those of $[\text{M}(\text{X})]^{2+}$ is presented in Table 4.4. The values for the difference in stabilities of the Ni^{2+} complexes of X and $\beta\text{CD-X}$ correspond to the effect of the βCD moiety on a six-coordinate, octahedral, metal centre. The value for the difference in stabilities of the Cu^{2+} complex with tren and $\beta\text{CD-tren}$ corresponds to the effect of the βCD substituent on a five-coordinate, trigonal bipyramidal metal centre. The values for the difference in stabilities of the Cu^{2+} complexes of trien and $\beta\text{CD-trien}$ and cyclen and $\beta\text{CD-cyclen}$ correspond to the effect of a βCD substituent on a five-coordinate, square pyramidal metal centre. The values for the difference in stabilities of the Zn^{2+} complexes of tren and $\beta\text{CD-tren}$ are intermediate those of the Ni^{2+} and Cu^{2+} complexes. This may result from the βCD substituent causing a change from five to six-coordination.¹ The value for the difference in stabilities of the Zn^{2+} complex of trien and $\beta\text{CD-trien}$ is inconclusive.

Further insight may be gained through a comparison of the $\log (\text{K}/\text{dm}^3 \text{ mol}^{-1})$ values for $[\text{M}(\beta\text{CD-X})]^{2+}$ with those of $[\text{M}(\beta\text{CD-X})\text{trp}]^+$ and are given in Table 4.5. The similar values for table entries 1, 2, 11 and 12 are consistent with similar coordination changes occurring for $[\text{Ni}(\beta\text{CD-tren})]^{2+}$ and $[\text{Zn}(\beta\text{CD-tren})]^{2+}$ on complexation of trp^- , and both metal centres being six-coordinate.² The similar values for table entries 7, 8, 13 and 14 may be consistent with similar coordination changes occurring for $[\text{Cu}(\beta\text{CD-trien})]^{2+}$ and $[\text{Zn}(\beta\text{CD-trien})]^{2+}$ on complexation of trp^- , and both metal centres being five-coordinate. The similar values for table entries 9, 10, 15 and 16 may indicate similar coordination changes are occurring for $[\text{Cu}(\beta\text{CD-cyclen})]^{2+}$ and $[\text{Zn}(\beta\text{CD-cyclen})]^{2+}$ on complexation of trp^- . This is a possible indication of the Zn^{2+} metal centre, being five-coordinate.

Table 4.4. The differences in $\log (K/\text{dm}^3 \text{ mol}^{-1})$ values for $[M(\beta\text{CD-X})]^{2+}$ compared with those of with those of $[M(X)]^{2+}$ where $X = \text{tren, trien and cyclen}$ and $M^{2+} = \text{Ni}^{2+}, \text{Cu}^{2+}$ and Zn^{2+} . These values indicate the effect of βCD substituent on the metal centre.

The differences in $\log (K/\text{dm}^3 \text{ mol}^{-1})$ between $[M(X)]^{2+}$ and $[M(\beta\text{CD-X})]^{2+}$ where $X =$			
M^{2+}	tren	trien	cyclen
Ni^{2+}	2.95	2.3	-
Cu^{2+}	1.21	9.40	9.80
Zn^{2+}	2.25	2.60	4.58

Table 4.5. The difference in $\log (K/\text{dm}^3 \text{ mol}^{-1})$ values for $[M(\beta\text{CD-X})]^{2+}$ compared with those of $[M(\beta\text{CD-X})\text{trp}]^+$. These values give an indication of the effect of binding (R/S)-tryptophan on the metal centre of $\beta\text{CD-X}$.

$[M(\beta\text{CD-X})]^{2+} \rightarrow [M(\beta\text{CD-X})\text{trp}]^+$		The difference in $\log (K/\text{dm}^3 \text{ mol}^{-1})$
1	$[\text{Ni}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Ni}(\beta\text{CD-tren})(R)\text{-trp}]^+$	3.45
2	$[\text{Ni}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Ni}(\beta\text{CD-tren})(S)\text{-trp}]^+$	3.55
3	$[\text{Ni}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Ni}(\beta\text{CD-trien})(R)\text{-trp}]^+$	4.60
4	$[\text{Ni}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Ni}(\beta\text{CD-trien})(S)\text{-trp}]^+$	4.71
5	$[\text{Cu}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-tren})(R)\text{-trp}]^+$	7.79
6	$[\text{Cu}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-tren})(S)\text{-trp}]^+$	7.89
7	$[\text{Cu}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-trien})(R)\text{-trp}]^+$	2.5
8	$[\text{Cu}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-trien})(S)\text{-trp}]^+$	2.8
9	$[\text{Cu}(\beta\text{CD-cyclen})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-cyclen})(R)\text{-trp}]^+$	6.77
10	$[\text{Cu}(\beta\text{CD-cyclen})]^{2+} \rightarrow [\text{Cu}(\beta\text{CD-cyclen})(S)\text{-trp}]^+$	6.76
11	$[\text{Zn}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-tren})(R)\text{-trp}]^+$	4.15
12	$[\text{Zn}(\beta\text{CD-tren})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-tren})(S)\text{-trp}]^+$	3.95
13	$[\text{Zn}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-trien})(R)\text{-trp}]^+$	2.76
14	$[\text{Zn}(\beta\text{CD-trien})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-trien})(S)\text{-trp}]^+$	2.39
15	$[\text{Zn}(\beta\text{CD-cyclen})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-cyclen})(R)\text{-trp}]^+$	6.67
16	$[\text{Zn}(\beta\text{CD-cyclen})]^{2+} \rightarrow [\text{Zn}(\beta\text{CD-cyclen})(S)\text{-trp}]^+$	6.68

The changes in band position and intensity, observed between the spectra of $[M(X)]^{2+}$ and $[M(\beta\text{CD-X})]^{2+}$, (where X = trien and cyclen), indicate that the presence of βCD does disrupt the binding ability of the X substituent. The similarity of the binary and ternary metallocyclodextrin spectra, in all but the case of $[\text{Ni}(\beta\text{CD-trien})]^{2+}$ and $[\text{Ni}(\beta\text{CD-trien})\text{trp}]^+$, suggest that the tryptophan guest does little to disrupt the binding of the metal. For the Ni^{2+} binary and ternary metallocyclodextrins of $\beta\text{CD-trien}$, the tryptophan does seem to induce a slight change in the binding of the metal centre.

The spectra of the binary and ternary metallocyclodextrins do not allow the determination of whether a cyclodextrin primary hydroxyl group is coordinating the metal centre, (held in close proximity by the polyamine substituent), (Section 4.2.2) or a water molecule. Thus, the stoichiometry of the binary metallocyclodextrins may be $[M(\beta\text{CD-X})]^{2+}$ and $[M(\beta\text{CD-X})\text{H}_2\text{O}]^{2+}$ for the five and six-coordinate species respectively. A similar situation may arise for the ternary metallocyclodextrins.

4.2.4. Ternary Metallocyclodextrins: The Complexation of Tryptophan/Tryptophanoate by the Zn^{2+} , Cu^{2+} and Ni^{2+} Binary Metallocyclodextrins of the Tetraazaalkyl-Monosubstituted- β -Cyclodextrins.

The results determined for the complexation of (*R*)- and (*S*)-tryptophan with the binary metallocyclodextrins of $\beta\text{CD-trienH}_n^{n+}$ and $\beta\text{CD-cyclenH}_n^{n+}$ from the experimental data (Figure 4.12 and Appendix C) are given in Table 4.6. The stabilities of $[\text{M}(\beta\text{CD-X})\text{trp}]^+$ are greater than those of either $[\beta\text{CD-X.trp}]^-$ or $[\text{M}(\text{trp})]^+$ (Table 4.3 and Table 4.7). This is consistent with the binding of the trp^- amino acid moiety by the metal centre and the hydrophobic interaction between the cyclodextrin cavity and the trp^- aromatic moiety acting together to stabilise the formation of $[\text{M}(\beta\text{CD-X})\text{trp}]^+$.

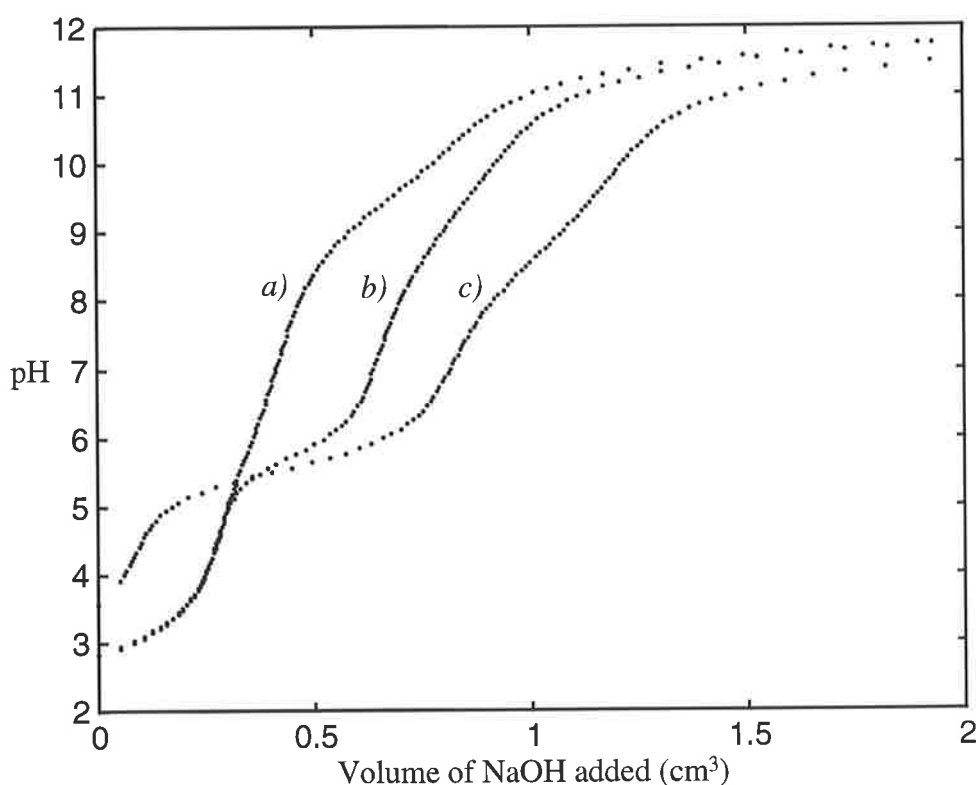


Figure 4.12. The full pH profiles of a) $\beta\text{CD-trienH}_n^{n+}$ and (*R*)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) $\beta\text{CD-trienH}_n^{n+}$, Zn^{2+} and (*R*)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) $\beta\text{CD-trienH}_n^{n+}$ and Zn^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

Table 4.6. The stability constants and acid dissociation constants (pK_a s) for the Ni^{2+} , Cu^{2+} and Zn^{2+} complexes of β CD-trien H_n^{n+} , β CD-cyclen H_n^{n+} and β CD-tren H_n^{n+} with (R)- and (S)-tryptophan at $I = 0.10$ ($NaClO_4$) and 298.2 K. The titrimetric data were fitted over the pH ranges 4.0 - 9.5, 4.6 - 9.5, 4.5 - 9.5 for β CD-trien H_n^{n+} with Ni^{2+} , Cu^{2+} and Zn^{2+} , respectively. Similarly, titrimetric data were fitted over the pH ranges 4.7 - 9.5 and 5.7 - 8.9 for β CD-cyclen H_n^{n+} with Cu^{2+} and Zn^{2+} , respectively.

Equilibrium	Log ($K/dm^3 mol^{-1}$)		
	$M^{2+} = Ni^{2+}$	$M^{2+} = Cu^{2+}$	$M^{2+} = Zn^{2+}$
$[M(\beta CD-trien)]^{2+} + (R)-trp^- = [M(\beta CD-trien).(R)-trp]^+$	6.90 ± 0.04	8.2 ± 0.3	6.64 ± 0.08
$[M(\beta CD-trien)]^{2+} + (S)-trp^- = [M(\beta CD-trien).(S)-trp]^+$	6.79 ± 0.04	7.9 ± 0.2	7.01 ± 0.07
$[M(\beta CD-cyclen)]^{2+} + (R)-trp^- = [M(\beta CD-cyclen).(R)-trp]^+$	-	6.84 ± 0.06	4.95 ± 0.05
$[M(\beta CD-cyclen)]^{2+} + (S)-trp^- = [M(\beta CD-cyclen).(S)-trp]^+$	-	6.85 ± 0.06	4.94 ± 0.04
$[M(\beta CD-tren)]^{2+} + (R)-trp^- = [M(\beta CD-tren).(R)-trp]^+$	8.2 ± 0.2	9.5 ± 0.3	8.1 ± 0.1
$[M(\beta CD-tren)]^{2+} + (S)-trp^- = [M(\beta CD-tren).(S)-trp]^+$	8.1 ± 0.2	9.4 ± 0.2	8.3 ± 0.1
$[M(\beta CD-trien)]^{2+} + (R)-trpH = [M(\beta CD-trien).(R)-trpH]^{2+}$	4.5 ± 0.2	4.4 ± 0.1	4.1 ± 0.1
$[M(\beta CD-trien)]^{2+} + (S)-trpH = [M(\beta CD-trien).(S)-trpH]^{2+}$	4.6 ± 0.1	4.2 ± 0.2	4.56 ± 0.05
$[M(\beta CD-cyclen)]^{2+} + (R)-trpH = [M(\beta CD-cyclen).(R)-trpH]^{2+}$	-	3.8 ± 0.1	2.68 ± 0.06
$[M(\beta CD-cyclen)]^{2+} + (S)-trpH = [M(\beta CD-cyclen).(S)-trpH]^{2+}$	-	4.10 ± 0.2	2.76 ± 0.04
$[M(\beta CD-tren)]^{2+} + (R)-trpH = [M(\beta CD-tren).(R)-trpH]^{2+}$	4.6 ± 0.2	4.3 ± 0.3	-
$[M(\beta CD-tren)]^{2+} + (S)-trpH = [M(\beta CD-tren).(S)-trpH]^{2+}$	4.3 ± 0.2	4.2 ± 0.2	-
$[M(\beta CD-trienH)]^{3+} + (R)-trpH = [M(\beta CD-trienH).(R)-trpH]^{3+}$	4.1 ± 0.1	3.95 ± 0.07	-
$[M(\beta CD-trienH)]^{3+} + (S)-trpH = [M(\beta CD-trienH).(S)-trpH]^{3+}$	4.6 ± 0.2	4.1 ± 0.1	-
$[M(\beta CD-cyclenH)]^{3+} + (R)-trpH = [M(\beta CD-cyclenH).(R)-trpH]^{3+}$	-	3.4 ± 0.2	-
$[M(\beta CD-cyclenH)]^{3+} + (S)-trpH = [M(\beta CD-cyclenH).(S)-trpH]^{3+}$	-	3.5 ± 0.1	-
$[M(\beta CD-trenH)]^{3+} + (R)-trpH = [M(\beta CD-trenH).(R)-trpH]^{3+}$	3.56 ± 0.07	4.4 ± 0.2	4.82 ± 0.06
$[M(\beta CD-trenH)]^{3+} + (S)-trpH = [M(\beta CD-trenH).(S)-trpH]^{3+}$	3.6 ± 0.3	4.4 ± 0.2	4.96 ± 0.05
	pK_a		
	$M^{2+} = Ni^{2+}$	$M^{2+} = Cu^{2+}$	$M^{2+} = Zn^{2+}$
$[M(\beta CD-trien).(R)-trpH]^{2+} = [M(\beta CD-trien).(R)-trp]^+ + H^+$	6.9 ± 0.1	6.10 ± 0.05	6.70 ± 0.06
$[M(\beta CD-trien).(S)-trpH]^{2+} = [M(\beta CD-trien).(S)-trp]^+ + H^+$	7.0 ± 0.2	6.2 ± 0.1	6.80 ± 0.06
$[M(\beta CD-cyclen).(R)-trpH]^{2+} = [M(\beta CD-cyclen).(R)-trp]^+ + H^+$	-	6.74 ± 0.07	6.97 ± 0.06
$[M(\beta CD-cyclen).(S)-trpH]^{2+} = [M(\beta CD-cyclen).(S)-trp]^+ + H^+$	-	6.9 ± 0.1	7.06 ± 0.03

Table 4.6 continued

	pK_a		
	$M^{2+} = Ni^{2+}$	$M^{2+} = Cu^{2+}$	$M^{2+} = Zn^{2+}$
$[M(\beta CD-tren).(R)-trpH]^{2+} = [M(\beta CD-tren).(R)-trp]^+ + H^+$	5.6	4.0	-
$[M(\beta CD-tren).(S)-trpH]^{2+} = [M(\beta CD-tren).(S)-trp]^+ + H^+$	5.4	4.0	-
$[M(\beta CD-trien).(R)-trpH]^{2+} = [M(\beta CD-trien).(R)-trp]^+ + H^+$	4.63 ± 0.06	5.43 ± 0.09	-
$[M(\beta CD-trien).(S)-trpH]^{2+} = [M(\beta CD-trien).(S)-trp]^+ + H^+$	4.97 ± 0.06	5.5 ± 0.1	-
$[M(\beta CD-cyclen).(R)-trpH]^{2+} = [M(\beta CD-cyclen).(R)-trp]^+ + H^+$	-	4.7 ± 0.1	-
$[M(\beta CD-cyclen).(S)-trpH]^{2+} = [M(\beta CD-cyclen).(S)-trp]^+ + H^+$	-	4.46 ± 0.09	-
$[M(\beta CD-tren).(R)-trpH]^{2+} = [M(\beta CD-tren).(R)-trp]^+ + H^+$	5.6	4.3	-
$[M(\beta CD-tren).(S)-trpH]^{2+} = [M(\beta CD-tren).(S)-trp]^+ + H^+$	5.6	4.3	-

Table 4.7. Stability constants for the complexes of tryptophan with Ni^{2+} , Cu^{2+} , and Zn^{2+} at $I = 0.10$ ($NaClO_4$) and 298.2 K.

Equilibrium	$\log (K/dm^3 mol^{-1})^a$		
	$M^{2+} = Ni^{2+}$	$M^{2+} = Cu^{2+}$	$M^{2+} = Zn^{2+}$
$M^{2+} + trp^- = [M(trp)]^+$	5.42	8.11	4.90
$[M(trp)]^+ + trp^- = [M(trp)_2]$	4.67	7.20	-
$[M(trp)OH] + H^+ = [M(trp)]^+$	9.1	7.28	-

^a Reference 4.

The stability constants for the binding of trp^- by the binary metallocyclodextrins, $[\text{M}(\beta\text{CD-X})]^{2+}$ follow the Irving-William series ($\text{Ni}^{2+} < \text{Cu}^{2+} > \text{Zn}^{2+}$). This variation in stability is similar to that for the formation of $[\text{M}(\text{trp})]^+$ and is consistent with the size⁴¹ and the electronic configuration⁴² of M^{2+} playing a major factor in the complexation of tryptophan.

Protonation of trp^- leads to an decrease in complex stability. TrpH is a monodentate ligand. Thus the stability of the complexes $[\text{M}(\beta\text{CD-X})\text{trp}]^+$, are significantly greater than those of $[\text{M}(\beta\text{CD-X})\text{trpH}]^{2+}$. The complexes of $[\text{M}(\beta\text{CD-X})\text{trpH}]^{2+}$ and $[\text{M}(\beta\text{CD-XH})\text{trpH}]^{3+}$, are of similar stability. In each case, trpH acts as a monodentate ligand. Complexation is stabilised by the interaction of the trpH aromatic moiety with the interior of the cyclodextrin cavity.

The stability constants for the binding of trp^- to $[\text{M}(\beta\text{CD-X})]^{2+}$ vary with changing X in the order $\text{cyclen} < \text{trien} < \text{tren}$. This is the same order observed for the binding of trpH to $\beta\text{CD-XH}^+$, (the only complex to be formed in common for all three cyclodextrins studied here), with changing X. These results suggest that although the presence of the metal ion acts to stabilise the trp^-/trpH complexes, the interaction between trp^-/trpH and the cyclodextrin itself is the governing factor determining the stability of the ternary metallocyclodextrin complexes formed. Despite this, there appears to be no significant enantioselectivity arising from the interaction of trp^- and trpH with the homochiral cyclodextrin annulus (Figures 4.13 and 4.14)

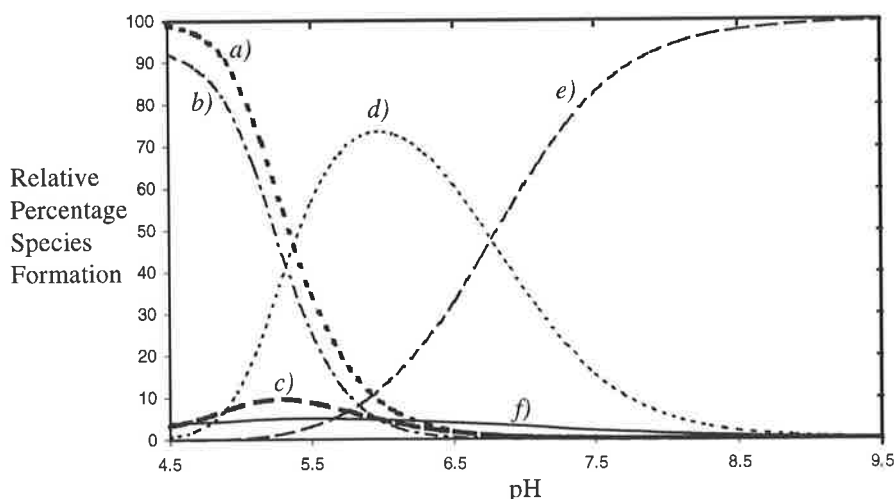


Figure 4.13. The percentage of species present in a solution $1 \times 10^{-3} \text{ mol dm}^{-3}$ of $[\beta\text{CD-trien}]$, $[\text{Zn}^{2+}]$ and $[(R)\text{-tryptophan}]$ between pH 4.5 and 9.5 calculated from the data in Table 4.6 and plotted relative to the concentration of all species being 100%. The curves represent a) Zn^{2+} b) $[\beta\text{CD-trienH}_3.(R)\text{-trpH}]^{3+}$ c) $[\beta\text{CD-trienH}_2.(R)\text{-trpH}]^{2+}$ d) $[\text{Zn}(\beta\text{CD-trien})(R)\text{-trpH}]^{2+}$ e) $[\text{Zn}(\beta\text{CD-trien})(R)\text{-trp}]^+$ and f) $(R)\text{-trpH}$. Only those species which formed above 5% are shown.

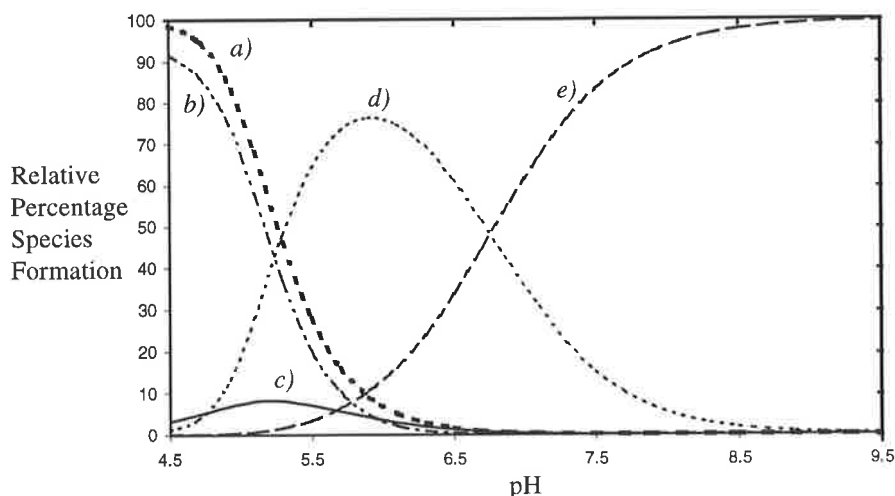


Figure 4.14. The percentage of species present in a solution $1 \times 10^{-3} \text{ mol dm}^{-3}$ of $[\beta\text{CD-trien}]$, $[\text{Zn}^{2+}]$ and $[(S)\text{-tryptophan}]$ between pH 4.5 and 9.5 calculated from the data in Table 4.6 and plotted relative to the concentration of all species being 100%. The curves represent a) Zn^{2+} b) $[\beta\text{CD-trienH}_3.(S)\text{-trpH}]^{3+}$ c) $[\beta\text{CD-trienH}_2.(S)\text{-trpH}]^{2+}$ d) $[\text{Zn}(\beta\text{CD-trien})(S)\text{-trpH}]^{2+}$ and e) $[\text{Zn}(\beta\text{CD-trien})(S)\text{-trp}]^+$. Only those species which formed above 5% are shown.

4.3. Summary and Conclusions.

The substitution of a primary hydroxyl group of β CD with a tetraamino group produces a modified cyclodextrin capable of binding a metal ion to form a binary metallocyclodextrin and simultaneously binding a metal ion and a guest aromatic amino acid to form a ternary metallocyclodextrin.

The stabilities of the binary metallocyclodextrins of β CD-tren, β CD-trien and β CD-cyclen vary with the Irving William series with a change in the nature of M^{2+} , as do the metal complexes of the free tetraamine analogues. The stabilities of the binary metallocyclodextrins of β CD-X are less than, and show a deviation in variation from, those of the metal complexes of X, with changing X. This is due to a combination of the reduced electron donating power of the amine group of the X substituent attached to β CD and the effect of the β CD moiety sterically hindering metal ion complexation.

The variation in stabilities of the ternary metallocyclodextrins follow the Irving William series with the changing nature of M^{2+} . In addition they follow the trend in stabilities of the 1:1 complexes of β CD-X with (*R/S*)-tryptophan with changing X revealing the interaction of the tryptophan with the cyclodextrin annulus being the governing factor in the ternary metallocyclodextrin stabilities. The stabilities of the ternary metallocyclodextrins are greater than those of the 1:1 complexes of both β CD-X and M^{2+} with (*R/S*)-tryptophan. This is due to the combined effects of the tryptophan guest interacting with the cyclodextrin annulus and the additional binding site introduced by the metal ion. While the 1:1 complexes of β CD-X and (*R/S*)-tryptophan showed a slight selectivity for the (*S*)-enantiomer, no enantioselectivity was observed for the ternary metallocyclodextrins.

No evidence for a change in coordination number upon formation of a ternary metallocyclodextrin from the binary metallocyclodextrin is found. The binary and ternary metallocyclodextrins of β CD-X appear to be 6-coordinate for Ni^{2+} . The binary and ternary metallocyclodextrins of β CD-X appear to be five-coordinate for Cu^{2+} with those of β CD-tren probably being trigonal bipyramidal and those for β CD-trien and β CD-cyclen being square pyramidal in geometry around the metal centre. The binary and ternary metallocyclodextrins of β CD-X with Zn^{2+} appear to be 6-coordinate, cis-diaquo-octahedral when X = tren, and five-coordinate, square pyramidal when X = trien and cyclen.

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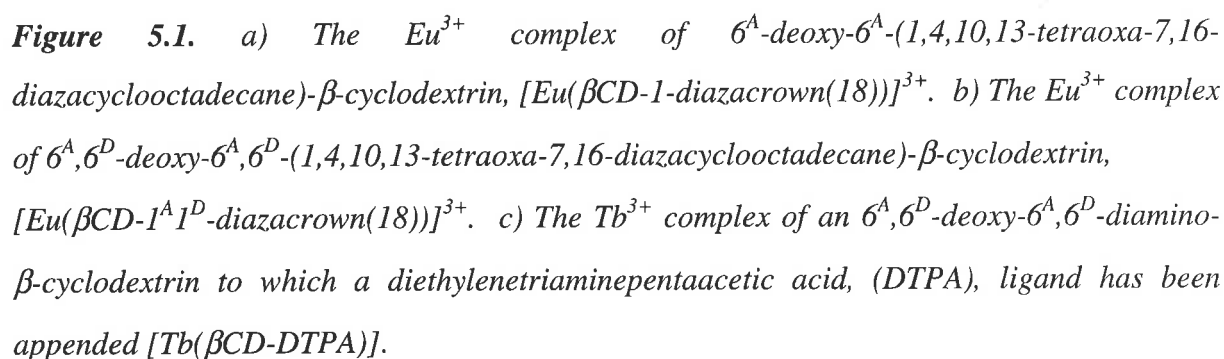
CHAPTER FIVE

6A-DEOXY-6A-(1,4,7,10-TETRAAZA-1,4,7-TRI(CARBOXYMETHYL)CYCLODODECANE)- β -CYCLODEXTRIN.**5.1. Introduction.**

Many polyazamacrocycles bearing pendant donor arms have been synthesised. This research has been undertaken primarily in order to seek and engender metal selectivity in the complexation reactions of these ligands as their cavity size and rigidity is varied.¹ The use of such ligands as receptors for organic molecules has also been explored.² Of particular interest has been the complexation of lanthanide ions. The conformational, complexing and thermodynamic properties of such lanthanide complexes have been exhaustively studied by techniques such as NMR spectroscopy,^{1b,3} X-ray crystallography⁴ and molecular modelling.⁴ Lanthanide complexes have demonstrated practical applications as chiral NMR shift reagents,⁵ magnetic resonance imaging reagents and as luminescent labels.⁶

Ligands that figure predominantly in the formation of such lanthanide complexes are based upon the macrocyclic skeleton 1,4,7,10-tetrazacyclododecane (cyclen) substituted at nitrogen by three or four pendant donor arms.⁶ The ligand 1,4,7,10-tetraaza-1,4,7,10-tetra(carboxymethyl)dodecane, (DOTA), appears repeatedly in the literature and is of special note as it has been reported to form a remarkably stable Ca^{2+} complex,^{1a} The anionic Gd^{3+} complex of DOTA is used as an extracellular contrast agent for general use in magnetic resonance imaging.⁶

Very few examples of lanthanide-cyclodextrin complexes exist in the literature. The first of such complexes have been studied for absorption-energy transfer-emission (AETE) phenomena, (Figure 5.1). AETE is the process whereby a light-harvesting subunit absorbs strongly at a suitable wavelength and efficiently transfers its excitation energy, through an intramolecular process, to a lanthanide ion. In accepting the energy, the lanthanide ion becomes excited to an emissive state. Loss of this energy gives rise to observable luminescence.⁶



A water quenching effect on the luminescence observed for the [Eu(β CD-1-diazacrown(18)).benzene]³⁺ complex paralleled that for the [Eu(diazacrown(18)).benzene]³⁺ complex. This led the authors to believe the 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane ligand, (diazacrown(18)) appended to the cyclodextrin was orientated away from the cyclodextrin annulus. This conclusion was supported by molecular mechanics calculations.

The complexation of anionic guests with a high affinity for the hydrophobic cyclodextrin annulus and an ability to ligate the Eu^{3+} centre, such as benzoate and deprotonated picolinic acid was investigated. The complexes formed between these anionic guests and the $[\text{Eu}(\beta\text{CD-1-diazacrown(18))}]^{3+}$ species displayed marked enhancement of Eu^{3+} luminescence in comparison to the $[\text{Eu}(\beta\text{CD-1-diazacrown(18))}.\text{benzene}]^{3+}$ complex. This was attributed to the increased association of these guests with the cyclodextrin annulus and the guest ligating the Eu^{3+} centre enforcing an orientation of the diazacrown(18) ligand towards the annulus.⁸

The linking of the diazacrown across the face of βCD gave rise to $6^{\text{A}},6^{\text{D}}$ -deoxy- $6^{\text{A}},6^{\text{D}}$ -(1,4,10,13-tetraoxa-7,16-diazacyclooctadecane)- β -cyclodextrin, ($\beta\text{CD-1}^{\text{A}}1^{\text{D}}$ -diazacrown(18)).⁸ The Eu^{3+} complex of $\beta\text{CD-1}^{\text{A}}1^{\text{D}}$ -diazacrown(18), $[\text{Eu}(\beta\text{CD-1}^{\text{A}}1^{\text{D}}$ -diazacrown(18))] $^{3+}$, put the metal centre in greater proximity to the cyclodextrin annulus in comparison to the $[\text{Eu}(\beta\text{CD-1-diazacrown(18))}]^{3+}$ complex. The luminescence observed for the benzene complex of $[\text{Eu}(\beta\text{CD-1}^{\text{A}}1^{\text{D}}$ -diazacrown(18))] $^{3+}$ was weaker than that for the $[\text{Eu}(\beta\text{CD-1-diazacrown(18))}.\text{benzene}]^{3+}$ complex. The energy transfer process was more efficient between the benzene and the Eu^{3+} centre in the $[\text{Eu}(\beta\text{CD-1}^{\text{A}}1^{\text{D}}$ -diazacrown(18))] $^{3+}$ complex due to the closer proximity of the metal centre to the annulus and hence the guest but the proximity of the $3+$ charge on the Eu^{3+} centre made the cyclodextrin annulus less hydrophobic in nature and decreased the association of the benzene with the annulus.

The Tb^{3+} complex of a $6^{\text{A}},6^{\text{D}}$ -deoxy- $6^{\text{A}},6^{\text{D}}$ -diamino- β -cyclodextrin to which a diethylenetriaminepentaacetic acid ligand has been appended, $[\text{Tb}(\beta\text{CD-DTPA})]$, (Figure 5.1c) reportedly gave very weak luminescence upon direct excitation⁹. This was markedly enhanced in the presence of aromatic and bicyclic hydrocarbons. Three factors were identified as contributing to this enhancement in luminescence: 1) the proximity of Tb^{3+} to the cyclodextrin annulus and hence its proximity to the guest due to ligation by the DTPA substituent; 2) the neutrality of the complex promoting the binding of guests to the hydrophobic annulus and 3) the good fit between the annulus and the guests used. Excitation of the Tb^{3+} ion occurs 12 μs after light is absorbed by a biphenyl group residing in the cyclodextrin annulus.¹⁰

Some lanthanide cyclodextrin-complexes have been utilised as chiral NMR shift reagents. It has been demonstrated that the addition of either Eu^{3+} or Dy^{3+} can enhance the spectroscopic enantiomeric resolution observed in the NMR spectra of an enantiomeric substrate and a chiral resolving agent (where there is a difference in the dissociation constants for the two enantiomers or a difference in their chemical shift in the bound state, or both). The spectroscopic resolution observed with cyclodextrin chiral resolving agents is small.^{5b} Two benefits are gained through directly coupling a lanthanide ion to a chiral NMR resolving agent such as a cyclodextrin: 1) where the ion is strongly bound by the chiral NMR resolving agent, there is a reduction in the number of equilibria in the substrate-chiral resolving agent-lanthanide mixture, and 2) structural information about the substrate complex formed can be determined due to the characteristic shifts induced by the lanthanide ion. Wenzel and coworkers have reported the synthesis of Dy^{3+} coupled cyclodextrin derivatives that enhance the enantiomeric resolution in the NMR spectra of substrates that associate with the cyclodextrin.¹¹

Several lanthanide-cyclodextrin complexes have been utilised as enzyme mimics for the cleavage of phosphodiester bonds. In DNA and RNA the ribonucleosides are linked by phosphodiester bonds and as a result phosphodiesterases have been widely used as RNA/DNA mimics. It is claimed that the half life of a phosphodiester bond in DNA (at pH=7 and 298.2K) is more than 200 million years.¹² Thus the development of a catalyst capable of increasing the cleavage of a phosphodiester bond has important applications in the field of biotechnology.

The highest rate accelerations for the cleavage of phosphodiester bonds thus far have been observed with Co^{3+} complexes,¹²⁻¹⁶ and with trivalent lanthanides, Ln^{3+} .^{12,13} The catalytically active form of an Ln^{3+} containing catalyst is believed to be the hydroxy complex.¹⁷ Water bonded to hydrated Ln^{3+} has a pK_a of approximately 9¹⁸ hence the formation of hydroxy complexes in basic aqueous solution. The hydroxy complex of Ln^{3+} is only produced at low concentrations due to the low solubilities of the Ln^{3+} hydroxy complexes, and their tendency to co-precipitate with inorganic salts such as nitrate or chloride.¹⁷ The use of a ligand that can protect the Ln^{3+} cation from precipitation and allow the hydroxy complex to form has been found to increase the activity of the Ln^{3+} centre/complex.¹⁷

Cyclodextrins have been found to enhance the solubility of Ln^{3+} in basic aqueous solution by the formation of complexes.¹⁹ Although a cyclodextrin may act to solubilise Ln^{3+} , it will not necessarily enhance the catalytic activity of the Ln^{3+} centre. An enhancement in catalytic activity was not observed for the cleavage of a monophosphoester bond by Ce^{3+} complexed with γCD although the solubility of Ce^{3+} was markedly enhanced.¹⁹ The rate of cleavage for the cyclodextrin complexed Ce^{3+} ion was lower than that of the uncomplexed ion.

Cyclodextrins are known to catalyse the cleavage of phosphodiester bonds. β -Cyclodextrin, (βCD), accelerates the cleavage of bis(4-nitrophenyl) hydrogenphosphate (BNPP) by 92 fold.²⁰ 6^A-Deoxy-6^A-(diethylenetriamine)- β -cyclodextrin, ($\beta\text{CD-dien}$), has been used to catalyse the cleavage of both BNPP and bis(2,4-dinitrophenyl) hydrogenphosphate (BDNPP)²¹ (Figure 5.2). The catalysis of BDNPP by $\beta\text{CD-dien}$ at pH 11 and pH 7 was 120 and 54 times more effective respectively than for an equimolar amount of free diethylenetriamine, indicating that the cyclodextrin residue played a significant role. $\beta\text{CD-dien}$ was also successfully recycled as a catalyst, which indicated a lack of formation of a stable intermediate in the catalytic cycle. The Zn^{2+} complex of $\beta\text{CD-dien}$ can cleave 2',3'cyclicmonophosphates of ribonucleotide dimers and ribonucleosides.²² The magnitude of the cleavage rate was found to be dependant on the kind of nucleic base adjacent to the phosphodiester bond cleaved.

The catalytic cleavage of BNPP by the Co^{3+} chloride complex of 6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecane)- β -cyclodextrin, ($\beta\text{CD-cyclen}$), has been reported by Czarnik et al.²³ In addition much development in this area of study has been accomplished by Breslow using bifunctional metallocyclodextrins,²⁴ bifunctional cyclodextrins,²⁵ and a range of tetraazamacrocyclic Zn^{2+} complexes attached to βCD .²⁶

Breslow has reported a lanthanide-cyclodextrin complex used to catalytically cleave a phosphodiester bond.²⁷ Breslow synthesised a bipyridine linked cyclodextrin dimer ($(\beta\text{CD-S})_2\text{-bipy}$, Figure 5.3), capable of complexing La^{3+} . The La^{3+} complex of $(\beta\text{CD-S})_2\text{-bipy}$ catalytically cleaved BNPP and methyl bis(4-nitrophenyl) hydrogenphosphate (a phosphate triester) with the aid of peroxide (which was believed to play an important mechanistic role (Figure 5.4)). Similarly an Eu^{3+} complex of a phenanthroline linked cyclodextrin dimer was found to enhance the cleavage of BNPP with respect to free Eu^{3+} .²⁸

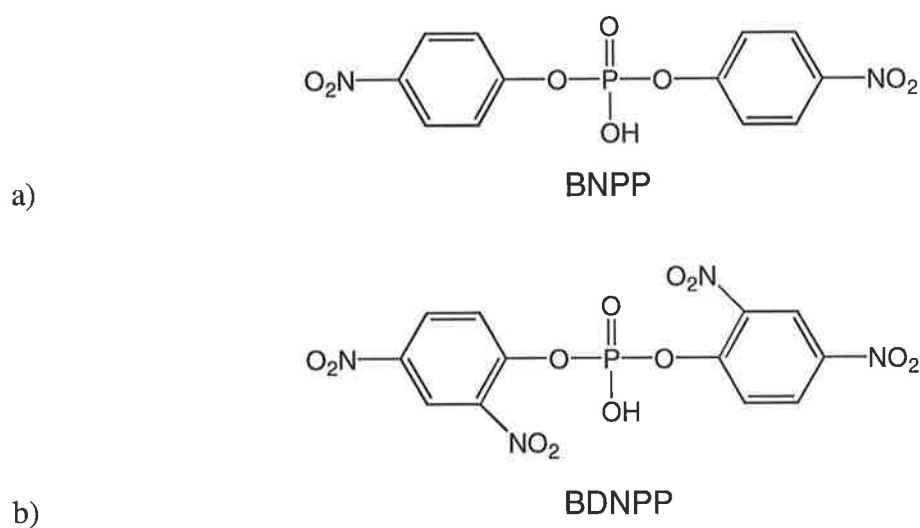


Figure 5.2. The structures of a) bis(4-nitrophenyl) hydrogen phosphate (BNPP) and b) bis(2,4-dinitrophenyl) hydrogen phosphate (BDNPP)

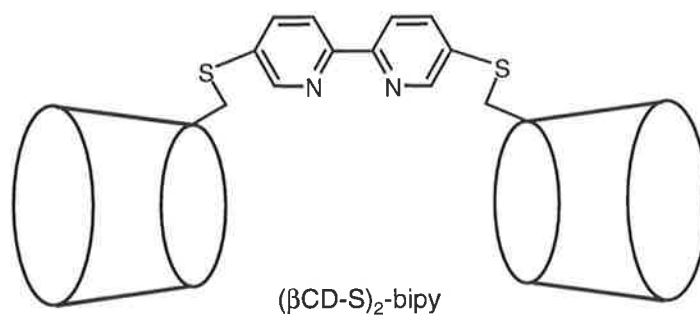


Figure 5.3. The bipyridine linked cyclodextrin dimer synthesised by Breslow ((β CD-S)₂-bipy).

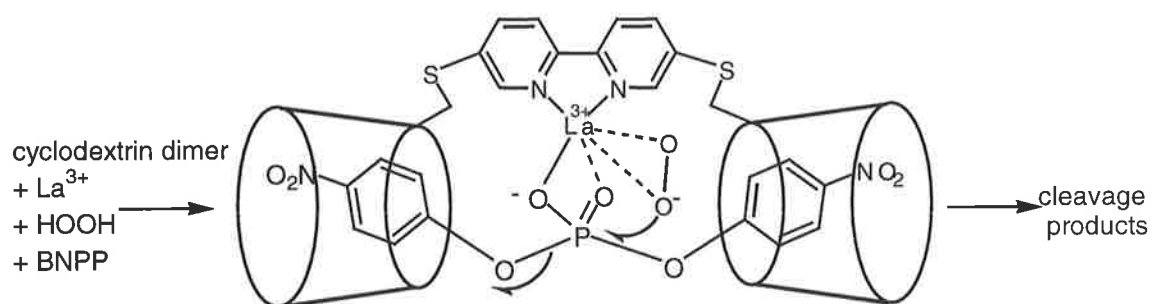


Figure 5.4. The mechanism proposed by Breslow²⁷ for the cleavage of BNPP with a lanthanide cyclodextrin dimer complex in the presence of hydrogen peroxide.

The work described in this chapter involves the synthesis of 6^A-deoxy-6^A-(1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane)- β -cyclodextrin (β CD-DO3A) and its Eu³⁺ complex, [Eu(β CD-DO3A)], and the investigation of their use as potential chiral NMR resolving agents. In addition, they were investigated for AETE properties and as potential catalysts of phosphate ester cleavage.

It was expected that [Eu(β CD-DO3A)] would be stable in aqueous solution as β CD-DO3A possesses oxygen and nitrogen donor atoms that are favoured by the hard, polarising Eu³⁺. The carboxylates, being anionic donors, should strongly ligate Eu³⁺ ensuring strong complexation.⁶ In addition the complexation of Eu³⁺ by β CD-DO3A was expected to help solubilise the hydroxy complex at higher pH. and the presence of the β CD residue was expected to enhance any catalytic activity of the Eu³⁺ complex.

The neutrality of [Eu(β CD-DO3A)] was expected to result in the hydrophobicity of the cyclodextrin annulus remaining largely intact. Dischino has reported that 1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane (DO3A) forms a neutral, stable, water soluble, Eu³⁺ complex.²⁹

It was envisioned that [Eu(β CD-DO3A)] would have two potential binding sites for guest molecules. The first binding site is the hydrophobic annulus of β CD itself. The second is a free coordination site on the Eu³⁺ ion. Lanthanide ions exhibit coordination numbers of eight and nine. Up to seven coordination sites may be occupied by β CD-DO3A which will leave at least one coordination site free for the potential binding of a suitable guest molecule.

5.2. Results and Discussion.

The synthesis of β CD-DO3A was achieved by the addition of acetate arms to β CD-cyclen. The preparation was similar to that employed in the literature to form DOTA from 1,4,7,10-tetraazacyclododecane, (cyclen), hence the procedure followed was similar (Section 6.5.1). An aqueous solution of chloroacetic acid, neutralised by sodium hydroxide, was added to an aqueous solution of 6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecan)- β -cyclodextrin, (β CD-cyclen). The resultant solution was raised to pH 11 with excess NaOH and heated with stirring for 8 hours in a sealed vessel at 80°C and the pH maintained by the addition of small amounts of NaOH as required. The reaction was then left stirring at room temperature until analysis by T.L.C. showed the presence of only one product and some native β CD (approx. 12 hours). The product was separated by ion exchange (product was eluted with 1.4% NH₄OH). The solvent was removed and the product washed with water to yield 0.35 grams of an off white solid.

5.2.1. The Acid Dissociation Constants of β CD-DO3A.

The acid dissociation constants of β CD-DO3A were determined by potentiometric titration using aqueous solutions of NaClO₄ and NEt₄ClO₄. The results are shown in Table 5.1 in addition to the literature results for similar titrations for the free DO3A ligand.²⁷

Inspection of the literature results for the DO3A system revealed that the presence of Na⁺ in the supporting electrolyte appears to have a large effect on the magnitude of the pK_as determined. No formal comment appears to have been made in the literature concerning this effect,³⁰ although it has not gone unnoticed by others.³¹ This same phenomenon was observed in this work for β CD-DO3A and may be attributable to the complexation of sodium ions in solution (present in large excess) by the DO3A moiety.

The differences between the pK_as of the tetraprotonated DO3A and β CD-DO3A are attributable to changes induced in the electronic character of the DO3A substituent of the latter species and changes in its solvation because of its proximity to the primary hydroxy groups of β CD as described earlier for the polyamino substituted cyclodextrins (Section 2.2.1).³²

Table 5.1. The pK_{as} of β CD-DO3A and DO3A in different aqueous media at 298.2 K

	β CD-DO3A ^a	DO3A ^b
	$I = 0.1 \text{ mol dm}^3 \text{ NaClO}_4$	$I = 0.1 \text{ mol dm}^3 \text{ NaCl}$
pK_{a1}	9.43 ± 0.05	10.51 ± 0.01
pK_{a2}	6.75 ± 0.05	9.08 ± 0.09
pK_{a3}	4.7 ± 0.2	4.36 ± 0.13
pK_{a4}	$(2.9 \pm 0.1)^c$	-
	$I = 0.1 \text{ mol dm}^3 \text{ NEt}_4\text{ClO}_4$	$I = 0.1 \text{ mol dm}^3 \text{ NMe}_4\text{Cl}$
pK_{a1}	11.5 ± 0.1	11.59 ± 0.03
pK_{a2}	9.50 ± 0.04	9.24 ± 0.03
pK_{a3}	4.99 ± 0.02	4.43 ± 0.07
pK_{a4}	3.74 ± 0.05	3.48 ± 0.01

^a This work. ^bReference 27. ^cResult from one titration only.

5.2.2. The Chiral Resolution of Racemic Guests by NMR Spectroscopy using β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$.

Enantiomeric resolution of (*R/S*)-histidinoate, (*R/S*)-tyrosinoate, (*R/S*)-phenylalaninoate, (*R/S*)-tryptophanoate and (*R/S*)-2-phenylpropanoate with β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$ in solution was investigated. The $[\text{Eu}(\beta\text{CD-DO3A})]$ complex was generated in situ. The $[\text{Eu}(\text{DO3A})]$ complex has a high stability ($\log K = 20.69$)³⁰ hence strong complexation of the Eu^{3+} ion by β CD-DO3A was anticipated. Europium (III) may be complexed by β CD-DO3A via the three carboxylate groups and the four amine groups (Figure 5.4a). A visual comparison of the structures of $[\text{Eu}(\beta\text{CD-DO3A})]$ and the Dy^{3+} complex of Wenzels cyclodextrin modified at the primary end ($[\text{Dy}(6\text{-}\beta\text{CD-en-DTPA})]$, Figure 5.4) revealed the length of the tether holding the lanthanide ion to the cyclodextrin annulus was shorter for the former, possibly bringing the lanthanide ion in closer proximity to the cyclodextrin annulus. The guests used have previously been shown to form inclusion complexes with β -cyclodextrins.³³⁻³⁵

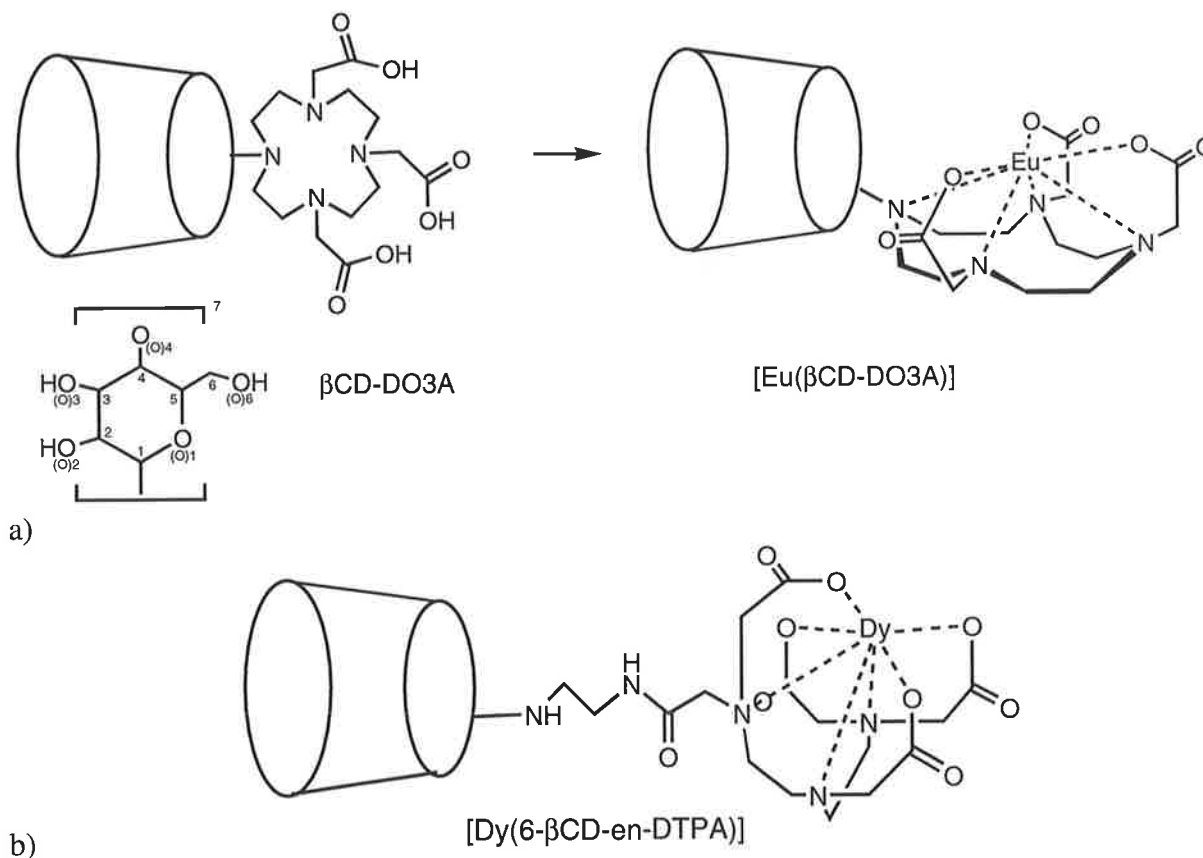
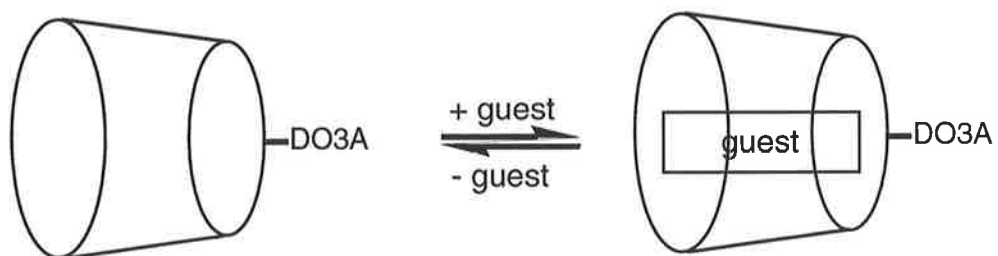
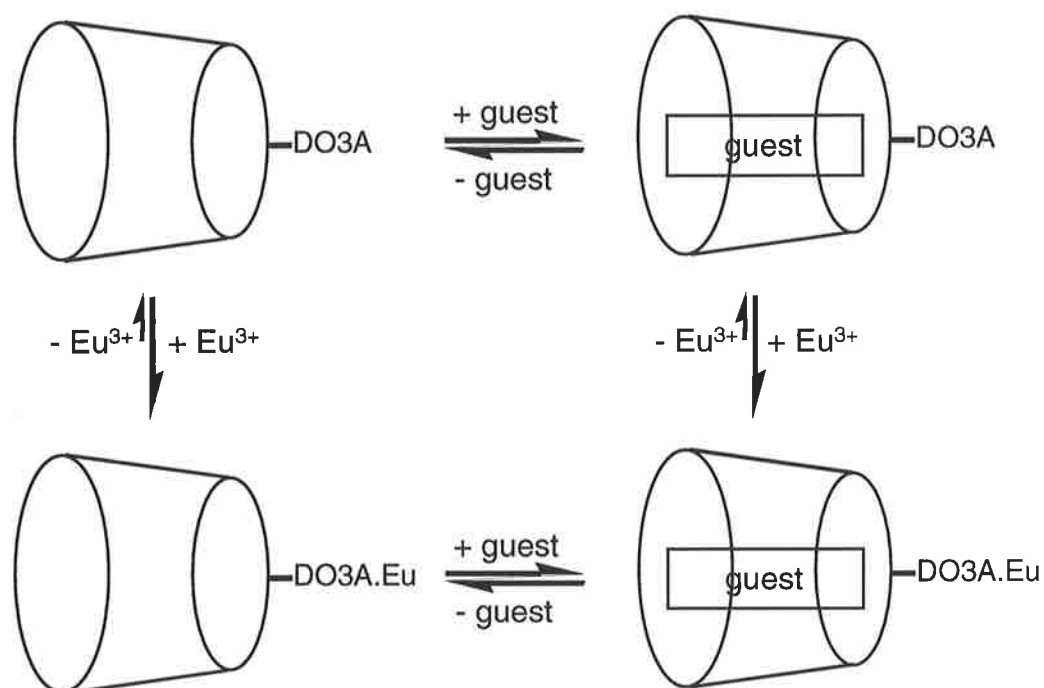
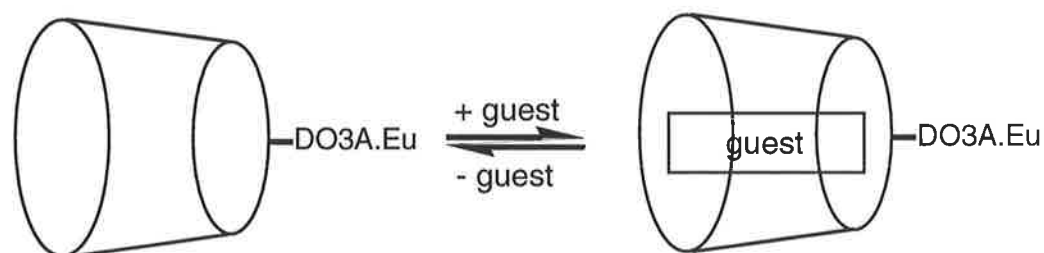


Figure 5.4. a) β CD-DO3A, shown as a schematic representation, and its Eu^{3+} complex. b) a schematic representation of the Dy^{3+} cyclodextrin complex (modified at the primary end of the cyclodextrin) used by Wenzel and co-workers ($[\text{Dy}(6\text{-}\beta\text{CD-en-DTPA})]$).¹¹

The ^1H NMR spectrum of $\beta\text{CD-DO3A}$, showed the acetate arm methylene signals overlapping the cyclen methylene signals giving a broad multiplet between $\delta 2.6 - 3.4$. It is possible that the DO3A ligand undergoes rapid conformational changes causing the observed broadening in the NMR spectrum. Addition of 1 mole equivalent of Eu^{3+} resulted in a significant shift and separation of the DO3A ^1H signals, (Appendix D, Figure D.6), characteristic of the ligand being complexed by a lanthanide ion. The cyclodextrin signals themselves became broadened.

Stock solutions of $0.040 \text{ mol dm}^{-3}$ $\beta\text{CD-DO3A}$ and each of the guests (at $\text{pH} = 10$ (NaOH), as the racemate) were prepared in D_2O . A grain of tetraethylammonium chloride was added to the stock solution of $\beta\text{CD-DO3A}$ to act as an internal reference.¹¹ The 500 MHz ^1H NMR spectrum of each guest was recorded in the presence and absence of $\beta\text{CD-DO3A}$ by pipetting 0.300 cm^3 of the stock solution of the guest into an NMR tube and adding either 0.300 cm^3 of D_2O or the $\beta\text{CD-DO3A}$ stock solution such that the resultant samples were all 0.02 mol dm^{-3} in the species of interest. To the NMR tube containing both $\beta\text{CD-DO3A}$ and the guest of interest, 0.010 cm^3 aliquots of a 0.12 mol dm^{-3} stock solution of Eu^{3+} were added sequentially to a total volume of 0.100 cm^3 . Final solutions were 0.700 cm^3 in volume and $0.017 \text{ mol dm}^{-3}$ in Eu^{3+} , $\beta\text{CD-DO3A}$ and the guest of interest. After each addition of Eu^{3+} , samples were allowed to equilibrate for 30 minutes within the probe of the NMR spectrophotometer before the ^1H NMR spectrum was recorded. Only those signals of the guest not overlapped by the cyclodextrin signals were examined for chiral resolution upon complexation by $\beta\text{CD-DO3A}$ and $[\text{Eu}(\beta\text{CD-DO3A})]$. Superimposing on the changes induced in the signals due to complexation by $[\text{Eu}(\beta\text{CD-DO3A})]$, was a dilution effect.

The equilibrium which exists in the initial solutions (containing 1:1 $\beta\text{CD-DO3A}$:guest) is represented by Scheme 5.1, the subsequent addition of Eu^{3+} to this solution gives rise to a number of equilibria represented in Scheme 5.2. Strong complexation of the Eu^{3+} resulted in the expectation of final solutions predominantly containing the equilibrium represented by Scheme 5.3.

Scheme 5.1.**Scheme 5.2.****Scheme 5.3.**

Initial experiments investigating the complexation of (*R/S*)-alaninoate with β CD-DO3A and [Eu(β CD-DO3A)] produced no observable change in the signals of (*R/S*)-alanine in the NMR spectra. (*R/S*)-Alaninoate lacks a phenyl moiety and was not expected to bind strongly if at all to the cyclodextrin annulus. From this experiment it was apparent that any interaction of alanine with either β CD-DO3A or [Eu(β CD-DO3A)] was weak and that the Eu³⁺ was preferentially bound by the DO3A ligand over (*R/S*)-alaninoate. Thus, any observed change in the NMR signals of the aromatic guests, (mentioned earlier), in the presence of β CD-DO3A and [Eu(β CD-DO3A)] will be due primarily to an interaction between these guests and the β -cyclodextrin annulus. A similar conclusion was reached by Wenzel.¹¹

A summary of the results obtained from the complexation of (*R/S*)-histidinoate, (*R/S*)-tyrosinoate, (*R/S*)-phenylalaninoate, (*R/S*)-tryptophanoate and (*R/S*)-2-phenylpropanoate (Figure 5.5) with β CD-DO3A and [Eu(β CD-DO3A)] are given in Table 5.2. The recorded spectra are collected in Appendix D. (Only those signals of the guests that were not overlapped by the cyclodextrin signals were examined for changes in the NMR spectra.) A downfield shift of any signal was attributed to both inclusion within the cyclodextrin annulus and to the proximity of Eu³⁺. Changes to the broadness of the signals due to the guest are indicative of the rate of exchange between the guest bound within the cyclodextrin annulus and the guest solvated in the bulk solution on the NMR timescale.

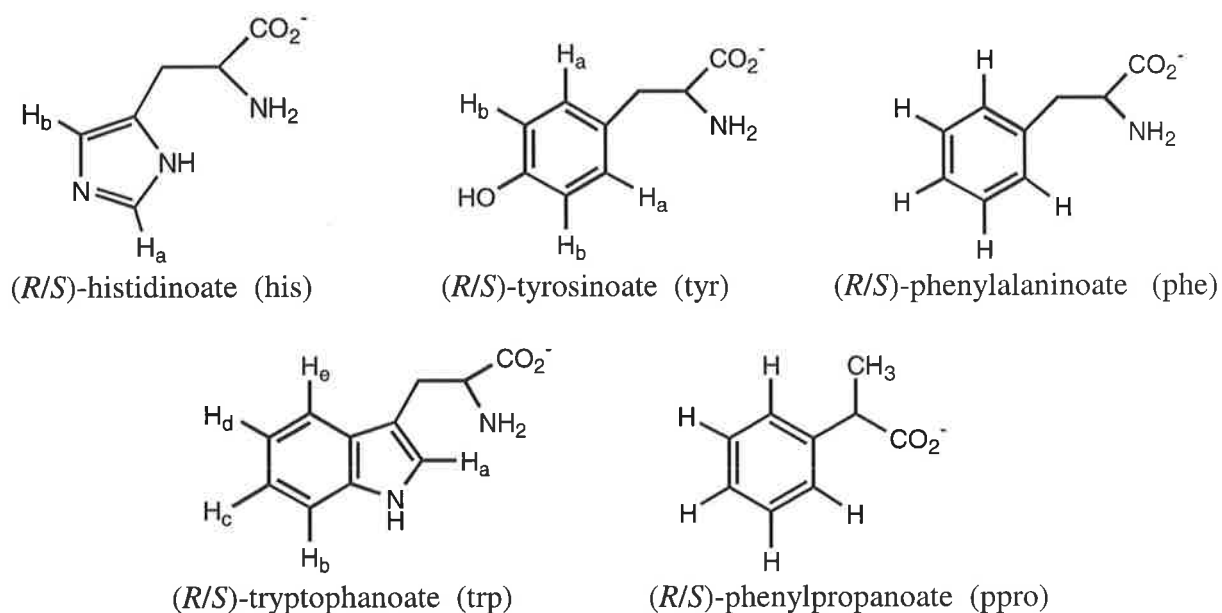


Figure 5.5. The structures of the aromatic guests studied showing the labelling of protons..

Table 5.2. Observed changes in the NMR spectra for the guests upon complexation with β CD-DO3A and [Eu(β CD-DO3A)].

		Signals Observed		
		Free Guest	Addition of 1 Equiv. β CD-DO3A	Addition of 1 Equiv. [Eu(β CD-DO3A)]
Guest ^a		Appearance, δ ppm	Appearance, δ ppm ($\Delta\delta$)	Appearance, δ ppm ($\Delta\delta$)
his	H _a	singlet, 7.59	singlet, 7.77 (+0.18)	doublet, 8.50 ^b (+0.91) [H _a (S), singlet, 8.48 (+0.89)] [H _a (R), singlet, 8.52 (+0.93)]
	H _b	singlet, 6.89	singlet, 6.96 (+0.07)	doublet, 7.35 ^b (+0.46). [H _b (S), singlet, 7.34 (+0.45)] [H _b (R), singlet, 7.37 (+0.48)]
tyr	H _a	doublet, 6.88 ^b	doublet/doublets, 7.01 ^b (+0.13) [H _a (S), doublet, 7.013 ^b (+0.133)] [H _a (R), doublet, 7.008 ^b (+0.127)]	NA. ^d
	H _b	doublet, 6.49 ^b	doublet/doublets, 6.65 ^b (+0.16) [H _b (S), doublet, 6.655 ^b (+0.165)] [H _b (R), doublet, 6.649 ^b (+0.154)]	NA. ^d
phe	Aromatic protons	multiplet, between 7.15 and 7.30	broad multiplet, no shift	broad multiplet, no shift
trp	H _b	doublet, 7.58 ^b	doublet, 7.59 ^b (+0.01)	broad singlet, 7.78 (+0.20)
	H _c	doublet, 7.34 ^b	doublet, 7.37 ^b (+0.03)	broad singlet, 7.55 (+0.21)
	H _a	singlet, 7.08	singlet, 7.18 (+0.10)	} signals coalesced to give broad doublet, 7.30 ^b
	H _c	triplet, 7.09 ^c	triplet, 7.08 ^c (-0.01)	
	H _d	triplet, 7.01 ^c	triplet, 6.99 ^c (-0.02)	
ppro	-CH ₃	doublet, 1.32 ^a	doublet of triplets (looks like overlapping doublet of doublet of doublets), 1.34 ^c (+0.02)	broad singlet, 1.43 (+0.11)
	Aromatic protons	multiplet, 7.23 ^a	broad doublet of triplets, 7.23 ^c (0.00) and 7.19 ^c (-0.04)	broad doublet, 7.47 (+0.24) and 7.32 (+0.13)

^aProtons are labelled as shown in Figure 5.5. ^bTaken from center of signal. ^cCentral peak of triplet. ^dPrecipitation observed.

5.2.2.1. Complexation by β CD-DO3A and Orientation of the Guest within the Cyclodextrin Annulus.

Chiral resolution was only observed for (*R/S*)-tyrosinoate in the presence of β CD-DO3A as indicated by the appearance of separate ^1H NMR H_a and H_b signals for the (*R*)- and (*S*)-tyrosinoate. The sharpness of the signals indicated (*R/S*)-tyrosinoate was in rapid exchange with the cyclodextrin annulus. The aromatic signals for (*R/S*)-tyrosinoate underwent the largest downfield shift of all of the guests studied upon complexation by β CD-DO3A, and appeared as a doublets of doublets (Figure 5.6). Repeating the experiment with 1:3 (*R*)-:(*S*)- tyrosinoate enabled the identification of the downfield doublet peaks as being those for the (*S*)- enantiomer. Chiral resolution resulted in a separation of 2.5 and 3.0 Hz for the H_a and H_b signals of (*R/S*)-tyrosinoate, respectively.

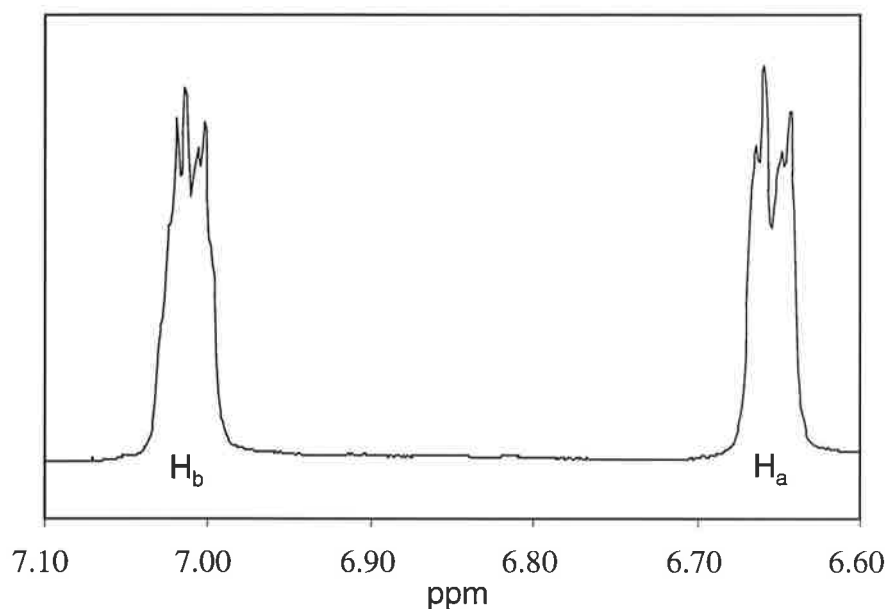


Figure 5.6. The observed ^1H NMR (500 MHz, D_2O) signals of (*R/S*)-tyrosinoate with 1 equivalent of β CD-DO3A added showing resolution of the (*R*)- and (*S*)- signals for the H_a and H_b protons.

The ^1H NMR signals due to the aromatic protons of (*R/S*)-histidinoate and (*R/S*)-tryptophanoate showed significant changes in their chemical shift upon complexation by $\beta\text{CD-DO3A}$. Both (*R/S*)-histidine and (*R/S*)-tryptophanoate appeared to be in rapid exchange between the cyclodextrin annulus and the bulk solution with these signals appearing sharply in the ^1H NMR spectra upon complexation by $\beta\text{CD-DO3A}$ whereas the broadness of the signals observed for (*R/S*)-2-phenylpropanoate indicated an intermediate rate of exchange. The observed broadening of the signals of (*R/S*)-phenylalaninoate also indicated an intermediate rate of exchange with the cyclodextrin annulus and there was no observable shift.

As discussed in previous chapters, cyclodextrin complexes of apolar guests may exist in two isomeric forms, one where the alignment of the dipoles of the host and guest is parallel, the second where it is antiparallel. The ^1H NMR spectra observed for the aromatic guests with $\beta\text{CD-DO3A}$ affords little information as to the orientation of the guest within the cyclodextrin annulus. The change in chemical shift induced by the presence of $\beta\text{CD-DO3A}$ on the ^1H NMR signals of the aromatic guests may give an indication as to which parts of guest are more deeply inserted into the cyclodextrin annulus, thus are shielded more effectively. The order of magnitude of the change of the chemical shift downfield observed for the ^1H NMR proton signals of (*R/S*)-tryptophanoate is $H_a > H_e > H_b > H_c > H_d$. While this allows no discernment of the orientation of the guest within the annulus it does suggest that (*R/S*)-tryptophanoate is complexed in such a way as to allow the deepest insertion of the H_a protons into the cyclodextrin annulus (Figure 5.7).

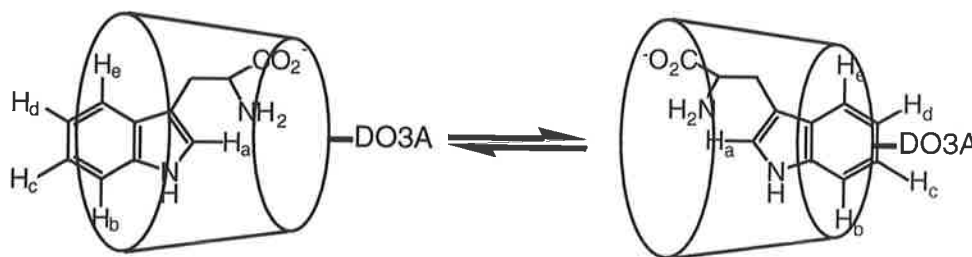


Figure 5.7. The two possible orientations of (*R/S*)-tryptophanoate within the annulus of $\beta\text{CD-DO3A}$ showing a possible depth of insertion based on the change in chemical shifts observed for the protons in the ^1H NMR spectrum upon complexation by $\beta\text{CD-DO3A}$

The ^1H NMR signal observed for the methyl group of (*R/S*)-2-phenylpropanoate upon complexation by $\beta\text{CD-DO3A}$ is shown in Figure 5.8. In the absence of $\beta\text{CD-DO3A}$ the ^1H NMR signal of the methyl protons of (*R/S*)-2-phenylpropanoate appear as a doublet. Upon complexation by $\beta\text{CD-DO3A}$, these signals appear as a poorly resolved doublet of triplets. Closer inspection reveals the central peak of the triplets is greater in size than the outer triplet peaks and each triplet more closely resembles an overlapping doublet of doublets. It may be concluded that upon complexation by $\beta\text{CD-DO3A}$, the ^1H NMR signal of the methyl protons of (*R/S*)-2-phenylpropanoate change from appearing as a doublet to a poorly resolved doublet of doublets of doublets. This is possible if both the (*R*)- and the (*S*)- enantiomers of 2-phenylpropanoate are complexed by $\beta\text{CD-DO3A}$ giving rise to two possible orientations within the $\beta\text{CD-DO3A}$ annulus, as mentioned above, and these all being magnetically inequivalent. The equilibrium expressed in Scheme 5.1 may be rewritten to take into account the two orientations of the aromatic guests within the cyclodextrin annulus and the chirality of the aromatic guests themselves to give rise to four equilibria and is represented in Scheme 5.4.

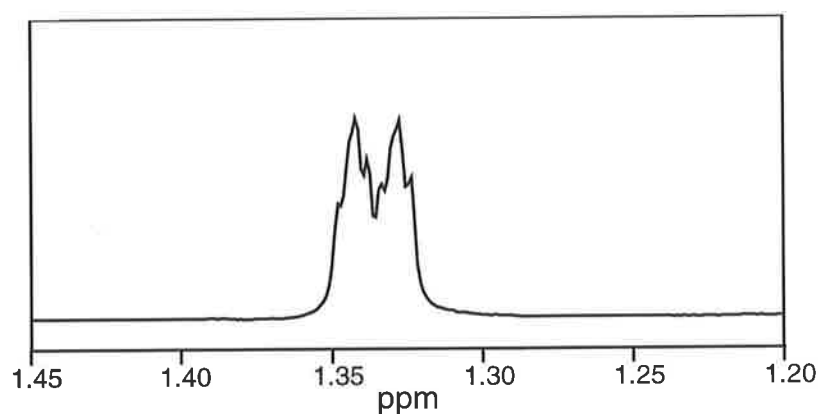
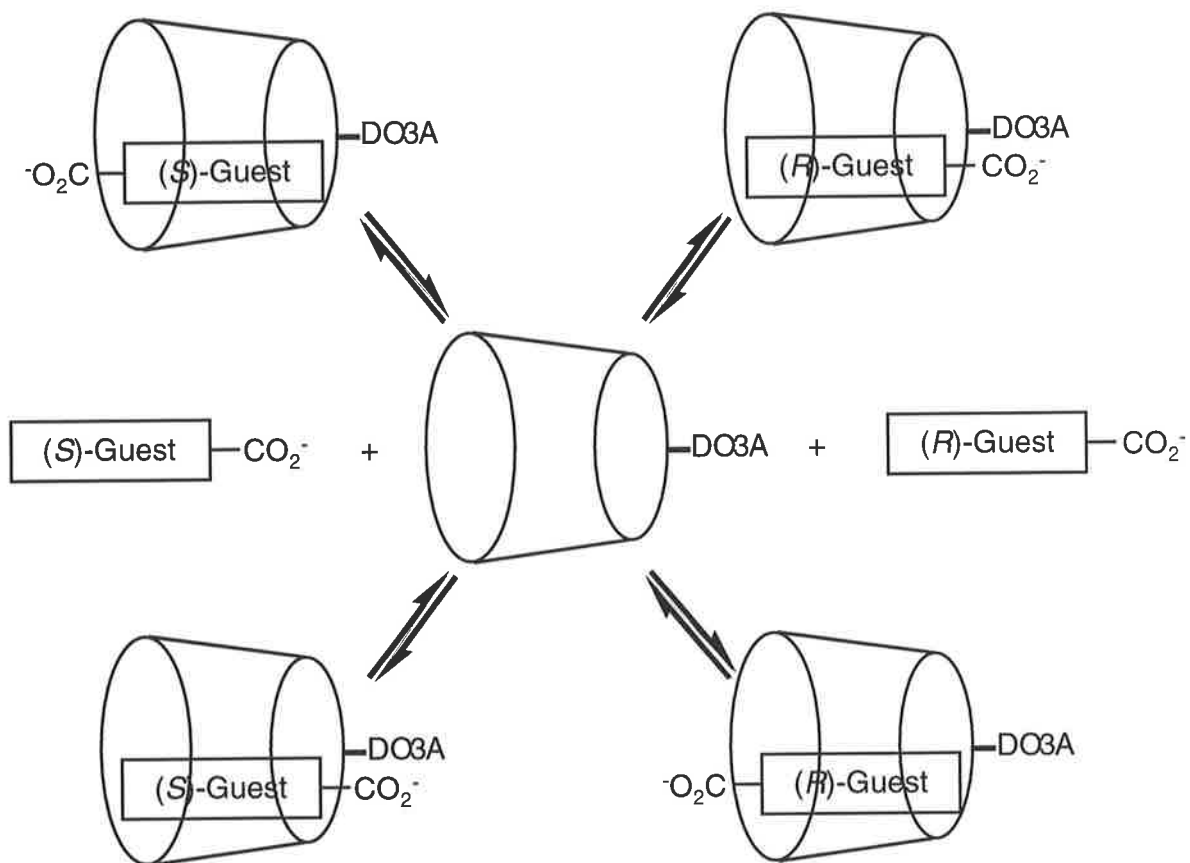


Figure 5.8. The observed ^1H NMR (500 MHz, D_2O) signal of the methyl group of (*R/S*)-2-phenylpropanoate with 1 equivalent of $\beta\text{CD-DO3A}$ added.

Scheme 5.4. The four equilibria that arise when taking into account the two orientations of the aromatic guests within the cyclodextrin annulus and the chirality of the aromatic guests themselves.



5.2.2.2. Complexation of the Guests by [Eu(β CD-DO3A)].

The gradual addition of Eu^{3+} to solutions containing β CD-DO3A and (*R/S*)-histidinoate, (*R/S*)-tyrosinoate, (*R/S*)-phenylalaninoate, (*R/S*)-tryptophanoate or (*R/S*)-2-phenylpropanoate, resulted in an initial broadening of the observed ^1H NMR signals of the guests. This is attributable to the aromatic guests undergoing an exchange between three environments, the annuli of β CD-DO3A and [Eu(β CD-DO3A)] and the bulk solution as represented in Scheme 5.2). Any broadness observed for the ^1H NMR signals of the guests in the final experiments, gave an indication of exchange rates of the aromatic guests with the annulus of [Eu(β CD-DO3A)] and the bulk solution alone, on the NMR timescale. The complete collection of the spectra recorded appears in Appendix D.

The addition of Eu^{3+} to solutions of β CD-DO3A with either (*R/S*)-tryptophanoate or (*R/S*)-2-phenylpropanoate resulted in a downfield shift of the ^1H NMR signals observed for the guests (Figures D.4 and D.5). In addition the broadness of the signals indicated an intermediate rate of exchange. Intermediate exchange rates and subsequent broadening of the ^1H NMR signals observed for (*R/S*)-phenylalaninoate resulted in the signals losing all definition (Figure D.3). No resolution was observed for (*R/S*)-tryptophanoate, (*R/S*)-2-phenylpropanoate or (*R/S*)-phenylalaninoate. Wenzel reported resolution of the ^1H NMR signals observed for (*R/S*)-tryptophan with his Dy^{3+} complexed mono-substituted cyclodextrin, modified at the secondary end ([Dy(2- β CD-en-DTPA)]) The [Dy(6- β CD-en-DTPA)] complex afforded no resolution of tryptophan¹¹

The stepwise addition of Eu^{3+} to the solution of β CD-DO3A and (*R/S*)-tyrosinoate resulted in a downfield shift and broadening of the observed signals (Figure D.2). There was also a corresponding decrease in the intensity of the observed signals. Prompt inspection of the NMR tube revealed the presence of a white precipitate, presumably the sodium salt of the [Eu(β CD-DO3A).(*R/S*)-tyrosinoate]⁻ complex. The precipitation was indicative of strong complexation but afforded no information regarding chiral resolution. Attempts to grow crystals suitable for X-Ray analysis proved unsuccessful.

Complete resolution was achieved for the ^1H NMR signals observed for (*R/S*)-histidinoate with $\beta\text{CD-DO3A}$ when 1 equivalent of Eu^{3+} was added (Figure D.1). Distinguishing between the (*R*)- and the (*S*)- enantiomers was made possible by repeating the experiment using a 1:3 ratio of the (*R*)-:(*S*)- histidinoate. The (*S*)- enantiomer was shown to give the upfield signals. This observed chiral resolution of 20 and 15 Hz for the H_a and H_b signals of (*R/S*)-histidinoate respectively in the ^1H NMR spectrum is larger than any observed for the systems studied by Wenzel.¹¹ This may be attributable to the closer proximity of Eu^{3+} to the β -cyclodextrin annulus. The observed chiral resolution coincides with the largest shift in the ^1H NMR signals observed for a guest upon complexation by $[\text{Eu}(\beta\text{CD-DO3A})]$ and the sharpness of the observed signals indicated rapid exchange. These results are clearly illustrated in Figure 5.9.

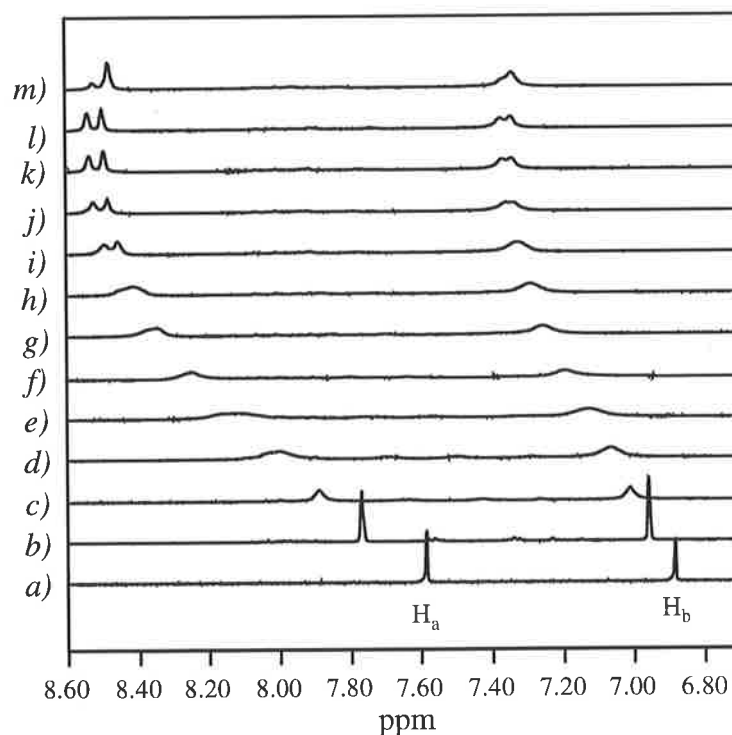


Figure 5.9. a) The observed ^1H NMR (500 MHz, D_2O) signals of a) (*R/S*)-histidine (0.02 mol dm^{-3}), then b) 1:1 (*R/S*)-histidine: $\beta\text{CD-DO3A}$ (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; i) 0.7 mole equivalents of Eu^{3+} ; j) 0.8 mole equivalents of Eu^{3+} ; k) 0.9 mole equivalents of Eu^{3+} ; l) 1 mole equivalent of Eu^{3+} , then m) 1:1:1 (*R/S*)-histidine: $\beta\text{CD-DO3A}:\text{Eu}^{3+}$ where (*R*)-:(*S*)-histidine is 1:3

5.2.3. The Absorption, Energy-Transfer, Emission properties of [Eu(β CD-DO3A)]

Experiments were designed to determine the change in emission at 616 nm (the wavelength corresponding to the $^5D_0$ - $^7F_2$ transition of Eu^{3+})⁷ with the increasing addition of tryptophanoate to solutions of [Eu(β CD-DO3A)]. Tryptophanoate was chosen as a guest for its known affinity for the β -cyclodextrin annulus (whose hydrophobicity remains intact due to the neutral complex formed between the cyclodextrin and Eu^{3+}) and for its potential to coordinate the Eu^{3+} centre. As mentioned previously (Section 5.1) guests that coordinate the Eu^{3+} centre result in an increase in Eu^{3+} luminescence compared to guests that do not.⁸

The excitation of [Eu(β CD-DO3A)] between 200 - 650 nm was monitored for emission at 616 nm. No signal was observed. Hence it was assumed that any signal observed for solutions containing [Eu(β CD-DO3A).tryptophanoate] could be attributed to the AETE phenomenon occurring between the guest and the Eu^{3+} centre. This was confirmed by the determination of the emission spectrum of [Eu(β CD-DO3A)] between 200 - 650 nm with excitation at both 220 nm and 280 nm (the previously determined absorbance wavelengths of tryptophanoate). Again no emissions characteristic of the Eu^{3+} energy levels were observed.

The initial excitation of a 1:0.25 solution of [Eu(β CD-DO3A)]:tryptophan between 200 - 650 nm (monitored for emission at 616 nm) gave two peaks that corresponded to the absorbance wavelengths of tryptophanoate (Figure 5.10). This appeared to indicate that energy transfer was occurring. Subsequent determination of the emission spectra over the same wavelength range with excitation at both 220 and 280 nm gave little to no emission at 616nm (the $^5D_0$ - $^7F_2$ transition of Eu^{3+}) contradicting the results of the excitation spectrum. There was, however, the beginning of a larger signal with $\lambda_{\text{max}} > 650$ nm and whose base encompassed the 616 nm point which could have given rise to the signals seen in the initial excitation spectra monitored at that wavelength. These experiments were repeated using only tryptophanoate and it was found that the spectra obtained were identical, the only excitation and emission being observed was that for the tryptophanoate itself.

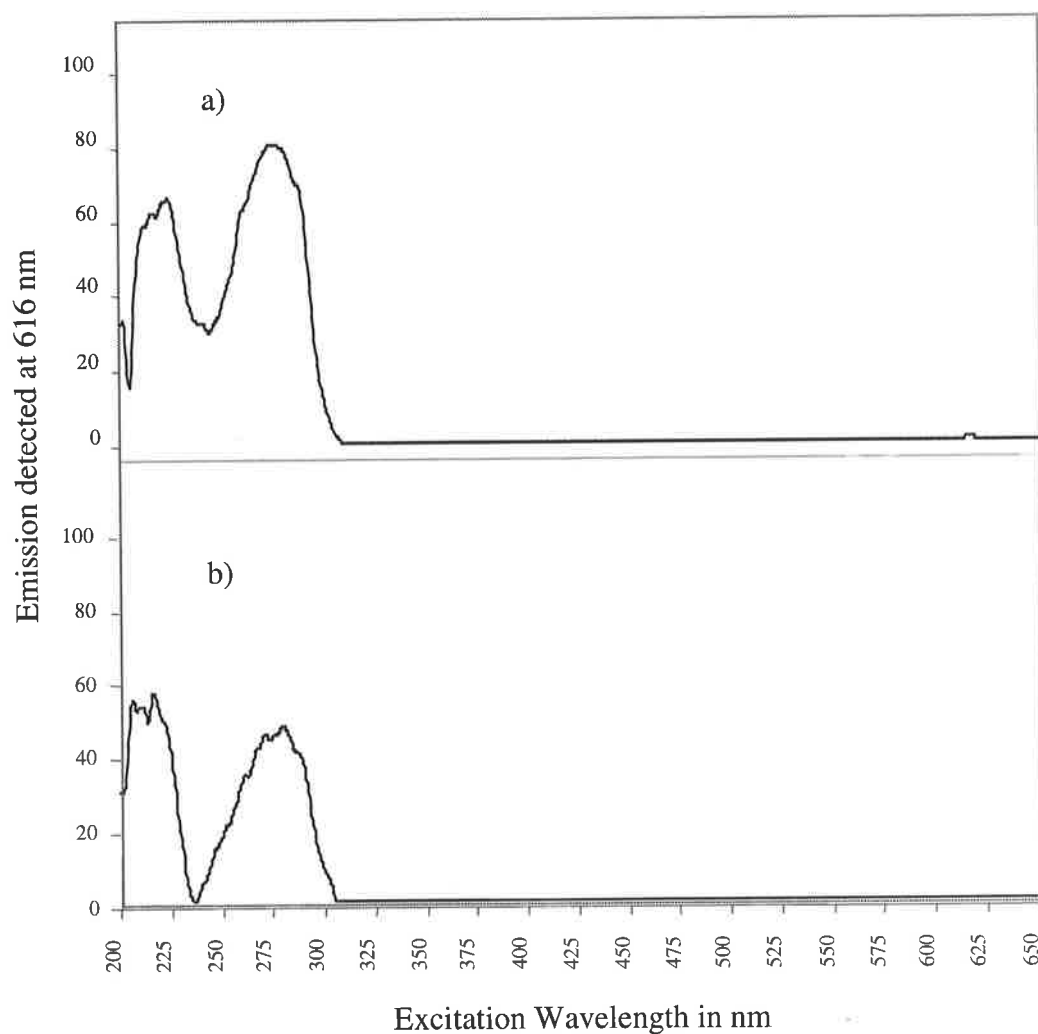


Figure 5.10. The excitation spectra of a) $2.5 \times 10^{-4} \text{ mol dm}^{-3} [\text{Eu}(\beta\text{CD.DO3A})]$ and $6.25 \times 10^{-5} \text{ mol dm}^{-3}$ tryptophanoate solution and b) $6.25 \times 10^{-5} \text{ mol dm}^{-3}$ tryptophanoate solution. Both were monitored for emission at 616 nm and both give signals corresponding to the absorbance wavelengths of tryptophan in the UV/Vis spectrum.

The lack of any AETE phenomenon for the [Eu(β CD-DO3A).tryptophan] system may be attributable to a) the tryptophanoate not binding the Eu^{3+} and hence it not being held in close enough proximity to allow efficient energy transfer and b) subsequent quenching at the Eu^{3+} centre by the D_2O solvent. From the NMR studies discussed earlier, we know that the tryptophanoate is complexed by [Eu(β CD-DO3A)] and is in slow exchange but whether or not tryptophanoate bound the Eu^{3+} was not discernible. Complexation is also evident from observation of the excitation spectra. The emission observed for tryptophan in the presence of [Eu(β CD-DO3A)] is of a greater intensity than in its absence. This can be attributed to [Eu(β CD-DO3A)] complexing the tryptophanoate and shielding it from collisions with other molecules, thus reducing its collisional frequency and enhancing the lifetime of the transition states giving rise to the emission observed. The possibility of quenching at the Eu^{3+} centre was investigated by repeating the initial experiments using DMSO but similar results were obtained proving DMSO no better a solvent for this system than D_2O . The solubility of [Eu(β CD-DO3A)] in other solvents hampered further efforts. These results were not too surprising as the structure of [Eu(β CD-DO3A)] somewhat resembles that of [Eu(β CD-1-diazacrown(18))] $^{3+}$ which also experienced quenching⁷ (Section 5.1).

5.2.4. [Eu(β CD-DO3A)] as a Potential Catalyst of RNA.

The reaction of [Eu(β CD-DO3A)] with bis(2,4-dinitrophenyl) hydrogenphosphate (BDNPP, Figure 5.2) were examined as a model for the reaction of phosphorylase enzymes. Reactions were carried out under pseudo-first order conditions ($[\text{Eu}(\beta\text{CD-DO3A})] \gg [\text{BDNPP}]$) and were monitored by following the formation of 2,4-dinitrophenylate by absorption at 400 nm. The first order rate constants for the cleavage of BDNPP by a variety of enzyme mimics at 333.2 K appears in Table 5.3.

Table 5.3. Rate constants for the cleavage of BDNPP by various enzyme mimics at 333.2 K

Species	pH	$k (\times 10^{-2} \text{ s}^{-1})$
$\beta\text{CD-dien}^{\text{a}}$	11	0.183
	7	0.0148
$[(\text{cyclen})\text{Co}(\text{OH})(\text{OH}_2)_2]^{2+\text{b}}$	7	1.1
$[(\text{tren})\text{Co}(\text{OH})(\text{OH}_2)_2]^{2+\text{b}}$	7	0.16
$[(\text{en})_2\text{Co}(\text{OH})(\text{NH}_3)]^{2+\text{c}}$	7	0.024
$[(\text{en})_2\text{Co}(\text{OH})(\text{OH})]^{2+\text{c}}$	7	0.042
$[(\text{trien})\text{Co}(\text{OH})(\text{OH}_2)_2]^{2+\text{c}}$	7	0.52
$[(\text{dien})\text{Co}(\text{OH})(\text{OH}_2)_2]^{2+\text{c}}$	7	8.6
none ^c	7	0.00021

^aReference 19, 0.10 mol dm⁻³. ^bReference 15, 0.010 mol dm⁻³. ^cReference 17, 0.010 mol dm⁻³

Reactions were carried out at 333.2 K by pipetting 1.5 cm^3 of a stock solution of $6.83 \times 10^{-4} \text{ mol dm}^{-3}$ $\beta\text{CD-DO3A}$ (pH = 9 borate buffer and $I = 0.1 \text{ mol dm}^{-3}$ NaClO_4) into a 1 cm pathlength short quartz cuvette placed in the heated cell block of a Carey 2000 spectrophotometer. Aliquots of a stock solution of 0.01 mol dm^{-3} Eu^{3+} were added ($0.025 - 0.100 \text{ cm}^3$) and the volume made up to 2 cm^3 by the addition of buffer solution, to give solutions of $[\text{Eu}(\beta\text{CD-DO3A})]$ over the range $0 - 5 \times 10^{-4} \text{ mol dm}^{-3}$ (assuming that all of the Eu^{3+} is complexed by $\beta\text{CD-DO3A}$). Solutions containing $[\text{Eu}^{3+}] > 2.5 \times 10^{-4} \text{ mol dm}^{-3}$ appeared cloudy, suggesting precipitation of either Eu^{3+} (as its hydroxy species) or the $[\text{Eu}(\beta\text{CD-DO3A})]$ complex. The solution was allowed to equilibrate for 30 minutes and then 0.05 cm^3 of a stock solution of BDNPP ($4.0 \times 10^{-4} \text{ mol dm}^{-3}$ in acetonitrile) was added to give a final solution that was 2.5% in acetonitrile and $1.0 \times 10^{-5} \text{ mol dm}^{-3}$ in BDNPP. The solution was mixed quickly and the increased absorption at 400 nm was recorded digitally for at least 7 half-lives as determined from the variation of the absorbance as a function of time (Figure 5.11). 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 observed first order rate constant (k_{obs}) was determined by fitting the collected data to a first order rate equation by conventional methods.

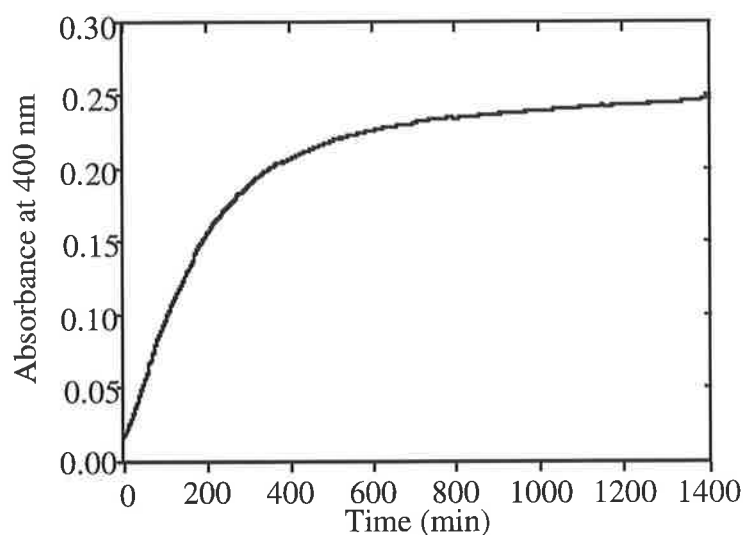


Figure 5.11. A plot of the absorbance versus time for the cleavage of BDNPP by $\beta\text{CD-DO3A}$ ($5 \times 10^{-4} \text{ mol dm}^{-3}$) with $1.25 \times 10^{-4} \text{ mol dm}^{-3}$ of Eu^{3+} .

The reaction of BDNPP with either the buffer or with a buffered solution of β CD-DO3A alone, was too slow to be followed by the procedure described above (half-life > 1000 minutes). The rate of formation of 2,4-dinitrophenylate from the reaction of BDNPP in pH = 9 borate buffer alone (k_o) and with a buffered solution of β CD-DO3A alone was determined by the addition of 1.5 cm³ of a solution 2×10^{-3} mol dm⁻³ in BDNPP to a 250 cm³ volumetric flask made up to the mark with buffer and containing the predetermined amount of β CD-DO3A by weight for the latter whilst keeping the solutions chilled. Samples of approximately 2.5 cm³ were then removed placed in vials and sealed. The vials were then placed in a water bath heated to an appropriate temperature to keep the solution inside the vials at a constant 333.2 K. A vial was removed every 2 hours for 80 hours and the sample frozen. Samples were later defrosted and their absorbance at 400 nm recorded. The last remaining samples were heated for a further 4 days to ensure complete cleavage of the ester. Under the conditions of this study, a value of 3.7×10^{-6} s⁻¹ was obtained for k_o and k_{obs} for the reaction of BDNPP in the presence of 5×10^{-4} mol dm⁻³ β CD-DO3A was 5.47×10^{-6} s⁻¹.

Solutions of Eu³⁺ in the range $1.25 - 5 \times 10^{-4}$ mol dm⁻³ in pH = 9 borate buffer and $I = 0.1$ mol dm⁻³ NaClO₄ all showed signs of cloudiness. The high salt content and pH of the buffered solutions favoured the formation of Eu(OH)₃ as a precipitate. In solutions containing both Eu³⁺ and β CD-DO3A, a lack of cloudiness was assumed to indicate the complete complexation of Eu³⁺ to form [Eu(β CD-DO3A)]. Analysis of the reaction products by TLC gave two spots corresponding to [β CD-DO3A] and [Eu(β CD-DO3A)]. An additional spot was identified as 2,4-dinitrophenolate. No complex between either of the cyclodextrins and either BDNPP or 2,4-dinitrophenolate was detected by TLC.

Table 5.4. The observed rate for the formation of 2,4-dinitrophenolate for the reaction of BDNPP by solutions containing β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$ where the total concentration of $\beta\text{CD-DO3A}$ is $5 \times 10^{-4} \text{ mol dm}^{-3}$ at 333.2 K, pH = 9 (borate buffer) and $I = 0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$.^a

Concentration of Eu^{3+} added ($\times 10^{-4} \text{ mol dm}^{-3}$)	k_{obs} ($\times 10^{-6} \text{ s}^{-1}$)
-	5.47
1.25	33.8
1.5	43.8
2.5	86.5
3.75 ^b	98.6
5 ^b	102

^a k_0 = of $3.7 \times 10^{-6} \text{ s}^{-1}$. ^b Some precipitation was observed.

Inspection of Table 5.4 reveals that the addition of Eu^{3+} to a solution of $\beta\text{CD-DO3A}$ results in a marked increase in the rate of formation of 2,4-dinitrophenolate in the reaction of BDNPP with $\beta\text{CD-DO3A}$, due to the formation of $[\text{Eu}(\beta\text{CD-DO3A})]$. A plot of k_{obs} verses the concentration of added Eu^{3+} (Figure 5.12) reveals that above $[\text{Eu}^{3+}] = 2.5 \times 10^{-4} \text{ mol dm}^{-3}$ there is little further enhancement of the rate of reaction with further additions of Eu^{3+} . This is most likely due to the precipitation of the added Eu^{3+} above a concentration of $2.5 \times 10^{-4} \text{ mol dm}^{-3}$.

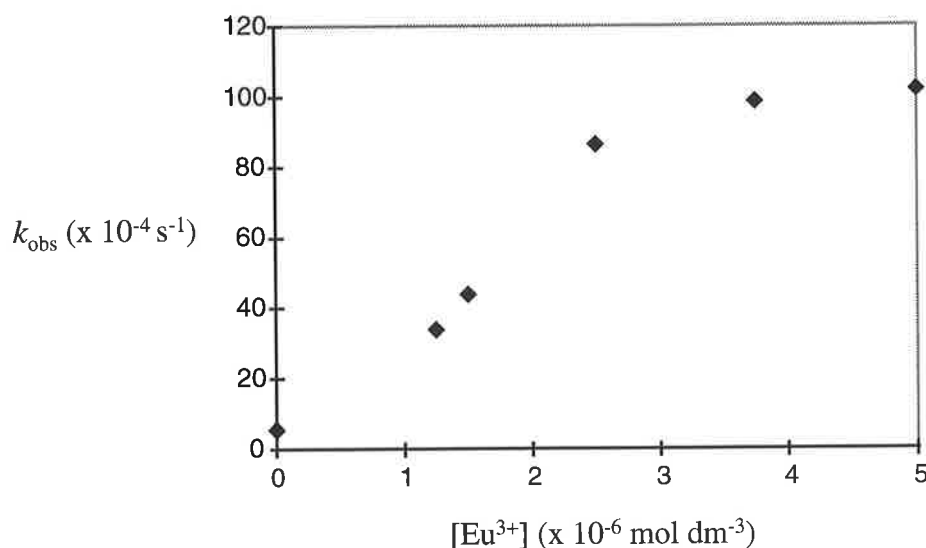
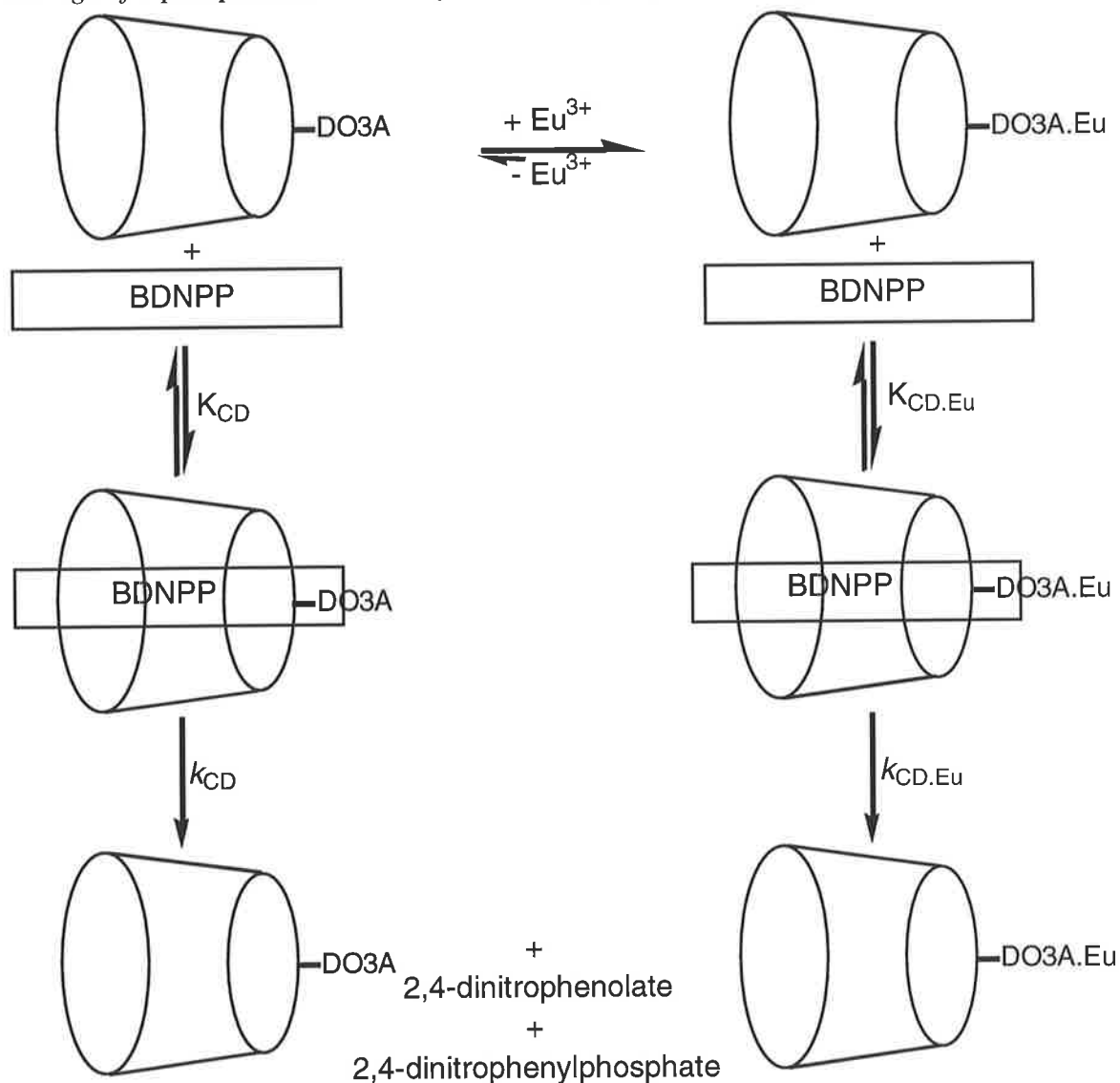


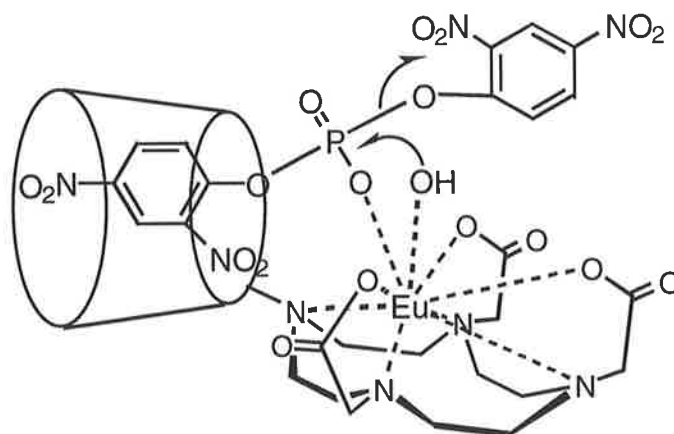
Figure 5.12. Plot of k_{obs} versus the concentration of Eu^{3+} added to the solution of $\beta\text{CD-DO3A}$ showing the effect of Eu^{3+} saturation on the observed rate.

No information is afforded as to the mechanism of the phosphodiester bond cleavage. The binding of Eu^{3+} to $\beta\text{CD-DO3A}$ significantly increases the rate of the reaction with BDNPP over that of $\beta\text{CD-DO3A}$ alone, indicating the Eu^{3+} plays an important part in the reaction. The mechanism for the cleavage of BDNPP by $\beta\text{CD-DO3A}$ and $[\text{Eu}(\beta\text{CD-DO3A})]$ may be a Michaelis-Menten type mechanism (Scheme 5.5) or a straight nucleophilic attack ($\text{S}_{\text{N}}2$). In the latter case, the reaction of BDNPP with $\beta\text{CD-DO3A}$ would proceed by the cleavage of a phosphodiester bond of BDNPP through a nucleophilic attack by either a hydroxy group of the βCD moiety or a carboxylate group of the DO3A. The addition of Eu^{3+} may enhance the rate of this reaction either by the formation of a hydroxy species capable of a nucleophilic attack on BDNPP or by acting to polarise the phosphate bond, making it more susceptible to nucleophilic attack. If the reaction of BDNPP with $\beta\text{CD-DO3A}$ proceeds by a Michaelis-Menten type mechanism, the nucleophilic attack on BDNPP by $\beta\text{CD-DO3A}$ would be preceded by the complexation of BDNPP in the cyclodextrin annulus. The presence of the Eu^{3+} may enhance the rate of reaction through the formation of a hydroxy species capable of a nucleophilic attack on BDNPP (as mentioned above) and/or by increasing the rate of binding of BDNPP to the cyclodextrin annulus. A Michaelis-Menten type mechanism is most likely based on the results of the previous NMR study where aromatic guests were found to include in the cyclodextrin annulus (Sections 5.2.2.1 and 5.2.2.2)

Scheme 5.1 a) The proposed mechanism for the cleavage of BDNPP by solutions containing β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$ where K_{CD} and $K_{\text{CD.Eu}}$ are the stability constants for the complexes formed by BDNPP with β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$ respectively. The rates of cleavage of the phosphodiester bond of BDNPP by β CD-DO3A and $[\text{Eu}(\beta\text{CD-DO3A})]$ are denoted by k_{CD} and $k_{\text{CD.Eu}}$, respectively. b) a possible mechanism for the cleavage of a phosphodiester bond of BDNPP by $[\text{Eu}(\beta\text{CD-DO3A})]$.



a)



b)

5.3. Summary and Conclusions.

Substitution of a primary hydroxyl group by a DO3A moiety produces a modified cyclodextrin whose acidic and metal binding properties mimic those of the free DO3A ligand.

β CD-DO3A is able to solubilise Eu^{3+} in basic aqueous solution in the absence of high salt content. In buffered solutions with high ionic strength Eu^{3+} may still form a hydroxy complex and precipitate out of solution in the presence of β CD-DO3A

The modified cyclodextrin, β CD-DO3A, is capable of binding a number of amino and carboxylic acids. The resolution by ^1H NMR spectroscopy of the aromatic proton signals of (*R/S*)-tyrosine is made possible using β CD-DO3A. Complexation of (*R/S*)-2-phenylpropanoate by β CD-DO3A produced ^1H NMR signals for the methyl protons of the guest suggestive of each enantiomer, existing in two possible orientations within the β CD-DO3A annulus, being magnetically inequivalent. No resolution is observed for the other guests studied. Complexation of Eu^{3+} by the modified cyclodextrin forms a stable metallocyclodextrin in most aqueous solutions.

The Eu^{3+} bound cyclodextrin, $[\text{Eu}(\beta\text{CD-DO3A})]$, is able to resolve the aromatic proton signals of (*R/S*)-histidine by ^1H NMR spectroscopy. No resolution is observed for the other guests studied. A stable complex, observable as a white precipitate, is formed between $[\text{Eu}(\beta\text{CD-DO3A})]$ and (*R/S*)-tyrosine.

Although retaining the hydrophobicity of its annulus and possessing vacant coordination sites at the Eu^{3+} centre, a tryptophanoate complex of $[\text{Eu}(\beta\text{CD-DO3A})]$ does not display any AETE properties in either D_2O or DMSO. This result is analogous to that for the benzene complex of $[\text{Eu}(\beta\text{CD-1-diazacrown(18))}]^{3+}$ which experienced quenching of its AETE phenomena by D_2O .⁷

A buffered solution of β CD-DO3A, ($\text{pH} = 9$ borate buffer, $I = 0.10 \text{ mol dm}^{-3}$ (NaClO_4)), moderately increases the rate of cleavage of BDNPP (an RNA mimic) in comparison with the buffer alone. The addition of Eu^{3+} to buffered solutions containing β CD-DO3A, and the subsequent formation of $[\text{Eu}(\beta\text{CD-DO3A})]$ results in a large increase in the rate of cleavage of BDNPP. In buffered solutions of constant ionic strength, β CD-DO3A ($5 \times 10^{-4} \text{ mol dm}^{-3}$) was not able to solubilise Eu^{3+} above a concentration of $2.5 \times 10^{-4} \text{ mol dm}^{-3}$.

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CHAPTER SIX

EXPERIMENTAL

6.1. General

6.1.1. Materials.

Unless otherwise stated, β CD-en, β CD-pn, β CD-bn, β CD-hn, β CD-dien, β CD-trien β CD-dipn, β CD-tacn, β CD-tacdo and β CD-cyclen were prepared as in the literature.¹ The cyclodextrins were dried to a constant weight over P_2O_5 and stored in the dark under refrigeration.

All solutions were prepared using deionised water that had been purified using a MilliQ-reagent system to produce water with a specific resistance of $>15\text{ M}\Omega\text{ cm}$. For solutions used for titrations the water was also boiled to remove CO_2 .

Any salts used were of analytical grade and were dried/stored over P_2O_5 under vacuum.

Thin Layer Chromatography (TLC) was carried out using Merck Kieselgel 60 F254 silica on aluminium sheets.

6.1.2. Potentiometric Titrations.

Potentiometric titrations were carried out using a Metrohm Dosimat E665 titrator, an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode filled with $0.10\text{ mol dm}^{-3}\text{ NaClO}_4$. Data was collected on either a Laser or Acer PC. The electrode was calibrated regularly and E_0 and pK_W values determined by titration of $0.01\text{ mol dm}^{-3}\text{ HClO}_4$ with standardised $0.1\text{ mol dm}^{-3}\text{ NaOH}$. Data was collected in mV units.

All titration solutions were thermostatted at $298.2 \pm 0.2\text{ K}$ in a 20 cm^3 water jacketed titration vessel, stirred magnetically, and saturated with nitrogen by passing a stream of nitrogen (previously passed through $1\text{ mol dm}^{-3}\text{ NaOH}$ to remove any trace CO_2 , then saturated with $0.10\text{ mol dm}^{-3}\text{ NaClO}_4$) through the solution for at least 15 minutes prior to the commencement of, and for the duration of the titration. At least three titrations were performed for each system investigated.

The apparent stability constants and pK_a s were derived from the experimental data using the program SUPERQUAD^{2,3} on either an Acer or a Digital Venturis 575 PC. SUPERQUAD^{2,3} uses a non-linear least squares method to minimise the sum of the differences between the measured and the theoretical data calculated from a given model. The fit of a single run was considered statistically acceptable if χ^2 was less than 12.6 at the 95% confidence level and at least two runs were averaged for each system.

Unless otherwise stated, all errors quoted for K (the mean of N runs) represent the standard deviation, $\sigma = ((\sum(K_i - K)^2)/(N-1))$ where K_i is a value from a single run for the best fit of the variation of pH with added volume of NaOH titrant obtained through SUPERQUAD^{2,3} and $i = 1, 2, \dots, N$.

6.2. The Complexation of Carboxylic Acids by 6^A-Deoxy-6^A-(ω -aminoalkyl)amino- β -Cyclodextrins

6.2.1. Materials and Solutions.

Benzoic acid, 4-methylbenzoic acid and (*R*)- and (*S*)-2-phenylpropanoic acid (Sigma) were used without further purification and stored under vacuum over P₂O₅. The enantiomeric purities of the (*R*)- and (*S*)-2-phenylpropanoic acid had been previously determined to be >97.5% and 98.5% respectively.⁴ These purity limits were taken into account when calculating the errors on the apparent stability constants for complexes with these enantiomers with the cyclodextrins studied.

The diamines 1,2-diaminoethane (en), 1,3-diaminopropane (pn), 1,4-diaminobutane (bn) and 1,6-diaminohexane (hn), were purchased from Aldrich and used without further purification.

Solutions of the individual acids, diamines and modified cyclodextrins were 0.002 mol dm⁻³ in the species of interest, 0.005 mol dm⁻³ in HClO₄ and 0.095 mol dm⁻³ in NaClO₄ in all titrations.

6.2.2. Determination of pK_a 's and Apparent Stability Constants.

The pK_a values of the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins and the analogous free diamines were determined by titration of standardised 0.1 mol dm⁻³ NaOH against 10.00 cm³ aliquots of the species of interest.

To determine the apparent stability constants for the complexation of a carboxylic acid and its conjugate base by β CD-X (where x = en, pn, bn or hn), the titration vessel contained 5.00 cm³ each of the acid and cyclodextrin of interest. This was then titrated with standardised 0.1 mol dm⁻³ NaOH

6.2.3. ¹H Nuclear Magnetic Resonance Spectroscopy and Molecular Modelling.

The ¹H NMR one dimensional and two dimensional ROESY (mixing time of 0.35 s) experiments were run on a Varian Inova 600 spectrometer as in the literature.¹⁹ All samples were 0.06 mol dm⁻³ in each of the β CD-X and were 0.06 mol dm⁻³ in the guest species where present. Solutions were filtered and degassed by freeze thawing before their spectra were recorded.

Gas phase force-field molecular modelling was performed on a Silicon Graphics Iris Indigo X2 400 Unix workstation. Structures of the cyclodextrin species of interest were produced by modification of the structure of native β CD, obtained from the Cambridge Crystallographic Data Base,⁶ using the builder module within the *Insight II* molecular modelling package Version 2.1.0, from Biosym Technologies.⁷ The guest molecules were created as needed using the same.

Structural minimisation of the species or complexes of interest were performed using *Discover* version 2.9 from Biosym Technologies.⁷ A CVFF forcefield programme incorporating the 6-12 ϵ function with geometric averages for the off diagonal heteronuclear interactions, was applied and the default settings for this forcefield within the *Discover* package were used.

The minimisation itself employed a steepest descents algorithm until the root mean square of the atomic derivatives (RMS) was < 10, a conjugate gradients algorithm was then employed until the RMS was < 1 and the final step employed a Quasi-Newton-Raphson algorithm until the minimum was obtained. Several local energy minima were obtained before the global minimum was reached.

The steepest descents algorithm relies on the gradient of the energy surface to find the minimum. The line search direction is simply taken as the gradient at the point of the surface at which the system lies. After each line search a new direction is chosen, being the gradient at the new point, and the process is repeated. The steepest descents method is useful for systems whose initial configurations are far from minimal but convergence near the minimum is slow as the gradient approaches zero.⁸

The conjugate gradients algorithm produces a basis set of mutually conjugate directions such that each line search continually refines the direction towards the minimum. For this algorithm, $\mathbf{h}_{i+1}$, the new direction vector starting from point $i+1$, is calculated by adding the gradient at point $i+1$, $\mathbf{g}_{i+1}$ to the previous direction $\mathbf{h}_i$ scaled by some constant γ_i such that:

$$\mathbf{h}_{i+1} = \mathbf{g}_{i+1} + \gamma_i \mathbf{h}_i$$

thus γ_i is a scalar defined as:

$$\gamma_i = \frac{\mathbf{g}_{i+1} \cdot \mathbf{g}_{i+1}}{\mathbf{g}_i \cdot \mathbf{g}_i}$$

No matter what the initial point is, γ times the old direction always provides a new direction conjugate to all previous directions the benefit being that no earlier progress by the algorithm is undone (unlike for steepest descents).⁸

The quasi-Newton-Raphson algorithm not only utilises the gradient to give a direction but also uses the curvature of the function/surface to predict where along the gradient the function will pass through a minimum. The benefits of this algorithm is that it rapidly converges near the minimum hence its employment after application of the Steepest Descents and conjugate gradients algorithms. This method is more computationally involved than the previous methods and if applied to a structure that is far from its minimum the minimisation process can become unstable.⁸

6.2.4. The Complexation of Adamantane-1-Carboxylate by the 6^A-deoxy-6^A-(ω -aminoalkyl)amino- β -cyclodextrins.

Adamantane-1-carboxylic acid (ACH, Aldrich) was used without further purification. Due to its low solubility in acidic aqueous solution, solutions containing both β CD-X and ACH were 0.002 mol dm⁻³ of the species of interest, 0.01 mol dm⁻³ of standardised NaOH and 0.09 mol dm⁻³ of NaClO₄. The burette was filled with 0.1 mol dm⁻³ standardised HClO₄.

An attempt was made to determine the pK_a of ACH by titrating a 10 cm³ aliquot of the

species containing solution. Upon nearing the pK_a of the acid it began to precipitate out of solution, thus this method proved to be unsuccessful. The pH at which the acid precipitated out of solution corresponded closely with the pK_a of the acid reported in the literature though this was determined from a titration of a 50% (vol.) aqueous ethanolic solution.⁹ This being the case it was decided to use the reported pK_a value of 6.90 ± 0.02 in any calculations to determine apparent stability constants for ACH containing complexes. To determine the apparent stability constants for the complexation of ACH and the modified cyclodextrins, the titration vessel contained 5 cm³ each of the species of interest. Again as the pH neared that of the pK_a of the ACH, precipitation occurred. Thus, this method was deemed inappropriate for determining the stabilities of the complexes formed.

6.3. The Complexation of Carboxylic Acids by 6^A-Deoxy-6^A-Triazaalkyl- and 6^A-Deoxy-6^A-Tetraazaalkyl- β -Cyclodextrins.

The carboxylic acids used were as in Section 6.2.1. Both 2-(2-aminoethylamino)ethylamine (dien), and 3-(3-aminopropylamino)propylamine (dipn), were purchased from Aldrich and used without further purification. 1,4,7,10-tetraazacyclododecane bis(dihydrogen sulfate) (cyclen.2H₂SO₄), was purchased from Aldrich and reacted with concentrated NaOH and the basic aqueous solution extracted with chloroform until no more amine was detected in the extractant by modified TLC analysis (amine was spotted on plate and undeveloped plate was dipped in 1% ninhydrin in ethanol, then heated, presence of the amine indicated by purple colouration of spot). The chloroform extracts were dried over anhydrous magnesium sulfate and the chloroform removed to yield 1,4,7,10-tetraazacyclododecane (cyclen). 2-(2-(2-Aminoethylamino)ethylamino)ethylamine tetrahydrochloride (trien.4HCl, Aldrich) was purified by two recrystallisations from ethanol-water.¹⁰ 1,4,7-Triazacyclononane trihydrochloride and 1,5,9-triazacyclododecane trihydrochloride were prepared as in the literature.^{11,12}

Solutions of the individual acids, tri- and tetraaza compounds and modified cyclodextrins were 0.002 mol dm⁻³ in the species of interest, 0.005 mol dm⁻³ in HClO₄ and 0.095 mol dm⁻³ in NaClO₄ in all titrations with the exception of solutions containing trien.4HCl, which were 0.002 mol dm⁻³ in the amine, 0.002 mol dm⁻³ in HClO₄ and 0.0098 mol dm⁻³ in NaClO₄ (thus overall acid concentration was 0.010 mol dm⁻³ in these solutions). In addition solutions containing β CD-trien were 0.010 mol dm⁻³ in HClO₄ and 0.090 mol dm⁻³ in NaClO₄.

The determination of pK_a s and the apparent stability constants for the complexes with the guest carboxylic acids was carried out as in Section 6.2.2

^{13}C NMR spectra were recorded on a Bruker ACP300 spectrometer operating at 75.47 MHz. The two dimensional ^1H ROESY NMR spectra were run as outlined in section 6.2.3.

6.5. The Formation of Metallo-Tetraazaalkyl-Monosubstituted- β -Cyclodextrins and Their Complexation of Tryptophan and Tryptophanoate in Aqueous Solution.

6.5.1. Materials and Solutions.

(*R*)- and (*S*)-tryptophan were purchased from Sigma and used without further purification, and were dried to constant weight and stored over P_2O_5 under vacuum. The enantiomeric purities of the amino acids had previously been determined to be $> 99\%$ ¹³ and these purity limits were used in the calculation of errors on the apparent stability constants for the complexes containing these enantiomers. Metal perchlorates (Fluka) were recrystallised from water, dried and stored over P_2O_5 under vacuum.

Solutions of $\text{Ni}(\text{ClO}_4)_2$, $\text{Zn}(\text{ClO}_4)_2$, and $\text{Cu}(\text{ClO}_4)_2$ (0.1 mol dm^{-3}) were prepared using CO_2 free Milli-Q water. The exact metal ion concentration of each solution was then determined by ion exchange chromatography and acid-base titration. A column of Dowex 50-X8 ion exchange resin (H^+ form, $1.5 \text{ cm} \times 10 \text{ cm}$) was prepared by first acidifying a slurry of the resin ($0.1 \text{ mol dm}^{-3} \text{ HCl}$) which was then loaded into a column and washed with water until the eluent was neutral. For each metal solution, 5 cm^3 was loaded onto the column and washed with water (approx. 50 cm^3) until the eluent was neutral. The eluent was then made up to a volume of 70 cm^3 and indicator (bromothymol blue, pH 6.0 - 7.6, yellow-green-blue) added. Titration of the eluent with a standardised solution of $0.1 \text{ mol dm}^{-3} \text{ NaOH}$ allowed the number of moles of acid present to be determined and hence the concentration of the metal ion solutions. This procedure was repeated for each metal solution until at least three similar results were obtained.

The $0.002 \text{ mol dm}^{-3}$ tryptophan, $\beta\text{CD-cyclen}$ and $\beta\text{CD-trien}$ stock solutions were $0.005 \text{ mol dm}^{-3}$ in HClO_4 and $0.095 \text{ mol dm}^{-3}$ in NaClO_4 for $\beta\text{CD-cyclen}$, and the tryptophan solutions used in titrations with this host, while the $\beta\text{CD-trien}$, and the tryptophan solutions used in titration with this host, were 0.01 mol dm^{-3} in HClO_4 and 0.09 mol dm^{-3} in NaClO_4 .

6.5.2. Determination of Apparent Stability Constants.

The apparent stability constants for the complexes formed between β CD-cyclen and β CD-trien and the amino acids were determined by titration of 5.00 cm³ each of the stock solutions of either (*R*)- or (*S*)-tryptophan and the cyclodextrin of interest.

The apparent stability constants for the formation of the different metallo-cyclodextrin complexes were determined, where possible, by the titration of 10.00 cm³ aliquots of the cyclodextrin of interest with 0.10 cm³ of metal perchlorate solution added. The complexation of Ni²⁺ by β CD-cyclen was too slow to be determined by this method.

The apparent stability constants for the formation of ternary complexes containing the cyclodextrin and metal of interest and either (*R*)- or (*S*)-tryptophan were determined by titration of 5.00 cm³ each of the stock solutions of either the (*R*)- or the (*S*)-enantiomer and either β CD-cyclen or β CD-trien with 0.10 cm³ of metal perchlorate solution added.

The program, SUPERQUAD^{2,3}, can only handle a maximum of 18 specified equilibria which characterise stability constants which are either fixed or to be refined. As a consequence, the predetermined species found to be present in solution in amounts less than 1.5% in the ternary system, assuming the formation of no ternary complexes, were omitted in the fitting of the ternary systems.

6.5.3. Absorbance Spectra of the Metal Complexes.

An aqueous solution of 0.025 mol dm⁻³ NaPIPES (Calbiochem) at an ionic strength of 0.10 mol dm⁻³ (NaClO₄) was adjusted to pH 8.0 by the addition of NaOH and used as a buffer in the preparation of the metal complex solutions.

All spectra were run in duplicate on a Cary 2200 spectrophotometer in 1 cm pathlength quartz cuvettes, thermostatted at 298.2 $\pm$ 0.1 K and run against reference solutions containing all components of the solution of interest except the metal salt.

6.6. 6^A-Deoxy-6^A-(1,4,7,10-tetraaza-1,4,7-tri(carboxymethyl)cyclododecane)- β -Cyclodextrin (β CD-DO3A).

6.6.1. Preparation of (β CD-DO3A).

An aqueous solution of chloroacetic acid (0.25 g, 2.5 mmol in 1 cm³ H₂O) was neutralised by the addition of an aqueous solution of sodium hydroxide (0.1 g, 2.5 mmol in 1 cm³ H₂O). This was then added to an aqueous solution of 6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecan)- β -cyclodextrin,¹ (β CD-cyclen), (0.4 g, 0.3 mmol in 5 cm³ H₂O). The resultant solution was raised to pH 11 with excess NaOH. The mixture was then heated and stirred for 8 hours in a sealed vessel at 80°C and the pH maintained by the addition of small amounts of NaOH as required. The reaction was then left stirring at room temperature until analysis by T.L.C. showed the presence of only one product and some native β CD (approx. 12 hours). The solution was acidified with HCl, diluted and loaded onto a column containing Dowex 50W X2 Cation Exchange Resin (H⁺ form, 1 cm x 15 cm). The column was then washed with H₂O (200 cm³) and the product eluted with 1.4% NH₄OH. The solvent was removed and the product washed with water to yield 0.35 grams of an off white solid. The triammonium salt was the expected isolated product but microanalysis revealed the product as the diammonium salt. Anal. Found: C, 43.18; H, 6.15; N, 5.41. Calcd. for C₅₆H₁₀₀N₆O₄₀.3H₂O: C, 43.35; H, 6.88; N, 5.41.

δ C (D₂O-NaOH, pH~14), 184.2, 176.6, 175.4 (C4 DO3A), 107.4, 107.0, 106.4 (C1), 86.7, 86.2 (C4), 78.7, 77.7, 77.5, 77.2 (C2, C3, C5), 75.3 (C5^A), 66.0 (C6), 62.3 (C6^A), 63.1, 62.1, 61.5, 59.2, 58.1, 57.3, 56.7, 56.1, 54.1, 48.9, 48.4 (C1, C2, C3 of DO3A);

δ H (D₂O, pH~9.5) 4.93 (s, 7H, H1), 3.92 - 3.64 (m, 26H, H3, H5, H6), 3.56 - 3.37 (m, 16H, H2, H4, H6^A), 3.37 - 2.40 (m, 22H, H-DO3A).

The Eu³⁺ complex of β CD-DO3A was prepared *in situ*.

δ H (250 MHz, D₂O, pH~9.5) of [Eu(β CD-DO3A)] 33.92 (s, 4H, cyclen-H_{ax}), 4.82 (s, 7H, H1), 4.2 - 2.3 (m, 42H, H3-H6), -1.26 (s, 4H, cyclen-H_{eq}), -6.93 (s, 4H, cyclen-H_{ax}'), -8.37 (s, 4H, cyclen-H_{eq}'), -14.94 (s, 4H, -CH₂CO closest to β CD), -16.46 (s, 2H, -CH₂CO furthest from β CD)

(T.L.C. used 1:1:8 chloroform: water: acetic acid and developed using 1% H₂SO₄ in ethanol and heat to char) Potentiometric titrations were performed using NaOH/NaClO₄ and NEtOH/NEt₄ClO₄ as in Section 6.1.2. SUPERQUAD^{2,3} was used to fit the data and account was made for the presence of ammonium.

6.6.2. The Chiral Resolution of Racemic Guests by NMR Spectroscopy using β CD-DO3A and [Eu(β CD-DO3A)].

Materials.

(*R/S*)-histidine, (*R/S*)-tyrosine, (*R/S*)-phenylalanine, (*R/S*)-tryptophan and (*R/S*)-phenylpropionic acid were obtained from Sigma Chemical Corp. Tetraethylammonium chloride was purchased from the British Drug Houses (BDH). Deuterium oxide (99.75%) was obtained from the Australian Nuclear Science and Technology Organisation (ANSTO).

Apparatus and Procedures.

NMR spectra were recorded on either a Varian Inova 500 spectrophotometer operating at 500.044 MHz for protons and 125.748 MHz for carbons, or a Varian Inova 600 spectrophotometer operating at 599.959 MHz for protons and 150.873 MHz for carbons. Stock solutions of 0.040 mol dm⁻³ β CD-DO3A and each of the guests (at pH = 10 (NaOH), as the racemate) were prepared in D₂O. A grain of tetraethylammonium chloride was added to the stock solution of β CD-DO3A to act as an internal reference.¹¹ The 500 MHz ¹H NMR spectrum of each guest was recorded in the presence and absence of β CD-DO3A by pipetting 0.300 cm³ of the stock solution of the guest into an NMR tube and adding either 0.300 cm³ of D₂O or the β CD-DO3A stock solution such that the resultant samples were all 0.02 mol dm⁻³ in the species of interest. To the NMR tube containing both β CD-DO3A and the guest of interest, 0.010 cm³ aliquots of a 0.12 mol dm⁻³ stock solution of Eu³⁺ were added sequentially to a total volume of 0.100 cm³. Final solutions were 0.700 cm³ in volume and 0.017 mol dm⁻³ in Eu³⁺, β CD-DO3A and the guest of interest. After each addition of Eu³⁺, samples were allowed to equilibrate for 30 minutes within the probe of the NMR spectrophotometer before the ¹H NMR spectrum was recorded. Only those signals of the guest not overlapped by the cyclodextrin signals were examined for chiral resolution upon complexation by β CD-DO3A and [Eu(β CD-DO3A)]. NMR spectra were recorded at 298.2 K and were referenced with respect to the central peak of the methylene triplet of tetraethylammonium chloride.¹⁴

6.6.3. The Absorption, Energy-Transfer, Emission properties of [Eu(β CD-DO3A)].

Materials and Solutions.

All solutions were made up with degassed D₂O or DMSO and were 2.5×10^{-4} mol dm⁻³ in [Eu(β CD-DO3A)] (made *in situ*, Eu³⁺ was as its triflate salt) and either 0.0 or 6.25×10^{-5} mol dm⁻³ in tryptophan.

Apparatus and Procedures.

Absorption spectra were recorded using a Cary 2200 spectrophotometer in 1 cm pathlength matched quartz cuvettes thermostatted at 298.2 ± 0.1 K against reference solutions containing D₂O or DMSO

Fluorescence spectra were recorded at 0.5 nm intervals in duplicate on a Perkin Elmer LS 50B luminescence spectrometer in a quartz cuvette of pathlength 1 cm thermostatted at 298.2 ± 0.1 K. A baseline was recorded of D₂O or DMSO prior to each set of measurements. The duplicate runs were averaged and the baseline subtracted to obtain the final spectra. An excitation slit of 3 nm and an emission slit of 4 nm were used for all the systems studied.

6.6.4. [Eu(β CD-DO3A)], a potential catalyst of RNA.

Materials and Solutions.

Bis(4-nitrophenyl) hydrogenphosphate (BNPP) was purchased from Aldrich and used without any further purification. Bis(2,4-dinitrophenyl) hydrogenphosphate (BDNPP) was made as in the literature,¹⁵ the crude product was recrystallised twice from acetone and dried over P₂O₅ under vacuum to yield 0.76 g of the pyridinium salt (lit. yield 1.42 g). The product was checked for purity by melting point (gave 156 - 158°, lit. m.p. 159 - 160°) and microanalysis. Anal. Found: C, 40.24; H, 2.18; N, 13.78; Calcd. for C₁₇H₁₁N₅O₁₂P: C, 40.24; H, 2.03; N, 13.67.

All solutions were prepared with boiled Milli-Q water. A 0.05 mol dm⁻³ borate buffer solution of pH 9 and I = 0.1 mol dm⁻³ NaClO₄ was prepared by adding 50 cm³ of a stock solution of 0.1 mol dm⁻³ H₃BO₃/NaClO₄ to 20.8 cm³ of 0.1 mol dm⁻³ NaOH and making the volume up to 100 cm³ with 0.1 mol dm⁻³ NaClO₄. A 2×10^{-3} mol dm⁻³ stock solution of BDNPP was prepared in freshly distilled acetonitrile, 2 cm³ of this was taken and made up to

10 cm³ to prepare a 4×10^{-4} mol dm⁻³ stock solution. Reaction solutions were all 5×10^{-4} mol dm⁻³ in cyclodextrin and 1×10^{-5} mol dm⁻³ in BDNPP.

Apparatus and Procedures.

Reactions were carried out at 333.2 K by pipetting 1.5 cm³ of a stock solution of 6.83×10^{-4} mol dm⁻³ β CD-DO3A (pH = 9 borate buffer and $I = 0.1$ mol dm⁻³ NaClO₄) into a 1 cm pathlength short quartz cuvette placed in the heated cell block of a Carey 2000 spectrophotometer. Aliquots of a stock solution of 0.01 mol dm⁻³ Eu³⁺ were added (0.025 - 0.100 cm³) and the volume made up to 2 cm³ by the addition of buffer solution, to give solutions of [Eu(β CD-DO3A)] over the range 0 - 5×10^{-4} mol dm⁻³ (assuming that all of the Eu³⁺ is complexed by β CD-DO3A). Solutions containing [Eu³⁺] > 2.5×10^{-4} mol dm⁻³ appeared cloudy, suggesting precipitation of either Eu³⁺ (as its hydroxy species) or the [Eu(β CD-DO3A)] complex. The solution was allowed to equilibrate for 30 minutes and then 0.05 cm³ of a stock solution of BDNPP (4.0×10^{-4} mol dm⁻³ in acetonitrile) was added to give a final solution that was 2.5% in acetonitrile and 1.0×10^{-5} mol dm⁻³ in BDNPP. The solution was mixed quickly and the increased absorption at 400 nm was recorded digitally for at least 7 half-lives as determined from the variation of the absorbance as a function of time (Figure 5.11). 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 observed first order rate constant (k_{obs}) was determined by fitting the collected data to a first order rate equation by conventional methods.

The rates for the uncatalysed cleavage of BDNPP and for that with β CD-DO3A was achieved by the addition of 1.5 cm³ of 2×10^{-3} mol dm⁻³ stock BDNPP to 250 cm³ of either the buffer or the cyclodextrin whilst keeping the solutions chilled. Samples of approx 2.5 cm³ were then taken and placed in vials that were then sealed. The vials were then placed in a water bath heated at 333.2 K and 1 vial removed every two hours for 80 hours. Upon removal each vial was frozen. Samples were later defrosted and the absorbance of the sample determined at 400 nm. The last remaining samples were left in the bath for a further 4 days to ensure complete cleavage of the ester.

Table 6.1. The amounts of Eu^{3+} added to the reaction solutions and the resultant amount of βCD -DO3A complexed.

Amount Eu^{3+} added (μL)	Amount of βCD - DO3A complexed (percent)
25	25
30	30
50	50
75	75 ^a
100	100 ^a

^aignoring any precipitation of $\text{Eu}(\text{OH})_3$

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APPENDIX A

The Titration Profiles for the Complexation of Aromatic Carboxylic Acids by the 6^A-Deoxy-6^A-(ω -aminoalkylamino)- β -Cyclodextrins

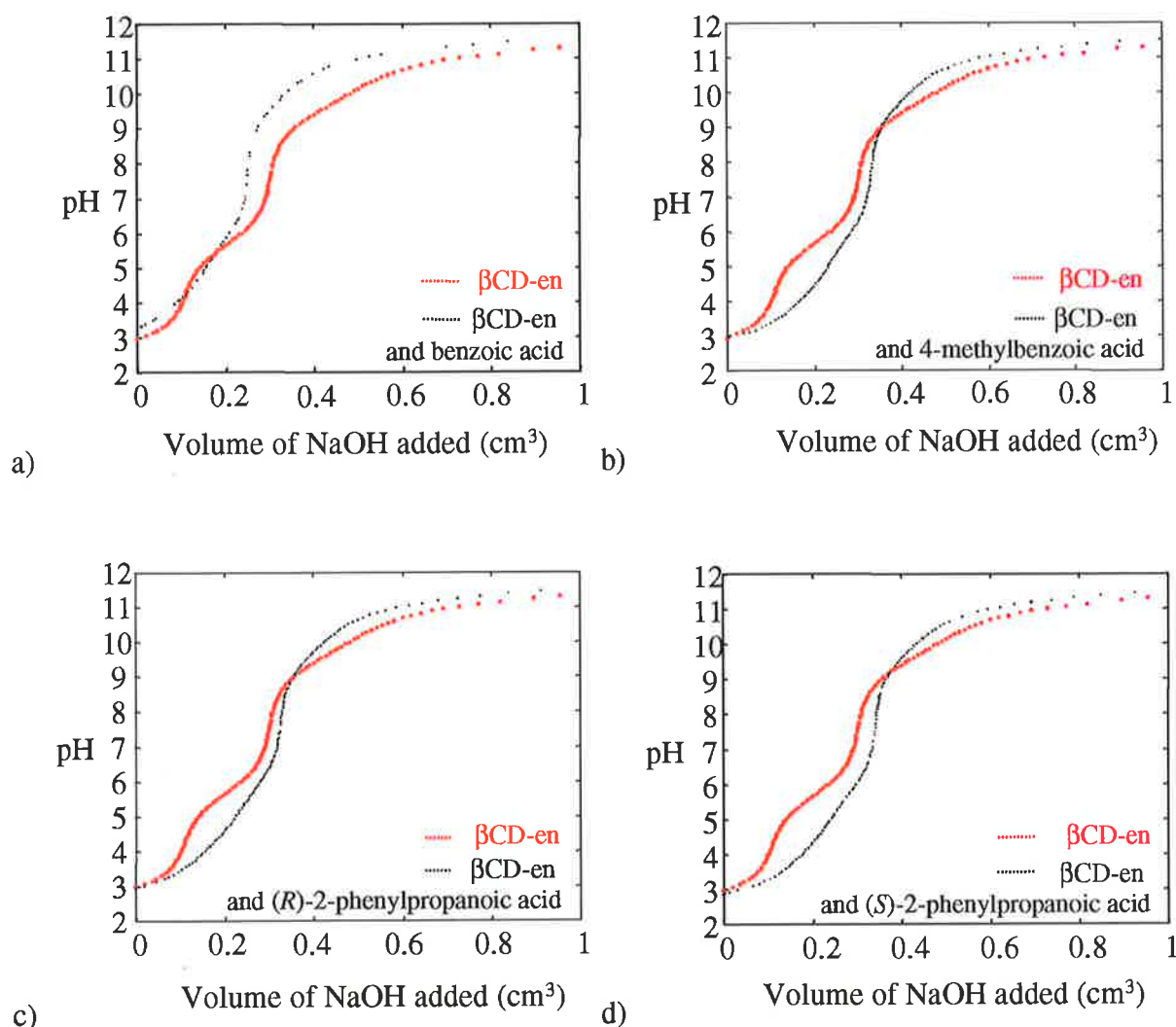


Figure A.1. The titration profiles of 6^A-deoxy-6^A-(aminoethyl)amino- β -cyclodextrin in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest acids (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) used in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. A comparison of titration profiles allowed determination of the complex formation and the derivation of stability constants. The curves represent raw data from titrations of β CD-en in the presence and absence of benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial volume of the titration solution was 10.00 cm^3 .

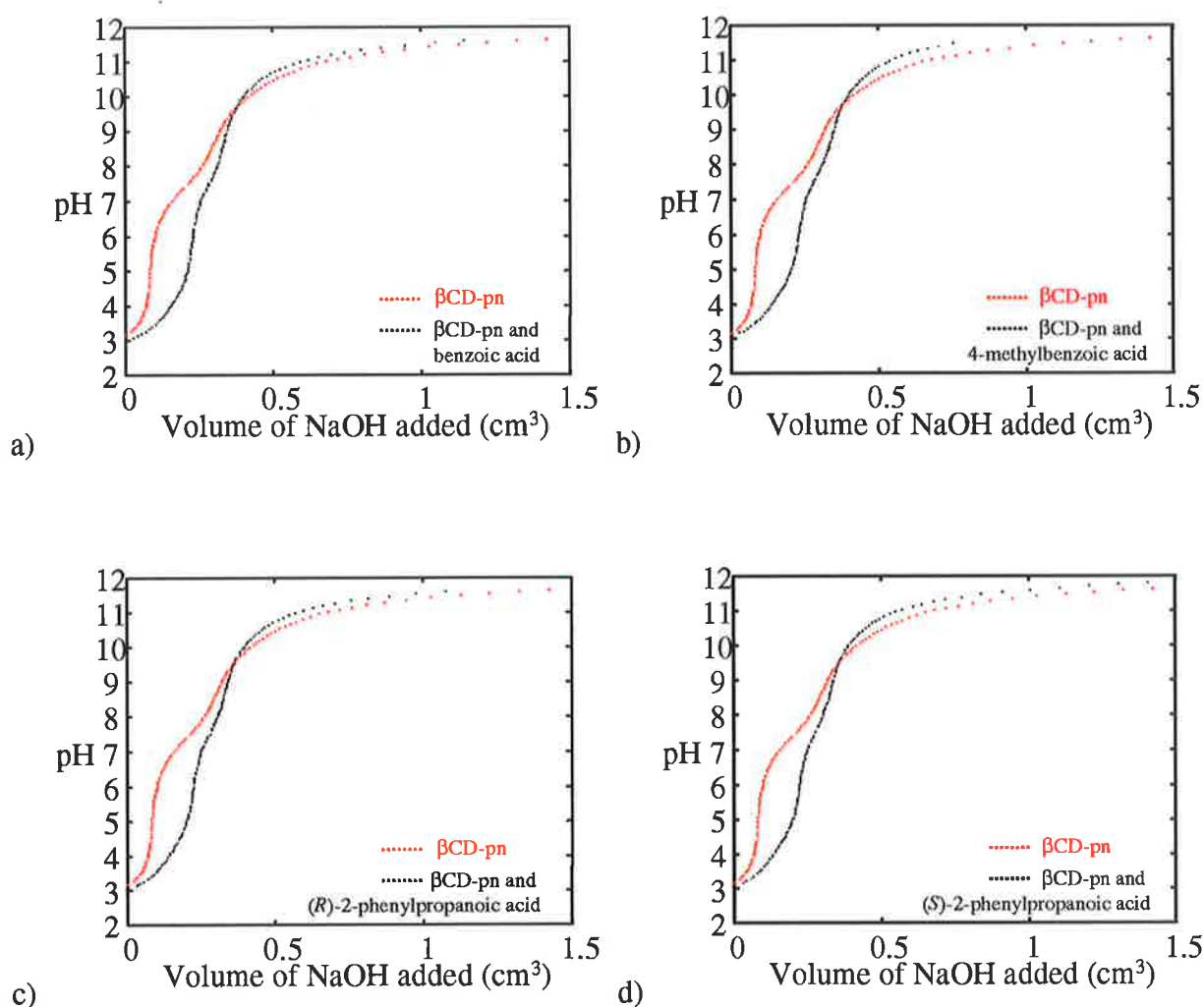


Figure A.2. The titration profiles of 6^A-deoxy-6^A-(aminopropyl)amino- β -cyclodextrin in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest acids (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) used in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. A comparison of titration profiles allowed determination of the complex formation and the derivation of stability constants. The curves represent raw data from titrations of β CD-pn in the presence and absence of benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial volume of the titration solution was 10.00 cm^3 .

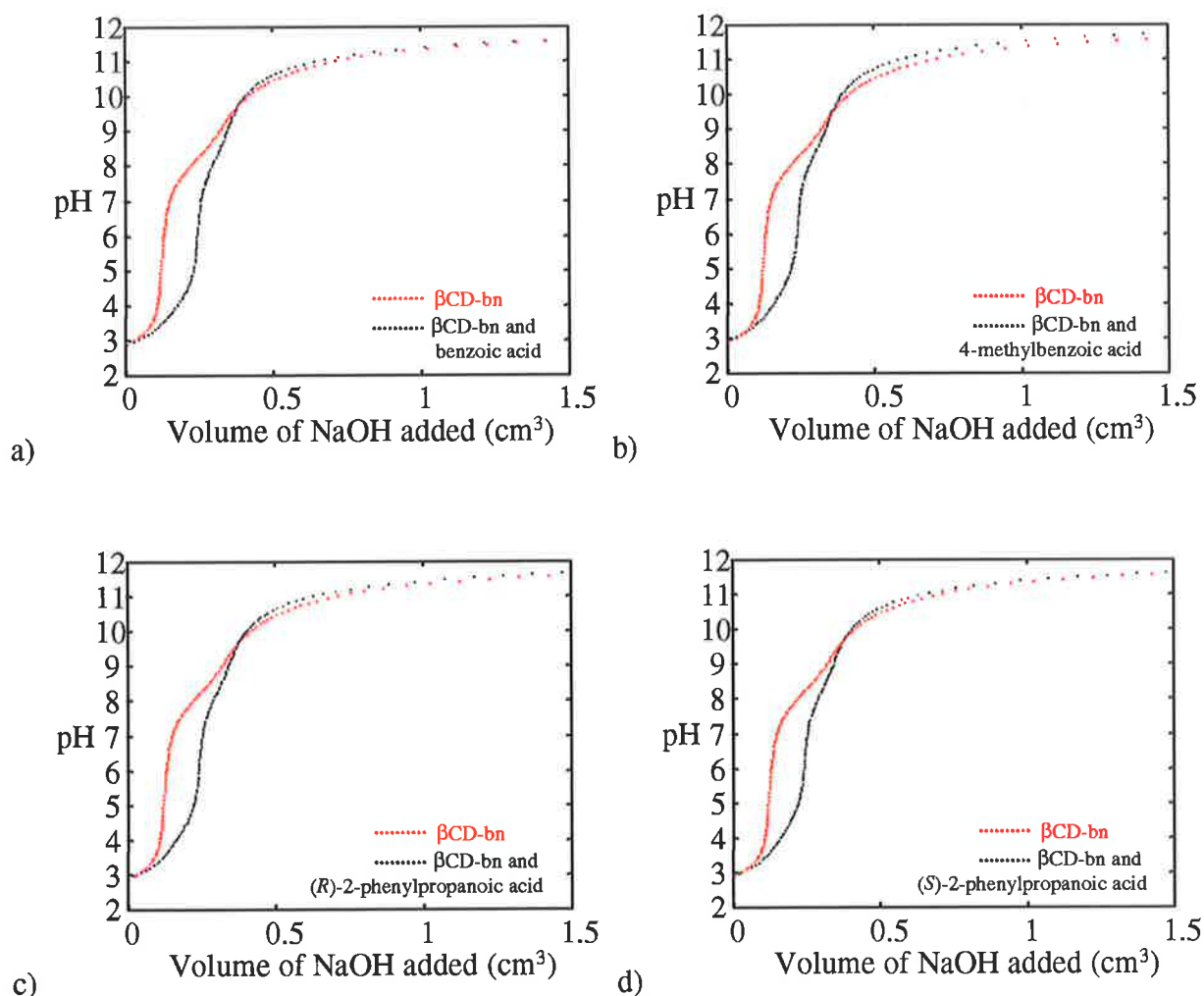


Figure A.3. The titration profiles of 6^A-deoxy-6^A-(aminobutyl)amino-β-cyclodextrin in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest acids (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) used in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. A comparison of titration profiles allowed determination of the complex formation and the derivation of stability constants. The curves represent raw data from titrations of βCD-bn in the presence and absence of benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial volume of the titration solution was 10.00 cm^3 .

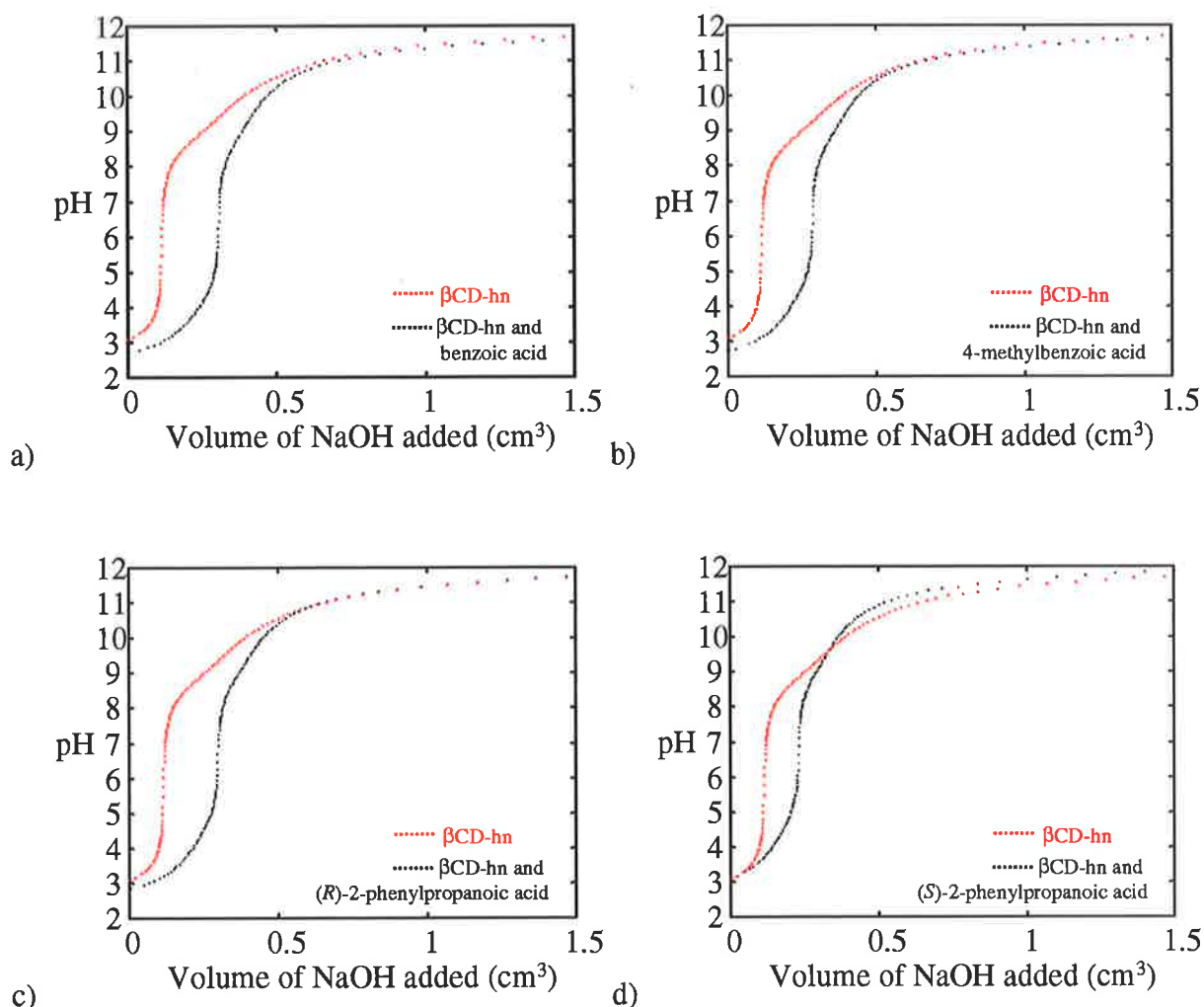


Figure A.4. The titration profiles of 6^A-deoxy-6^A-(aminohexyl)amino- β -cyclodextrin in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest acids (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) used in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. A comparison of titration profiles allowed determination of the complex formation and the derivation of stability constants. The curves represent raw data from titrations of β CD-hn in the presence and absence of benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.10 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial volume of the titration solution was 10.00 cm^3 .

APPENDIX B

The Titration Profiles for the Complexation of Aromatic Carboxylic Acids by 6^A-Deoxy-6^A-Triazaalkyl- and 6^A-Deoxy-6^A-Tetraazaalkyl- β -Cyclodextrins

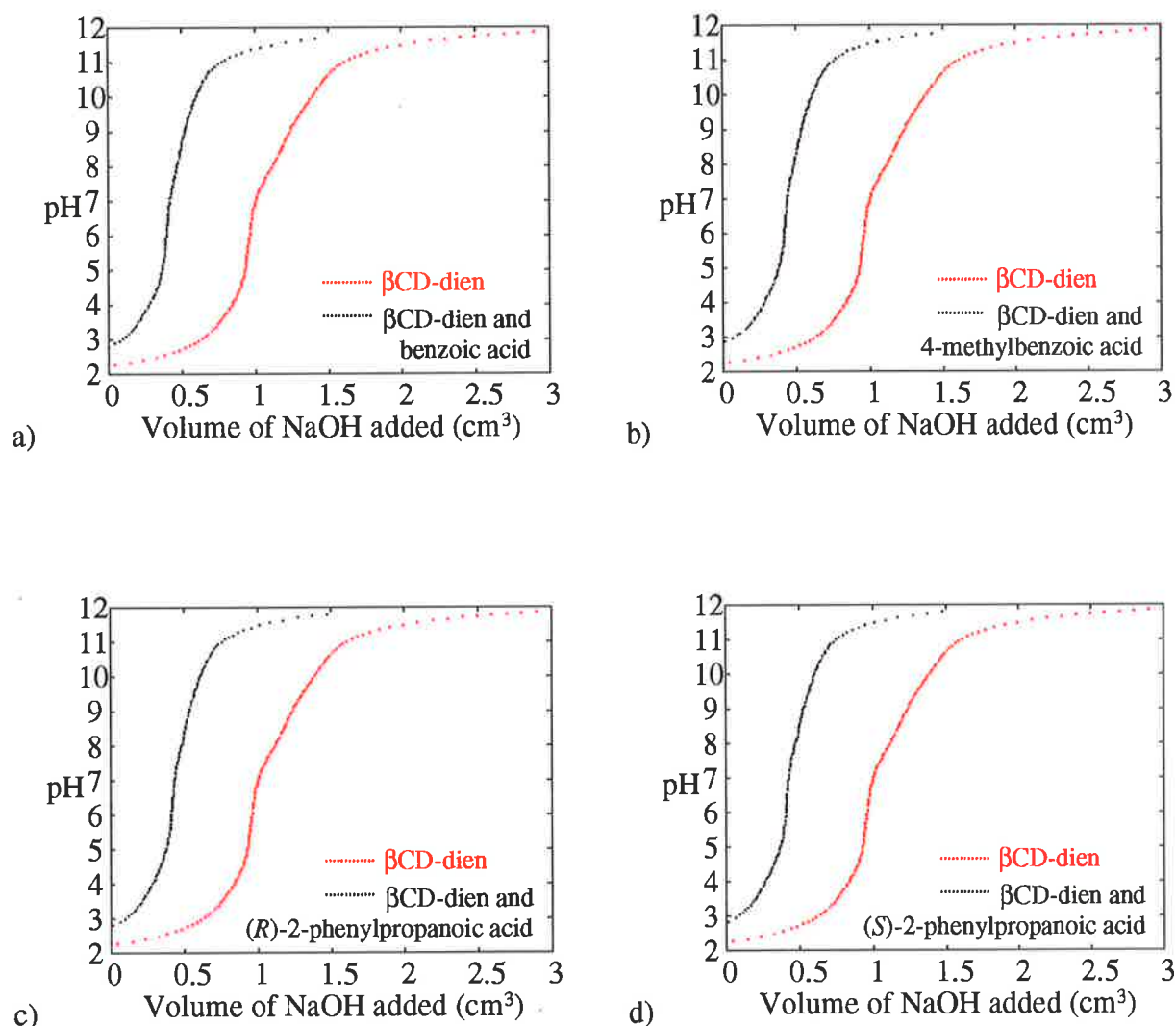


Figure B.1. The titration profiles of β CD-dien H_n^{n+} in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and $0.005 \text{ mol dm}^{-3}$ of HClO_4) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and $0.010 \text{ mol dm}^{-3}$ of HClO_4) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$). The curves represent raw data from titrations of β CD-dien in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The volumes of the initial titration solutions were 10.00 cm^3 .

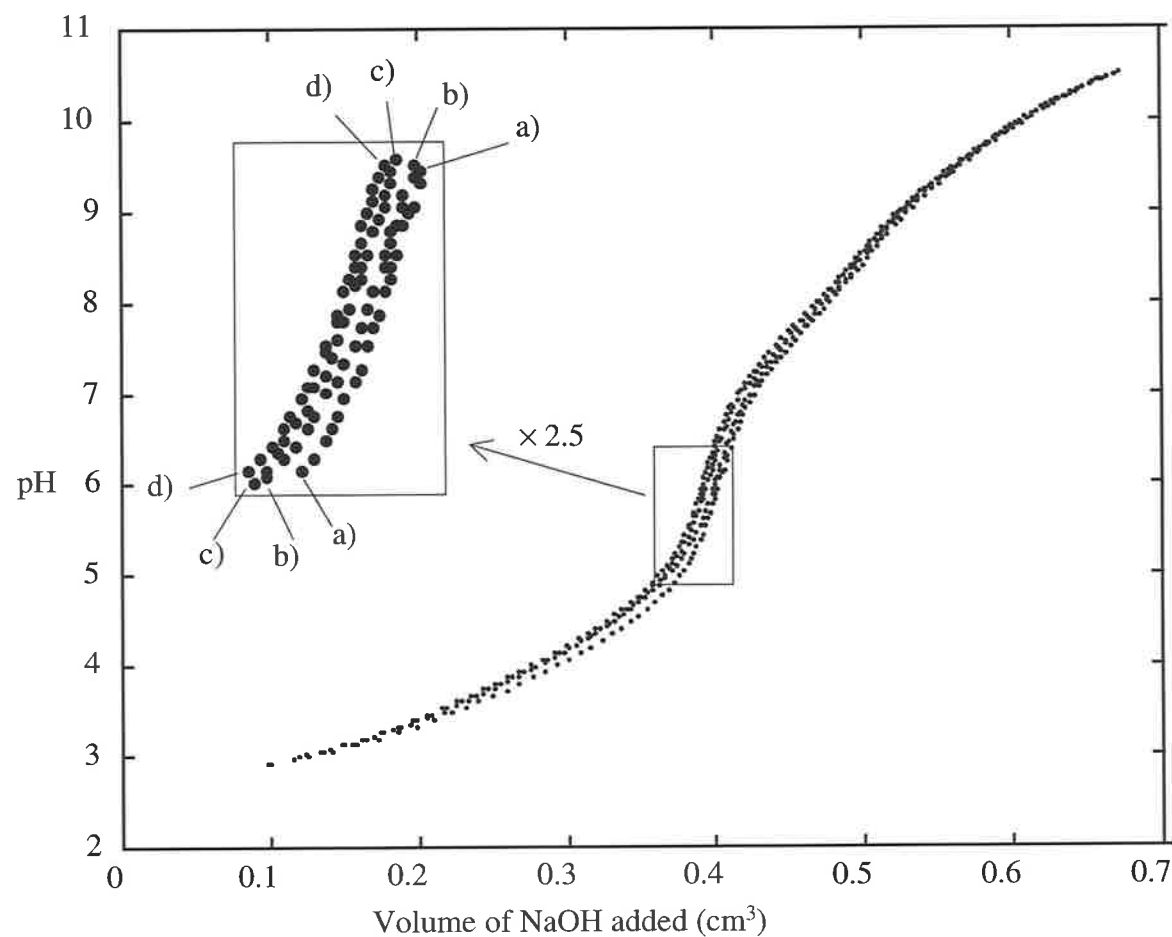


Figure B.2. The titration profiles of $\beta\text{CD-dienH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-dienH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K .

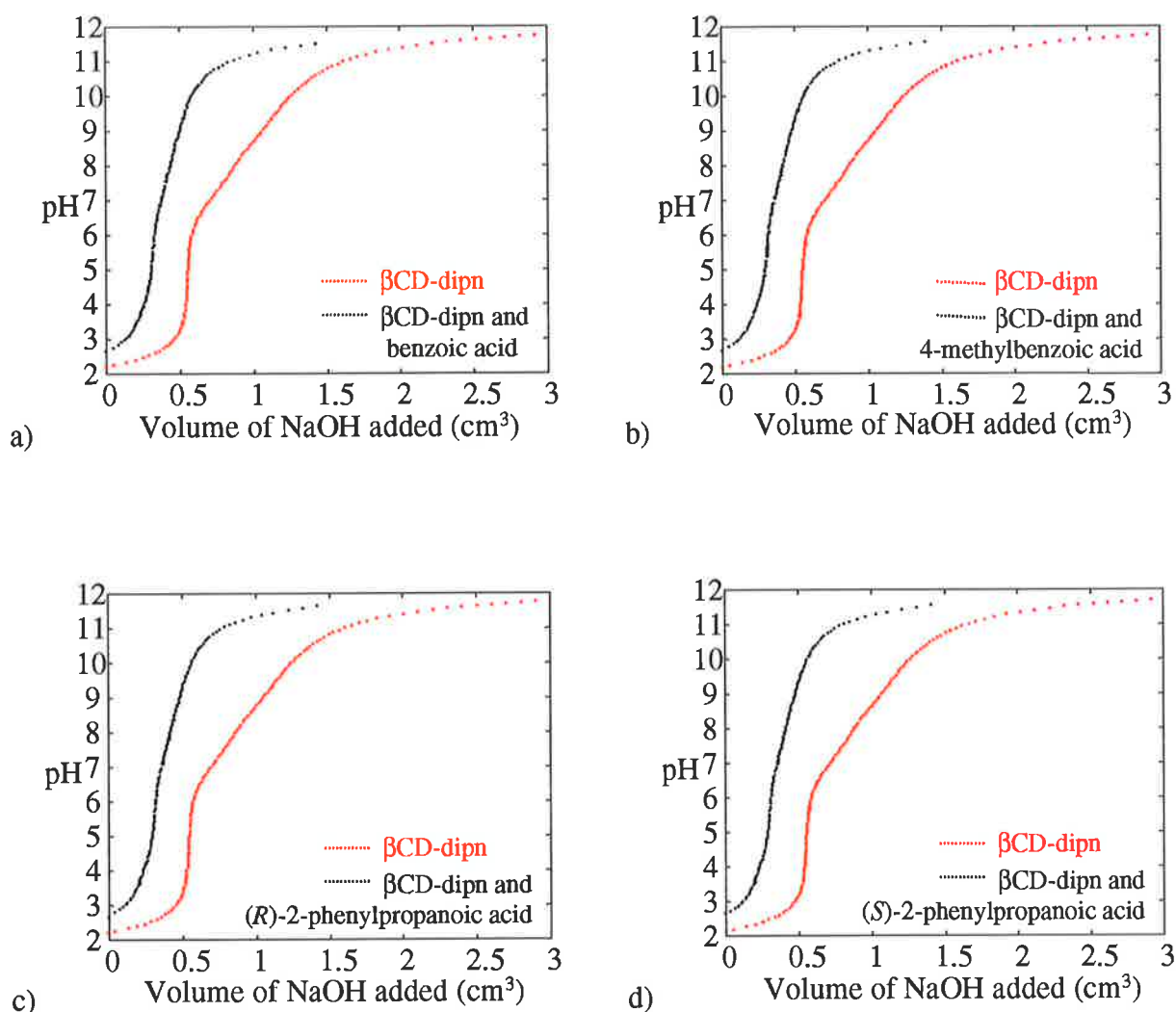


Figure B.3. The titration profiles of $\beta\text{CD-dipnH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and $0.005 \text{ mol dm}^{-3}$ of HClO_4) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and $0.001 \text{ mol dm}^{-3}$ of HClO_4) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$). The curves represent raw data from titrations of $\beta\text{CD-dipnH}_n^{n+}$ in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The volumes of the initial titration solutions were 10.00 cm^3 .

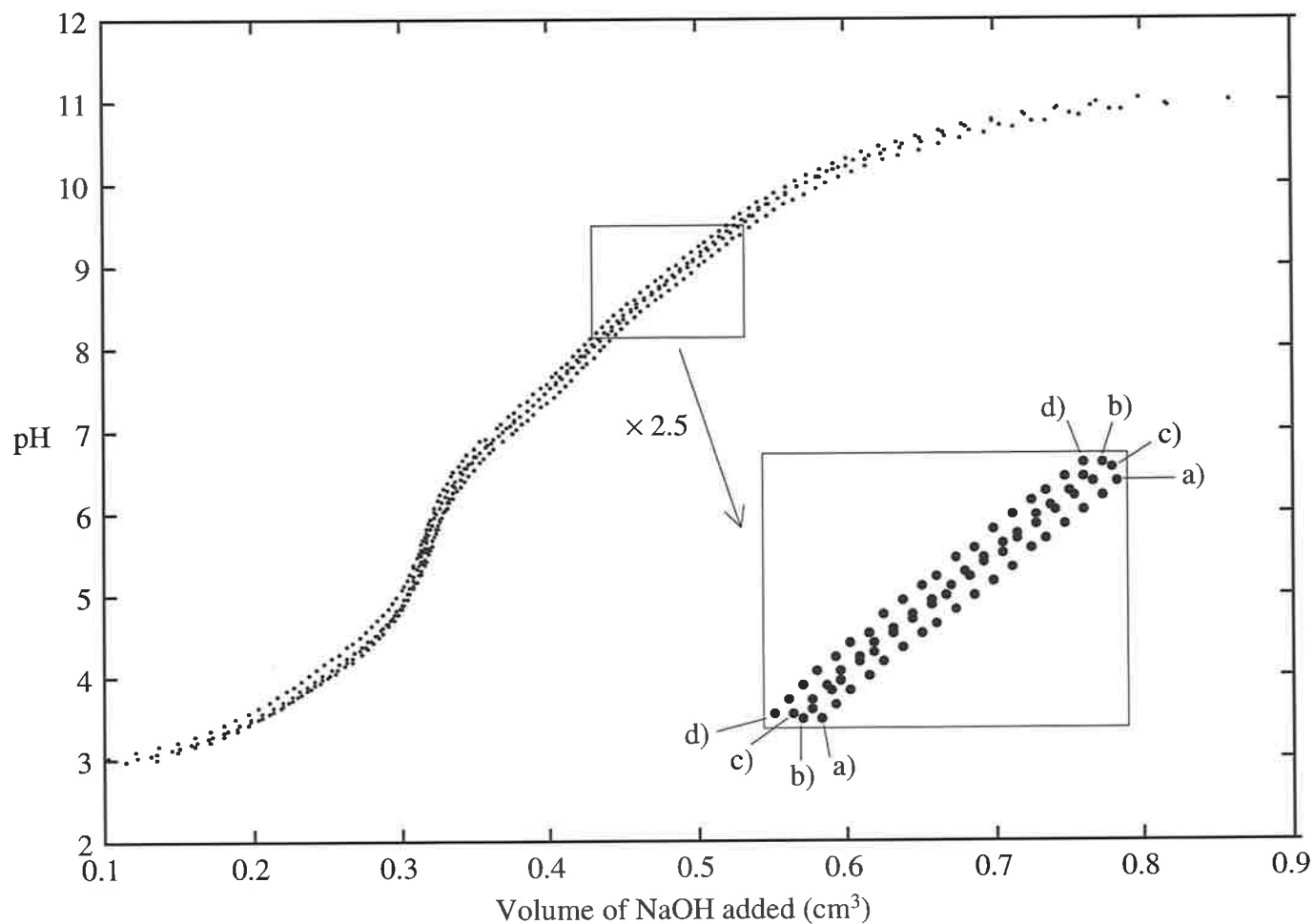


Figure B.4. The titration profiles of $\beta\text{CD-dipnH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-dipnH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K.

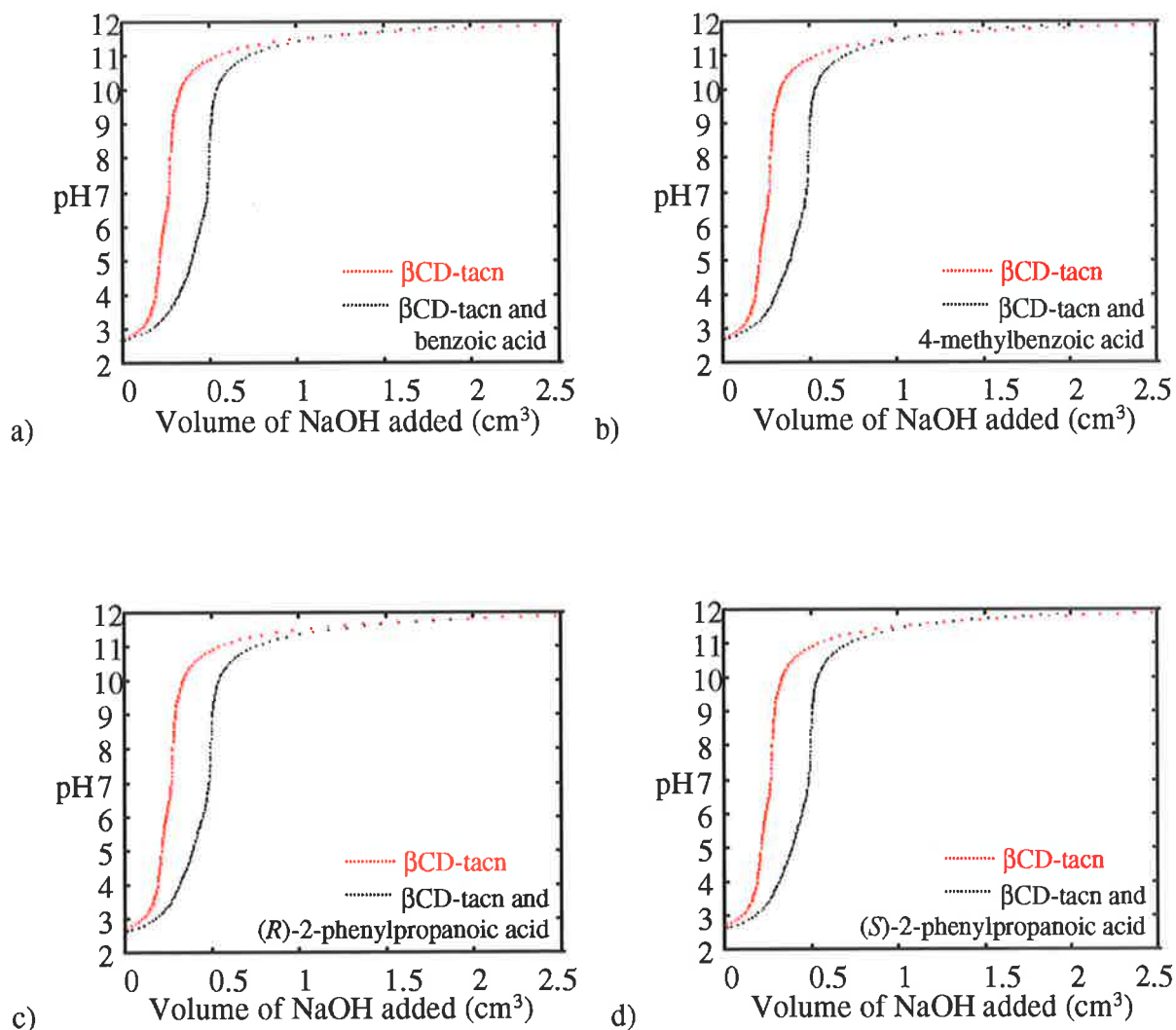


Figure B.5. The titration profiles of $\beta\text{CD-tacnH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. The curves represent raw data from titrations of $\beta\text{CD-tacnH}_n^{n+}$ in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The volumes of the initial titration solutions were 10.00 cm^3 .

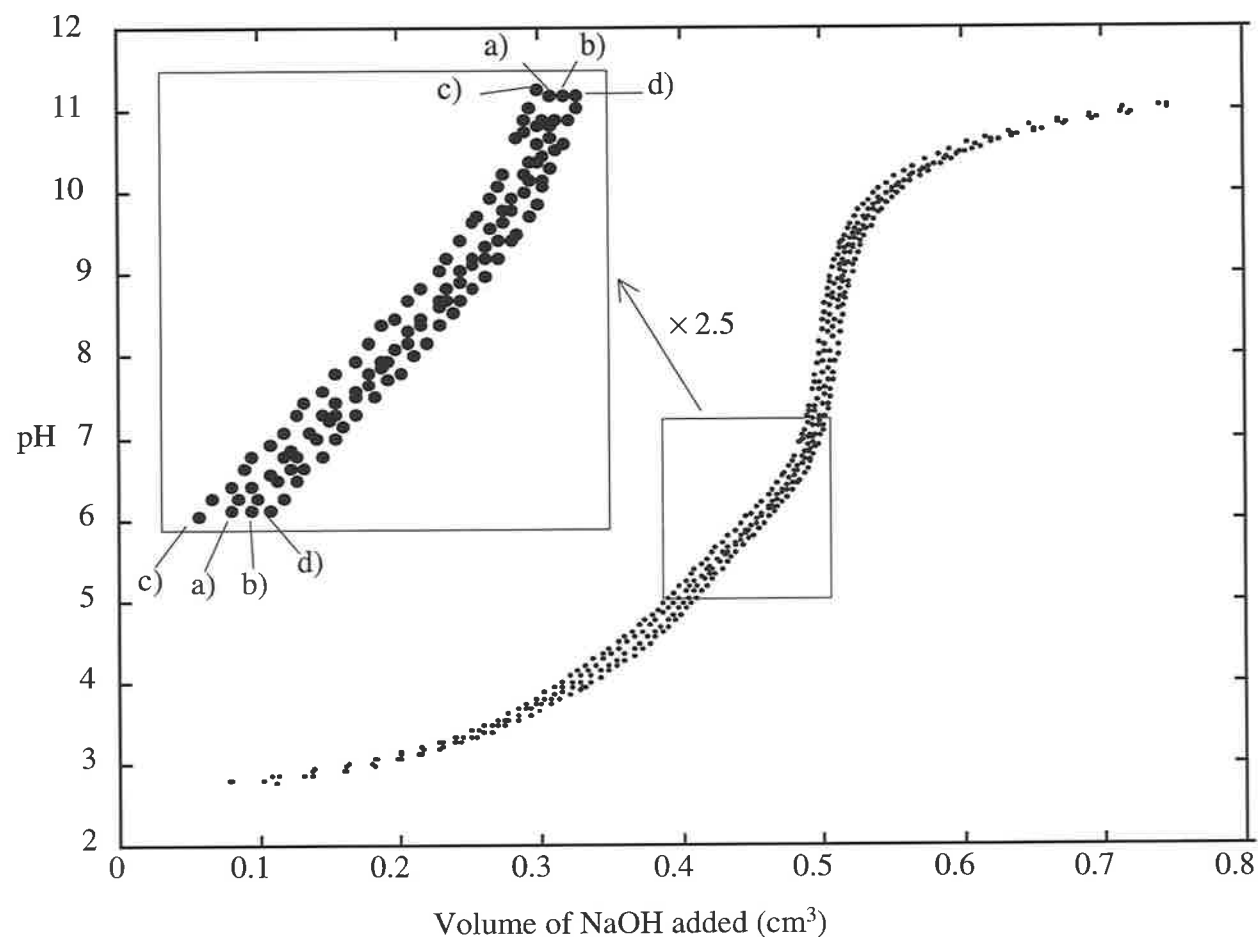


Figure B.6. The titration profiles of $\beta\text{CD-tacnH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-tacnH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K .

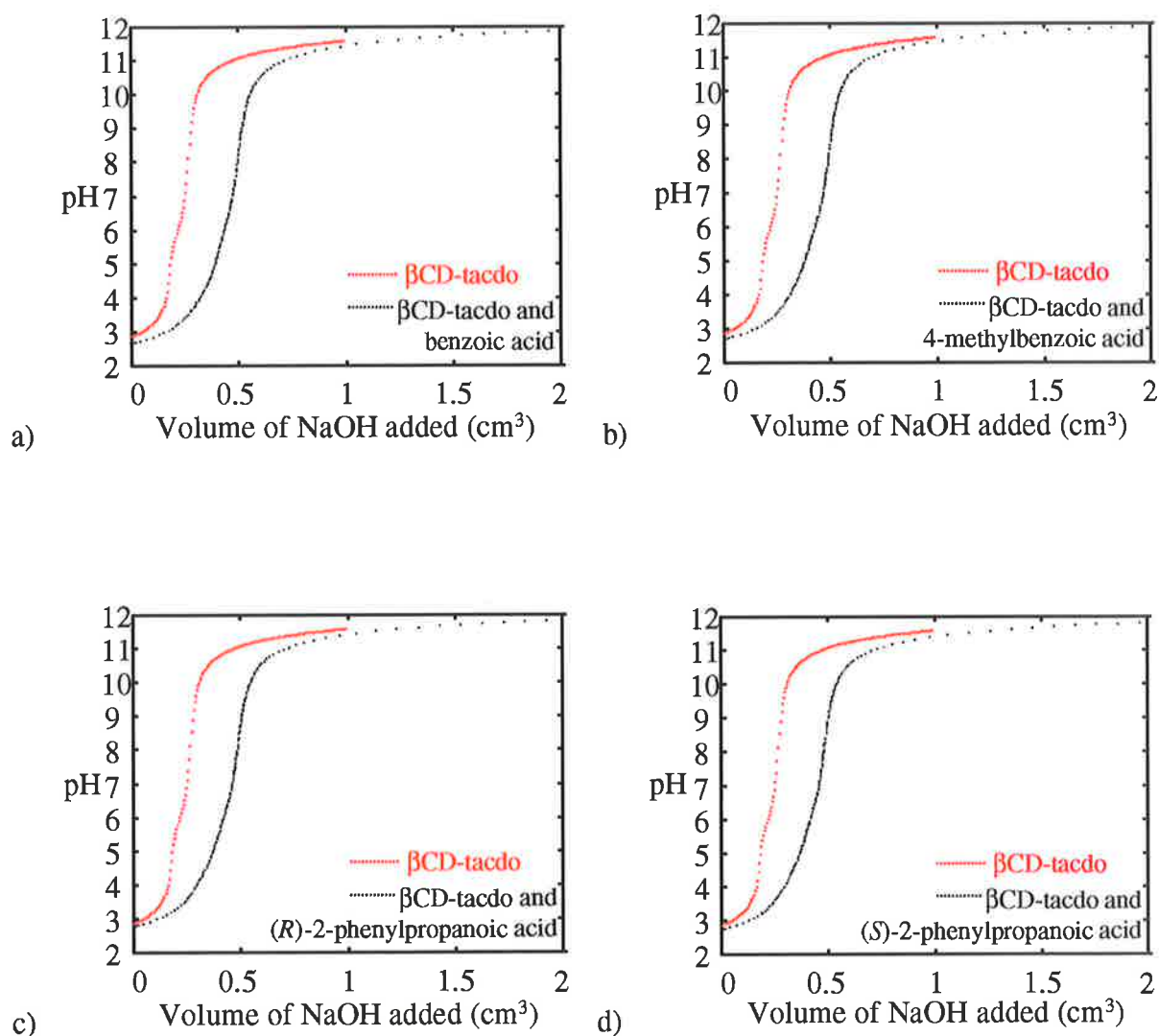


Figure B.7. The titration profiles of β CD-tacdo H_n^{n+} in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. The curves represent raw data from titrations of β CD-tacdo H_n^{n+} in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K. The volumes of the initial titration solutions were 10.00 cm^3 .

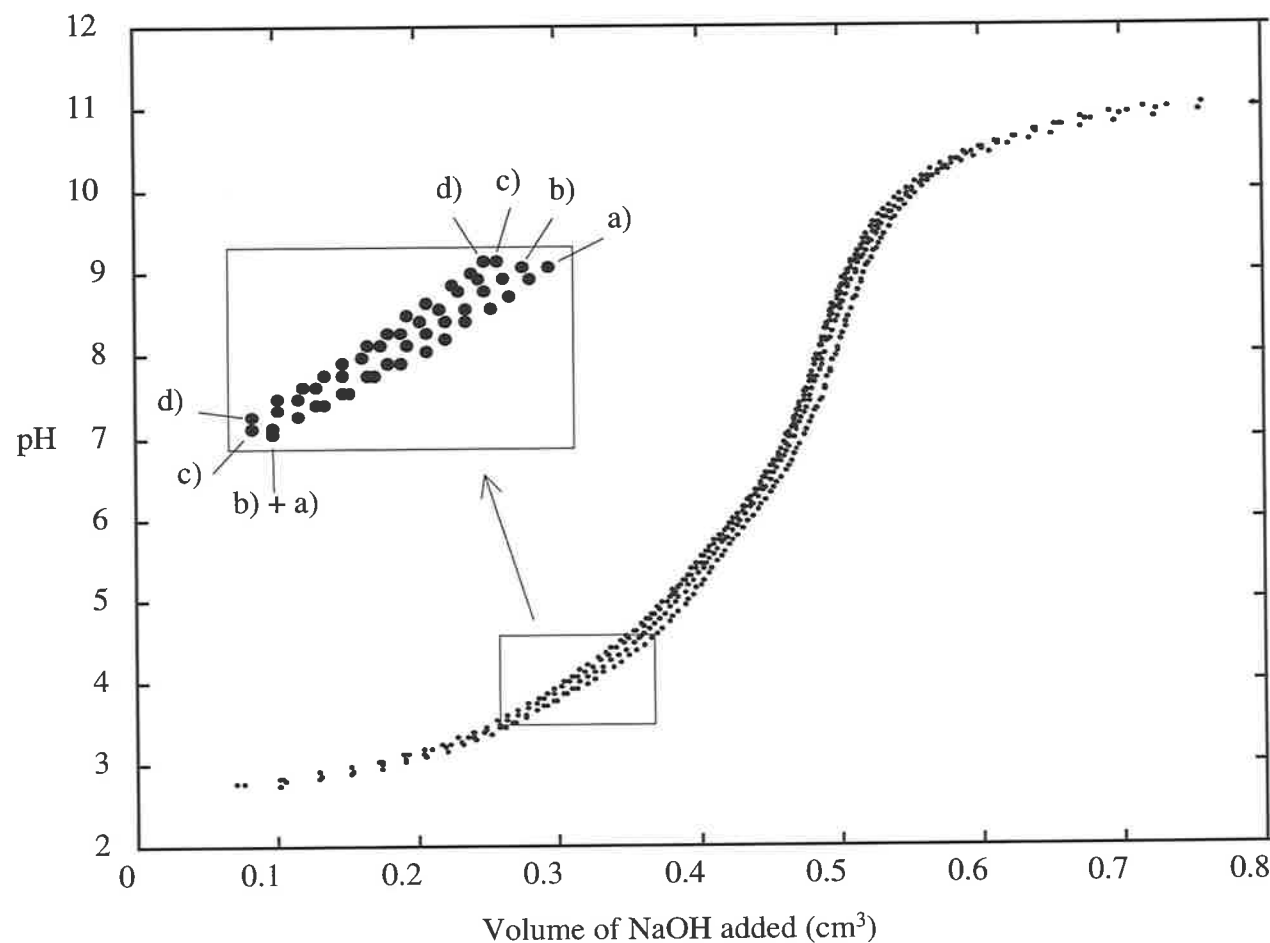


Figure B.8. The titration profiles of $\beta\text{CD-tacdoH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-tacdoH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K .

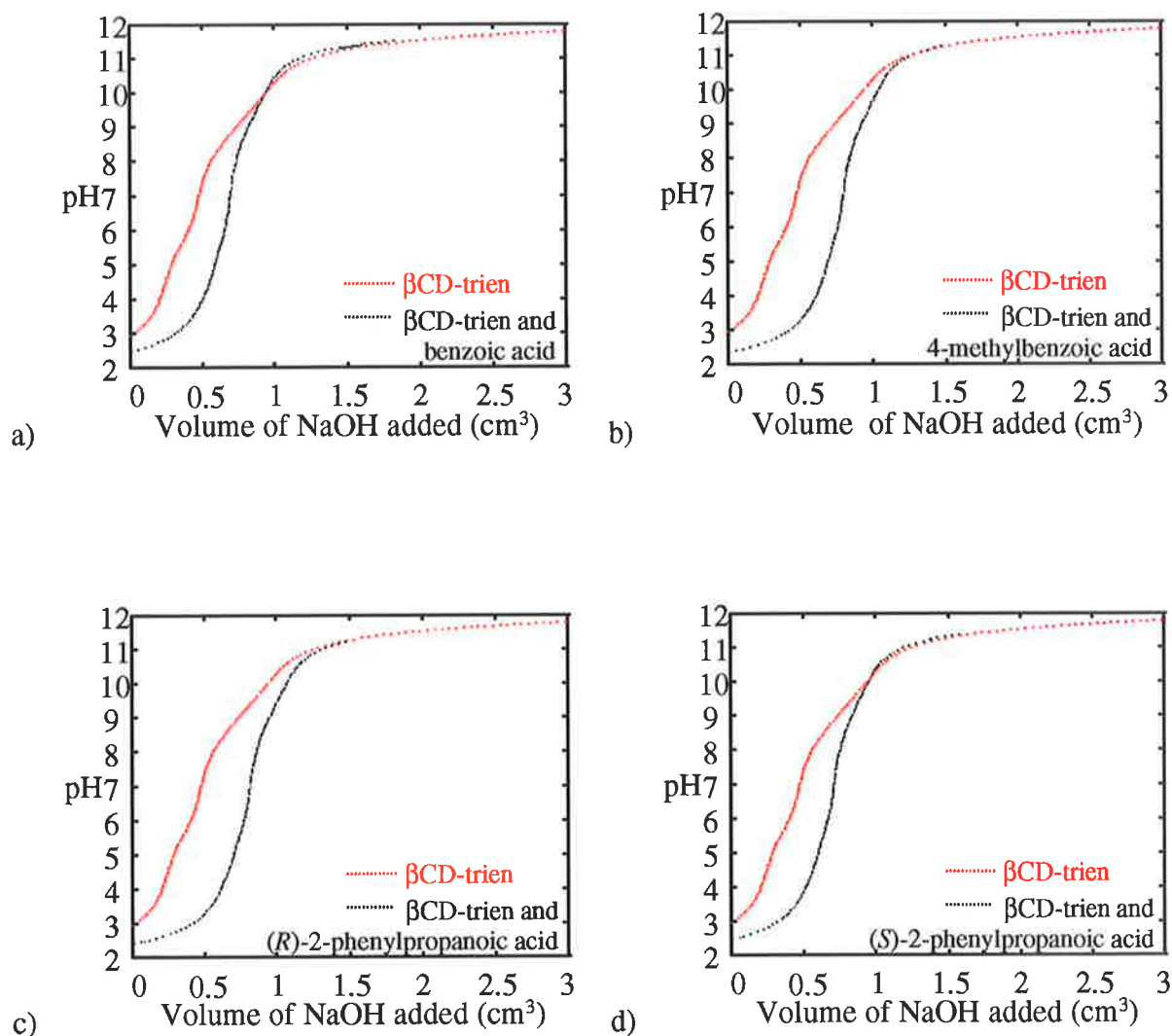


Figure B.9. The titration profiles of $\beta\text{CD-trienH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $2 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in the presence of $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$. The curves represent raw data from titrations of $\beta\text{CD-trienH}_n^{n+}$ in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The volumes of the initial titration solutions were 10.00 cm^3 .

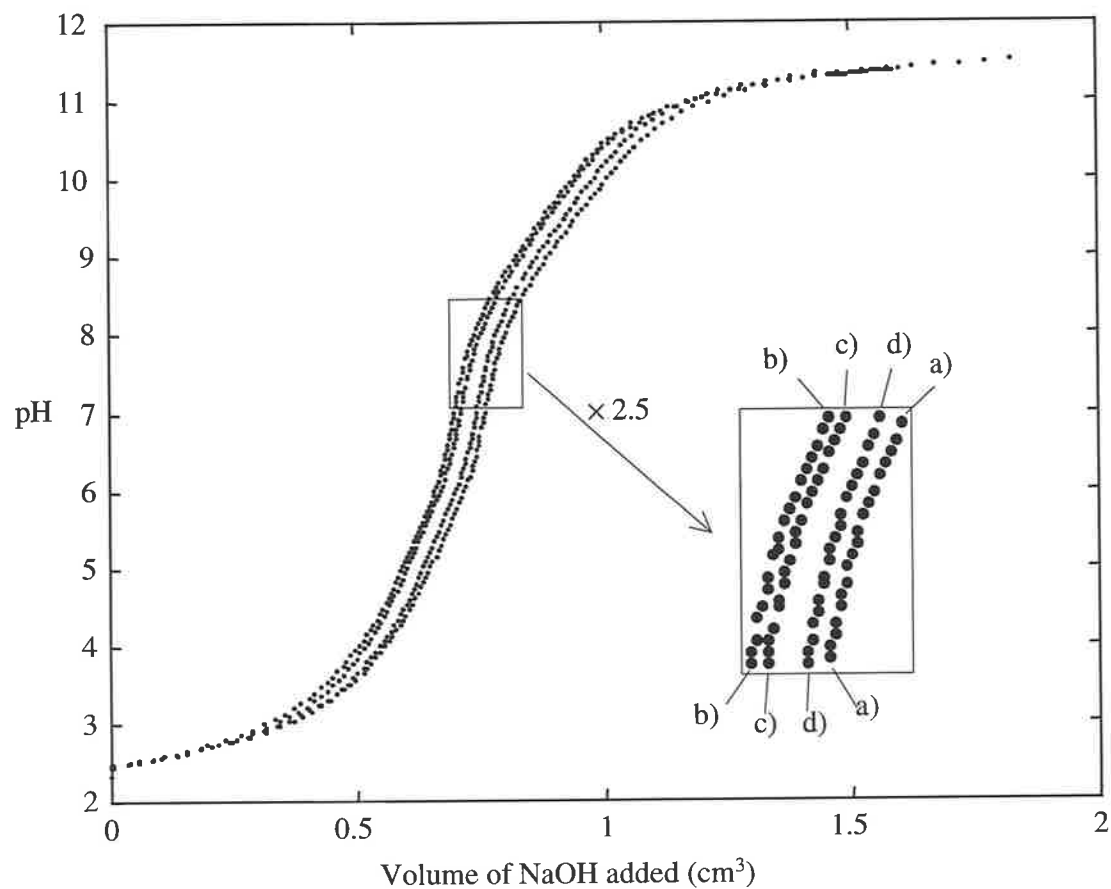


Figure B.10. The titration profiles of $\beta\text{CD-trienH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions 0.01 mol dm^{-3} in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-trienH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K .

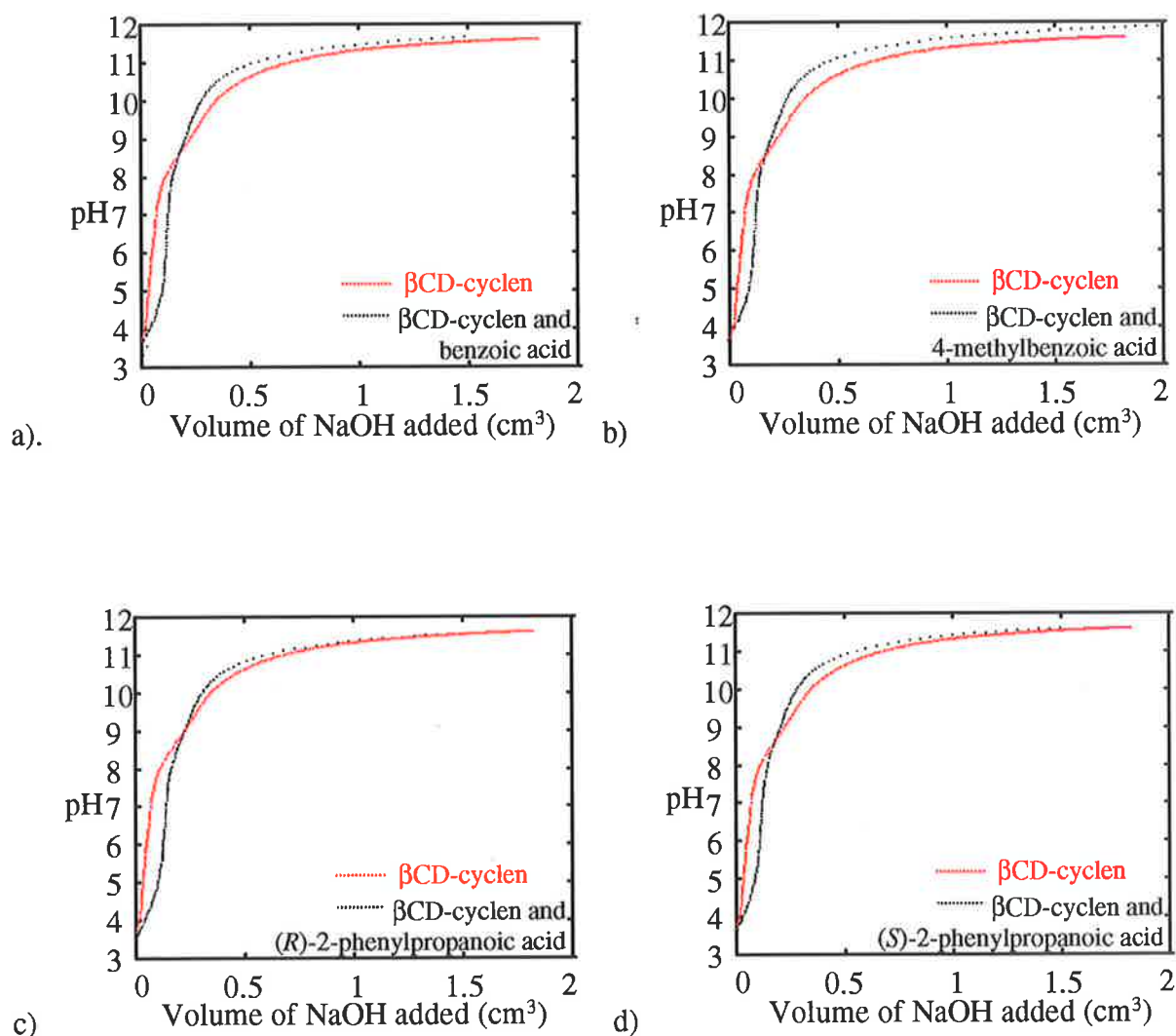


Figure B.11. The titration profiles of $\beta\text{CD-cyclenH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) and absence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in the presence of $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$. The curves represent raw data from titrations of $\beta\text{CD-cyclenH}_n^{n+}$ in the presence and absence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The volumes of the initial titration solutions were 10.00 cm^3 .

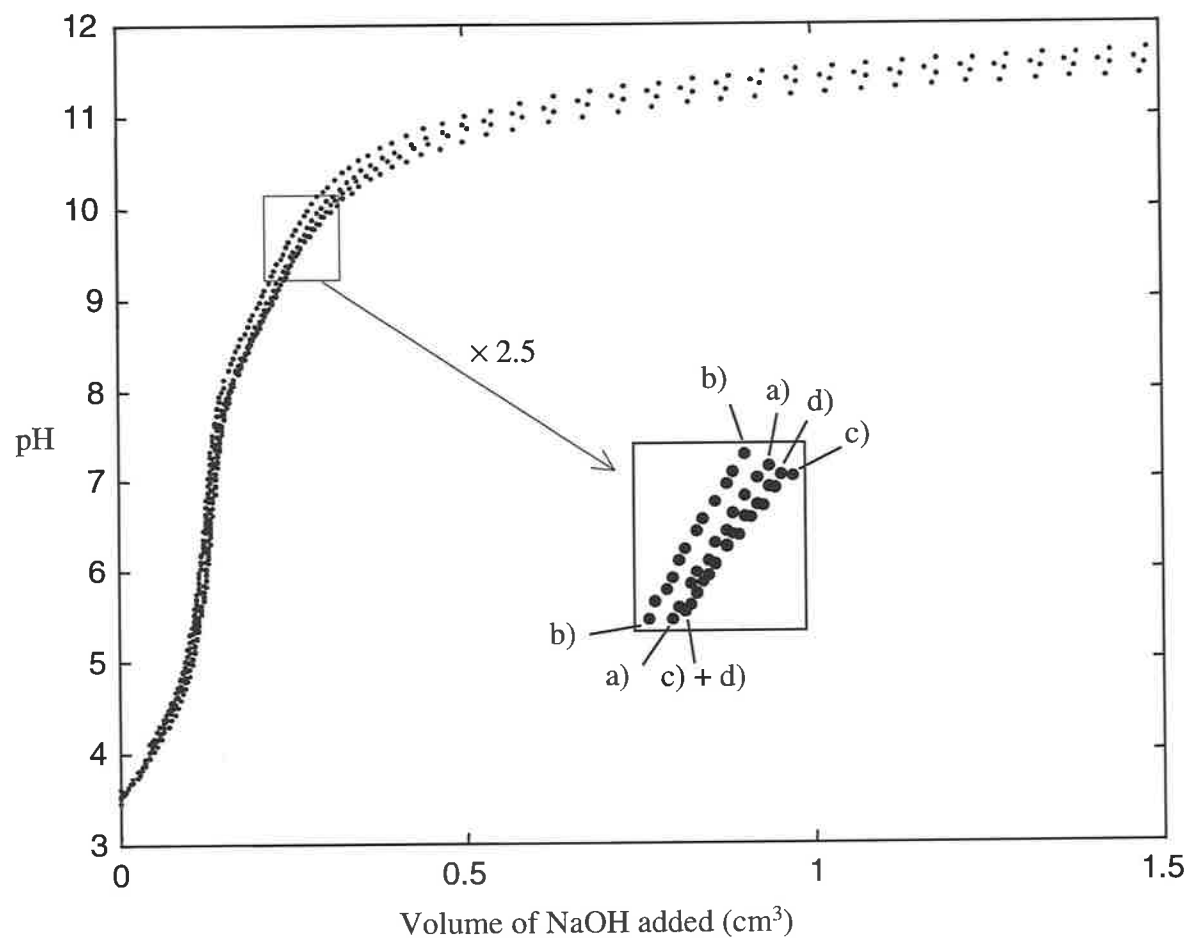


Figure B.12. The titration profiles of $\beta\text{CD-cyclenH}_n^{n+}$ in the presence (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) of each of the guest aromatic carboxylic acids used (at $1 \times 10^{-3} \text{ mol dm}^{-3}$) in solutions $0.005 \text{ mol dm}^{-3}$ in HClO_4 . The curves represent raw data from titrations of $\beta\text{CD-cyclenH}_n^{n+}$ in the presence of a) benzoic acid, b) 4-methylbenzoic acid, c) (R)-2-phenylpropanoic acid and d) (S)-2-phenylpropanoic acid, performed at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K.

APPENDIX C

The Titration Profiles for the Complexation of (*R/S*)-tryptophan/tryptophanoate and M^{2+} by the 6^A-Deoxy-6^A-Tetraazaalkyl- β -Cyclodextrins where $M^{2+} = Ni^{2+}$, Cu^{2+} and Zn^{2+} .

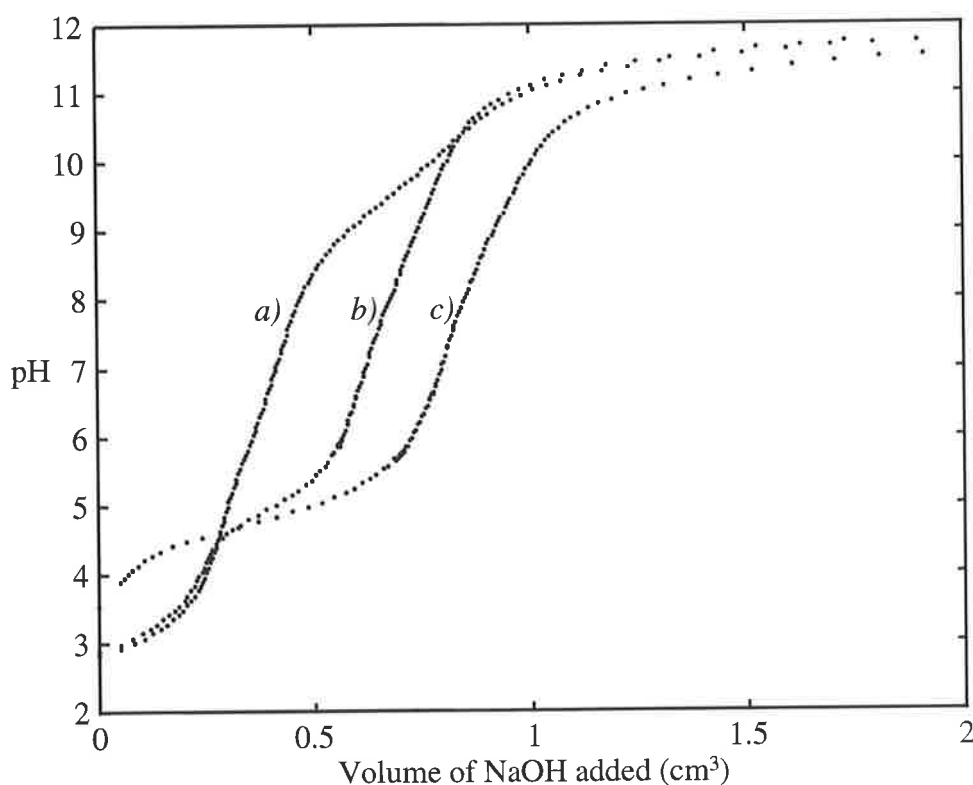


Figure C.1. The full pH profiles of a) β CD-trien H_n^{n+} and (*R*)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-trien H_n^{n+} , Ni^{2+} and (*R*)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-trien H_n^{n+} and Ni^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

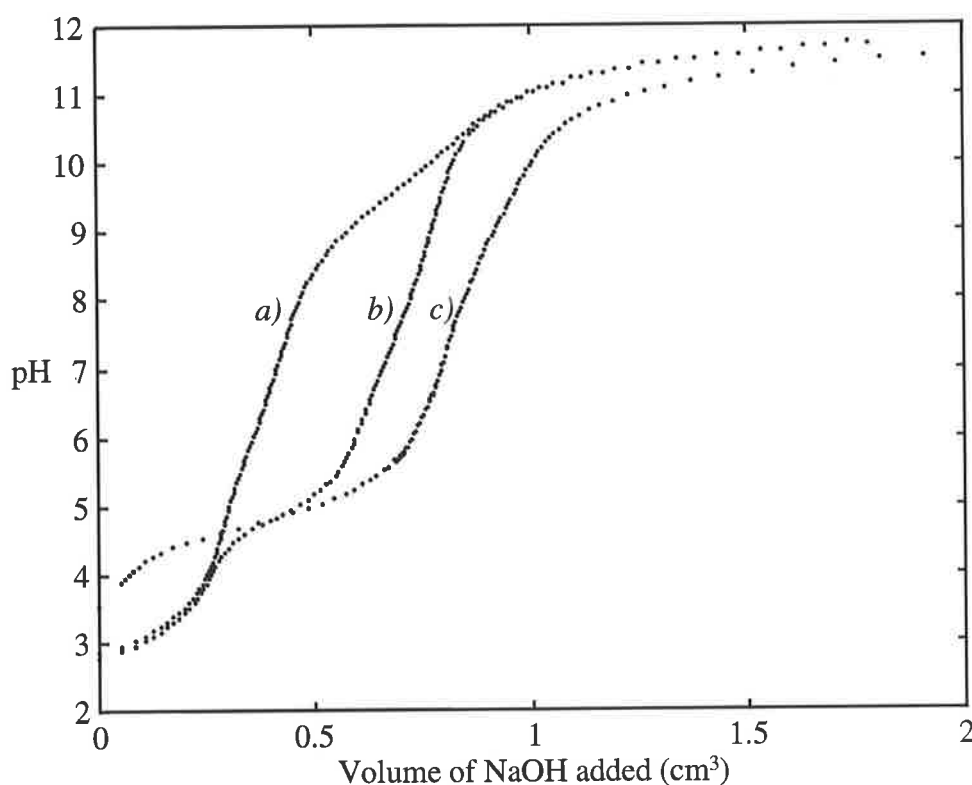


Figure C.2. The full pH profiles of a) β CD-trien H_n^{n+} and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-trien H_n^{n+} , Ni^{2+} and (S)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-trien H_n^{n+} and Ni^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

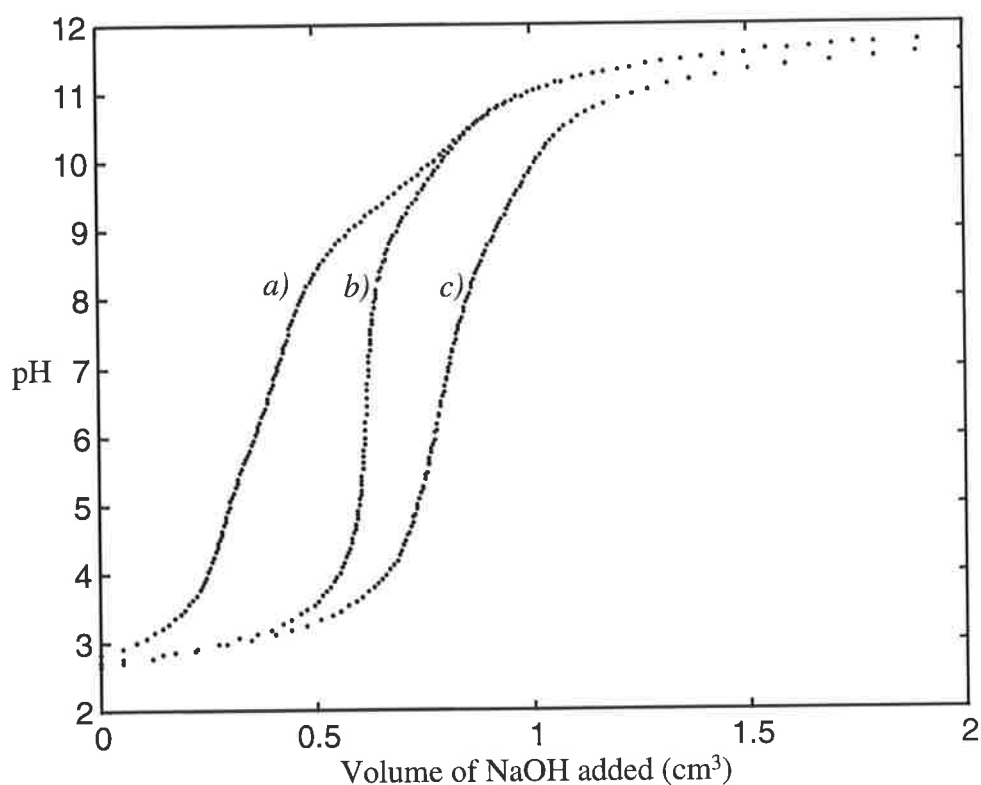


Figure C.3. The full pH profiles of a) β CD-trien H_n^{n+} and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-trien H_n^{n+} , Cu^{2+} and (R)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-trien H_n^{n+} and Cu^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

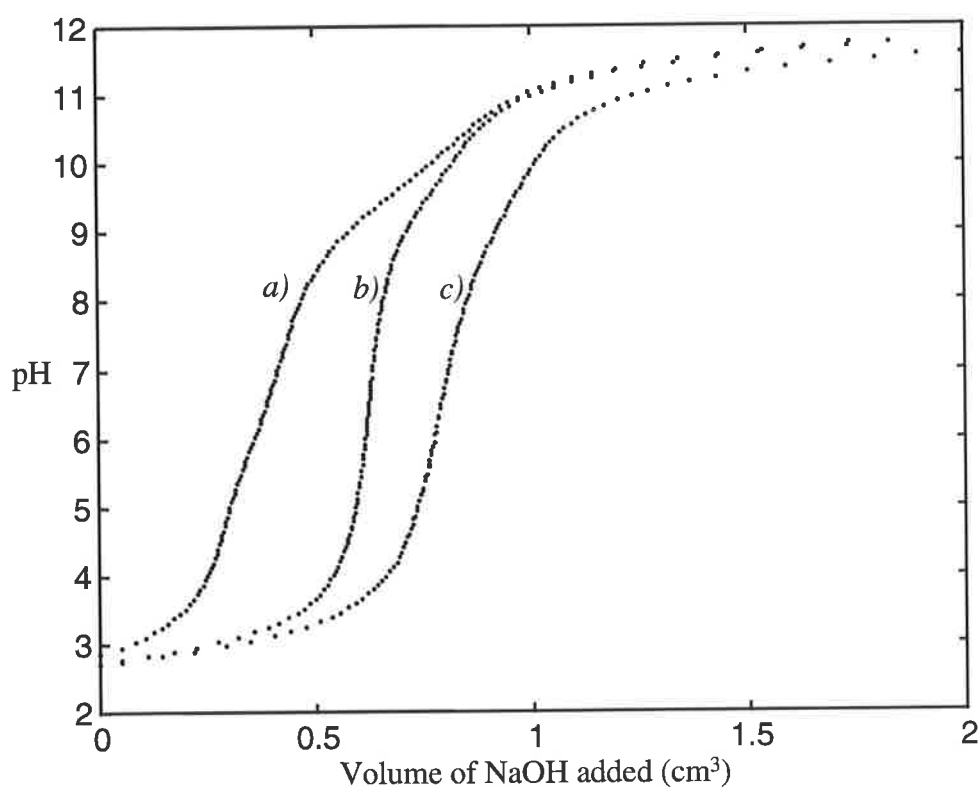


Figure C.4. The full pH profiles of a) β CD-trienH_nⁿ⁺ and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-trienH_nⁿ⁺, Cu²⁺ and (S)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-trienH_nⁿ⁺ and Cu²⁺ both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO₄) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

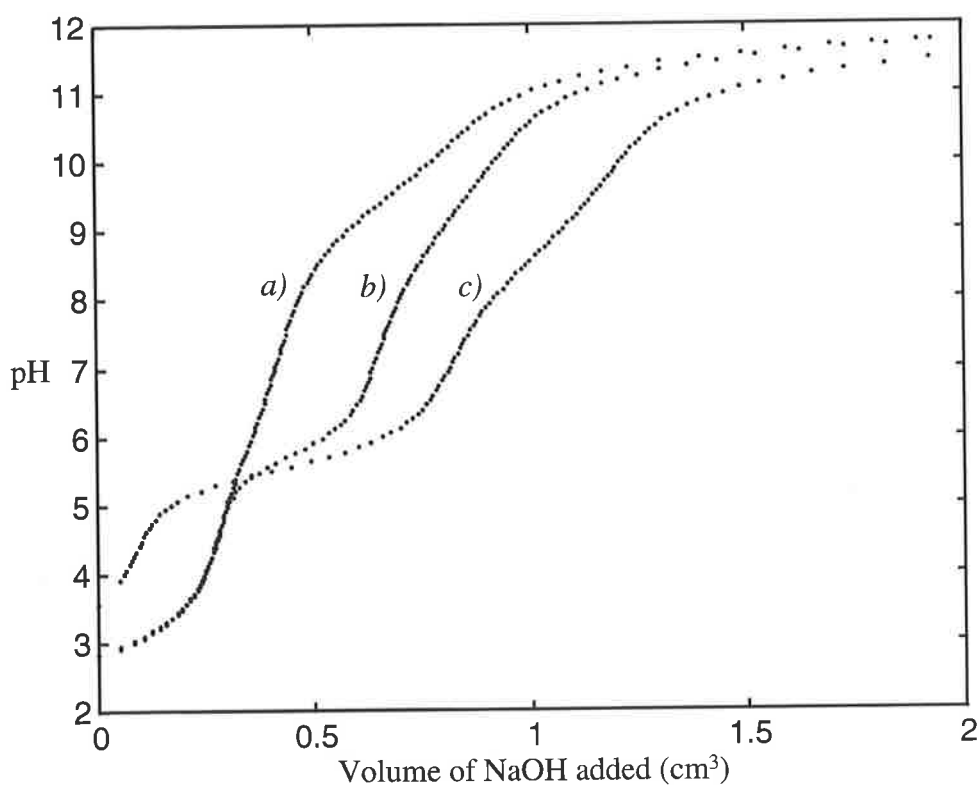


Figure C.5. The full pH profiles of a) β CD-trien H_n^{n+} and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-trien H_n^{n+} , Zn^{2+} and (R)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-trien H_n^{n+} and Zn^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

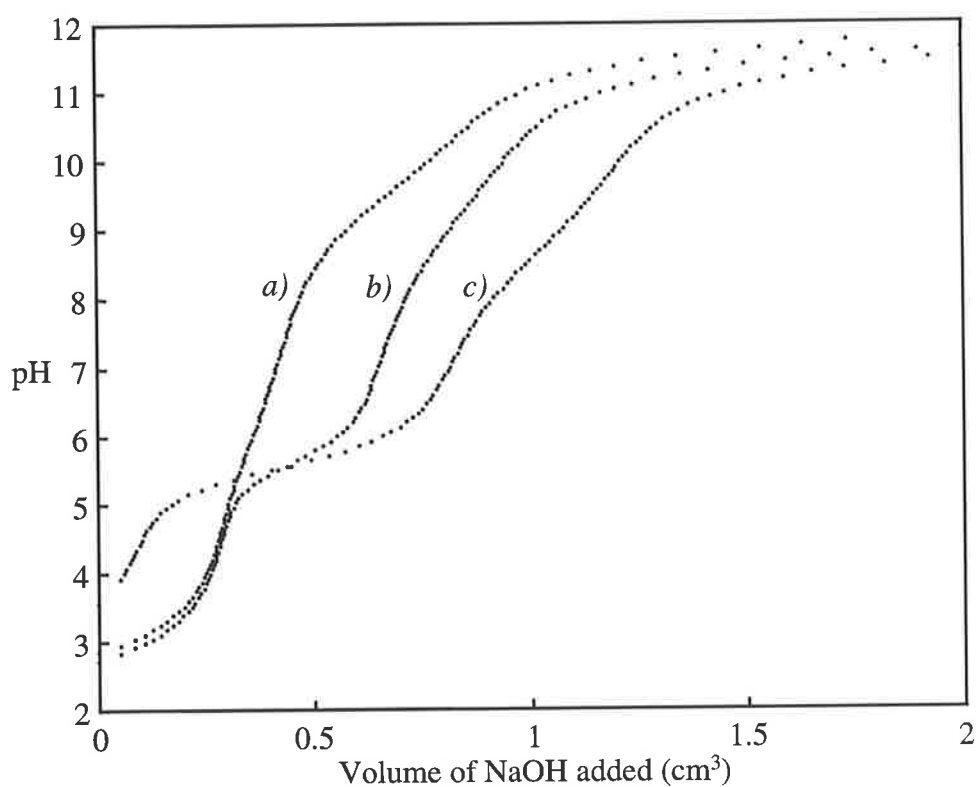


Figure C.6. The full pH profiles of a) $\beta\text{CD-trienH}_n^{n+}$ and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) $\beta\text{CD-trienH}_n^{n+}$, Zn^{2+} and (S)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) $\beta\text{CD-trienH}_n^{n+}$ and Zn^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.010 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3}$ (NaClO_4) and 298.2 K . The initial titration solutions were 10 cm^3 in volume.

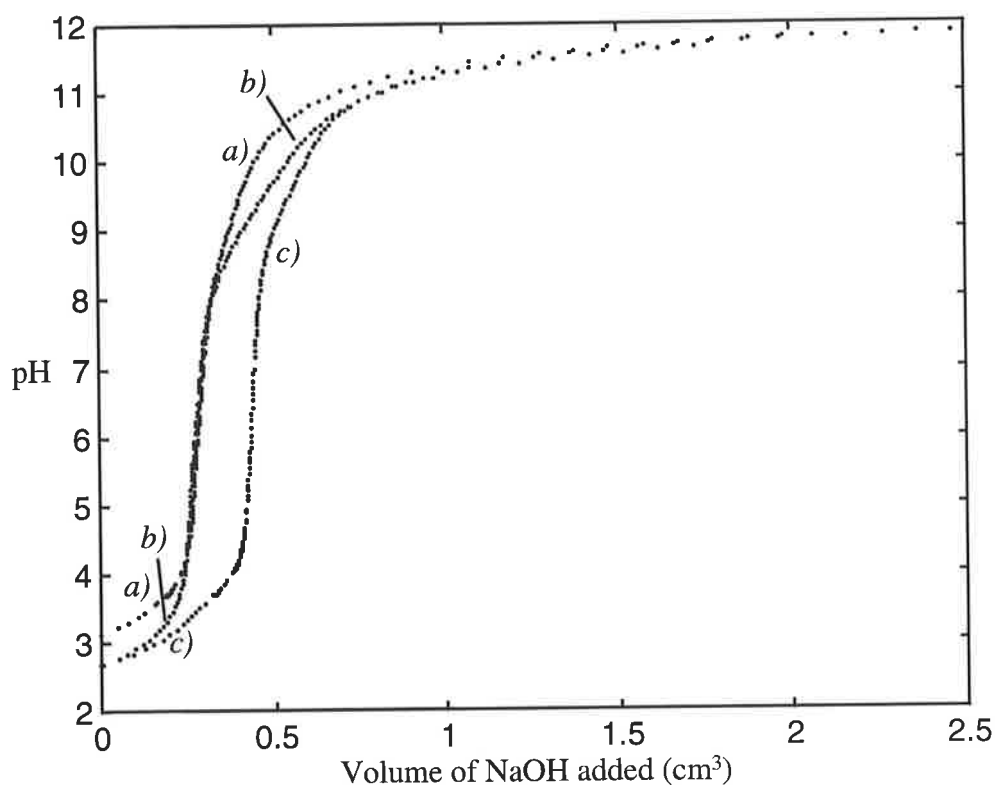


Figure C.7. The full pH profiles of a) $\beta\text{CD-cyclenH}_n^{n+}$ and Cu^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ b) $\beta\text{CD-cyclenH}_n^{n+}$ and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) $\beta\text{CD-cyclenH}_n^{n+}$, Cu^{2+} and (R)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial titration solutions were 10 cm^3 in volume.

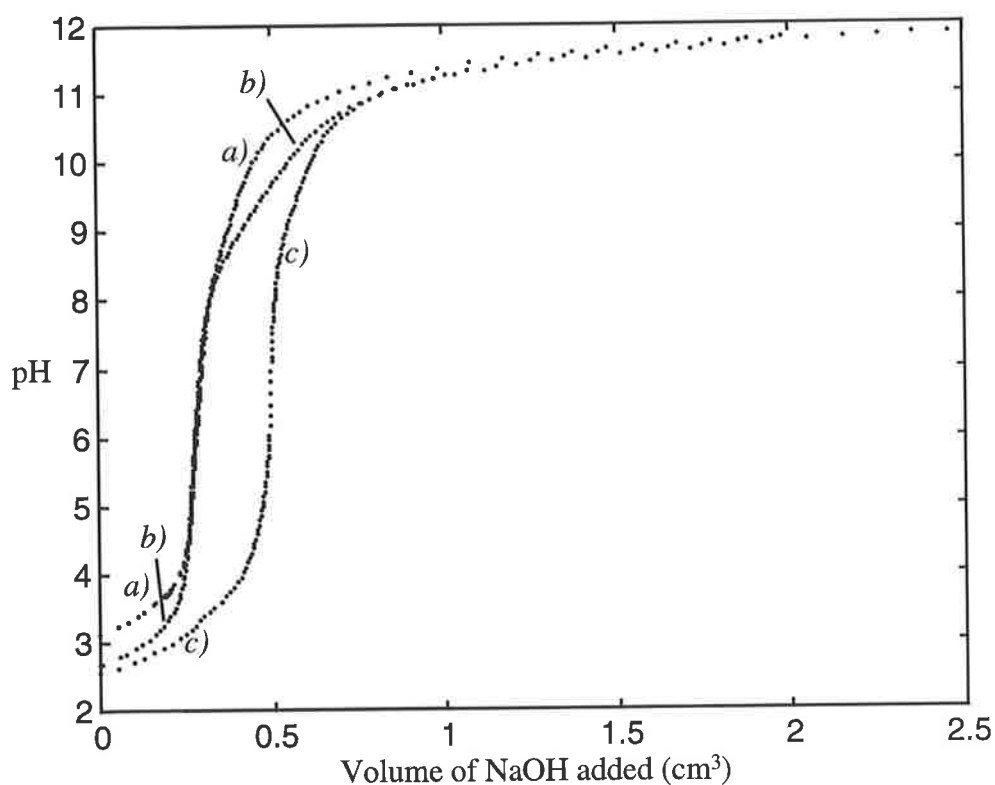


Figure C.8. The full pH profiles of a) $\beta\text{CD-cyclenH}_n^{n+}$ and Cu^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ b) $\beta\text{CD-cyclenH}_n^{n+}$ and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ c) $\beta\text{CD-cyclenH}_n^{n+}$, Cu^{2+} and (S)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial titration solutions were 10 cm^3 in volume.

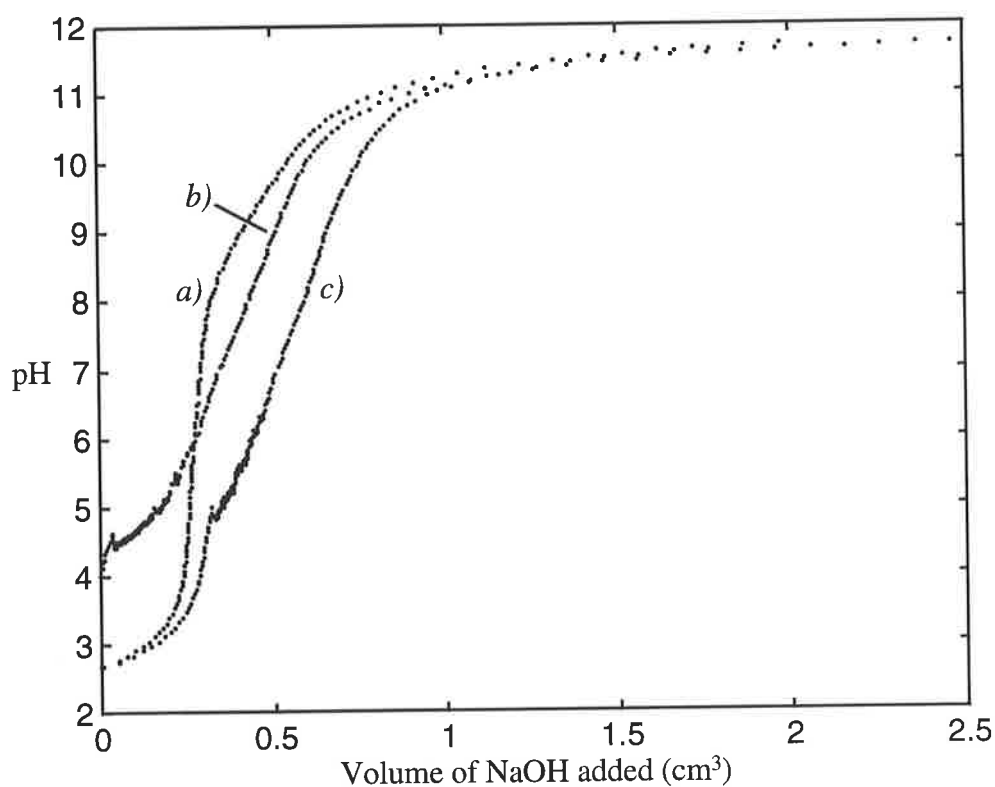


Figure C.9. The full pH profiles of a) β CD-cyclen H_n^{n+} and (R)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-cyclen H_n^{n+} and Zn^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-cyclen H_n^{n+} , Zn^{2+} and (R)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K. The initial titration solutions were 10 cm^3 in volume.

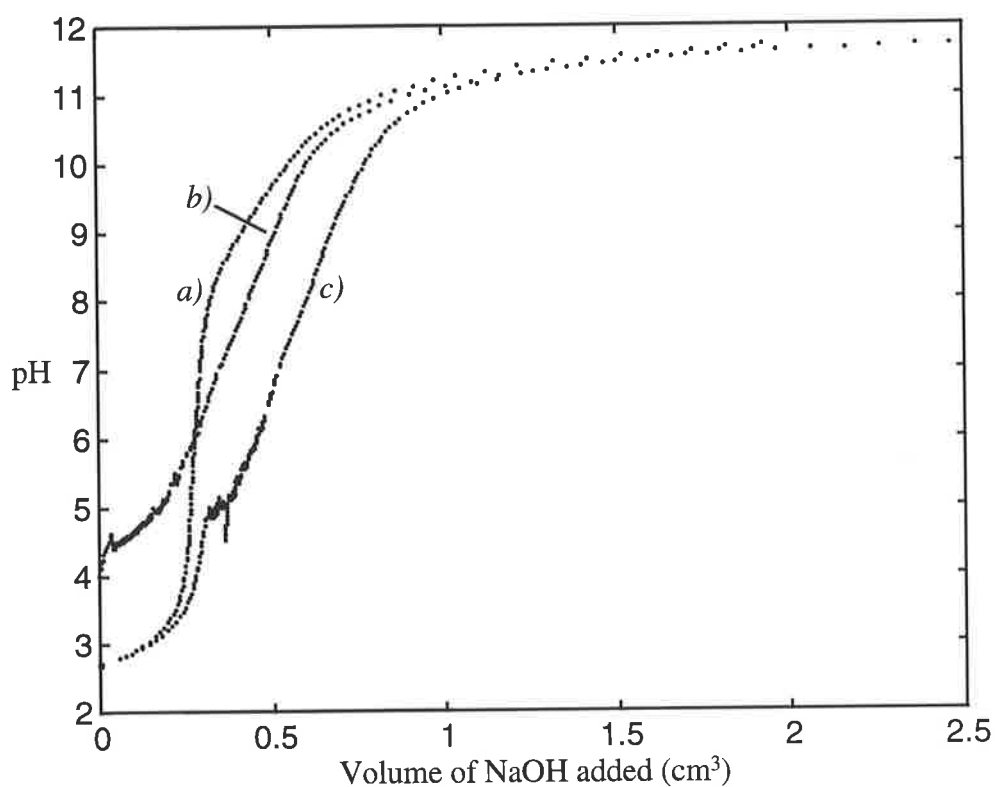


Figure C.10. The full pH profiles of a) β CD-cyclen H_n^{n+} and (S)-tryptophan both at $1 \times 10^{-3} \text{ mol dm}^{-3}$ b) β CD-cyclen H_n^{n+} and Zn^{2+} both at $2 \times 10^{-3} \text{ mol dm}^{-3}$ c) β CD-cyclen H_n^{n+} , Zn^{2+} and (S)-tryptophan all at $1 \times 10^{-3} \text{ mol dm}^{-3}$ and in aqueous $0.005 \text{ mol dm}^{-3} \text{ HClO}_4$ at $I = 0.1 \text{ mol dm}^{-3} (\text{NaClO}_4)$ and 298.2 K . The initial titration solutions were 10 cm^3 in volume.

APPENDIX D

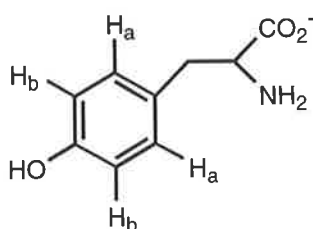
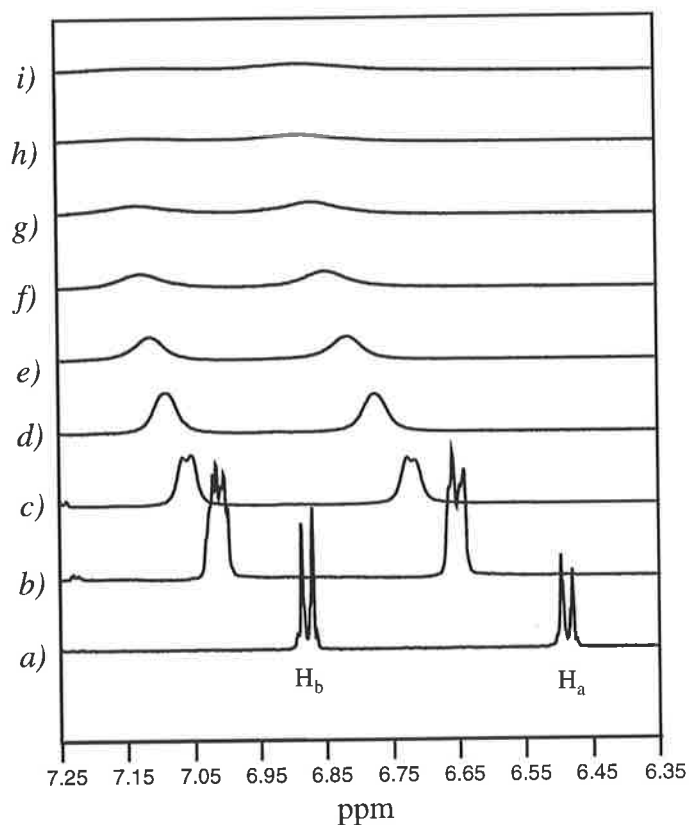


Figure D.1. The ^1H NMR spectra of (R/S)-tyrosine with $\beta\text{CD-DO3A}$ and Eu^{3+} . The observed ^1H NMR (500 MHz, D_2O) signals of a) (R/S)-tyrosine (0.02 mol dm^{-3}), then b) 1:1 (R/S)-tyrosine: $\beta\text{CD-DO3A}$ (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; and i) 0.7 mole equivalents of Eu^{3+} .

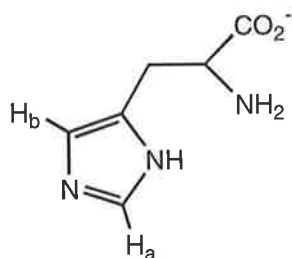
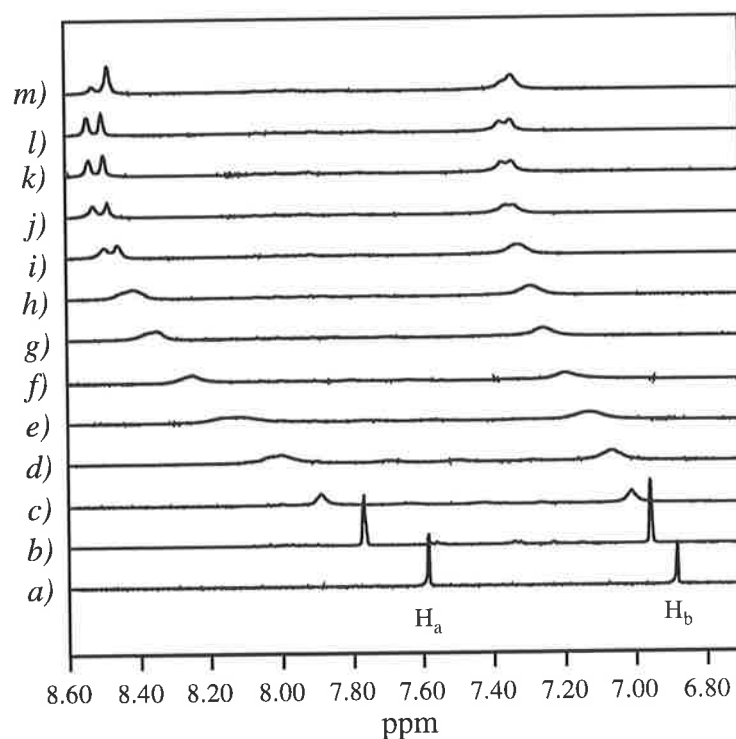


Figure D.2. The ^1H NMR spectra of (R/S)-histidine with $\beta\text{CD-DO3A}$ and Eu^{3+} . The observed ^1H NMR (500 MHz, D_2O) signals of a) (R/S)-histidine (0.02 mol dm^{-3}), then b) 1:1 (R/S)-histidine: $\beta\text{CD-DO3A}$ (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; i) 0.7 mole equivalents of Eu^{3+} ; j) 0.8 mole equivalents of Eu^{3+} ; k) 0.9 mole equivalents of Eu^{3+} ; l) 1 mole equivalent of Eu^{3+} , then m) 1:1:1 (R/S)-histidine: $\beta\text{CD-DO3A}:\text{Eu}^{3+}$ where (R)-:(S)-histidine is 1:3

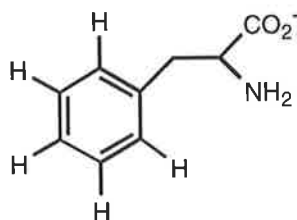
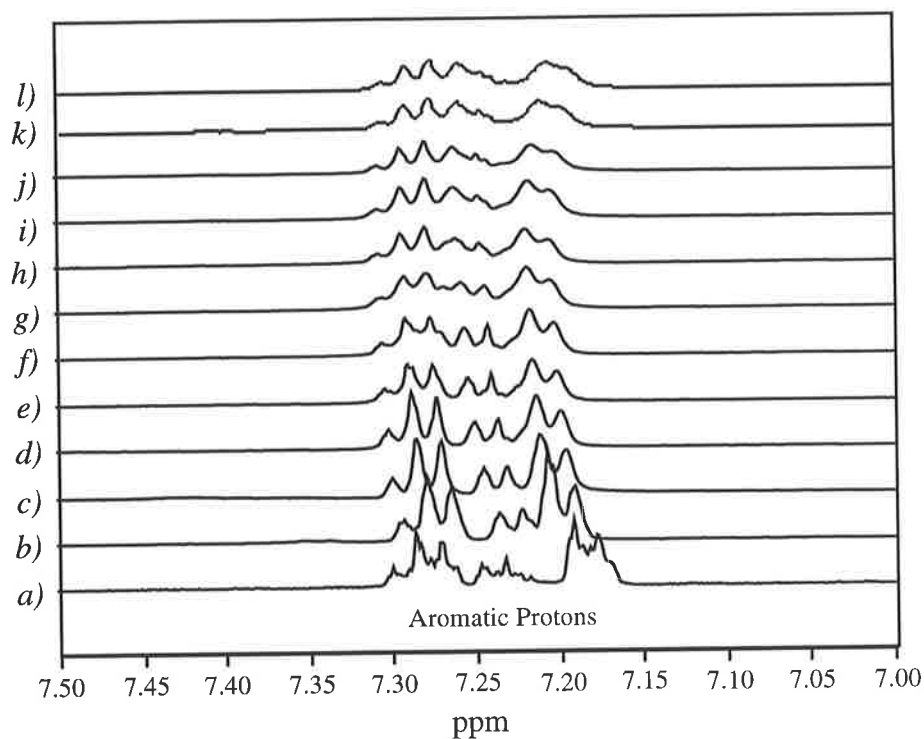


Figure D.3. The NMR spectra of (R/S)-phenylalanine with β CD-DO3A and Eu^{3+} . The observed ^1H NMR (500 MHz, D_2O) signals of a) (R/S)-phenylalanine (0.02 mol dm^{-3}), then b) 1:1 (R/S)- phenylalanine: β CD-DO3A (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; i) 0.7 mole equivalents of Eu^{3+} ; j) 0.8 mole equivalents of Eu^{3+} , k) 0.9 mole equivalents of Eu^{3+} and l) 1 mole equivalent of Eu^{3+} .

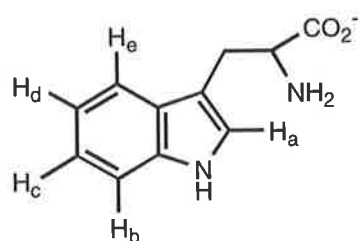
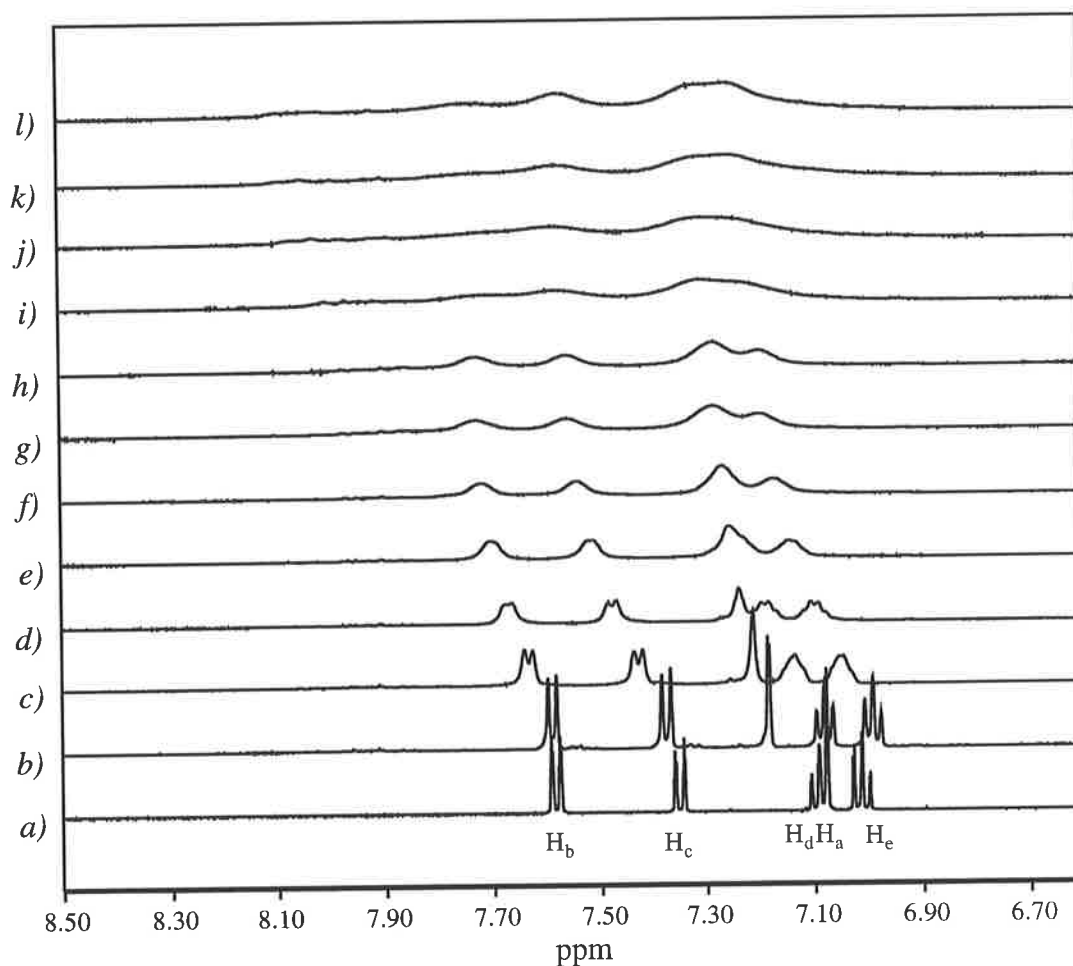


Figure D.4. The ^1H NMR spectra of (R/S)-tryptophan with $\beta\text{CD-DO3A}$ and Eu^{3+} . The observed ^1H NMR (500 MHz, D_2O) signals of a) (R/S)-tryptophan (0.02 mol dm^{-3}), then b) 1:1 (R/S)-tryptophan: $\beta\text{CD-DO3A}$ (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; i) 0.7 mole equivalents of Eu^{3+} ; j) 0.8 mole equivalents of Eu^{3+} ; k) 0.9 mole equivalents of Eu^{3+} and l) 1 mole equivalent of Eu^{3+} .

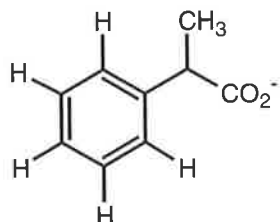
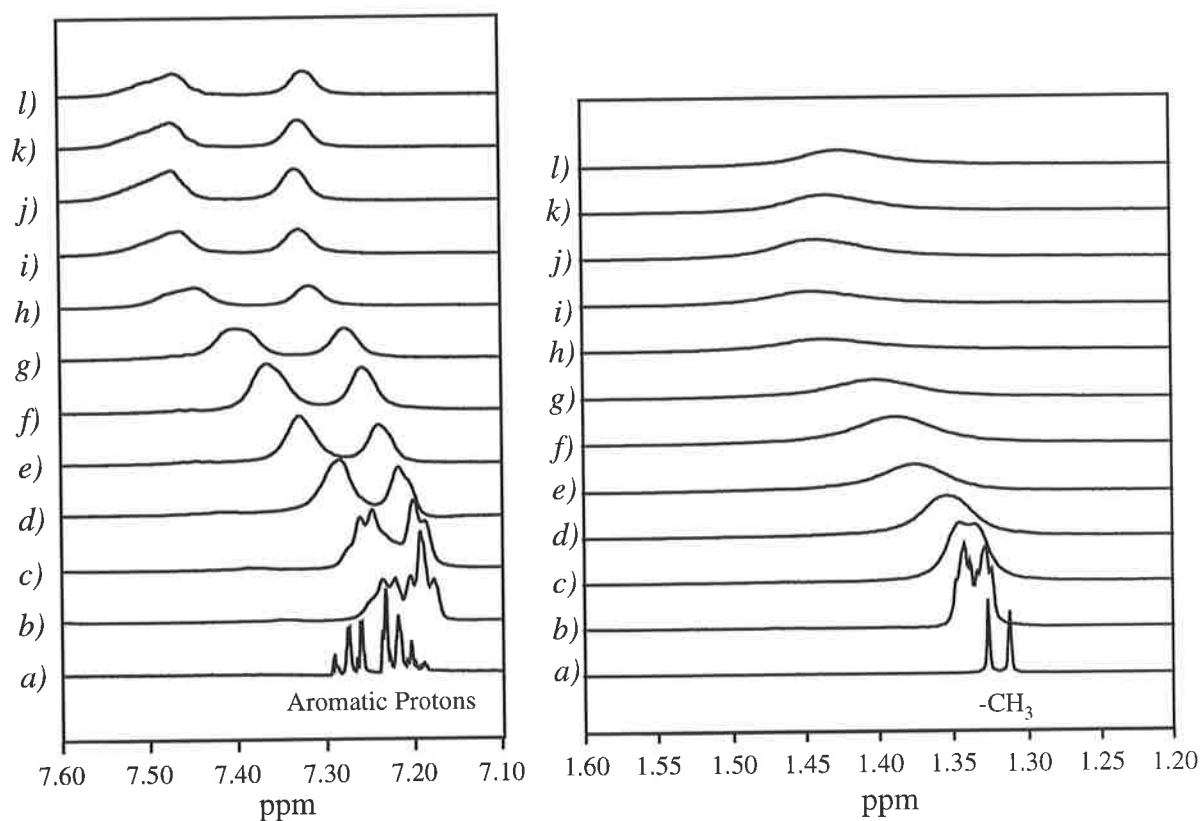


Figure D.5. The ^1H NMR spectra of (R/S)-2-phenylpropanoate with $\beta\text{CD-DO3A}$ and Eu^{3+} . The observed ^1H NMR (500 MHz, D_2O) signals of a) (R/S)-2-phenylpropanoate (0.02 mol dm^{-3}), then b) 1:1(R/S)-2-phenylpropanoate: $\beta\text{CD-DO3A}$ (0.02 mol dm^{-3}) with c) 0.1 mole equivalents of Eu^{3+} ; d) 0.2 mole equivalents of Eu^{3+} ; e) 0.3 mole equivalents of Eu^{3+} ; f) 0.4 mole equivalents of Eu^{3+} ; g) 0.5 mole equivalents of Eu^{3+} ; h) 0.6 mole equivalents of Eu^{3+} ; i) 0.7 mole equivalents of Eu^{3+} ; j) 0.8 mole equivalents of Eu^{3+} ; k) 0.9 mole equivalents of Eu^{3+} and l) 1 mole equivalent of Eu^{3+} .

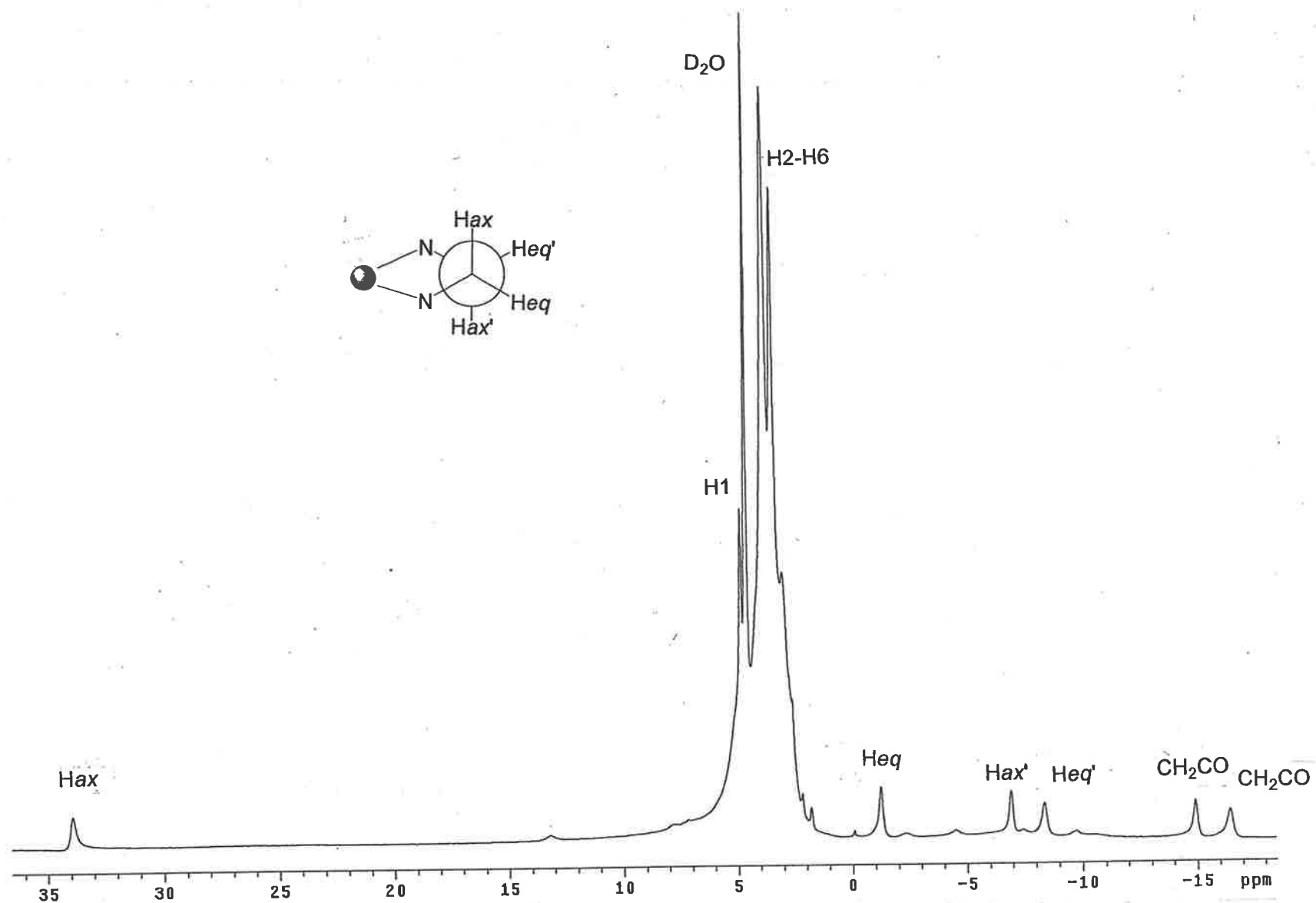


Figure D.6. The ^1H NMR (250 MHz, D_2O) spectra $[\text{Eu}(\beta\text{CD-DO3A})]$: δH (250 MHz, D_2O , pH~9.5) of $[\text{Eu}(\beta\text{CD-DO3A})]$ 33.92 (s, 4H, cyclen-Hax), 4.82 (s, 7H, H1), 4.2 – 2.3 (m, 42H, H3-H6), -1.26 (s, 4H, cyclen-Heq), -6.93 (s, 4H, cyclen-Hax'), -8.37 (s, 4H, cyclen-Heq'), -14.94 (s, 4H, -CH₂CO closest to βCD), -16.46 (s, 2H, -CH₂CO furthest from βCD)

PUBLICATIONS

Some of the work in this thesis appears in the following publications.

“Metallocyclodextrins of 6^A-(2-(2-(2-Aminoethylamino)ethylamino)ethylamino)-6^A-deoxy-β-cyclodextrin and 6^A-deoxy-6^A-(1,4,7,10-tetraazacyclododecanyl)-β-cyclodextrin: Their Formation and Complexation of (R)- and (S)-Tryptophan and Tryptophanoate in Aqueous Solution”

S. D. Kean, C. J. Easton and S. F. Lincoln (*in preparation*).

“Host-Guest Complexation of Aromatic Carboxylic Acids and their Conjugate Bases by β-Cyclodextrins Mono-Substituted at C(6) by Linear and Cyclic Polytriamines in Aqueous Solution”

S. D. Kean, B. L. May, P. Clements, S. F. Lincoln and C. J. Easton.

“Deacylation of 4-nitrophenyl acetate by 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-Cyclodextrins”

K. Redman, B. L. May, S. D. Kean, P. Clements, C. J. Easton and S. F. Lincoln.

J. Chem. Soc., Perkin Trans. 2, **1999**, 1711.

“Host-Guest Complexation of Aromatic Carboxylic Acids and their Conjugate Bases by 6^A-(ω-aminoalkylamino)-6^A-deoxy-β-Cyclodextrins in Aqueous Solution”

S. D. Kean, B. L. May, P. Clements, S. F. Lincoln and C. J. Easton.

J. Chem. Soc., Perkin Trans. 2, **1999**, 1257.

“Preparation and Characterisation of 6^A-Polyamine-Mono-Substitued-β-Cyclodextrins”

B. L. May, S. D. Kean, S. F. Lincoln and C. J. Easton.

J. Chem. Soc., Perkin Trans. 1, **1997**, 3157.

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

1 PERKIN

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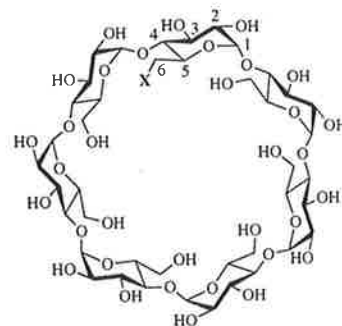
General syntheses for eleven β -cyclodextrins (cyclomaltoheptaoses) mono-substituted at the C6 position by a polyamine are described. The basis of the synthesis is the reaction of 6^A-O-(4-methylphenylsulfonyl)- β -cyclodextrin in the presence of KI in 1-methylpyrrolidin-2-one solution. This produces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

Introduction

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

We require a range of β -CDXs which can be produced in reasonable yield and high purity for our host-guest complex and metallocyclodextrin studies. Some of these β -CDXs have been reported previously. However, in our hands, the products obtained through these preparations usually required lengthy purification and this provided the impetus for our quest for an improved general synthetic method. Two major routes have been previously reported for the syntheses of the required β -CDXs. For the liquid polyamines, heating either β -cyclodextrin (β -CD),¹⁸ 6^A-O-(4-methylphenylsulfonyl)- β -cyclodextrin (β -CDtos)¹² or 6^A-deoxy-6^A-iodo- β -cyclodextrin (β -CDI)¹⁹ in excess polyamine in a sealed tube yields β -CDX which requires purification by lengthy chromatographic separation. For either the more expensive liquid or solid polyamines, reaction of β -CDtos^{10-13,16,20} with the polyamine in *N,N*-dimethylformamide (DMF) under similar conditions yields β -CDX, but we found it difficult to avoid some formylation of the β -CDX which necessitated tedious separations using this method. We now report a simple general procedure for the synthesis of some reported β -CDXs where X is either the 2-aminoethylamino,^{11,18} 3-amino-propylamino,⁷⁻⁹ 2-(2-aminoethylamino)ethylamino,^{12,14,19} 2-[2-(2-aminoethylamino)ethylamino]ethylamino,¹² 2-[bis(2-aminoethyl)amino]ethylamino¹⁰ or 1,4,7,10-tetraazacyclododecan-1-yl^{13,15} group bonded through nitrogen to the β -CD C6 carbon which in most cases have not been fully characterised, and some new β -CDXs that yield clean products under mild conditions.

The β -CDX's protonated amine groups exhibit a wide range of pK_as which are likely to have a major influence on host-guest complexation and metal ion coordination reactions. Accordingly, a systematic study of pK_a variation with the nature of X has been carried out in parallel with a study of the ¹³C



β -CDen; X = NH(CH₂)₂NH₂
 β -CDpn; X = NH(CH₂)₃NH₂
 β -CDbn; X = NH(CH₂)₄NH₂
 β -CDhn; X = NH(CH₂)₆NH₂
 β -CDdien; X = NH(CH₂)₂NH(CH₂)₂NH₂
 β -CDdipn; X = NH(CH₂)₃NH(CH₂)₃NH₂
 β -CDtrien; X = NH(CH₂)₂NH(CH₂)₂NH(CH₂)₂NH₂
 β -CDtren; X = NH(CH₂)₂N[(CH₂)₃NH₂]₂

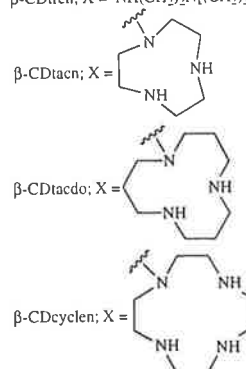


Fig. 1 Schematic representations of the β -CDXs prepared. The individual C and H atoms of the polyamino substituent are labelled 1, 2, ... n as distance from the β -CD moiety increases.

NMR spectral variation of β -CDXs with pH to gain an insight into the factors influencing these characteristics.

Results and discussion

Preparative aspects

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

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

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

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

NMP is a dipolar aprotic solvent that has been shown to be superior to DMF for nucleophilic substitutions of toluene-*p*-sulfonates²⁵ but is more stable than DMF under either acid or base conditions.²⁶ When β -CDtos was heated at 70 °C for 4 h with 3.3 equiv. of tris(2-aminoethyl)amine and 0.1 equiv. of KI (to generate β -CDI *in situ*) in NMP, pure β -CDtren was obtained in 60% yield following a single precipitation with ethanol and product separation through ion exchange chromatography. There was no evidence for reaction between tris(2-aminoethyl)amine or β -CDtren and NMP. The formation of β -CDI in the reaction was shown by TLC of the reaction mixture during the course of the reaction. A series of β -CDXs, having either linear, branched or cyclic polyamine substituents, was prepared under the same conditions (Table 1). All of the β -CDXs prepared by this procedure were shown to be pure by TLC, ¹H and ¹³C NMR spectroscopy and microanalysis. (A referee has pointed out that the cyclic solvent, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone, has been employed in the nucleophilic substitution of a modified cyclodextrin.²⁷)

pK_a Variations

The two pK_a s of β -CDXs increase as X is systematically changed from 1,2-diaminoethane (en) to 1,6-diaminohexane (hn) while the difference between the two pK_a s decreases, and a similar trend is seen for the free diamine analogues (Table 2). The latter observation is attributable to increases in charge separation in the diprotonated species decreasing electrostatic repulsion as the diamine increases in size. The increase in pK_a magnitude coincides with increases in hydrophobicity as the

Table 2 pK_a s^a for some protonated 6 α -polyamine-substituted β -cyclodextrins and the corresponding free polyamines in aqueous NaClO₄ (*I* = 0.10 mol dm⁻³) at 298.2 K

Species	pK_a	Species	pK_a
β -CDenH ₂ ²⁺	9.42 $\pm$ 0.01	enH ₂ ²⁺	9.97 $\pm$ 0.03
	5.70 $\pm$ 0.02		7.16 $\pm$ 0.02
β -CDpnH ₂ ²⁺ ^b	9.90 $\pm$ 0.1	pnH ₂ ²⁺	10.56 $\pm$ 0.02
	7.39 $\pm$ 0.04		8.97 $\pm$ 0.01
β -CDbnH ₂ ²⁺	10.26 $\pm$ 0.02	bnH ₂ ²⁺	10.91 $\pm$ 0.02
	8.06 $\pm$ 0.01		9.49 $\pm$ 0.01
β -CDhnH ₂ ²⁺	10.27 $\pm$ 0.03	hnH ₂ ²⁺	11.01 $\pm$ 0.06
	8.72 $\pm$ 0.01		10.04 $\pm$ 0.03
β -CDdienH ₃ ³⁺	9.52 $\pm$ 0.02	dienH ₃ ³⁺	9.78 $\pm$ 0.01
	7.63 $\pm$ 0.03		8.99 $\pm$ 0.03
	3.88 $\pm$ 0.07		4.32 $\pm$ 0.03
β -CDdipnH ₃ ³⁺	10.06 $\pm$ 0.02	dipnH ₃ ³⁺	10.56 $\pm$ 0.05
	8.44 $\pm$ 0.03		9.44 $\pm$ 0.06
	6.72 $\pm$ 0.03		7.54 $\pm$ 0.06
β -CDtrienH ₄ ⁴⁺	9.33 $\pm$ 0.02	trienH ₄ ⁴⁺	9.83 $\pm$ 0.04
	8.22 $\pm$ 0.03		8.93 $\pm$ 0.05
	5.61 $\pm$ 0.03		5.40 $\pm$ 0.05
	3.13 $\pm$ 0.07		3.0 $\pm$ 0.1
β -CDtrenH ₄ ⁴⁺ ^c	9.85 $\pm$ 0.02	trenH ₄ ⁴⁺ ^d	10.14
	8.99 $\pm$ 0.09		9.43
	6.89 $\pm$ 0.05		8.41
	2.6 $\pm$ 0.3		
β -CDtacnH ₃ ³⁺	10.0 $\pm$ 0.1	tacnH ₃ ³⁺	10.69 $\pm$ 0.02
	5.89 $\pm$ 0.07		7.01 $\pm$ 0.01
	2.4 $\pm$ 0.2		
β -CDtadoH ₃ ³⁺	11.24 $\pm$ 0.04	tadoH ₃ ³⁺ ^e	12.60
	5.85 $\pm$ 0.03		7.57
	2.8 $\pm$ 0.1		2.41
β -CDcyclenH ₄ ⁴⁺	10.40 $\pm$ 0.01	cyclenH ₄ ⁴⁺ ^f	10.6
	8.62 $\pm$ 0.02		9.6

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

aliphatic chain lengthens and indicates a decrease in the ability of surrounding water to accept a proton from the protonated amine as overall hydration decreases. The two pK_a s of β -CDXs 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 solvation experienced by the protonation sites in β -CDX and the free diamine or a combination of both. In addition, the diamine moiety in β -CDX is bound adjacent to the ring of six primary hydroxy groups delineating the narrow end of the cyclodextrin annulus such that hydrogen bonding between them and the amine nitrogens may decrease the basicity of the latter. This is supported to some extent through the observation that in basic solution more fine structure is seen in the 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 -CDtado and β -CDcyclen in Figs. 2 and 3.) A similar interpretation has been presented for β -CDdien (where pK_a magnitude increases in the sequence -NH₂⁺ < β -CD-NH₂⁺ < -(CH₂)₂-NH₂⁺(CH₂)₂- as identified by ¹³C NMR spectroscopy¹⁹) which together with its β -CDdipn homologue shows similar trends (Table 2) to those discussed above. Generally, similar trends in pK_a magnitudes are observed for the polyamine β -CDX as for their diamine analogues and their origins are probably similar.

¹³C NMR Spectra

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

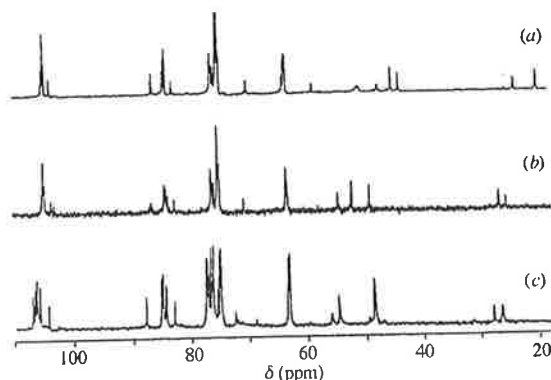


Fig. 2 75.47 MHz ^{13}C NMR spectra of β -CDtacdo in (a) 0.1 mol dm^{-3} $\text{HCl-D}_2\text{O}$, (b) $\text{HCl-D}_2\text{O}$, pH ~ 8.5 and (c) 0.1 mol dm^{-3} $\text{NaOH-D}_2\text{O}$ †

† δ_{C} (0.1 mol dm^{-3} $\text{HCl-D}_2\text{O}$) 104.9, 104.7, 104.6, 103.7 (C1), 86.3 (C4^A), 84.3, 84.0, 82.8 (C4), 76.1, 76.0, 75.9, 75.6, 75.2, 75.0, 74.7, 74.6 (C2, C3, C5), 70.0 (C5^A), 63.8, 63.3 (C6), 58.6, 50.9 (broad), 47.5, 45.2, 43.9 (C6^A, taedoC1, taedoC3, taedoC4), (25.4), 23.7, 19.9 (taedoC2, taedoC5); δ_{C} ($\text{HCl-D}_2\text{O}$, pH ~ 8.5) 104.8, 104.5, 103.4, 103.0 (C1), 86.4 (C4^A), 84.1, 83.9, 83.7, 83.6, 82.5 (C4), 76.1, 76.0, 75.9, 75.6, 74.9, 74.7 (C2, C3, C5), 70.5 (C5^A), 63.3, 63.2 (C6), 54.3 (C6^A), 51.9, 49.0 (taedoC1, taedoC3, taedoC4), 26.6, 25.4 (taedoC2, taedoC5); δ_{C} (0.1 mol dm^{-3} $\text{NaOH-D}_2\text{O}$) 106.9, 106.6, 106.4, 106.3, 105.8, 105.7, 104.3 (C1), 87.7 (C4^A), 85.2, 85.1, 85.0, 84.9, 84.5, 84.3, 82.9 (C4), 77.4, 77.2, 77.1, 77.0, 76.9, 76.8, 76.7, 76.5, 76.3, 76.1, 75.4, 75.1, 74.9 (C2, C3, C5), 72.5 (C5^A), 63.4, 63.1 (C6), 55.9 (C6^A), 54.6 (taedoC1), 48.7, 48.6 (taedoC3, taedoC4), 28.0, 26.5 (taedoC2, taedoC5).

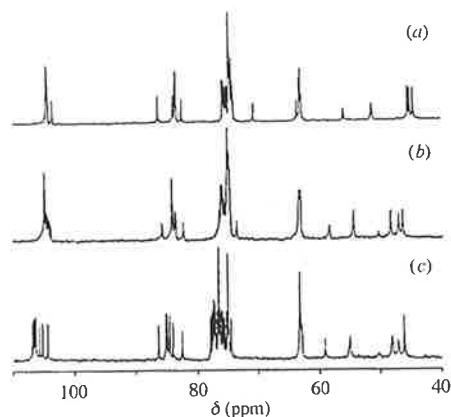


Fig. 3 75.47 MHz ^{13}C NMR spectra of β -CDcyclen in (a) 0.1 mol dm^{-3} $\text{HCl-D}_2\text{O}$, (b) $\text{HCl-D}_2\text{O}$, pH ~ 9 and (c) 0.1 mol dm^{-3} $\text{NaOH-D}_2\text{O}$ §

§ δ_{C} (0.1 mol dm^{-3} $\text{HCl-D}_2\text{O}$) 104.5, 104.4, 104.3, 103.6 (C1), 86.4 (C4^A), 84.0, 83.7, 83.6, 82.6 (C4), 75.9, 75.8, 75.6, 75.5, 75.3, 75.2, 74.9, 74.8, 74.7, 74.6, 74.4, 74.3, 74.2 (C2, C3, C5), 70.9 (C5^A), 63.6, 63.2, 63.0 (C6), 56.2 (C6^A), 51.5 (cyclenC1), 45.7, 45.4, 44.9 (cyclenC2-4); δ_{C} ($\text{HCl-D}_2\text{O}$, pH ~ 9) 104.9, 104.6, 104.3, 104.2, 103.8 (C1), 85.6 (C4^A), 84.1, 83.6, 83.5, 82.2 (C4), 76.4, 76.1, 76.0, 75.8, 75.5, 75.4, 75.2, 75.1, 74.9, 74.6, 74.5, 73.5 (C2, C3, C5), 63.1, 63.0, 62.8 (C6), 58.3 (C6^A), 54.3, (50.2), 48.2, 46.9, 46.2 (cyclenC); δ_{C} (0.1 mol dm^{-3} $\text{NaOH-D}_2\text{O}$) 106.9, 106.8, 106.6, 106.4, 105.7, 105.3, 104.4 (C1), 86.35 (C4^A), 85.2, 85.0, 84.7, 84.6, 84.0, 82.6 (C4), 77.9, 77.7, 77.5, 77.4, 77.2, 77.1, 76.8, 76.6, 76.4, 76.1, 76.0, 75.8, 75.6, 75.2, 75.1, 74.6 (C2, C3, C5), 63.1, 62.9, 62.8 (C6), 59.0 (C6^A), 55.0, (50.2), 48.0, 47.1, 46.1 (cyclenC).

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

The ^{13}C NMR spectra of β -CDtacdo and β -CDcyclen at dif-

ferent pHs appear in Figs. 2 and 3, respectively, and illustrate the substantial spectral changes which occur with change in pH. At pH 1 resolution of the ^{13}C resonances of fully protonated β -CDtacdoH₃³⁺ and β -CDcyclenH₄⁴⁺ is relatively small consistent with the polyamine substituent swinging out from the β -CD moiety so that it interacts weakly if at all with the primary hydroxy groups and the differentiation of the seven glucopyranose units is minimised. At the highest pH, where β -CDtacdo and β -CDcyclen exist as the deprotonated neutral species, all seven C1 and C4 resonances are observed consistent with the polyamine substituents hydrogen bonding with the primary hydroxy groups of β -CD and maximising the differentiation between the seven glucopyranose units. This interpretation is in agreement with that presented for the similarly pH dependent ^{13}C NMR spectra of β -CDdien.¹⁹

Experimental

Materials and instrumental methods

The polyamines 1,2-diaminoethane (en), 1,3-diaminopropane (pn), 1,4-diaminobutane (bn), 1,6-diaminohexane (hn), 2-(2-aminoethylamino)ethylamine (dien), 3-(3-aminopropylamino)-propylamine (dipn), tris(2-aminoethyl)amine (tren) and 1,4,7,10-tetraazacyclododecane bis(dihydrogen sulfate) (cyclen·2H₂SO₄) were purchased from Aldrich and used without further purification. 2-[2-(2-Aminoethylamino)ethylamino]ethylamine tetrahydrochloride (trien·4HCl, Aldrich) was purified by two recrystallisations from ethanol-water.²¹ 1,4,7-Triazacyclononane·3HCl and 1,5,9-triazacyclododecane·3HCl were prepared as in the literature.^{22,23} HPLC grade 1-methylpyrrolidin-2-one (NMP, Aldrich) was dried by distillation from CaH₂ at reduced pressure. β -Cyclodextrin was a gift from Nihon Shokuhin Kako Co. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F₂₅₄ silica on aluminium sheets and samples were eluted using a mixture of propan-2-ol-ethyl acetate-water-ammonium hydroxide (7:7:5:4). Compounds containing amino groups were detected by dipping the developed plate into a solution of 1% ninhydrin in ethanol and heating the plate. Cyclodextrins were detected by dipping the developed plate into a solution of 1.5% H₂SO₄ in ethanol and heating the plate. *R_f* values are reported as *R_c* (retention relative to β -CD).

Titration curves were carried out using a Metrohm Dosimat E665 titrator, an Orion SA 720 potentiometer, and an Orion 8172 Ross Sureflow combination pH electrode which was filled with 0.10 mol dm^{-3} NaClO₄. During all titrations a stream of fine nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} NaOH to remove any last traces of CO₂ and then 0.10 mol dm^{-3} NaClO₄ to ensure a constant water vapour pressure) was passed through the titration solution which was magnetically stirred and thermostatted at 298.2 ± 0.1 K in a water-jacketed 20 cm³ titration vessel which was closed to the atmosphere with the exception of a small exit for the nitrogen stream. Deionised water, purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 M Ω cm, was used in the preparation of all solutions after boiling to remove CO₂. Standardised 0.100 mol dm^{-3} NaOH was titrated against 10.00 cm³ aliquots of solutions (0.002 mol dm^{-3} in the species of interest, 0.005 mol dm^{-3} in HClO₄ and 0.095 mol dm^{-3} in NaClO₄ in all titrations). The *pK_a*s were determined using the programme SUPERQUAD²⁴ on a Digital Venturis 575 computer.

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

General procedure for preparation of amino-substituted β -cyclodextrins

A solution of β -CDtos¹¹ (2.0 g, 1.55×10^{-3} mol), KI (0.025 g, 0.15×10^{-3} mol) and the amine (5×10^{-3} mol) in dry NMP (5

cm³) was stirred at 70 °C in a lightly stoppered flask for 4–8 h. The resultant light yellow solution was cooled to room temperature and diluted with ethanol (100 cm³). The resulting precipitate was collected by vacuum filtration, washed successively with ethanol (100 cm³) and diethyl 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 × 4.5 cm) of H⁺ form BioRex 70, 100–200 mesh (Biorad). The column was washed with water (400 cm³) and β-CDX was eluted with 1 mol dm⁻³ NH₄OH. Fractions containing β-CDX were combined and evaporated to dryness under vacuum. The residue was dissolved in water and the solution evaporated under reduced pressure to remove excess ammonia (this procedure was repeated several times). The product was dried under vacuum over P₂O₅ to give β-CDX in yields of 25–60%. Specific preparative descriptions and characterisation data of β-CD-tren, previously prepared by other methods,¹⁰ and previously unreported β-CD-tacdo are provided below. Similarly detailed preparative and characterisation data for the remaining β-CDXs shown in Fig. 1 are provided as supplementary data.[†]

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

A mixture of β-CDtos (2.048 g, 1.59 × 10⁻³ mol), tris(2-aminoethyl)amine (0.74 g, 5.07 × 10⁻³ mol) and KI (0.024 g) in NMP (5 cm³) was treated according to the general procedure to give β-CDtren as a white powder (1.192 g, 59%). *R*_f 0.31; Electrospray-MS *m/z* 1263 (M⁺) [Found: C, 43.84; H, 7.58; N, 4.40. Calc. for β-CDtren·3H₂O (C₄₈H₉₂N₄O₃₄): C, 43.76; H, 7.04; N, 4.25%]; δ_H(D₂O–NaOH, pH ~14) 5.00 (br s, 7H + solvent, H1), 3.5–3.8 (m, 26H, H3, H5, H6), 3.1–3.4 (m, 13H, H2, H4), 3.02 (t, *J* 9.0, 1H, H4^A), 2.85 (d, *J* 12.0, 1H, H6^A), 2.2–2.7 (m, 13H, H6^A, trenH); δ_H(D₂O, pH ~9) 5.05 (br s, 7H, H1), 3.8–4.0 (m, 26H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.41 (t, *J* 9.0, 1H, H4^A), 3.05 (d, *J* 11.4, 1H, H6^A), 2.4–2.9 (m, 13H, H6^A, trenH); δ_H(D₂O–HCl, pH ~1) 5.00 (s, 7H, H1), 4.10 (t, *J* 9.0, 1H, H5^A), 3.6–4.0 (m, 25H, H3, H5, H6), 3.4–3.6 (m, 14H, H2, H4), 2.9–3.4 (m, 14H, H6^A, trenH); δ_C(D₂O–NaOH, pH ~14) 107.0, 106.6, 106.4, 105.2 (C1), 87.6 (C4^A), 85.0, 84.8, 84.5, 83.9 (C4), 77.3, 76.4, 76.3, 75.2, 74.9 (C2, C3, C5), 70.9 (C5^A), 63.0 (C6), 59.8 (trenC3,3'), (56.9), 55.1 (C6^A), 50.5 (trenC2), 46.2 (trenC1), 41.0 (trenC4,4'); δ_C(D₂O, pH ~9) 104.7, 104.3 (C1), 86.4 (C4^A), 84.0, 83.6 (C4), 75.9 (C2), 74.9 (C3), 74.7 (C5), 73.3 (C5^A), 63.1 (C6), 58.7 (trenC3,3'), 55.7 (trenC2), 52.0 (C6^A), 48.7 (trenC1), 40.7 (trenC4,4'); δ_C(D₂O–HCl, pH ~1) 104.5, 103.8 (C1), 85.8 (C4^A), 84.2, 83.8, 83.4 (C4), 75.8, 75.5, 75.0, 74.8, 74.5 (C2, C3, C5), 70.2 (C5^A), 63.6, 63.1 (C6), 52.8 (trenC3,3'), 51.5 (C6^A), 51.3 (trenC2), 47.0 (trenC1), 38.6 (trenC4,4').

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

A mixture of 1,5,9-triazacyclododecane·3HCl²³ (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 at room temp. for 90 min. 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 β-CDtos (2.081 g, 1.61 × 10⁻³ mol) and KI (0.030 g) were added to the solution. The resultant mixture was treated according to the general procedure to give β-CDtacdo as a white powder (0.709 g, 34%). *R*_f 0.75; Electrospray-MS *m/z* 1288 (M⁺) [Found: C, 45.28; H, 7.34; N, 3.15. Calc. for β-CDtacdo·4H₂O (C₅₁H₉₇N₃O₃₈): C, 45.03; H, 7.18; N, 3.08%]; δ_H(D₂O–NaOH, pH ~14) 4.9 (br s, 7H + solvent, H1), 4.14 (t, *J* 6.0, 1H, H5^A), 3.7–4.0 (m, 25H, H3, H5, H6), 3.17 (t, *J* 6.0, 1H, H4^A), 2.88 (d, *J* 15, 1H, H6^A), 2.64 (m, 13H, H6^A, tacdoH1, tacdoH3, tacdoH4), 1.66 (m, 6H,

tacdoH2, tacdoH5); δ_H(D₂O–HCl (1:1), pH ~8.5] 5.09 (s, 7H + solvent, H1), 4.26 (t, *J* 9.0, 1H, H5^A), 3.8–4.2 (m, 25H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.39 (t, *J* 9.0, 1H, H4^A), 2.5–3.2 (m, 14H, H6^A, tacdoH1, tacdoH3, tacdoH4), 1.6–2.0 (m, 6H, tacdoH2, tacdoH5); δ_H(D₂O–HCl (1:2), pH ~6.0] 5.07 (br s, 7H, H1), 4.25 (t, *J* 9.0, 1H, H5^A), 3.8–4.1 (m, 25H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.43 (t, *J* 9.0, 1H, H4^A), 2.7–3.3 (m, 14H, H6^A, tacdoH1, tacdoH3, tacdoH4), 1.7–2.2 (m, 6H, tacdoH2, tacdoH5); δ_H(D₂O–HCl, pH ~1) 5.0 (br s, 7H + solvent, H1), 4.33 (br t, 1H, H5^A), 3.7–4.0 (m, 25H, H3, H5, H6), 3.2–3.6 (m, 27H, H2, H4, H6^A, tacdoH1, tacdoH3, tacdoH4), 2.2 (br, 6H, tacdoH2, tacdoH5).

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

2 PERKIN

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A potentiometric titration study of the complexation of the guests benzoic acid, 4-methylbenzoic acid and (*RS*)-2-phenylpropanoic acid and their conjugate bases by host 6^A-(ω -aminoalkylamino)-6^A-deoxy- β -cyclodextrins, where the ω -aminoalkylamino groups are -NH(CH₂)_nNH₂ (*n* = 2, 3, 4 and 6), is reported. Of the 64 host-guest complexes whose formation is statistically possible over the pH range 2.0–12.0 studied, 35 were detected. Their stability constants range from $140 \pm 35 \text{ dm}^3 \text{ mol}^{-1}$ for the [βCDNH(CH₂)₂NH₂·4-methylbenzoate] complex to $1760 \pm 150 \text{ dm}^3 \text{ mol}^{-1}$ for the [βCDNH(CH₂)₆NH₂·(*S*)-2-phenylpropanoate][−] complex at 298.2 K and *I* = 0.10 mol dm^{−3} (NaClO₄). The charge and hydrophobicity of both host and guest appear to be significant factors in the variation of host-guest complex stability. Qualitative structural information on the host-guest complexes obtained from 600 MHz ¹H NMR ROESY spectroscopy is generally consistent with the structures generated by molecular modelling.

Introduction

The range of cyclodextrins (CDs), their modified forms and the host-guest complexes formed by them is extensive.^{1–16} In most cases, entry of a hydrophobic moiety of the guest into the hydrophobic region of the host CD annulus occurs during host-guest complexation, and the CD may discriminate between guests on the basis of their size, hydrophobicity, charge and chirality. We are particularly interested in the effects of variation of charge and hydrophobicity of both the host CD and the guest on complex stability. Our reported syntheses of 6^A-(ω -aminoalkylamino)-6^A-deoxy-βCDs (βCDNH(CH₂)_nNH₂) (Fig. 1) and the determination of the p*K*_as of their protonated forms¹⁷ facilitate a systematic study of the effects of the simultaneous variation of these factors in both host and guest on complex stability. We have studied the relative importance of these factors in βCDNH(CH₂)_nNH₂ (*n* = 2, 3, 4 and 6) where the hydrophobicity of the -NH(CH₂)_nNH₂ substituent increases with *n*, and a variation of charge occurs through amine protonation.¹⁴ The guests selected for this study, benzoic acid, 4-methylbenzoic acid and (*R*)- or (*S*)-2-phenylpropanoic acid and their conjugate bases, also exhibit differences in hydrophobicity and charge depending on protonation, and in the case of the (*R*)- or (*S*)-2-phenylpropanoic acids and their conjugate bases, differences in chirality. We have previously reported² the complexation of these guests by βCD and 6^A-amino-6^A-deoxy-βCD which provides a comparison with our new studies.

Experimental

The modified βCDs were prepared as previously described,¹⁷ and were dried to constant weight and stored over P₂O₅ prior to use. The carboxylic acids were high quality commercial grade materials. Deionized water was purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm and, after boiling to remove CO₂, was used in the prepar-

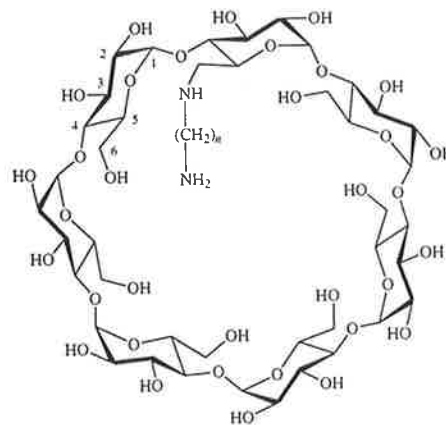


Fig. 1 6^A-(ω -Aminoalkylamino)-6^A-deoxy- β -cyclodextrins where *n* = 2, 3, 4 or 6.

ation of all solutions. A Metrohm Dosimat E665 titrator, an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode filled with 0.10 mol dm^{−3} NaClO₄ were used in all titrations. During each titration a fine stream of nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{−3} NaOH to remove any CO₂ traces, and then through aqueous 0.10 mol dm^{−3} NaClO₄) was passed through the titration solution which was magnetically stirred and thermostatted at $298.2 \pm 0.1 \text{ K}$ in a water-jacketed 20 cm³ titration vessel which was closed to the atmosphere with the exception of a small vent for the nitrogen stream. In each titration, 10 cm³ of a solution $1.0 \times 10^{-3} \text{ mol dm}^{-3}$ in [βCDNH(CH₂)_nNH₂] and [carboxylic acid] and $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ in [HClO₄] and *I* = 0.10 mol dm^{−3} (NaClO₄) were measured into the titration vessel and allowed 30 min to reach thermal equilibrium. Sodium hydroxide solution (0.10 mol dm^{−3}) was the titrant. All titrations were carried out in duplicate at least and a typical titration curve is shown in Fig. 2. Complex stability constants were derived from the titration data using the program SUPERQUAD.¹⁸

[†] β-Cyclodextrin = cycloheptamaltose.

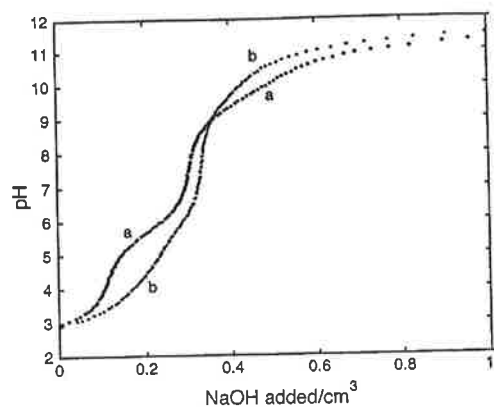


Fig. 2 The titration curves for a) a 0.010 mol dm⁻³ solution of β CDNH-(CH₂)₆NH₂, 0.020 mol dm⁻³ in [HClO₄], and b) a 0.010 mol dm⁻³ solution of both β CDNH(CH₂)₆NH₂ and 4-methylbenzoic acid, 0.020 mol dm⁻³ in [HClO₄] against 0.100 mol dm⁻³ NaOH, at 298.2 K where $I = 0.10$ mol dm⁻³ (NaClO₄).

The ¹H NMR 1D and 2D ROESY (mixing time of 0.35 s)¹⁹ experiments were run on a Varian Inova 600 spectrometer. Either β CDNH(CH₂)₆NH₂ alone or equimolar amounts of the guest species and β CDNH(CH₂)₆NH₂ were dissolved in D₂O to give total concentrations of 0.06 mol dm⁻³ of each species, and the pH was adjusted to ≥ 11.5 . The resultant solutions were filtered and degassed by freeze-thawing before the spectra were recorded. The spectral assignments below are according to the glucopyranose numbering system H1–H6 (where a superscript A specifies the glucopyranose unit bearing the 1,6-diamino-hexyl substituent) in Fig. 1. The hexyl protons are labelled Ha–Hf as distance increases from the secondary amine group, and aromatic guest protons are labelled as Ho and Hm where the former is adjacent to the carboxylate group.

The spectral assignments for β CDNH(CH₂)₆NH₂ alone at pH ≥ 11.5 are: 1D ¹H spectrum: δ_H 4.80 (s, 7H + solvent, H1); 3.5–3.8 (m, 26H, H3, H5, H6); 3.2–3.4 (m, 13H, H2, H4); 3.11 (t, $J = 9.3$ Hz, 1H, H4^A); 2.93 (d, $J = 12.4$ Hz, 1H, H6^A); 2.70 (m, 1H, H6^A); 2.65 (m, 2H, Hf); 2.46 (m, 2H, Ha); 1.40 (br s, 4H, Hb, He); 1.26 (br s, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.26 (Hc,d) shows cross-peaks with 1.40 (Hb,e), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 1.40 (Hb,e) shows cross-peaks with 1.26 (Hc,d), 2.46 (Ha), 2.65 (Hf), 3.7 (H5), 3.8 (H3); 2.46 (Ha) shows cross-peaks with 1.26 (Hc,d), 1.4 (Hb,e), 3.9 (H5^A); 2.65 (Hf) shows cross-peaks with 1.26 (Hc,d), 1.4 (Ha,e), 3.7 (H5), 3.8 (H3). These cross-peaks are absent from the 2D-ROESY spectrum obtained after acidification of the sample solution to pH 1 with hydrochloric acid.

The stability constants discussed below indicate that the [β CDNH(CH₂)₆NH₂·4-methylbenzoate]⁻ complex constitutes 85% of the total [β CDNH(CH₂)₆NH₂] and [4-methylbenzoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ¹H spectrum: δ_H 7.80 (d, $J = 7.8$ Hz, 2H, Ho); 7.29 (d, $J = 7.8$ Hz, 2H, Hm); 5.00 (m, 7H + solvent, H1); 3.6–3.9 (m, 26H, H3, H5, H6); 3.4–3.6 (m, 13H, H2, H4); 3.26 (t, $J = 9.6$ Hz, 1H, H4^A); 2.99 (d, $J = 13.2$ Hz, 1H, H6^A); 2.71 (m, 1H, H6^A); 2.64 (t, $J = 7.2$ Hz, 2H, Hf); 2.45 (t, $J = 7.2$ Hz, 2H, Ha); 2.41 (s, 3H, Me-); 1.4–1.5 (m, 4H, Hb, He); 1.2–1.3 (m, 4H, Hc, Hd). The 2D ROESY spectrum shows the following cross-peaks: δ_H 1.2–1.3 (Hc,d) shows cross-peaks with 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 1.4–1.5 (Hb,e) shows cross-peaks with 1.2–1.3 (Hc,d), 2.45 (Ha), 2.64 (Hf), 3.8 (H5), 3.9 (H3); 2.41 (Me) shows a cross-peak with 7.29 (Hm); 2.45 (Ha) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.64 (Hf) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 3.8 (H5), 3.9 (H3); 2.71 (H6^A) shows a cross-peak with 3.26 (H4^A); 3.26 (H4^A) shows a cross-peak with 2.71 (H6^A); 3.8 (H5) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5

(Hb,e), 7.29 (Ho), 7.80 (Hm); 3.9 (H3) shows cross-peaks with 1.2–1.3 (Hc,d), 1.4–1.5 (Hb,e), 2.45 (Ha), 2.64 (Hf), 7.29 (Ho), 7.80 (Hm); 7.29 (Ho) shows cross-peaks with 2.41 (Me-), 3.8 (H5), 3.9 (H3), 7.80 (Hm); 7.80 (Hm) shows cross-peaks with 3.8 (H5), 3.9 (H3), 7.29 (Ho).

The stability constants discussed below indicate that the [β CDNH(CH₂)₆NH₂·(S)-2-phenylpropanoate]⁻ complex constitutes 90% of the total [β CDNH(CH₂)₆NH₂] and [(S)-2-phenylpropanoate] at the pH ≥ 11.5 of the NMR study. The spectral assignments are: 1D ¹H spectrum: δ_H 7.30 (m, 4H, Hm, Ho); 7.22 (m, 1H, Hp); 4.92 (br s, 7H + solvent, H1); 3.6–4.0 (m, 26H, H3, H5, H6); 3.58 (q, $J = 7.2$ Hz, α CH); 3.2–3.5 (m, 13H, H2, H4); 3.19 (t, $J = 9.6$ Hz, 1H, H4^A); 2.93 (d, $J = 12.6$ Hz, 1H, H6^A); 2.67 (dd, $J = 12.6$, 9.5 Hz, 1H, H6^A); 2.62 (t, $J = 7.2$ Hz, 2H, Hf); 2.45 (t, $J = 7.8$ Hz, 2H, Ha); 1.39 (m, 4H, Hb,e); 1.36 (d, $J = 7.2$ Hz, 3H, Me); 1.25 (m, 4H, Hc,d). 2D ROESY cross-peaks: δ_H 1.25 (Hc,d) shows cross-peaks with 1.39 (Hb,e), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 1.36 (Me) shows a cross-peak with 3.58 (α CH); 1.39 (Hb,e) shows cross-peaks with 1.25 (Hc,d), 2.45 (Ha), 2.62 (Hf), 3.7 (H5), 3.75 (H3); 2.45 (Ha) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.8 (H5^A); 2.62 (Hf) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 3.7 (H5), 3.75 (H3); 2.67 (H6^A) shows cross-peaks with 2.93 (H6^A), 3.19 (H4^A); 2.93 (H6^A) shows cross-peaks with 2.67 (H6^A), 3.7 (H5), 3.8 (H5^A); 3.19 (H4^A) shows cross-peaks with 2.67 (H6^A), 3.75 (H3), 3.8 (H5^A); 3.58 (α CH) shows cross-peaks with 1.36 (Me), 7.30 (Ho, Hm); 3.7 (H5) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (Ho, Hm); 3.75 (H3) shows cross-peaks with 1.25 (Hc,d), 1.39 (Hb,e), 2.62 (Hf), 7.30 (Ho, Hm); 3.8 (H5^A) shows cross-peaks with 2.45 (Ha), 2.93 (H6^A), 3.19 (H4^A); 7.30 (Ho, Hm) shows cross-peaks with 3.58 (α CH), 3.75 (H3). Very similar spectra were recorded for the β CDNH(CH₂)₆NH₂·(R)-2-phenylpropanoate complex.

Molecular modelling²⁰ was carried out using a Silicon Graphics Iris Indigo X2 400 Unix workstation. Computational results were obtained using the force-field programme CVFF with the 6-12 e function with geometric averages for the heteronuclear interactions. Energy minimisations were performed with the Discover programme, using a steepest descents algorithm until the root mean square of the residuals (RMS) derived < 10 , whereafter a conjugate gradients algorithm was used until RMS < 1 and the global minimisation was obtained using a quasi Newton–Raphson algorithm. Several local energy minima were found before the global minimum was reached. Graphical displays were printed through the Insight II molecular modelling programme.

Results and discussion

Complex stabilities

The various weak secondary bonding interactions between the CD host and the guest can sum to produce quite high stabilities in CD complexes. It has been concluded from a wide range of studies dominated by natural CDs that the complexation process involves conformational change in the CD host and the guest accompanied by dehydration of both to an extent depending on the nature of both entities.^{1,3,11,13,15,16} These aspects of CD complexation have been extensively discussed, and as a consequence only those interactions which appear to dominate the variations in stability observed in this study are discussed in detail here. On a statistical basis, 64 different host–guest complexes could be formed between the four host β CDNH₂·(CH₂)_nNH₃²⁺ ($n = 2, 3, 4$ and 6) and the four guest carboxylic acids and their respective conjugate bases, but only 35 of these complexes were detected under the conditions of this study. The complex stability range is encompassed by stability constants $K = 140$ and 1760 dm³ mol⁻¹ for equilibria (7) and (16) when $n = 2$ and 6, respectively, as shown in Table 1. While each pos-

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

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

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

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

A broad pattern emerges in the variation of complex stability in Table 1. This may be represented through Fig. 3 where the rectangle represents the set of stability constants for a carboxylic acid and its carboxylate being complexed by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ and its protonated analogues. Generally, the most stable complexes occur in the top left and bottom right corners of the rectangle (with the exception of $[\beta\text{CDNH}_2(\text{CH}_2)_2\text{NH}_3 \cdot 4\text{-methylbenzoic acid}]^{2+}$ which was not detected), while there is an absence of detected complexes about the diagonal running from the bottom left to the top right of the rectangle. This

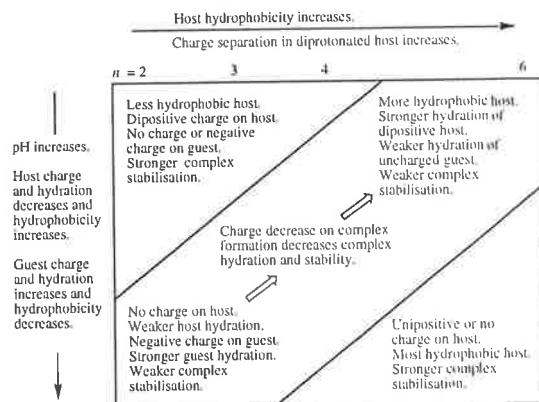


Fig. 3 A schematic representation of the variation of complex stability and the factors contributing to it.

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

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

The β CD annulus and the two amine groups represent constant structural features in the four β CDNH(CH₂)_nNH₂ hosts. Hence, variations in effects i)–iv) generated by the CD hosts arise predominantly from the changes in the length of the –NH(CH₂)_nNH₂ substituent indicated by *n*. The phenyl ring and the carboxylic acid group are invariant in the four guest carboxylic acids, and therefore differences in their complexation characteristics arise from differences in hydrophobicity and stereochemistry engendered by the CH₃– and CH₃CH< moieties in 4-methylbenzoic acid and (*R*)- or (*S*)-2-phenylpropanoic acid, respectively, when compared with those of benzoic acid.

Descending the vertical axis of Fig. 3 is equivalent to increasing solution pH so that the host's charge decreases with decreasing protonation and its hydrophobicity increases as a consequence. Correspondingly, the guest becomes negatively charged with the probable result that its hydration increases and its hydrophobicity decreases. On the horizontal axis, host hydrophobicity increases and the charge separation in the diprotonated host increases as *n* in β CDNH(CH₂)_nNH₂ increases. Thus, in the upper left hand corner of Fig. 3, the host has a dipositive charge and the guest has either no charge or a negative charge, and it appears that either a charge–dipole or a charge–charge interaction stabilises the complex. The intensity of this interaction probably diminishes as *n* increases and the charges in β CDNH₂(CH₂)_nNH₃²⁺ move further apart so that the stabilities of the complexes diminish in the upper right hand corner of Fig. 3. (The orientation of the carboxylic acid or carboxylate guest within the annulus may also change with variation of β CDNH(CH₂)_nNH₂ charge as its protonation changes as indicated by the molecular modelling studies discussed below.)

At the lower left hand corner of Fig. 3, the host is uncharged, the guest is negatively charged and no complexes are observed. (This is consistent with the observation that the analogous complexes formed between β CD and β CDNH₂ and the same carboxylates as those studied here are characterised by stability constants in the range 13–110 dm³ mol^{–1} while the complexes formed by β CD, β CDNH₂ and β CDNH₃⁺ with the analogous acids are characterised by stability constants in the range 340–1680 dm³ mol^{–1}.)² However, as hydrophobicity increases with *n* as Fig. 3 is traversed from left to right, complexation of the carboxylate guest strengthens to give more stable complexes in the lower right hand corner of Fig. 3. While it is possible that the increase in host hydrophobicity is the main source of complex stabilisation, it also appears (from the NMR and molecular modelling studies discussed below) that the –NH(CH₂)_nNH₂ substituent enters the annulus in basic solution both in β CDNH(CH₂)_nNH₂ and in its carboxylate complexes.²¹ As the β CDNH(CH₂)_nNH₂ carboxylate complexes are some of the more stable species, it seems that this latter effect stabilises the host–guest complex while the short –NH(CH₂)₂–NH₂ substituent is less effective in this role. Similar self-complexations are observed for the pendant naphthyl group and pendant dansyl group of 6^A-N-[N'-(5-dimethylamino-1-naphthylsulfonyl)diaminoethane]-6^A-deoxy- β -cyclodextrin and 3^A-O-naphthylmethyl- β -cyclodextrin, respectively.^{22,23} The charged –NH₂(CH₂)_nNH₃²⁺ substituent does not appear to enter the β CDNH₂(CH₂)_nNH₃²⁺ annulus as is discussed below.

Superimposed on these effects is that of hydration. The hydration of β CDNH(CH₂)_nNH₂ and its protonated analogues probably has two main components: water occupying the annulus but interacting weakly with the methylene, methine and ether oxygen moieties defining the hydrophobic centre of the annulus, and water hydrogen bonding with the hydroxy groups at either end of the annulus. (From 6–6.5 H₂O have been observed in the β CD annulus in solid state neutron and X-ray diffraction studies.^{24–27}) The displacement of water from the

annulus by the hydrophobic moiety of the guest during complexation represents a major hydration change and probably makes a significant contribution to the free energy of complexation.^{1,3,6,15,16} Depending on whether it is β CDNH(CH₂)_nNH₂ or one of its protonated analogues which acts as the host, and whether the carboxylic acid or its carboxylate acts as the guest, the extent of hydration may either increase or decrease on formation of the complex. The complete or partial cancellation of host charge by that of the guest is likely to produce a decrease in the overall hydration of the complex compared with that of its charged components, and a consequent lessening of complex stability. This occurs in the centre of Fig. 3 and is exemplified by equilibria (2) and (3), (6) and (7), (10) and (11), and (14) and (15) in Table 1. Hydration effects on stability are likely to be less for equilibria (1), (4), (5), (8), (9), (12), (13) and (16) where no change in overall charge occurs on complexation. Thus, a degree of complex stabilisation may be achieved through minimising the interactions of the hydrophobic moieties of the host and guest with water through partial encapsulation of the hydrophobic guest moiety in the hydrophobic annulus of the host while retaining the hydration of their hydrophilic moieties.

There is no apparent systematic stereochemical effect of the variation of *n* in β CDNH(CH₂)_nNH₂ and its conjugate acids and of the stereochemistry of the carboxylic acids and the conjugate bases other than those subsumed into the discussion of factors i)–iv) above. However, there is a small chiral discrimination in equilibria (9) and (13) when *n* = 2 and 3, where [β CDNH(CH₂)_nNH₃·(*R*)-2-phenylpropanoic acid]²⁺ is more stable than its (*S*)-analogue when *n* = 2 and *vice versa* when *n* = 3. Although small, these differences are consistent with *n* of the –NH₂(CH₂)_nNH₃²⁺ substituent influencing guest orientation as is also the case for the –NH(CH₂)_nNH₂ substituent where *n* = 3 and 6 in equilibria (12) and (16).

NMR structural studies

The detailed assignment of the ¹H NMR spectrum of β CDNH(CH₂)₆NH₂ at pH $\geq$ 11.5 (Fig. 4) is presented in the Experimental section, and the cross-peaks observed in the ROESY spectrum (Table 2) provide structural information. The cross-peaks arising from interaction between Hb–Hf of the –NH(CH₂)₆NH₂ substituent and H3 and H5 of the β CD annulus and Ha–Hc and H5^A are consistent with complexation of –NH(CH₂)₆NH₂ inside the annulus as shown schematically in Fig. 5. These cross-peaks are absent from the ROESY spectrum obtained after acidification of the sample solution to pH 1 with hydrochloric acid consistent with the protonated –NH₂(CH₂)₆NH₃²⁺ substituent being excluded from the annulus as a result of its decreased hydrophobicity.

The titration data discussed above indicate that the [β CDNH(CH₂)₆NH₂·4-methylbenzoate][–] complex constitutes 85% of the total [β CDNH(CH₂)₆NH₂] and [4-methylbenzoate] at the pH $\geq$ 11.5 of the NMR study. The ¹H chemical shifts and the spectral resolution of the –NH(CH₂)₆NH₂ substituent methylene protons of β CDNH(CH₂)₆NH₂ differ from those observed for the [β CDNH(CH₂)₆NH₂·4-methylbenzoate][–] complex (Fig. 5 and Experimental) consistent with the methylene chain of –NH(CH₂)₆NH₂ being inside the β CD annulus and parallel to the face of the aromatic ring where they experience an anisotropic field arising from the high π electron density of the guest. Cross-peaks between Ha–Hf of –NH(CH₂)₆NH₂ and H3 and H5, and H_o and H_m of the 4-methylbenzoate and H3 and H5 (Fig. 6 and Table 2) are consistent with the simultaneous complexation of both entities in the β CD annulus. However, there are no cross-peaks due to interactions between the –NH(CH₂)₆NH₂ substituent and 4-methylbenzoate. These data are consistent with either a single complex where the carboxylate protrudes from either the primary or the secondary face (Fig. 5) of the β CD annulus (respectively delineated by primary and

Table 2 ^1H NMR cross-peaks^a observed for $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ and its 4-methylbenzoate and (*S*)-2-phenylpropanoate complexes

$\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$	
Annular protons	6-Aminohexyl substituent protons
	Ha Hb Hc Hd He Hf
H3	+ ++ ++ ++ ++ ++
H5	+ ++ ++ ++ ++ +
H5 ^A	++ + +
$[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$	
Annular protons	6-Aminohexyl substituent and 4-methylbenzoate protons
	Ha Hb Hc Hd He Hf Ho Hm Me
H	++ ++ ++ ++ ++ ++ + ++ +
H3	++ ++ ++ ++ ++ ++ + ++ +
H5	++ ++ ++ ++ ++ ++ + ++ +
$[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2 \cdot (S)\text{-2-phenylpropanoate}]^-$	
Annular protons	6-Aminohexyl substituent and (S)-2-phenylpropanoate protons
	Ha Hb Hc Hd He Hf Ho, Hm Me
H3	+ + + + + ++ +
H5	+ + + + + +
H5 ^A	++

^a The intensity of the cross peaks increases from + to ++. The concentrations of $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ and either 4-methylbenzoate or (S)-2-phenylpropanoate, when present, were 0.06 mol dm⁻³.

^a The intensity of the cross peaks increases from + to ++. The concentrations of $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ and either 4-methylbenzoate or (*S*)-2-phenylpropanoate, when present, were 0.06 mol dm^{-3} .

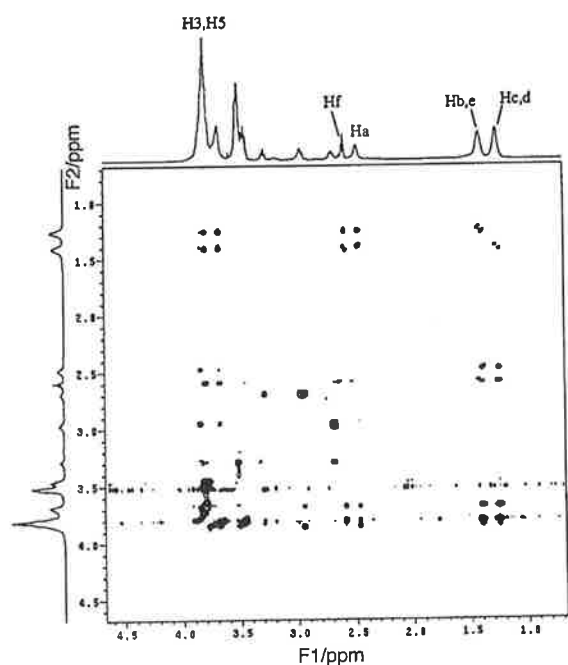


Fig. 4 The ^1H 600 MHz ROESY NMR spectrum of $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$. Cross-peaks are formed between Hb-Hf and H3, Ha-Hf and H5 and Ha-Hc and H5^A.

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

The titration data above also indicate that the $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2 \cdot (\text{S})\text{-2-phenylpropanoate}]^-$ complex constitutes 90% of the total $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2]$ and $[(\text{S})\text{-2-phenylpropanoate}]$ at the $\text{pH} \geq 11.5$ of the NMR study. In the ROESY spec-

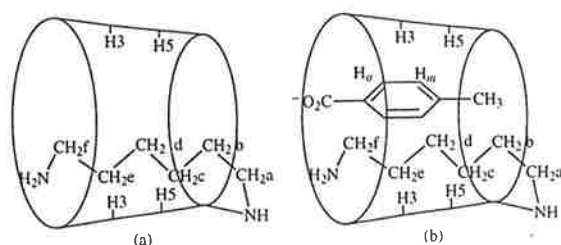


Fig. 5 Schematic representations of the structures of (a) the intramolecular complex formed by $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ and (b) the intermolecular $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ complex.

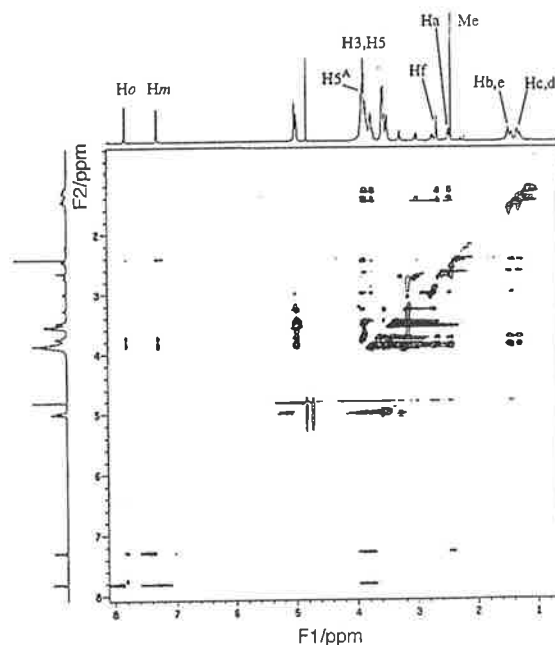


Fig. 6 The ^1H 600 MHz ROESY NMR spectrum of the $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ complex showing cross-peaks formed between Ha to Hf and H3 and H5, Ho and Hm of 4-methylbenzoate and H3 and H5, and Me of 4-methylbenzoate and H3 and H5.

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

Molecular modelling

Gas phase force-field molecular modelling²⁰ produced the global energy minimised ($877.1 \text{ kJ mol}^{-1}$) structure of $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ with the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent complexed inside the βCD annulus as shown in Fig. 7. Similar modelling showed that the $-\text{NH}_2(\text{CH}_2)_6\text{NH}_3^{2+}$ substituent of $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_3^{2+}$ does not enter the βCD annulus. Both of these

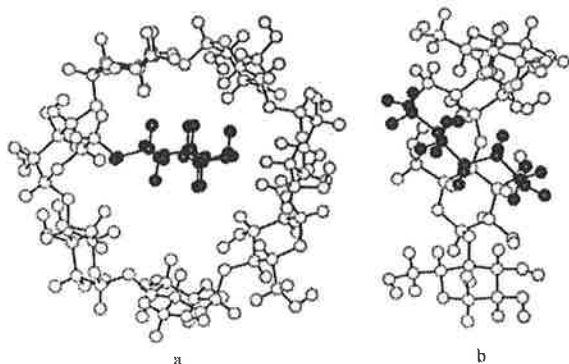


Fig. 7 The global energy minimised structure of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ viewed from a) the primary face of the annulus and b) from the side with three glucopyranose units cut away. The $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent is shown in dark shading.

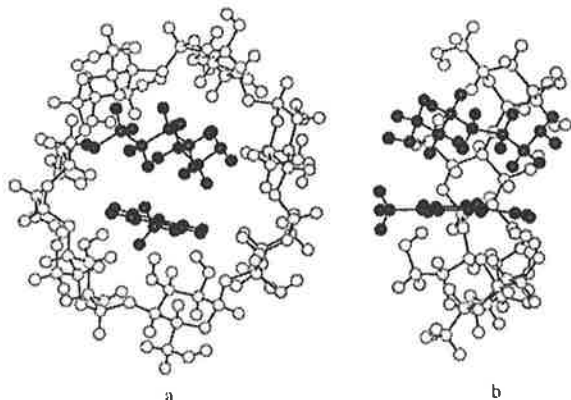


Fig. 8 The global energy minimised structure of $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ viewed from a) the primary face of the βCD annulus and b) from the side with three glucopyranose units cut away. The $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent and 4-methylbenzoate are shown in dark shading.

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

Modelling the $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ complex produced a global energy minimum ($797.4 \text{ kJ mol}^{-1}$) for the structure shown in Fig. 8 where 4-methylbenzoate is oriented with its carboxylate group towards the secondary face of the βCD annulus and the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent is also complexed inside the βCD annulus consistent with the NMR data. This orientation of the carboxylate towards the secondary face is also found in adamantane-1-carboxylate complexes of αCD and βCD ¹⁰ and the cyclohexanecarboxylate complex of βCD .¹¹ When the 4-methylbenzoate orientation is reversed so that the carboxylate group is oriented towards the primary face the complex energy is $870.4 \text{ kJ mol}^{-1}$ consistent with this being a less favoured orientation. The modelled structure of the $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_3 \cdot 4\text{-methylbenzoate}]^+$ complex shows the 4-methylbenzoate guest to have its carboxylate group in the vicinity of the primary face of the βCD annulus. This reversal of orientation, compared with that in the $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ complex, is consistent with the charge of the $-\text{NH}_{(1+m)}(\text{CH}_2)_2\text{NH}_{(2+n)}^{(m+n)+}$ substituent (where $m = 0$ or 1 and $n = 0$ or 1) substantially influencing guest orientation through electrostatic interactions. This has also been found to occur in modelling studies of the 4-methylbenzoate complex of protonated heptakis(6-amino-6-deoxy)- β -cyclodextrin¹² and also its amino acid complexes.¹⁴ While our modelling studies show the probable orienting effects of charge in $[\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_3 \cdot 4\text{-methylbenzoate}]^+$ and $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$, the latter complex was not detected in solution as discussed above.

the latter complex was not detected in solution as discussed above.

Molecular modelling also shows that both the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent and (*S*)-phenylpropanoate are complexed within the βCD annulus in the $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot (\text{S})\text{-phenylpropanoate}]^-$ complex. The carboxylate group is oriented towards the secondary face of the βCD annulus. The $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot (\text{R})\text{-phenylpropanoate}]^-$ complex is found to have a similar structure to that of its (*S*)-analogue with some differences in orientation of the guest within the βCD annulus. The globalised energy minima of the $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot (\text{S})\text{-phenylpropanoate}]^-$ complex and its (*R*)-analogue are 738.5 and $812.3 \text{ kJ mol}^{-1}$, respectively, showing the (*S*)-diastereomer to be the more stable in the gas phase.

Conclusion

The stabilities of the host-guest complexes formed between the 6^A-(ω -aminoalkylamino)-6^A-deoxy- β -cyclodextrin hosts and their protonated forms [where the ω -aminoalkylamino groups are $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ and $n = 2, 3, 4$ and 6] and the guests, benzoic acid, 4-methylbenzoic acid and (*R*)- or (*S*)-2-phenylpropanoic acid and their conjugate bases, vary significantly. This is consistent with the charge and hydrophobicity of the host and the guest generating significant secondary interactions which affect complex stability. The ¹H NMR studies show that the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ self-complexes inside the βCD annulus, and that in $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot 4\text{-methylbenzoate}]^-$ and its (*S*)-2-phenylpropanoate analogue both the guest and the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent are simultaneously complexed within the βCD annulus. Gas phase force-field modelling also show this to occur for $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ and these two complexes, where in the latter two cases the carboxylate group is oriented towards the secondary face of the annulus. The entry of the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent into the βCD annulus may significantly affect complex stability, as may also be the case for the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituents where $n = 2, 3$ and 4 if they also enter the βCD annulus. In contrast the fully protonated $-\text{NH}_2(\text{CH}_2)_n\text{NH}_3^+$ substituent does not enter the βCD annulus according to the ¹H NMR and molecular modelling studies.

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Deacylation of 4-nitrophenyl acetate by 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins†

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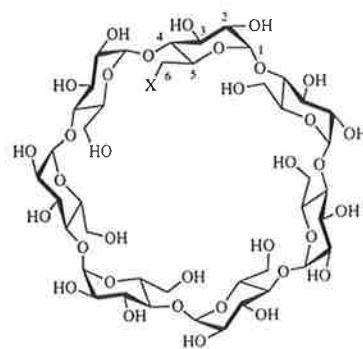
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The deacylation of 4-nitrophenyl acetate (*p*NPA) in aqueous solution to give 4-nitrophenolate is significantly accelerated by the 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins [βCDNH(CH₂)_nNH₂] which are themselves acylated to give predominantly βCDNH(CH₂)_nNHCOCH₃. The deacylation is characterised by *k*_d*K* = 27.4, 35.5, 24.5 and 16.0 dm³ mol⁻¹ s⁻¹ at 298.2 K in aqueous 0.05 mol dm⁻³ borate buffer and *I* = 0.10 mol dm⁻³ (NaClO₄) when *n* = 2, 3, 4 and 6, respectively, where *k*_d (s⁻¹) is the rate constant for *p*NPA deacylation through a βCDNH-(CH₂)_nNH₂·*p*NPA complex characterised by a stability constant *K* (dm³ mol⁻¹). The inhibition of the deacylation by adamantane-1-carboxylate (AC⁻) is consistent with a mechanism where AC⁻ competes with *p*NPA in entering the βCDNH(CH₂)_nNH₂ annulus through the formation of a βCDNH(CH₂)_nNH₂·AC⁻ complex. The latter complex has been qualitatively studied by ¹H NMR ROESY methods, and its structure and that of βCDNH(CH₂)_nNH₂·*p*NPA have also been force-field modelled. The possibility of the operation of an S_N2 mechanism as an alternative explanation for the deacylation data is also considered.

Introduction

Natural and modified cyclodextrins (CDs) possess annuli which accommodate a wide range of guest species in the formation of complexes.¹⁻⁶ To some extent, these complexes resemble Michaelis complexes formed between enzymes and substrates and have been studied both as possible enzyme mimics and because of their intrinsic interest as in the classical studies of α- and β-cyclodextrin (αCD and βCD) by Bender and co-workers.⁷ Breslow and co-workers have been at the forefront of studies of substantially modified CDs that accelerate reactions of guest species in their complexes by a thousand-fold or so in Michaelis–Menten catalytic cycles.⁸ Catalytic studies have also been reported by Tee and co-workers for both natural and modified CDs.⁹ A key aspect of such studies is the identification of the mechanistic steps in the catalytic cycle. In this study, our main interest is to identify the mechanistic steps of the reaction acceleration process and the factors determining the effect of the CD modification up to the formation of the first covalent bond between the modified CD and the guest species. This identifies the mode of nucleophilic attack of the modified cyclodextrin on the guest to give a product which may be likened to the intermediate product in an enzymatic cycle prior to its breakdown and regeneration of the active enzyme.

For our study we have chosen the 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins (Fig. 1), βCDNH(CH₂)_nNH₂ where *n* is either 2, 3, 4 or 6,^{10,11} which we find generate the dominant acylated products βCDNH(CH₂)_nNHCOCH₃ and 4-nitrophenolate through reaction with 4-nitrophenyl acetate (*p*NPA). [Deacylation of *p*NPA by βCDNH(CH₂)₆NH₂ also yields βCDNH(CH₂)₆NH₂ acylated at a secondary hydroxy group as a minor product.] These modified CDs provide an opportunity to study the effect of the flexibility of their diamine substituents on the nucleophilic attack on *p*NPA, and also the influence of protonation of the amine groups on this process.



X = NH(CH₂)_nNH₂ where *n* = 2, 3, 4 or 6

Fig. 1 The 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins, βCDNH(CH₂)_nNH₂, studied.

Experimental

Materials and instrumental methods

The preparations of βCDNH(CH₂)_nNH₂ were as previously described.¹⁰ They were dried to constant weight and stored over P₂O₅ prior to use, and gave good microanalyses and clean ¹³C NMR spectra. 4-Nitrophenyl acetate was prepared by standard methods and recrystallised from ethanol. Adamantane-1-carboxylic acid (Aldrich) was used as received. Borate buffer, prepared from Na₂B₄O₇·7H₂O (BDH Analar), and HEPES (CalBiochem) were made up as described in the literature.¹² All pH measurements were made with an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode. Aqueous solutions were prepared in deionised water, purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm which was boiled to remove CO₂. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F₂₅₄ silica on aluminium sheets. Samples were eluted using a mixture of PrOH–EtOH–H₂O–NH₄OH

† β-Cyclodextrin = cyclomaltoheptaose.

(7:7:5:4). Compounds containing amino groups were detected by dipping the developed plate into a solution of 1% ninhydrin in ethanol and heating the plate. CDs were detected by dipping the developed plate into a solution of 1.5% H₂SO₄ in ethanol and heating the plate. Values of R_f are reported as R_c (retention relative to β CD).

The deacylation of *p*NPA was followed spectrophotometrically. In a typical hydrolysis run, 2 cm³ of a solution of β CDNH(CH₂)_{*n*}NH₂ in the concentration range (5.36–99.3) $\times 10^{-5}$ mol dm⁻³ buffered in borate buffer (0.05 mol dm⁻³) was allowed to attain the reaction temperature of 298.2 K over 30 min in a quartz 1 cm pathlength cell at 298.2 K in the thermostatted cell block of a Varian Cary 2200 spectrophotometer. A 50 μ m³ aliquot of 2.0×10^{-4} mol dm⁻³ *p*NPA in acetonitrile was then added with a micropipette and rapidly mixed to make the reaction solution 5.0×10^{-6} mol dm⁻³ in *p*NPA, and the increase of absorbance at 400 nm was recorded digitally for at least 7 reaction half-lives as *p*NPA deacylated to 4-nitrophenolate. The observed first order deacylation rate constant, k_{obs} , was determined by fitting the 3000 digital points to a first order rate equation by conventional methods. The rate constant, k_n , for *p*NPA deacylation was similarly determined in the presence of buffer alone. Deacylation rates were also determined in the presence of NH₂(CH₂)_{*n*}NH₂ (*n* = 2, 3 and 6) and in the presence of β CDNH(CH₂)_{*n*}NH₂ and adamantane-1-carboxylate (AC⁻). All rate constants were determined in triplicate at least.

The ¹H (300 MHz) and ¹³C (74.57 MHz) NMR spectra of β CDNH(CH₂)_{*n*}NHCOCH₃ were run on a Varian Gemini 300 spectrometer, and ¹H ROESY (mixing time of 0.35 s)¹³ 600 MHz spectra of the β CDNH(CH₂)_{*n*}NH₂·AC⁻ complex were run on a Varian Inova 600 spectrometer. The spectral assignments listed below are given according to the numbering in Fig. 1 and the alphabetical labelling of the methylene groups in the diaminoalkyl substituent from (a) for that adjacent to the secondary amino group. The 600 MHz ¹H NMR spectrum of a solution 0.1 mol dm⁻³ in β CDNH(CH₂)₆NH₂ and AC⁻ in D₂O at pH ≥ 11 , where the dominant species is the [β CDNH(CH₂)₆NH₂·AC⁻] complex, is characterised by: δ_{H} 4.65 (m, 7H, H1), 3.81 (t, *J* 10.2 Hz, 1H, H5^A), 3.5–3.8 (m, 25H, H3, H5, H6), 3.2–3.4 (m, 13H, H2, H4), 3.06 (t, *J* 10.2 Hz, 1H, H4^A), 2.92 (d, *J* 14.0 Hz, 1H, H6^A), 2.58 (dd, *J* 14.0, 10.2 Hz, 1H, H6^A), 2.42 (m, 2H, hexyl Hf), 2.23 (dt, *J* 5.4, 10.8 Hz, 1H, hexyl Ha), 2.14 (m 1H, hexyl Ha'), 1.99 (br s, 3H, AC⁻ H3), 1.76 (br s, 6H, AC⁻ H2), 1.69 (br d, *J* 10.8 Hz, 3H, AC⁻ H4), 1.45 (br d, *J* 10.8 Hz, 3H, AC⁻ H4'), 1.0–1.4 (m, 8H, hexyl Hb–e). In the ¹H ROESY spectrum, δ_{H} 1.45 (AC⁻ H4') shows cross-peaks with 1.69 (AC⁻ H4), 1.99 (AC⁻ H2), 3.53 (H5); 3.7 (H3), 1.69 (AC⁻ H4) shows cross-peaks with 1.45 (AC⁻ H4'), 1.99 (AC⁻ H2), 3.53 (H5); 3.7 (H3); 1.76 (AC⁻ H2) shows cross-peaks with 1.99 (AC⁻ H2); 3.53 (H5), 3.7 (H3); 1.99 (AC⁻ H3) shows cross-peaks with 1.45 (AC⁻ H4'), 1.69 (AC⁻ H4), 1.76 (AC⁻ H2), 3.53 (H5), 3.7 (H3); 2.23 (hexyl Ha) shows cross-peaks with 2.42 (hexyl Hf), 2.92 (H6^A), 3.81 (H5^A); 2.42 (hexyl Hf) shows cross-peaks with 2.23 (hexyl Ha), 3.81 (H5^A); 2.58 (H6^A) shows cross-peaks with 2.92 (H6^A), 3.06 (H4^A); 2.92 (H6^A) shows cross-peaks with 2.58 (H6^A), 3.81 (H5^A); 3.53 (H5) shows cross-peaks with 1.45 (AC⁻ H4'), 1.69 (AC⁻ H4), 1.76 (AC⁻ H2), 1.99 (AC⁻ H3); 3.7 (H3) shows cross-peaks with 1.45 (AC⁻ H4'), 1.69 (AC⁻ H4), 1.76 (AC⁻ Hb), 1.99 (AC⁻ H3); 3.81 (H5^A) shows cross-peaks with 2.23 (hexyl Ha), 2.42 (hexyl Hf), 2.92 (H6^A).

Molecular modelling¹⁴ was carried out using a Silicon Graphics Iris Indigo X2 400 Unix workstation. Computational results were obtained using the force-field programme CVFF incorporating the 6–12 ϵ function with geometric averages for the heteronuclear interactions. Energy minimisations were performed with the Discover programme, using a steepest descents algorithm until the root mean square of the residuals (RMS) derived was <10 , whereafter a conjugate gradients algorithm was used until RMS < 1 and the global minimisation

was obtained using a quasi-Newton–Raphson algorithm. Several local energy minima were found before the global minimum was reached. Graphical displays were printed through the Insight II molecular modelling programme.

Acylation reactions

To aid the identification of the products of deacylation of *p*NPA accelerated by β CDNH(CH₂)_{*n*}NH₂, it was necessary to prepare the most probable products as described below. The primary aim of these preparations was to use the products as aids to identification of the products obtained in the kinetic studies under aqueous conditions. Hence, the preparations were not optimised for yield.

Acylation of 6^A-(2-aminoethyl)amino-6^A-deoxy- β -cyclodextrin

A mixture of β CDNH(CH₂)₂NH₂ (0.100 g, 0.085×10^{-3} mol) and *p*NPA (0.015 g, 0.082×10^{-3} mol) in 1-methyl-2-pyrrolidone (NMP, 2 cm³) was stirred at room temperature for 18 hours. TLC showed the presence of β CD and a new spot ($R_c = 1.06$). 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 β CDNH(CH₂)₂NHCOCH₃ were combined and dried to give 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 β CDNH(CH₂)₂NHCOCH₃·HCl·6H₂O (C₄₆H₉₁ClN₂O₄₁) C, 40.52; H, 6.72; N, 2.05%); δ_{H} (D₂O) 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, CH₂NCOCH₃), 3.06 (d, *J* 11.7 Hz, 1H, H6^A), 2.77 (m, 3H, H6^A, CH₂NH), 1.99 (s, 3H, methyl); δ_{C} (D₂O) 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 (ethyl Ca), 41.26 (ethyl Cb), 24.63 (methyl).

Acylation of 6^A-(3-aminopropyl)amino-6^A-deoxy- β -cyclodextrin

A mixture of β CDNH(CH₂)₃NH₂ (0.096 g, 0.086×10^{-3} mol) and *p*NPA (0.016 g, 0.088×10^{-3} mol) in NMP (2 cm³) was stirred at room temperature for 18 hours. TLC showed the presence of β CD and a new spot ($R_c = 1.1$). The yellow solution was diluted with 1 mol dm⁻³ hydrochloric acid (30 cm³) and washed with dichloromethane (5×10 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 4–14, containing β CDNH(CH₂)₃NHCOCH₃, were combined and evaporated to dryness to give 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 β CDNH(CH₂)₃NHCOCH₃·HCl·5H₂O (C₄₇H₉₁ClN₂O₄₀) C, 41.52; H, 6.74; N, 2.06%); δ_{H} (D₂O, 25 °C) 5.06 (s, 7H, H1), 3.8–4.0 (m, 26H, H3, H5, H6), 3.5–3.7 (m, 14H, H2, H4), 3.0–3.5 (m, 5H), 2.80 (m, 0.3H), 2.58 (t, *J* 6.9 Hz, 0.6H), 2.00, 1.98 (s, 3H, methyl ratio 2:1), 1.68 (m, 2H, propyl Hb); δ_{C} (D₂O) 176.69, 167.46 (C=O), 104.82, 104.33 (C1), 86.57 (C4^A), 83.92, 83.52 (C4), 75.94, 74.95, 74.67 (C2, C3, C5), 72.67 (C5^A), 65.38, 63.03 (C6), 51.86 (C6^A), 48.52 (propyl Ca), 39.95 (propyl Cc), 30.93 (propyl Cb), 24.64 (methyl); δ_{H} (D₂O, 50 °C) 5.35 (s, 7H, H1), 4.0–4.3 (m, 26H, H3, H5, H6), 3.8–4.0 (m, 13H, H2, H4), 3.68 (t, *J* 8.9 Hz, 1H, H4^A), 3.47 (t, *J* 6.9 Hz, 2H, CH₂NAc), 3.30 (d, *J* 11.9 Hz, 1H, H6^A), 3.04 (dd, *J* 11.9, 7.1 Hz, 1H, H6^A), 2.87 (t, *J* 6.9 Hz, 2H, CH₂NH), 2.26 (s, 3H, methyl), 1.94 (br s, 2H, propyl Hb).

Acylation of 6^A-(6-aminohexyl)amino-6^A-deoxy-β-cyclodextrin

A mixture of βCDNH(CH₂)₆NH₂ (0.101 g, 0.082 × 10⁻³ mol) and *p*NPA (0.014 g, 0.078 × 10⁻³ mol) in NMP (2 cm³) was stirred at room temperature for 18 hours. TLC showed the presence of βCD and a new spot (*R*_f = 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 βCDNH-(CH₂)₆NHCOCH₃ were combined and dried to give a white powder (0.018 g, 18%); *R*_f = 1.2; Electrospray-MS 1275 (M⁺) (Found C, 42.84; H, 6.64; N, 1.99. Calculated for βCDNH-(CH₂)₆NHCOCH₃·HCl·5H₂O (C₅₀H₉₇ClN₂O₄₀) C, 42.84; H, 6.97; N, 2.00%; δ_H(D₂O) 5.07 (s, 7H, H1), 3.5–4.0 (m, 39H, H2, H3, H4, H5, H6), 3.40 (t, *J* 9.0 Hz, 1H, H4^A), 3.16 (t, *J* 7.2 Hz, 2H, CH₂NHCOCH₃), 3.05 (d, *J* 12.6 Hz, 1H, H6^A), 2.76 (m, 1H, H6^A), 2.58 (t, *J* 7.2 Hz, 2H, CH₂NH), 1.99 (s, 3H, methyl), 1.2–1.6 (m, 8H, hexyl Hb, hexyl Hc, hexyl Hd, hexyl He); δ_C(D₂O) 176.51 (C=O), 104.81, 104.73, 104.61, 104.43, 103.36 (C1), 85.72 (C4^A), 84.03, 83.89, 83.75, 82.83 (C4), 76.48, 76.04, 75.85, 75.65, 74.79, 74.45 (C2, C3, C5), 71.24 (C5^A), 63.04, 62.90 (C6), 50.47 (C6^A), 49.20 (hexyl Ca), 42.11 (hexyl Cf), 31.26, 29.92, 28.81, 28.70 (hexyl Cb, hexyl Cc, hexyl Cd, hexyl Ce), 24.76 (methyl).

Results and discussion

Deacylation kinetics in the presence of βCDNH(CH₂)_nNH₂

The deacylation kinetic data are first discussed in terms of the mechanism proposed in Fig. 2 where a rapid preequilibrium between βCDNH(CH₂)_nNH₂ (1) and *p*NPA (2) results in the reactive complexes 3a and 3b which lead to the products βCDNH(CH₂)_nNHCOCH₃ (4) and βCDNH(CH₂)_nNH₂ acylated at a secondary hydroxy group (5). The *p*NPA deacylation rate varies with pH according to eqn. (1) where *k*₀ is the rate constant observed at a particular pH in either 0.05 mol dm⁻³ borate or HEPES aqueous buffer at 298.2 K and *I* = 0.10 mol dm⁻³ (NaClO₄) over the pH range 6.08–10.03 (Table 1). The observed rate constant for the deacylation of *p*NPA in excess [βCDNH(CH₂)_nNH₂]_{total} at a given pH is *k*_{obs}, and *k*_{obs} - *k*₀ is the acceleration of the deacylation of *p*NPA (Table 1). The βCDNH(CH₂)_nNH₂ bearing the shortest and longest substituents (*n* = 2 and 6) were selected to determine the form of the deacylation pH dependence which is considered to encompass the characteristics of the βCDNH(CH₂)_nNH₂ series. This deacylation is greatly accelerated as pH is increased when *n* = 2 and 6 consistent with βCDNH(CH₂)_nNH₂ being the dominant nucleophile such that *k*_{obs} - *k*₀ varies according to eqns. (2) and (3), where [3] refers to either 3a or 3b or both depending on their reactivities, to give *k*_d*K* = 27.4 ± 0.2 and 16.0 ± 0.2 dm³ mol⁻¹ s⁻¹ when *n* = 2 and 6, respectively. (When the *k*_{obs} - *k*₀ data is fitted to an equation analogous to eqn. (2), but including a term in [βCDNH(CH₂)_nNH₃⁺], the derived rate constants characterising βCDNH(CH₂)_nNH₃⁺ are <5% of that characterising βCDNH(CH₂)_nNH₂. Accordingly βCDNH(CH₂)_nNH₃⁺ is judged to be an insignificant participant in the deacylation process.) Thus *k*₀ and the rate are given by eqns. (1) and (2),

$$k_0 = 5.43 \times 10^{(0.823\text{pH} - 12)} \quad (1)$$

$$\text{rate} = (k_{\text{obs}} - k_0)[p\text{NPA}]_{\text{total}} = k_d[3] = k_d K[p\text{NPA}][1] = k_d K[p\text{NPA}][1]_{\text{total}} / (1 + [\text{H}^+]/K_a) \quad (2)$$

where [1] ≈ [1]_{total} because [1]_{total} ≫ [pNPA]_{total}, and *K* = [3]/([pNPA][1]) and *K*_a is the acid dissociation constant of βCDNH-(CH₂)_nNH₃⁺. Given eqn. (3), it follows that when 1 ≫ *K*[1],

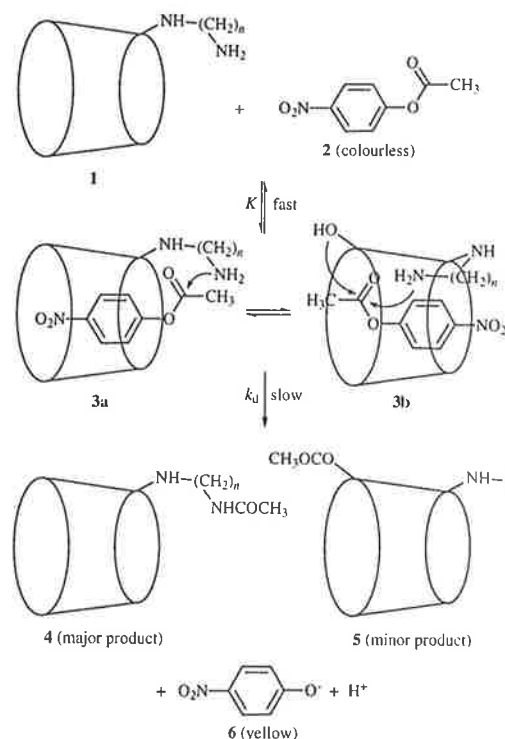


Fig. 2 Scheme for the deacylation of *p*NPA, 2, by βCDNH-(CH₂)_nNH₂, 1. The truncated cones represent the βCD annulus where the wide face is delineated by 14 secondary hydroxy groups and the narrow face by 6 primary hydroxy groups and the secondary amine group of the (ω-aminoalkyl)amino substituent. Two opposed orientations are shown for *p*NPA in the annulus in the complexes, 3a and 3b, where the curved arrows indicate possible directions of nucleophilic attack. The minor product, 5, was detected when *n* = 6.

Table 1 Variation of the rate constants^a for the deacylation of *p*NPA in the absence (*k*₀) and the presence of βCDNH(CH₂)_nNH₂ (*k*_{obs}) in aqueous buffered solutions at 298.2 K and *I* = 0.10 mol dm⁻³ (NaClO₄)

pH	<i>n</i> = 2 ^b		pH	<i>n</i> = 6 ^c	
	<i>k</i> ₀ /10 ⁻⁵ s ⁻¹	<i>k</i> _{obs} /10 ⁻³ s ⁻¹		<i>k</i> ₀ /10 ⁻⁵ s ⁻¹	<i>k</i> _{obs} /10 ⁻³ s ⁻¹
6.08 ^d	0.055	0.13	7.89 ^e	1.71	0.083
6.40 ^d	0.10	0.19	8.37 ^e	4.32	0.25
7.04 ^d	0.34	0.40	8.73 ^e	8.67	0.58
7.88 ^d	1.66	0.91	9.10 ^e	17.7	1.13
8.04 ^e	2.24	1.48	9.41 ^e	28.4	1.86
8.44 ^d	4.78	2.88	9.73 ^e	57.9	3.63
8.78 ^e	9.11	4.61	10.03 ^e	97.3	5.91
9.31 ^e	24.9	9.79	10.3 ^e	187	10.8
9.81 ^e	64.1	16.48			
10.03 ^e	97.3	19.49			

^a Each rate constant represents the average of three determinations. The error is < ± 4%. ^b [βCDNH(CH₂)₂NH₂] = 8.12 × 10⁻⁴ mol dm⁻³. ^c [βCDNH(CH₂)₆NH₂] = 9.77 × 10⁻⁴ mol dm⁻³. ^d HEPES buffer. ^e Borate buffer.

$$k_{\text{obs}} - k_0 = k_d K[p\text{NPA}][1] / ([p\text{NPA}] + [3]) = k_d K[1] / (1 + K[1]) \quad (3)$$

(*k*_{obs} - *k*₀)/[1] ≈ (*k*_{obs} - *k*₀)/[1]_{total} ≈ *k*_d*K* consistent with the linear dependence of *k*_{obs} - *k*₀ on [1]_{total} at pH 9.10 and 298.2 K seen in Fig. 3 where *n* = 2, 3, 4 and 6. The slopes: (*k*_{obs} - *k*₀)/[βCDNH(CH₂)_nNH₂]_{total} = 7.03 ± 0.05, 3.57 ± 0.02, 1.15 ± 0.04, and 0.90 ± 0.01 dm³ mol⁻¹ s⁻¹ for *n* = 2, 3, 4 and 6, respectively. When these values are corrected to reflect the proportions of [βCDNH(CH₂)_nNH₂]_{total} existing as the uncharged and

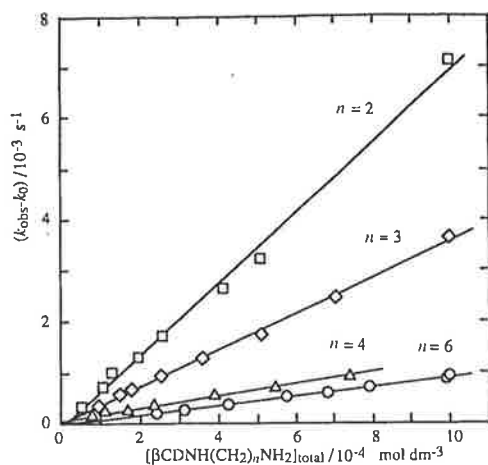


Fig. 3 The variation of $k_{\text{obs}} - k_0$ for the deacylation of $p\text{NPA}$ with $[\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2]_{\text{total}}$ for $n=2$ ($\square$), 3 ($\diamond$), 4 ($\triangle$) and 6 ($\circ$) at 298.2 K in borate buffer at pH 9.10 and $I=0.10 \text{ mol dm}^{-3}$ (NaClO_4). The solid lines represent linear least-square fits of $(k_{\text{obs}} - k_0)/[\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2]_{\text{total}} = k_d K$ to the data.

dominant nucleophile at pH 9.10, $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$, $k_d K = 25.5 \pm 0.2$, 35.5 ± 0.1 , 24.5 ± 0.8 , and $26.4 \pm 0.1 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ when $n=2, 3, 4$ and 6 . The $k_d K$ obtained when $n=2$ is in reasonable agreement with that obtained from the pH dependency studies. However, $k_d K = 25.0 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ is greater than $k_d K = 16.0 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ obtained from the pH dependency studies. The reason for this is probably that when $n=2$ the $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ constitutes 27.54% of the total at pH 9.10 whereas when $n=6$ $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ constitutes 3.40% of the total and as a consequence small errors in pH measurement have a substantial effect on the calculation of $k_d K$ at pH 9.10 in the latter case. Because the pH range employed in the pH dependence studies extends to higher pH values the $k_d K$ derived from them when $n=6$ is probably more reliable. [At pH 9.10, the percentage of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ existing in the un-, mono- and di-protonated states are: 27.54, 72.43 and 0.04; 10.05, 87.54 and 2.41; 4.70, 85.49 and 9.82; and 3.40, 63.35 and 33.25, where $n=2, 3, 4$ and 6 , respectively. The pK_a s of $\beta\text{CDNH}_2(\text{CH}_2)_n\text{NH}_3^{2+}$ are: 5.70 and 9.42, 7.39 and 9.90, 8.06 and 10.26, and 8.72 and 10.27 where $n=2, 3, 4$ and 6 , respectively.¹⁰ The product identification studies discussed below show that the dominant CD product of the deacylation reactions is $\beta\text{CDNH}_2(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$ (4) and that when $n=6$, $\leq 25\%$ of the CD product could be $\beta\text{CDNH}_2(\text{CH}_2)_n\text{NH}_2$ acylated at a secondary hydroxy group (5).

The reported acceleration of $p\text{NPA}$ deacylation caused by 6 α -amino-6 α -deoxy- β -cyclodextrin¹⁵ (βCDNH_2 , $X = \text{NH}_2$ in Fig. 1), where $k_d K = 7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ (calculated from $k_d K = 6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ reported for pH 10 and $pK_a = 9.20$ for βCDNH_3^+) is less than those caused by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ by ≥ 2 . [It is not possible to apportion the increased reactivity of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ between k_d and K as these parameters were not separately determined.] Overall, this is consistent with the flexible $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent allowing the primary amine to more effectively make a nucleophilic attack on the carbonyl carbon of $p\text{NPA}$. This flexibility allows the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent to enter the annulus of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ and those of its complexes with benzoate, 4-methylbenzoate and (*RS*)-2-phenylpropanoate as shown from ^1H NMR and modelling studies,¹¹ and the modelling studies discussed below are consistent with this occurring in the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2/p\text{NPA}$ complex. [The deacylation of 3-nitrophenyl acetate ($m\text{NPA}$) in the presence of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ occurs much more slowly than does that of $p\text{NPA}$ and produces 25% $\beta\text{CDNH}(\text{CH}_2)_6\text{NHC}(\text{CH}_3)_3$ and 75% $\beta\text{CDNH}_2(\text{CH}_2)_6\text{NH}_2$ acylated at a secondary hydroxy group

at pH 9.10. Acylation at a secondary hydroxy group is also the dominant pathway through which βCD ⁷ and βCDNH_2 ¹⁵ deacylate $m\text{NPA}$.]

An alternative explanation of the kinetic data is that deacylation occurs through an $\text{S}_{\text{N}}2$ mechanism such that eqn. (4)

$$\text{rate} = (k_{\text{obs}} - k_0)[p\text{NPA}]_{\text{total}} = k_2[p\text{NPA}]_{\text{total}}[\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2]_{\text{total}}/(1 + [\text{H}^+]/K_a) \quad (4)$$

applies and $k_2 = 27.4, 35.5, 24.5$ and $16.0 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ when $n=2, 3, 4$ and 6 , respectively. This is further discussed under Deacylation inhibition.

Deacylation kinetics in the presence of βCD and $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$

The effects of the components of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ on the deacylation of $p\text{NPA}$ may be assessed to some extent through a direct comparison of the effect of βCD and $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ on the deacylation of $p\text{NPA}$ with that of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$. Thus, at pH 9.10 in borate buffer, $I=0.10 \text{ mol dm}^{-3}$ (NaClO_4) at 298.2 K, $k_{\text{obs}} - k_0 = (0.14 \pm 0.01) \times 10^{-3} \text{ s}^{-1}$ in the presence of excess $[\beta\text{CD}] = 1.00 \times 10^{-3} \text{ mol dm}^{-3}$, which compares with $k_{\text{obs}} - k_0 = (7.03 \pm 0.28) \times 10^{-3}$, $(3.57 \pm 0.14) \times 10^{-3}$, $(1.15 \pm 0.046) \times 10^{-3}$ and $(0.90 \pm 0.04) \times 10^{-3} \text{ s}^{-1}$ under the same conditions for $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ where $n=2, 3, 4$ and 6 , respectively ($k_0 = 0.20 \times 10^{-3} \text{ s}^{-1}$). This provides an empirical indication of the acceleration of the $p\text{NPA}$ deacylation caused by the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent. A similar empirical comparison of the effect of the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent may be made on the basis of $k_{\text{obs}} - k_0 = (0.69 \pm 0.03) \times 10^{-3}$, $(1.15 \pm 0.05) \times 10^{-3}$ and $(1.08 \pm 0.04) \times 10^{-3} \text{ s}^{-1}$ where $n=2, 3$ and 6 , respectively, for the deacylation of $p\text{NPA}$ in the presence of excess $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ ($[\text{NH}_2(\text{CH}_2)_n\text{NH}_2]_{\text{total}} = 1.00 \times 10^{-3} \text{ mol dm}^{-3}$) under the same conditions as the deacylations discussed above. At pH 9.10 the percentages of these diamines existing as $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ and $\text{NH}_2(\text{CH}_2)_n\text{NH}_3^+$ are 11.8 and 87.2, 2.0 and 56.3, and 0.1 and 10.3 where $n=2, 3$ and 6 , respectively. [The pK_a s of $\text{NH}_3(\text{CH}_2)_n\text{NH}_3^{2+}$ are 7.16 and 9.97, 8.97 and 10.56, and 10.04 and 11.01 where $n=2, 3$ and 6 , respectively.¹⁰] It is assumed that $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ and possibly $\text{NH}_2(\text{CH}_2)_n\text{NH}_3^+$ also, are nucleophiles for $p\text{NPA}$ on which basis it is seen that when the relative proportions of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ and $\text{NH}_2(\text{CH}_2)_n\text{NH}_2$ and $\text{NH}_2(\text{CH}_2)_n\text{NH}_3^+$ at pH 9.10 are taken into account, the free diamines approach or exceed the nucleophilicities of their $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ analogues toward $p\text{NPA}$.

Acylated product identification

Deacylation of $p\text{NPA}$ by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ gives 4-nitrophenolate and a dominant modified CD identified as $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$. This was shown by preparing samples of $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$, where $n=2, 3$ or 6 , by reacting $p\text{NPA}$ and $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ in 1-methyl-2-pyrrolidone as discussed under Experimental. The $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$ products gave good analyses, and the substitution site was identified from ^1H NMR spectra. The resonances of the methylene group adjacent to the amide nitrogen in $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$ showed a downfield shift of 0.5–0.6 ppm (see Experimental) by comparison with the resonances of the methylene group adjacent to the primary nitrogen in $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$.¹⁰ The methylene group adjacent to the secondary nitrogen and the 6 α proton of the βCD moiety showed no significant change in δ compared with that in $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$.¹⁰ This was taken as evidence that the acylation site was the primary amine in $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$. TLC analyses of $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$, of a reacted aqueous reaction mixture (buffered at pH 9.10 with borate, where $[p\text{NPA}]$ approached $[\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2]_{\text{total}}$), $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$, and βCD were run simultaneously. The $\beta\text{CDNH}(\text{CH}_2)_n\text{NHC}(\text{CH}_3)_3$ and the major products from the aqueous reaction

mixture had identical R_f values quite different from those for $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ and βCD . On this basis the predominant acylated product from the aqueous reaction was identified as $\beta\text{CDNH}(\text{CH}_2)_n\text{NHCOCH}_3$. In the case of the TLC study of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ system, a faint spot was observed just ahead of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NHCOCH}_3$ spot, and was thought to be $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ acylated at a secondary hydroxy group, consistent with its identification by ^1H NMR spectroscopy as discussed below.

The ^1H NMR spectra of the reaction mixture of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ and either *p*NPA or *m*NPA in borate buffer at pH 9.10, freeze dried and redissolved in D_2O , showed the methyl resonance of $\beta\text{CDNH}(\text{CH}_2)_n\text{NHCOCH}_3$ at 1.99 ppm and that of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ acylated at a secondary hydroxy group at 2.17 ppm in the ratios 25:75 and 75:25, respectively. The latter resonance disappeared when the pH of the solution was raised ≥ 12 consistent with rapid deacylation of the second product. The assignment of acylation occurring at a secondary hydroxy group is based on a similar assignment for the deacylation of *p*NPA and *m*NPA by βCD where a deprotonated secondary hydroxy group ($\text{p}K_a \approx 12$) is thought to be the nucleophile.⁷

Deacylation inhibition

At pH 9.10 in borate buffer at 298.2 K, the acylation of $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ is inhibited in the presence of adamantane-1-carboxylate. When $[\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2]_{\text{total}} = 1.00 \times 10^{-3} \text{ mol dm}^{-3}$, $k_{\text{obs}} - k_0 = (6.33 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$ which decreases to $(3.14 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$ when $[\text{AC}^-]_{\text{total}} = 5.0 \times 10^{-4} \text{ mol dm}^{-3}$ where $n = 2$. The corresponding values are $(2.73 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$ and $(1.65 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$ where $n = 3$, and $(0.69 \pm 0.03) \times 10^{-3} \text{ s}^{-1}$ and $(0.53 \pm 0.03) \times 10^{-3} \text{ s}^{-1}$ where $n = 6$. In terms of the mechanism shown in Fig. 2, these decreases in $k_{\text{obs}} - k_0$ by 50.4, 39.6 and 23.2% where $n = 2, 3$ and 6, respectively, are consistent with AC^- competing with *p*NPA for complexation by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ and forming a $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2 \cdot \text{AC}^-$ complex. This decreases the proportion of *p*NPA complexed as $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2 \cdot \text{pNPA}$ which is the Michaelis-like complex through which *p*NPA deacylation is thought to occur (3a and 3b in Fig. 2). Thus, while inhibition is quantitative for $\beta\text{CDNH}(\text{CH}_2)_2\text{NH}_2$ which has the shortest substituent, the decreasing effectiveness of the deacylation inhibition by AC^- as n increases to 3 and 6 is attributable to AC^- competing less effectively with the simultaneous complexation of *p*NPA and the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent as the bulk and hydrophobicity of the latter increases with n . Evidence for the simultaneous complexation of *p*NPA and the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent is adduced from reported NMR studies¹⁰ showing the simultaneous complexation of either benzoate, 4-methylbenzoate or (*R,S*)-2-phenylpropanoate and the $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$, and is consistent with the modelling studies discussed below.

An alternative explanation of the inhibition data is that AC^- is complexed quantitatively in the $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ annulus and the ability of the expelled $-\text{NH}(\text{CH}_2)_n\text{NH}_2$ substituent to make a nucleophilic attack on *p*NPA outside the annulus increases with its length so that the decreased deacylation occurring with decreasing $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2 \cdot \text{pNPA}$ formation is partially offset by this alternative $\text{S}_{\text{N}}2$ reaction pathway. Should the alternative $\text{S}_{\text{N}}2$ deacylation mechanism operate both in the absence and the presence of AC^- such that $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$ operates as a sterically hindered diamine the effect of the complexation of AC^- on the magnitude k_2 is not readily predicted. However, it seems unlikely that the quantitative inhibition by AC^- observed for $\beta\text{CDNH}(\text{CH}_2)_2\text{NH}_2$ would occur.

Adamantane-1-carboxylic acid ($\text{p}K_a = 6.90$ in 50% aqueous ethanol)¹⁶ has a low solubility in aqueous solution which effectively limits aqueous studies to the more soluble AC^- . Because

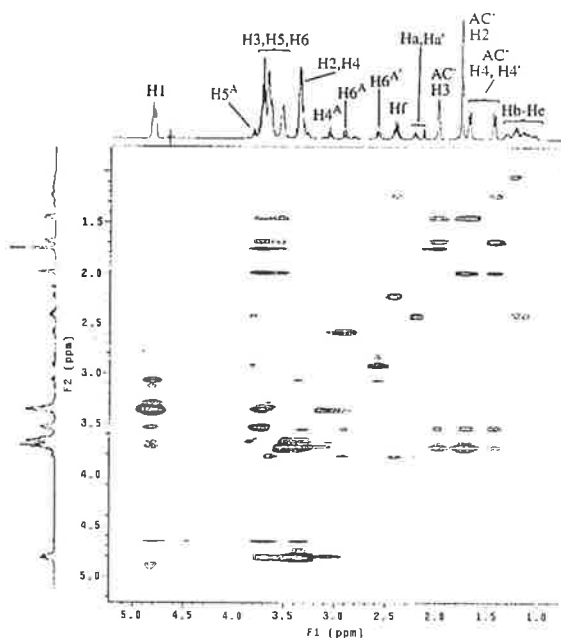


Fig. 4 600 MHz ^1H NMR NOESY spectrum of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot \text{AC}^-$ in D_2O at pH ≥ 11 .

of the consequently restricted pH range of study, a direct determination of the stability of the $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2 \cdot \text{AC}^-$ complexes by the potentiometric titration methods which we have previously employed for complexation of carboxylic acids and their conjugate bases by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$,¹¹ is precluded. However, calorimetric studies¹⁷ give a stability constant $K = 1.96 \times 10^4 \text{ mol dm}^{-3}$ for the $\beta\text{CD} \cdot \text{AC}^-$ complex and it appears from the NMR studies discussed below that similarly stable AC^- complexes are formed by $\beta\text{CDNH}(\text{CH}_2)_n\text{NH}_2$.

^1H NMR studies of the complexation of AC^- by $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$

The ^1H NMR spectrum of an equimolar 0.1 mol dm^{-3} solution of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ and AC^- (Fig. 4 and *Experimental*) shows increased differentiation of the ^1H resonances of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ by comparison with those reported¹¹ in the absence of AC^- consistent with the substantial formation of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot \text{AC}^-$ complex. [In particular, the fine structure exhibited by the resonances of hexyl Hb–He contrasts with the two broad resonances for hexyl Hb and He, and hexyl Hc and Hd observed in the absence of AC^- where $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ is complexed inside the annulus.¹¹] Reported 600 MHz ^1H NOESY NMR studies¹¹ are consistent with strong interaction between the protons (hexyl Ha–Hf) of the four inner methylene groups of the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ moiety and the H3 and H5 protons on the inside of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ annulus as shown by strong cross-peaks. These interactions are absent from the NOESY ^1H NMR spectrum of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot \text{AC}^-$. However, significant cross-peaks are observed between Ha and Hf (Table 2 and *Experimental*) consistent with the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ moiety assuming a coiled configuration outside the annulus adjacent to the primary face as shown in Fig. 5. Strong cross-peaks are observed for the interactions between H3 and H5 and AC^- H2–H4 (Table 2 and *Experimental*) consistent with AC^- occupying the annulus. While the deacylation of *p*NPA precludes ROESY NMR studies show that its complexes, such studies of the unreactive 4-methylbenzoate and (*R*)- and (*S*)-2-phenylpropanoate complexes of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ all exist with both the guest and the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent complexed inside the annulus as is also shown through modelling these complexes.¹¹ Support

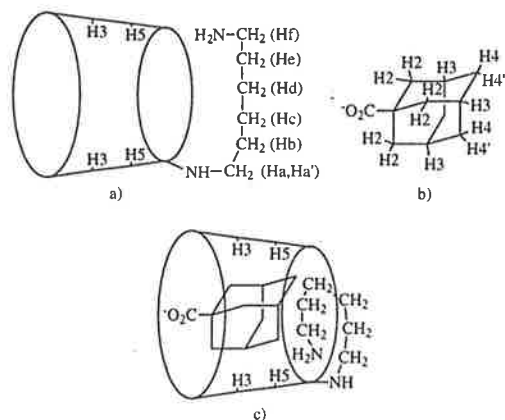


Fig. 5 Representations of: a) $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ showing the hydrogen numbering scheme, b) AC^- showing the hydrogen numbering scheme, and c) the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot \text{AC}^-$ complex showing a possible orientation of AC^- .

Table 2 ^1H NMR Cross-peaks^a observed for the adamantane-1-carboxylate complex of $[\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2]$

	$\text{AC}^- \text{H2}$	$\text{AC}^- \text{H3}$	$\text{AC}^- \text{H4}$	Ha	Hf
H3	++	++	++		
H5	+	+	+		
Ha					+
Hf				+	

^a The intensity of the cross-peaks increases from + to ++.

for similar structures for the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot p\text{NPA}$ complex is adduced from the modelling studies below.

Modelling of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ complexes

Force field modelling in the gas phase shows the complex with $p\text{NPA}$ inside the annulus and oriented with its acetate group adjacent to the secondary face of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$, where the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent is also complexed inside the annulus (Fig. 6), to have a globally minimised energy of 971.6 kJ mol^{-1} . The carbonyl carbon of $p\text{NPA}$ and the nitrogen of the primary amine group are in close proximity (3.65 Å). The analogous complex where the $p\text{NPA}$ is orientation is reversed has a slightly lower energy of 958.9 kJ mol^{-1} , and the carbonyl carbon of $p\text{NPA}$ and the nitrogen of the primary amine group are more distant from each other (5.13 Å). However, the small energy difference between the isomeric complexes is consistent with the coexistence of both species. (These complexes correspond to 3a and 3b, respectively, in Fig. 2.) The isomers of both complexes where the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent is outside the annulus are relatively unstable as shown by their higher energies of 1224.8 and 1243.0 kJ mol^{-1} , respectively. Thus, the two lower energy forms of the $p\text{NPA}$ complex of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ where the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent is complexed inside the annulus modelled in the gas phase appear plausible representations of the $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot p\text{NPA}$ ground state Michaelis complexes in solution which lead to transition states whose $\Delta\Delta G^\ddagger$ determines the product ratio.^{18,19} Modelling of the complexes of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ and $m\text{NPA}$ yields global energy minimised structures of $m\text{NPA}$ oriented with its acetate group adjacent to the primary and secondary faces of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ with energies of 941.9 and 874.6 kJ mol^{-1} , respectively.

Modelling also shows the complexation of AC^- by $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2$ to exclude the $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent from the annulus to yield complexes with energies of 1025.5 kJ mol^{-1} where the carboxylate group is oriented towards the secondary

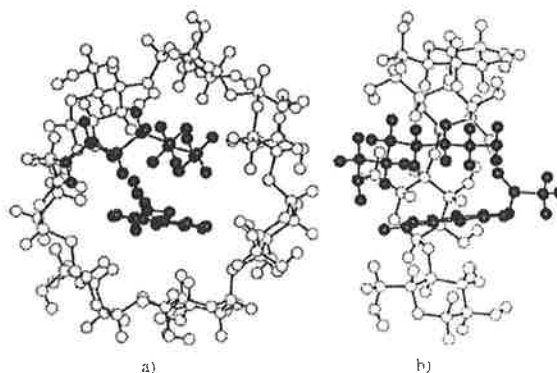


Fig. 6 The global energy minimised structure of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot p\text{NPA}$ viewed from a) the primary end of the annulus and b) from the side with three glucopyranose units cut away. The $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent and $p\text{NPA}$ are shown in dark shading.

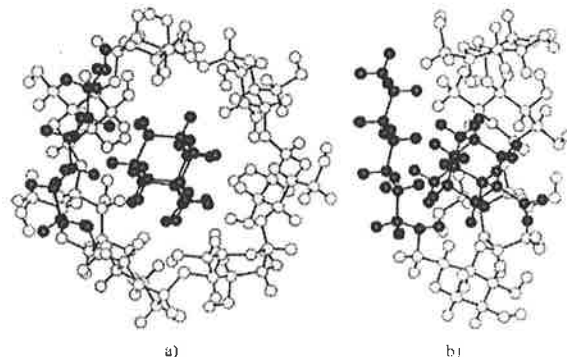


Fig. 7 The global energy minimised structure of $\beta\text{CDNH}(\text{CH}_2)_6\text{NH}_2 \cdot \text{AC}^-$ viewed from a) the primary end of the annulus and b) from the side with three glucopyranose units cut away. The $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent and AC^- are shown in dark shading.

face (Fig. 7) and 1111.4 kJ mol^{-1} where the AC^- orientation is reversed. The $-\text{NH}(\text{CH}_2)_6\text{NH}_2$ substituent lies along one side of the rim of the primary face in each case and does not show the close proximity of the methylene groups adjacent to the primary and secondary amine groups that appears to be consistent with the interpretation of the ^1H NMR ROESY data. This difference may reflect the influence of hydration on the substituent orientation in aqueous solution.

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