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Synthesis of Modified Cyclodextrins and Studies of Their Inclusion Complexes

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Contents

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I finally wish to dedicate this thesis to my parents, Harry and Anna Papageorgiou, who have worked so hard in their lives to give their children the opportunities that others wish for. Their continual support, advice, encouragement, love and understanding, helped me take my education to a higher level.

Statement

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ABSTRACT

The stability constants of the inclusion complexes of the conjugate bases of *para*-fluorobenzoic acid and the corresponding *ortho*-isomer, and the methyl esters of those acids, with β-cyclodextrin and the conjugate acids of 6^A-amino-6^A-deoxy-β-cyclodextrin and 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin, in pH 6.0 phosphate buffer, have been determined through the application of ^{19}F nuclear magnetic resonance spectroscopy. In addition the ^{19}F chemical shifts of the fluoro substitutents of the guests in their fully complexed states have been derived. The stability constant of the inclusion complex of β-cyclodextrin with the *ortho*-substituted anion is 19 ± 3 mol^{-1} dm^3, with the *para*-substituted anion is 50 ± 2 mol^{-1} dm^3, with the *ortho*-substituted ester is 253 ± 11 mol^{-1} dm^3 and with the *para*-substituted ester is 228 ± 7 mol^{-1} dm^3. The stability constant of the inclusion complex of the conjugate acid of 6^A-amino-6^A-deoxy-β-cyclodextrin with the *ortho*-substituted anion is 65 ± 2 mol^{-1} dm^3, with the *para*-substituted anion is 69 ± 4 mol^{-1} dm^3, with the *ortho*-substituted ester is 152 ± 7 mol^{-1} dm^3 and with the *para*-substituted ester is 128 ± 7 mol^{-1} dm^3. The stability constant of the inclusion complex of the conjugate acid of 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin with the *ortho*-substituted anion is 32 ± 3 mol^{-1} dm^3, with the *para*-substituted anion is 19 ± 5 mol^{-1} dm^3, with the *ortho*-substituted ester is 69 ± 2 mol^{-1} dm^3, and with the *para*-substituted ester is 59 ± 2 mol^{-1} dm^3.

The results of this study show that the factors affecting complexation include the charge and extent of hydration of the hosts and guests, the antiparallel alignment of the dipole moments of the hosts and guests in the inclusion complexes, and ionic interactions between the hosts and guests. Complexes of the conjugate acid of 6^A-amino-6^A-deoxy-β-cyclodextrin with the esters are less stable than those of β-cyclodextrin. This is probably a reflection of the decreased hydrophobicity of the annulus of the modified cyclodextrin, resulting from the

effect of hydration of the protonated amino substituent to impinge on the character of the cyclodextrin cavity. The stability constants of complexes of the esters with the conjugate acid of 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin are even lower. The synthesis of 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin occurs with inversion of stereochemistry at C-2 and C-3 of the modified glucopyranose unit,[79] with the result that the amino substituent intrudes into the cavity of the cyclodextrin. The consequent hydration of the protonated substituent will decrease the hydrophobicity of the cyclodextrin annulus, to an even greater extent than for the conjugate acid of 6^A-amino-6^A-deoxy-β-cyclodextrin.

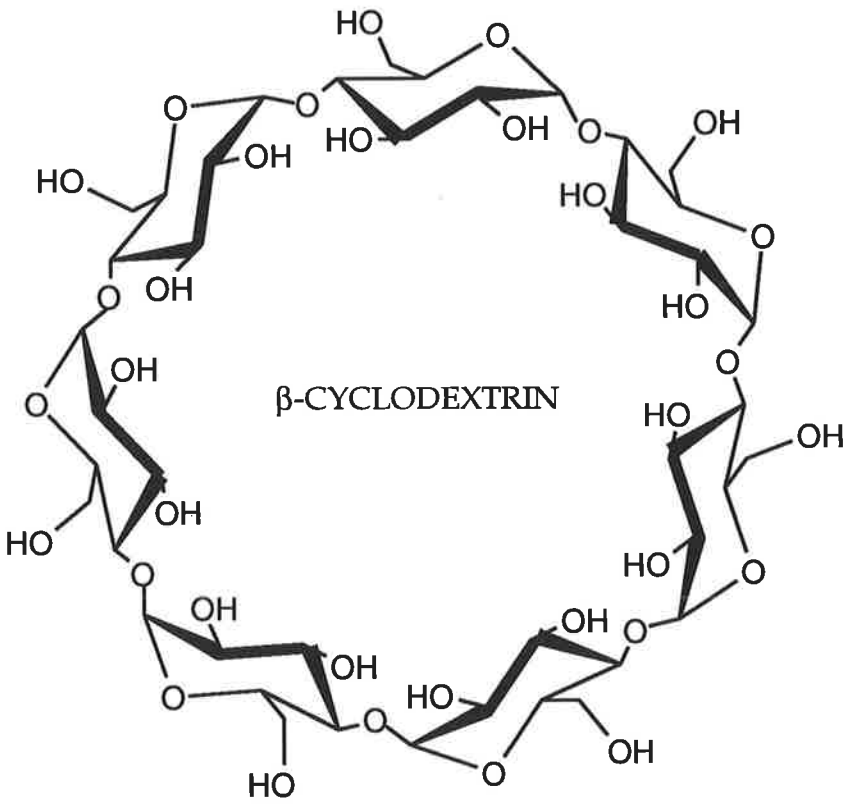
C-3 and C-6 substituted cyclodextrin derivatives of 2-phenylpropanoic acid and the non-steroidal antiinflammatory drug Ibuprofen have been prepared *via* reactions of *meta*-nitrophenyl esters. In addition, C-6 cyclodextrin derivatives of the above acids have been prepared by the hydrolysis and subsequent decarboxylation of malonate substituted cyclodextrin derivatives. The diastereoselectivity of these reactions was determined by nuclear magnetic resonance spectroscopic analysis of the products. A modest diastereoselectivity of 2:1 was typically seen in these reactions.

Stability constants of the 1:1 inclusion complexes of C-3-C-3, C-3-C-6 and C-6-C-6 substituted diamide linked β-cyclodextrins with the biaromatic species, 6-(*para*-toluidino)-2-naphthalenesulfonic acid in aqueous buffer at pH 6.9 have been determined through the application of fluorescence spectroscopy. The stability constants of the C-3-C-3 succinamide and oxalamide linked cyclodextrin inclusion complexes are 8,800 and 5,500 mol^{-1} dm^3 respectively, and the stability constant of the analogous C-3-C-6 substituted succinamide linked cyclodextrin complex is 11,050 mol^{-1} dm^3. The stability constant of the C-6-C-6 substituted malonamide linked cyclodextrin complex is 12,000 mol^{-1} dm^3, and the stability constant of the analogous C-6-C-6 substituted urea linked cyclodextrin complex is 55,000 mol^{-1} dm^3. These compare with a stability constant of the corresponding β-cyclodextrin complex of 2,800 mol^{-1} dm^3.

The results of this work indicate that the extent of cooperative binding by the cyclodextrin annuli of the C-3-C-3 and C-6-C-3 diamide linked β-cyclodextrins is only modest, and the extent of cooperative binding shown is lower than that exhibited by the corresponding C-6-C-6 diamide linked cyclodextrins. The extent of cooperative binding by the cyclodextrin annuli of the C-3-C-3 substituted linked β-cyclodextrins decreases as the length of the bridge connecting the annuli is shortened. However the extent of cooperative binding by the cyclodextrin annuli of the C-6-C-6 substituted diamide linked cyclodextrins increases as the length of the tether is shortened. Presumably the inclusion complex conformations of the C-3-C-3 and C-3-C-6 substituted diamide linked β-cyclodextrins are more strained than those of the corresponding C-6-C-6 substituted diamide linked cyclodextrins especially when the tether joining the annuli of these dimers is shortened. The strain may be attributed to the stereochemistry of the cyclodextrin substitution, where the substituents of the C-3 modified cyclodextrins point toward the interior of the cyclodextrin annuli, analogous to the stereochemistry of the amino group of their precursor, 3^{A}-amino-3^{A}-deoxy-($2^{A}S$,$3^{A}S$)-β-cyclodextrin. In direct contrast, the substituents of C-6-C-6 substituted diamide linked cyclodextrins are attached to sterically non-hindered methylene carbons which are free to point away from the cyclodextrin cavity, resulting in less strained systems.

INTRODUCTION

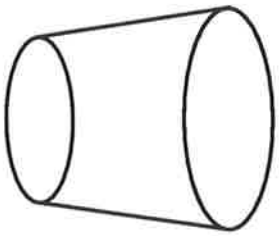
Cyclodextrins[1] are oligosaccharides produced by the action of the amylase of *Bacillus macerans* on starch related compounds. They consist of D-(+)-glucopyranose units joined to each other by α-(1,4)-linkages. Cyclodextrins are toroidal in shape with all the glucose units in substantially undistorted C1 (D) chair conformations.[1] The interior of the torus consists only of a ring of C-H groups, a ring of glucosidic oxygens and another ring of C-H groups, rendering the interior of the torus relatively non-polar compared to water. There are three predominant types of cyclodextrins; α-cyclodextrin (α-CD) consists of six D-(+)-glucopyranose units, β-cyclodextrin (β-CD) (1) consists of seven and γ-



(1)

cyclodextrin (γ-CD) has eight. Cyclodextrins are conveniently depicted by a

truncated cone (2) where the narrow end represents the primary hydroxyl groups attached at C-6 positions and the wide end represents the secondary hydroxyl groups attached at C-2 and C-3 positions.



(2)

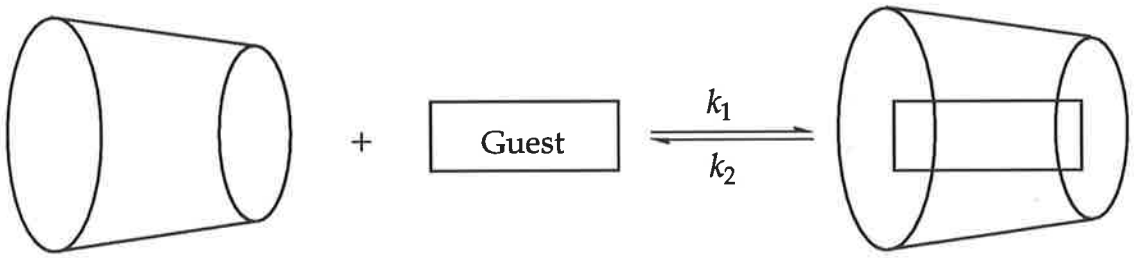
Physical properties such as water solubility, optical rotation and dimensional data of α-, β- and γ-cyclodextrin have been documented in the past (Table 1).[1]

Table 1 Properties of α-, β- and γ-cyclodextrin.

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Molecular weight	972	1135	1297
Water solubility (g/100 ml)	14.5	1.85	23.2
Specific rotation $[\alpha]^{D}_{25}$	150.5	162.5	177.4
Internal diameter (Å)	4.5	7.0	8.5
Depth (Å)	6.7	7.0	7.0

The most interesting property of cyclodextrins is that they contain a hydrophobic cavity which can encapsulate a guest molecule to form an inclusion complex (Scheme 1). The molecular ratio of guest to cyclodextrin is usually found to be 1 : 1,[2,3] however this can change depending on the shape and geometry of the guest and the cyclodextrin. Inclusion complex formation in aqueous media usually involves the displacement of water molecules from the cavity of the cyclodextrin and subsequent replacement with the guest molecule.

A vast array of guest molecules has been seen to include in the cavities of cyclodextrins, ranging from polar amines, acids and ions to hydrophobic non-polar aliphatic and aromatic hydrocarbons.[4,5] It has been clearly shown that a favourable enthalpy change and an unfavourable entropy change accompanies



Host Inclusion complex

Stability constant of inclusion complex = $K = k_1/k_2$

Scheme 1

complexation between a cyclodextrin and a guest.[1] Van der Waals interactions between guest and host, and hydrogen bonding between the hydroxyl groups of cyclodextrins and hydrogen bonding participating groups of the guest, are thought to be behind the favourable enthalpy change.[1] X-Ray crystallography has revealed that the macrocyclic conformation of α-CD is unsymmetrical when water is included in it's cavity.[1] One glucose unit in α-CD is distorted so that the water molecules can be accommodated.[6-8] Saenger *et al.*,[8] revealed by X-ray crystallography that exactly two water molecules are included in the cavity of α-CD and four water molecules are located on the outside of the cavity in the α-CD-hexahydrate complex. An interesting process was observed when a guest molecule replaced the water in the cavity of α-cyclodextrin; the cyclodextrin subsequently adopted an unstrained favourable cyclic form.[6,7] This may also contribute to the driving force for complexation to occur in an aqueous medium.

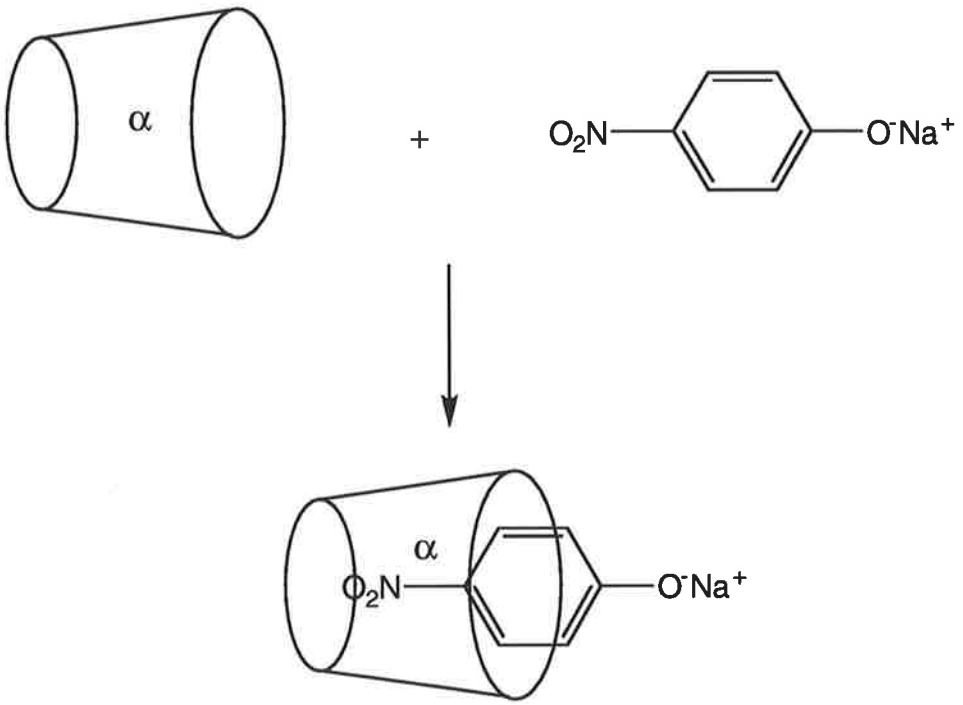
Inclusion complex formation can be detected using a variety of analytical methods. Spectroscopic techniques such as nuclear magnetic

resonance (nmr) spectroscopy have been exploited on a regular basis in order to detect and study complex formation.[9-15] When a guest molecule is included into the cavity of a cyclodextrin, the chemical shifts of signals of it's atoms, carbon (^{13}C) and proton (^{1}H), can change due to the change in environment. The chemical shift of signals of cyclodextrin hydrogens also can change upon inclusion of various aromatic compounds. Stability constants of inclusion complexes can be determined by the use of ^{1}H and ^{13}C nmr spectroscopy. By measuring the change in chemical shift of a guest's proton or carbon over a range of cyclodextrin concentrations, the binding constant of the guest molecule can be derived.

Complex formation was shown by ^{1}H nmr spectroscopy when substituted benzoic acids were added to solutions of α-CD.[9] The hydrogens attached to the C-3 and C-5 carbons of the glucose units moved substantially upfield. These hydrogens point in to the cyclodextrin cavity, and were thought to be located nearest to the aromatic ring of the guest which exhibited a shielding effect.[9] The hydrogens attached to the C-1, C-2 and C-4 carbons showed a minimal change in chemical shift probably because they point to the cyclodextrin exterior.

The orientation of a guest molecule entering the cavity of a cyclodextrin has also been determined by the use of nmr spectroscopy. Bergeron *et al.*,[13-15] studied the complexation of sodium *para*-nitrophenolate with α-CD in aqueous solution. Upon complexation, the hydrogens attached to the C-3 carbons of α-CD shifted upfield, however the hydrogens attached to the C-5 carbons of α-CD showed a negligible shift. The *meta* protons of the guest molecule were deshielded more than the *ortho* protons.[13] ^{13}C nmr spectroscopy also showed a substantial deshielding effect on the *meta* carbons compared to a small shielding effect on the *ortho* carbons.[14] A proton homonuclear Overhauser experiment involving irradiation of the hydrogens attached to the C-3 carbons of α-CD gave rise to a larger effect on the *para*-nitrophenolate

resonances than irradiation of any other cyclodextrin hydrogens. However, even though a large effect was seen for the *meta* protons of the guest no such effect was seen for the *ortho* protons.[13] Bergeron *et al.*,[13,14] concluded that the guest compound enters the cavity of α-CD from the secondary hydroxyl side with the nitro group first, where the *meta* protons of the guest are found to be nearest the hydrogens attached to the C-3 carbons of the cyclodextrin (Scheme 2).

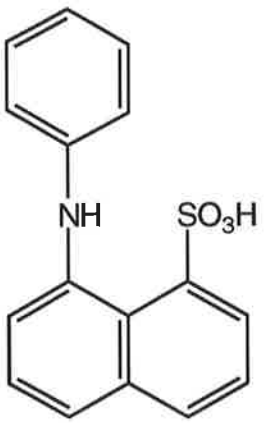


Scheme 2

Ultraviolet visible spectroscopy has also been used extensively for the study of complex formation.[16-22] Ultraviolet visible spectra of aromatic compounds usually change upon inclusion of the compounds into the cavities of cyclodextrins. Stability constants of inclusion complexes can be derived from measuring the absorption spectra of a range of solutions comprising a standard guest concentration and different cyclodextrin concentrations.

Certain guest molecules have been found to exhibit fluorescence in the presence of cyclodextrins in aqueous media.[19,23] This property is known to be a good indication of complex formation, the intensity of fluorescence being

proportional to the concentration of inclusion complex present. 1-Anilino-8-naphthalenesulfonic acid (ANS) (3) and 6-(*para*-toluidino)-2-naphthalenesulfonic acid (TNS) (4) are fluorescent in organic solvents; in water fluorescence is quenched, however, upon addition of an appropriate cyclodextrin, fluorescence is regained. Similarly to the case with ultraviolet visible spectroscopy previously described, stability constants of inclusion complexes formed between the fluorophores (3) and (4) and a cyclodextrin can be derived from measuring the fluorescence spectra of a range of solutions consisting of a constant fluorophore concentration with differing cyclodextrin concentrations.



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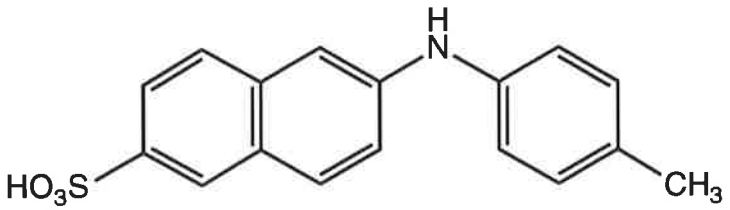
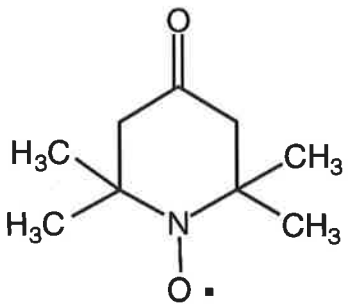


(4)

Inclusion complex formation between an organic acid and a cyclodextrin can also be detected using titration.[1,24] The pK_as of various included organic acids such as phenols and carboxylic acids have been determined.[24] It is evident that the pK_a of an organic acid changes upon complexation with a cyclodextrin. An example of a decrease in pK_a of an organic acid as a result of complexation with a cyclodextrin was clearly seen when *para*-nitrophenol was titrated in the presence of α-CD. The pK_a of complexed *para*-nitrophenol was found to be one unit lower than that of non-complexed *para*-nitrophenol.[24] In direct contrast, the pK_as of carboxylic acids

have been seen to increase as a result of complexation.[24] The pK_a of cinnamic acid in the presence of α-CD was found to be 1.37 units higher than that of the free acid.[24]

Inclusion complex formation has also been followed using electron spin resonance spectroscopy, by employment of spin-labelled substrates such as 2,2,6,6-tetramethyl-4-oxopiperidinyl-1-oxyl (5).[25-29] Upon inclusion of the nitroxide (5) into the cavity of β-CD (1), the isotropic nitrogen hyperfine coupling constant decreased, indicating the movement of the radical (5) to an environment less polar than water.[28]



(5)

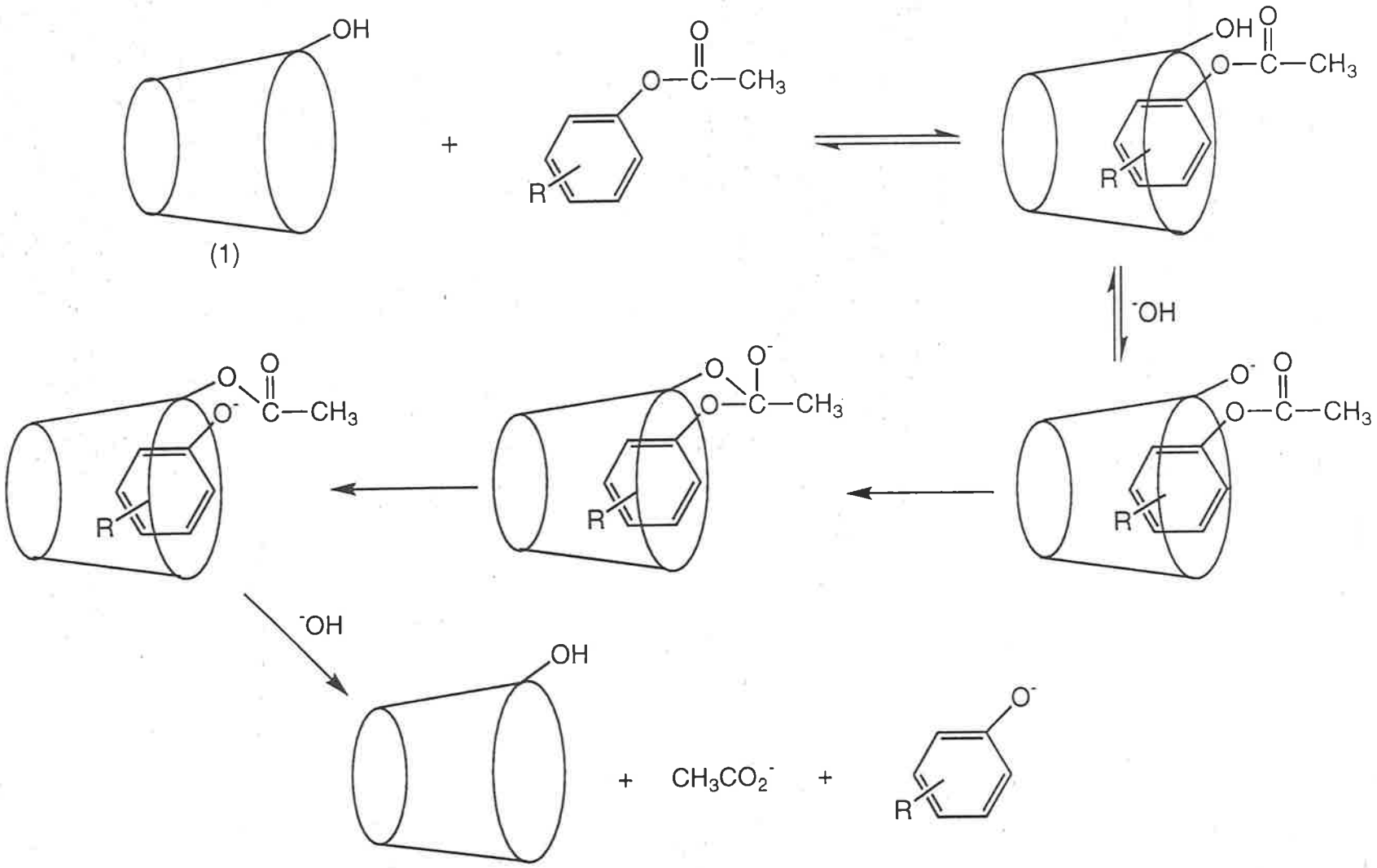
Optical rotation[30] has also been used in the detection of complex formation. Breslow *et al.*,[30] determined the binding constants of a series of guests with β-CD (1) in dimethyl sulphoxide, by plotting the change in optical rotation of a 5 mM β-CD (1) solution as a function of added guest concentration.

High performance liquid chromatography (HPLC) is an accurate tool which has been exploited on a number of occasions to study the interaction of guest molecules with cyclodextrins.[31] A steady state concentration of the guest is passed through an appropriate HPLC column under such conditions that the guest interacts with the stationary phase of the column and has an R_f value of approximately 0.25. Injection of a known quantity of a host onto the column results in the complexation of a discreet quantity of the guest, depending on the stability constant of the inclusion complex. Where the host does not interact

with the column, the complexed material is more rapidly eluted from the column and can be detected as an enhanced concentration of guest at shorter elution time and a depleted concentration of guest at the normal retention time of that material. The process is repeated for various concentrations of guest in the running solvent. A relationship between the number of moles of guest bound per moles of host present (r) and the concentration of the guest ([L]) may be found, where a plot of r/[L] versus [L] yields the stability constant of the complex of that guest with that host, as the intercept on the r/[L] axis.[31]

Cyclodextrins are well known for their ability to bind guest molecules and catalyse reactions of those guests. They have therefore been used as models of enzyme binding and catalysis.[32,33] Features of enzyme-catalysed reactions such as saturation, substrate specificity, substrate-enzyme complexation and competitive inhibition have all been mimicked by cyclodextrins.[16,25,26,34-46] The mechanism of enzyme-catalysis can proceed in either of two ways; namely through covalent and non-covalent catalysis. There are numerous examples throughout the literature in which cyclodextrins accelerate or catalyse reactions in these ways.

The hydrolysis of phenyl esters catalysed by cyclodextrins was extensively studied throughout the 1960's by Bender *et al.*[16,37,38,45,46] The authors established that the rates of hydrolysis of *meta*-substituted phenyl acetates were more rapid than the rates of hydrolysis of the corresponding *para*-isomers in the presence of β-CD (1). Experiments have indicated that the hydrolysis of phenyl acetates by β-CD (1) proceeds through covalent catalysis and follows classical Michaelis-Menten kinetics (Scheme 3).[16,46] Bender *et al.*,[16,46] determined that acetyl transfer in the complex was dependent on hydroxide ion to the first order. It had been established that the secondary hydroxyl groups present in β-CD (1) are more acidic than the primary hydroxyls, due to hydrogen bonding effects by neighbouring secondary hydroxyl groups.[34,46,47] As a result the secondary hydroxyls were thought to be selectively deprotonated. It is the deprotonated

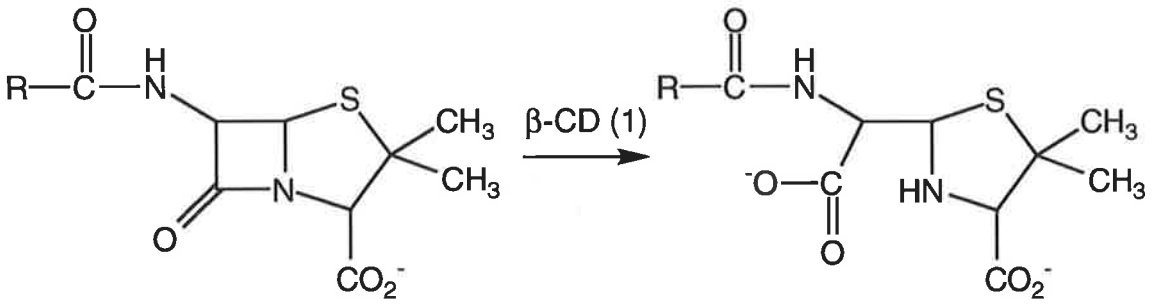


A substituent attached to the wide end of the cone replaces a C-3 or C-2 secondary hydroxy group of β-CD (1). A substituent attached to the narrow end of the cone replaces a C-6 primary hydroxy group of β-CD (1). In this case a substituent attached to the wide end replaces a C-3 hydroxy group of β-CD (1).

Scheme 3

cyclodextrin which was considered to be responsible for acetyl transfer within the β-CD (1) phenolic ester complex. In support of this conclusion, when all the primary hydroxyl groups of β-CD (1) were protected and the catalytic properties of the analogue were examined, ester hydrolysis was seen to be accelerated by the same magnitude as with the parent β-CD (1).[48] In direct contrast, when all the primary and all the C-3 secondary hydroxyls of β-CD (1) were protected, no enhancement in ester hydrolysis by the cyclodextrin derivative was observed, in fact hydrolysis times were extended.[49] The results of these experiments indicate strongly that the cyclodextrin C-3 secondary hydroxyl groups must be the catalytically active groups. The enhanced rate of hydrolysis of *meta*-substituted phenyl acetates compared to the *para*-isomers must be a result of the specific geometrical requirements demanded by the cyclodextrin cavity. In the inclusion complexes, the carbonyl group carbon of a *para*-substituted phenyl acetate is probably located further from the reactive C-3 alkoxide of β-CD (1) compared to the carbon of the carbonyl group of the *meta*-isomer, making the reaction less facile.

β-CD (1) has been shown to enhance the rates of hydrolysis of strained cyclic amides such as penicillins (6) (Scheme 4), *via* covalent



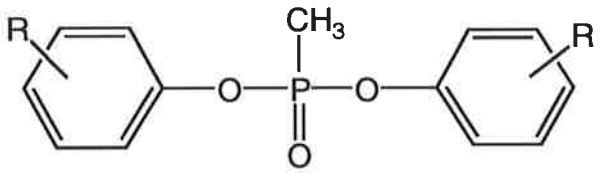
(6)

Scheme 4

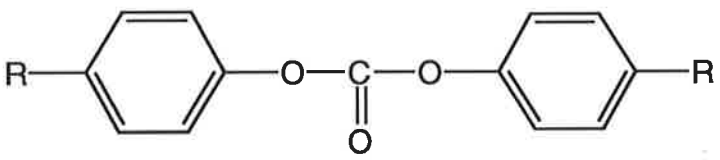
catalysis.[39,40] A 21-89 fold enhancement in the rates of hydrolysis of penicillins (6) has been noted. Enhancements in the rates of hydrolysis of *N*-acyl-

imidazoles and acetanilides in the presence of cyclodextrins have also been reported.[50,51] Komiyama *et al.*,[51] first observed the enhancement of hydrolysis of trifluoroacetanilide, *meta*-nitrotrifluoroacetanilide and *para*-nitrotrifluoroacetanilide in the presence of α-CD. In fact *para*-nitrotrifluoroacetanilide was hydrolysed in the presence of α-CD, at pH 6, sixteen fold faster compared to the hydrolysis in the absence of α-CD. The hydrolysis of the less reactive amide, *para*-nitroacetanilide, was inhibited by α-CD.[51] The hydrolyses of amides in the presence of cyclodextrins proceed *via* a mechanism analogous to that previously discussed for the hydrolysis of phenolic esters.[51]

Brass *et al.*,[42] explored the hydrolyses of the phosphonates (7-9) in the presence of α-CD and the carbonates (10) and (11) in the presence of β-CD (1). Hydrolysis of the phosphonate (7) in the presence of α-CD showed a 35.1 fold increase, cleavage of the *meta*-nitrated derivative (8) showed a 66.1 fold increase and cleavage of the *para*-isomer (9) exhibited an 8.35 fold enhancement. Similarly the rates of hydrolysis of the carbonates (10) and (11) were enhanced in the presence of β-CD (1), however, the magnitude of enhancement was only modest in each case.[42]



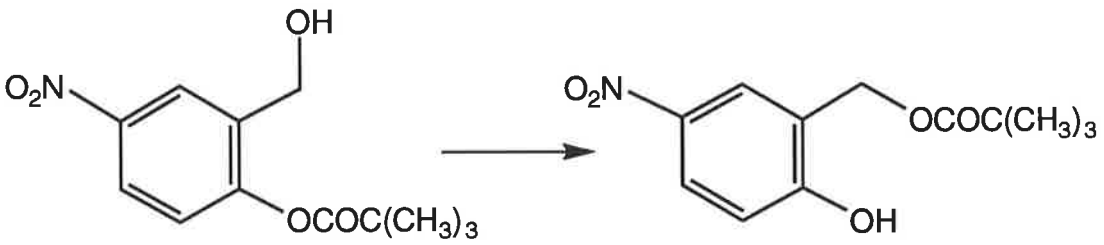
R	
(7)	H
(8)	*m*-NO_2
(9)	*p*-NO_2



R	
(10)	H
(11)	NO_2

Non-covalent catalysis by cyclodextrins has been documented extensively throughout the literature.[1] This involves inclusion of the guest in the cavity of the cyclodextrin and reaction of the included guest.[1] Catalysis may

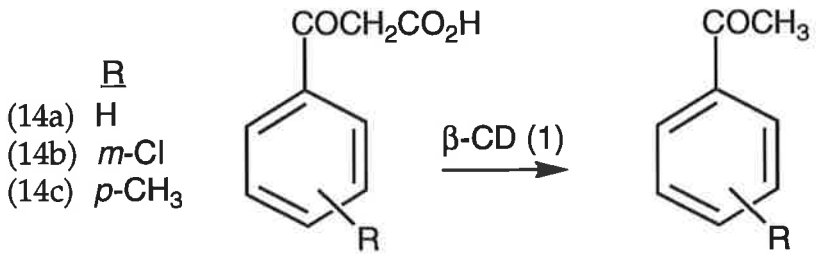
be attributed to the change in chemical environment of the included guest, to the preassembly of reagents within the cyclodextrin cavity and/or to the binding of a particularly reactive conformation of the guest. Griffith's *et al.*,[52] showed that formation of the ester (13) by intramolecular acyl migration in the alcohol (12) (Scheme 5) occurred six times faster when α-CD was present. The



(12) (13)

Scheme 5

enhancement in rate was attributed to inclusion of the alcohol (12) in a reactive conformation with the hydroxyl group near the carbonyl carbon of the ester moiety. Another example of non-covalent catalysis by cyclodextrins has been seen in the decarboxylation of the benzoylacetic acids (14a-c) (Scheme 6).[53] β-CD

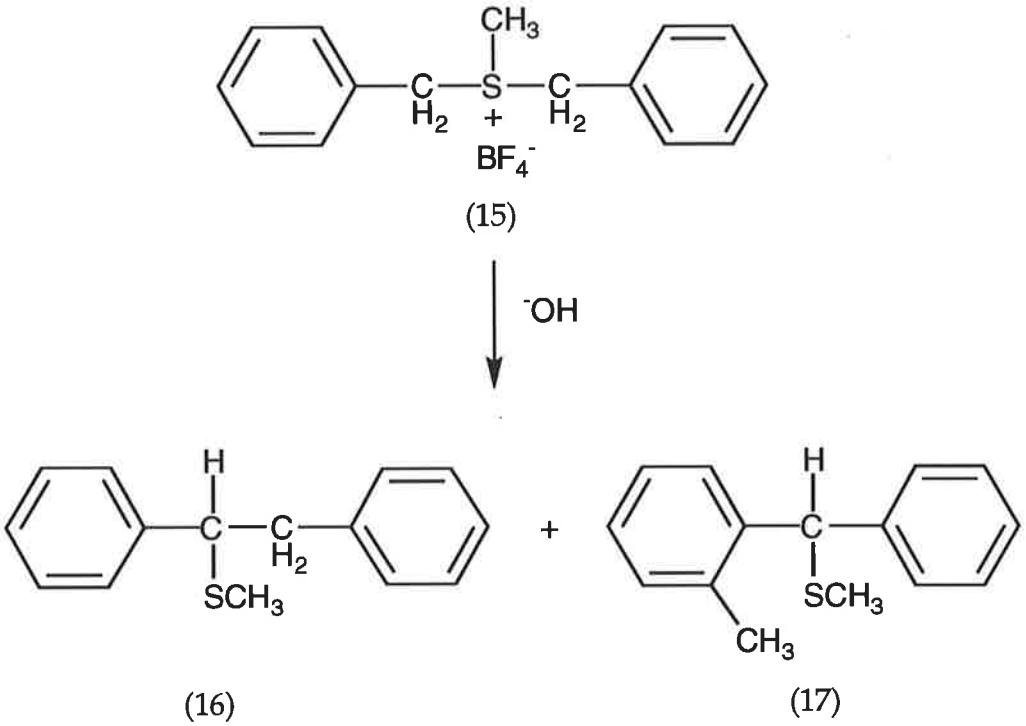


Scheme 6

(1) enhanced the rates of reaction by a factor of approximately five. This catalysis was again attributed to the inclusion of particularly reactive conformers of the β-keto-acids (14a-c).[53]

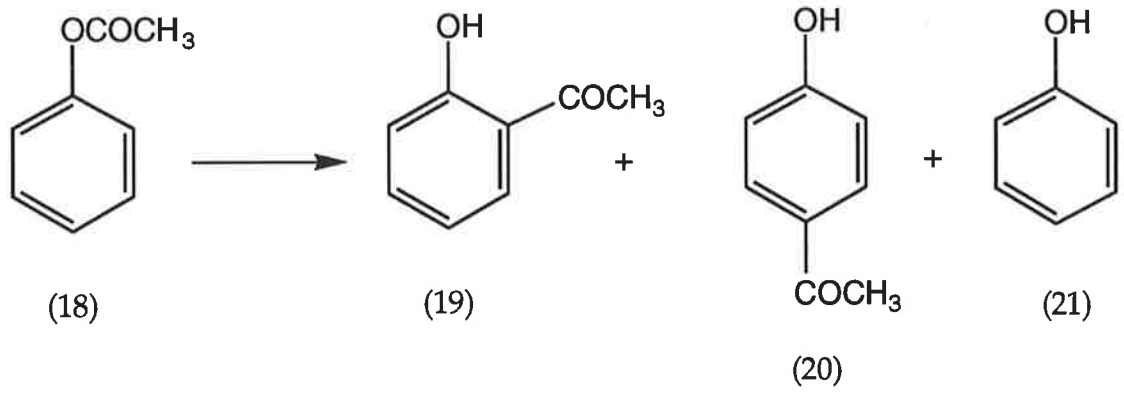
β-CD (1) has also been observed to favour particular reaction pathways of certain guests.[54,55] The reaction of dibenzylmethylsulfonium

tetrafluoroborate (15) in alkaline solution produces the rearranged species (16) and (17) (Scheme 7) in a ratio of 3:1.[54] However in the presence of β-CD (1), the product ratio was altered in favour of the latter product (17).[54] Another example



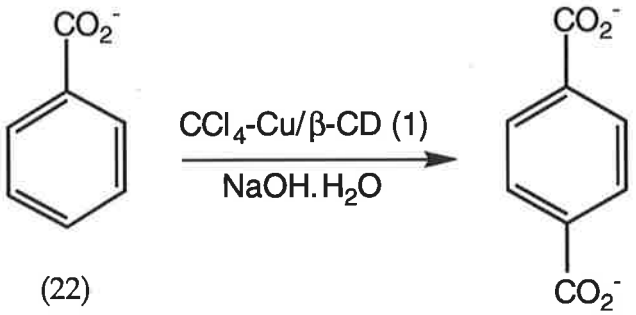
Scheme 7

of β-CD (1) favouring a particular reaction pathway is in the photochemical Fries rearrangement of phenyl acetate (18) (Scheme 8).[55] Photoirradiation of phenyl acetate (18) yields the *ortho*-hydroxyketone (19) and the *para*-isomer (20) in a



Scheme 8

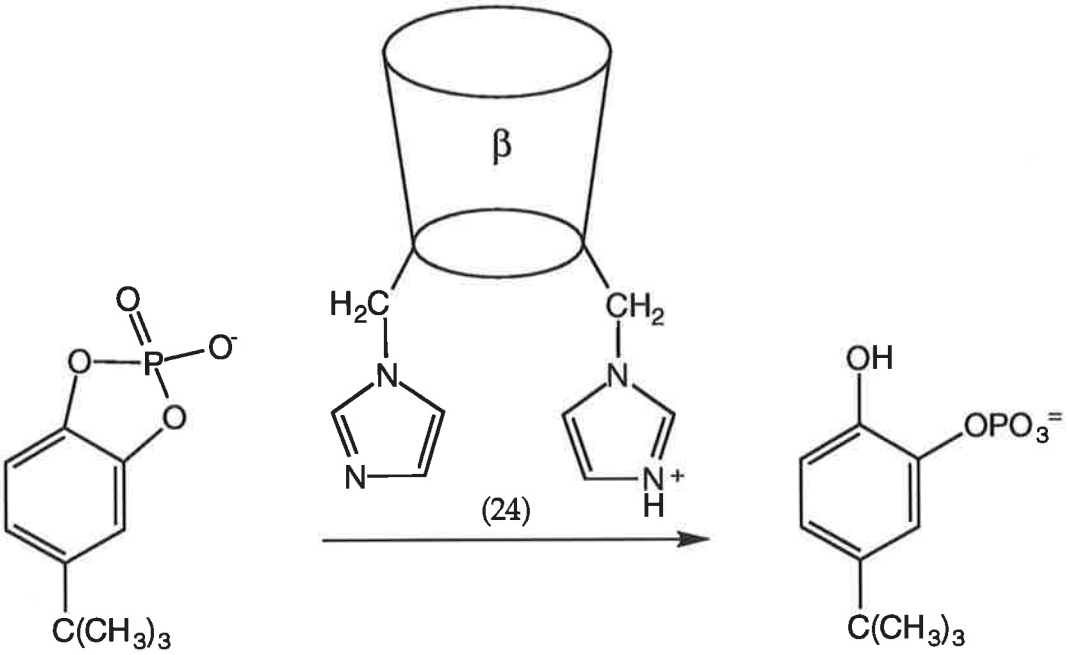
ratio of 1:1, plus some phenol (21). In the presence of β-CD (1), formation of the *para*-isomer (20) was favoured and formation of phenol (21) was suppressed.[55] Recently Hirai *et al.*,[56] showed that β-CD (1) catalyses the electrophilic carboxylation of a deactivated aromatic guest. They found that benzoate (22) could not be carboxylated in the presence of carbon tetrachloride and copper powder in aqueous sodium hydroxide. However in the presence of β-CD (1) under the same conditions, benzoate (22) was successfully converted to terepthalic acid, in 31% yield with 95% regioselectivity.[56]



(22) (23)

Scheme 9

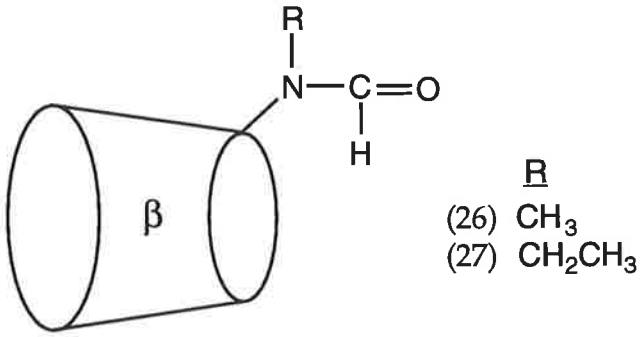
Modified cyclodextrins[57-59] have been found to catalyse reactions of particular substrates. Breslow *et al.*,[57] showed that the cyclodextrin derivative (24) catalysed the hydrolysis of the cyclic phosphate (25) (Scheme 10). Features of the catalysis were shown to be comparable to that of the enzyme ribonuclease. Breslow *et al.*,[57] found that when the *tert*-butylphenyl group of the cyclic phosphate (25) is included in the cavity of the cyclodextrin derivative (24), the cyclic phosphate group is accessible to the imidazole rings. Catalysis was thus attributed to hydrogen bonding of the protonated and non-protonated imidazole groups to the cyclic phosphate group of the guest (25). In direct contrast β-CD (1) did not catalyse the hydrolysis of the cyclic phosphate (25).



(25)

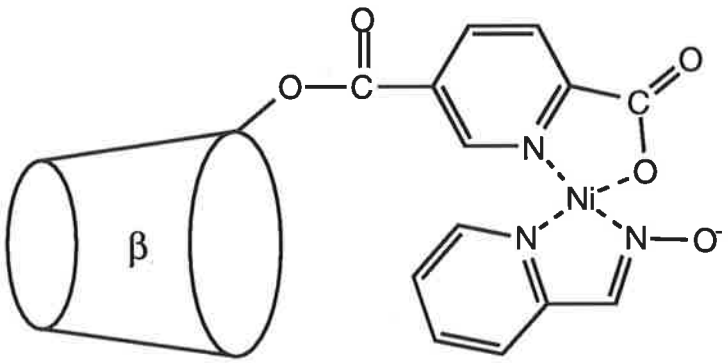
Scheme 10

Another example of catalysis exhibited by modified cyclodextrins was demonstrated by Breslow *et al.*[58] The authors studied the hydrolysis of various esters in the presence of the modified cyclodextrins (26) and (27).



It was concluded that the enhanced catalytic activity shown by the modified cyclodextrins (26) and (27) as compared to β-CD (1) was due to an improvement in geometry of the inclusion complexes of the modified cyclodextrins (26) and (27) as compared to that seen for the β-CD (1)-ester complexes.[58]

In the late 1960's Breslow *et al.*,[59] prepared the metallocyclodextrin (28) as a model for a metalloenzyme. The authors determined that the metallocyclodextrin (28) catalysed the hydrolysis of *para*-nitrophenyl acetate. The increased reactivity noted in the presence of the modified cyclodextrin (28) was stated to be a result of binding and reaction within the modified cyclodextrin (28)-ester complex.[59]



(28)

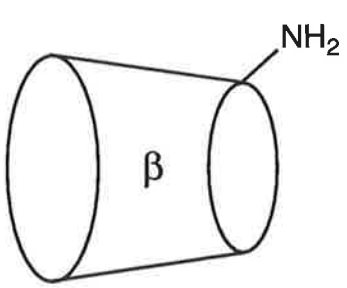
Cyclodextrins have been extensively used in the pharmaceutical industry to solubilize and stabilize drugs.[60-73] A restriction to the use of β-CD (1) in this area is its low solubility in aqueous media, which is limited to 1.85 g/100 ml.[60] Methylation of β-CD (1) has given rise to two compounds; namely heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DMCD) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMCD).[60] The aqueous solubility of DMCD and to a lesser extent TMCD is many fold higher than that of β-CD (1) (Table 2).[60,61-63] The enhancement of the aqueous solubility of DMCD and TMCD as compared to that of β-CD (1) at first seems odd, since ethers in general are known to be considerably less polar than alcohols. The low aqueous solubility of β-CD (1) is thought to arise from extensive hydrogen-bonding between the hydroxy groups of individual molecules of β-CD (1) in the crystal structure.[60] Methylation of the hydroxy groups of β-CD (1) results in a breakdown of the crystal lattice by disrupting this hydrogen-bonding.[60] This is thought to be the cause of the

enhancement of the aqueous solubility of DMCD and TMCD as compared to β-CD (1). Hydroxyalkylated cyclodextrins have also been synthesized and studied.[60,64-66] 2-Hydroxypropyl-β-CD (2HPCD) has been found to have greater aqueous solubility than β-CD (1) (Table 2).[60,61-63] 2HPCD has been observed to solubilize various proteins.[60] Ovine growth hormone (OGH) is practically insoluble in pH 7.6 buffer at room temperature, however, upon addition of OGH at a concentration of 2.5 mg/100 ml to an aqueous solution of 40% w/v 2HPCD, the protein was found to completely dissolve.[60] Even though alkylated and hydroxyalkylated β-cyclodextrins have greater solubility in aqueous media as compared to β-CD (1) their use in pharmaceutical applications is limited. The *O*-alkylated cyclodextrins DMCD, TMCD and 2HPCD are more lipophilic than β-CD (1) as a result of the modifications, and attract lipids, making them more toxic than β-CD (1).[60]

Table 2 Physical properties of some cyclodextrins.

	β-CD (1)	DMCD	TMCD	2HPCD
Degree of substitution		14	21	2.5-11.3
Aqueous solubility (g/100 ml)	1.85	57	31	> 50

The modified cyclodextrins 6^A-amino-6^A-deoxy-β-cyclodextrin (β-CD-6-NH_2) (29) and 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (β-CD-3-NH_2) (30), where one C-6 hydroxy substituent of β-CD (1) is replaced by an amino



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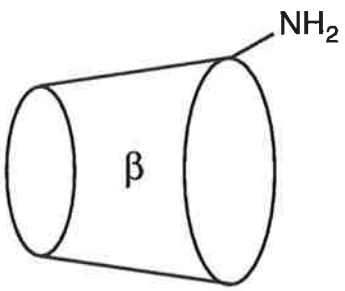


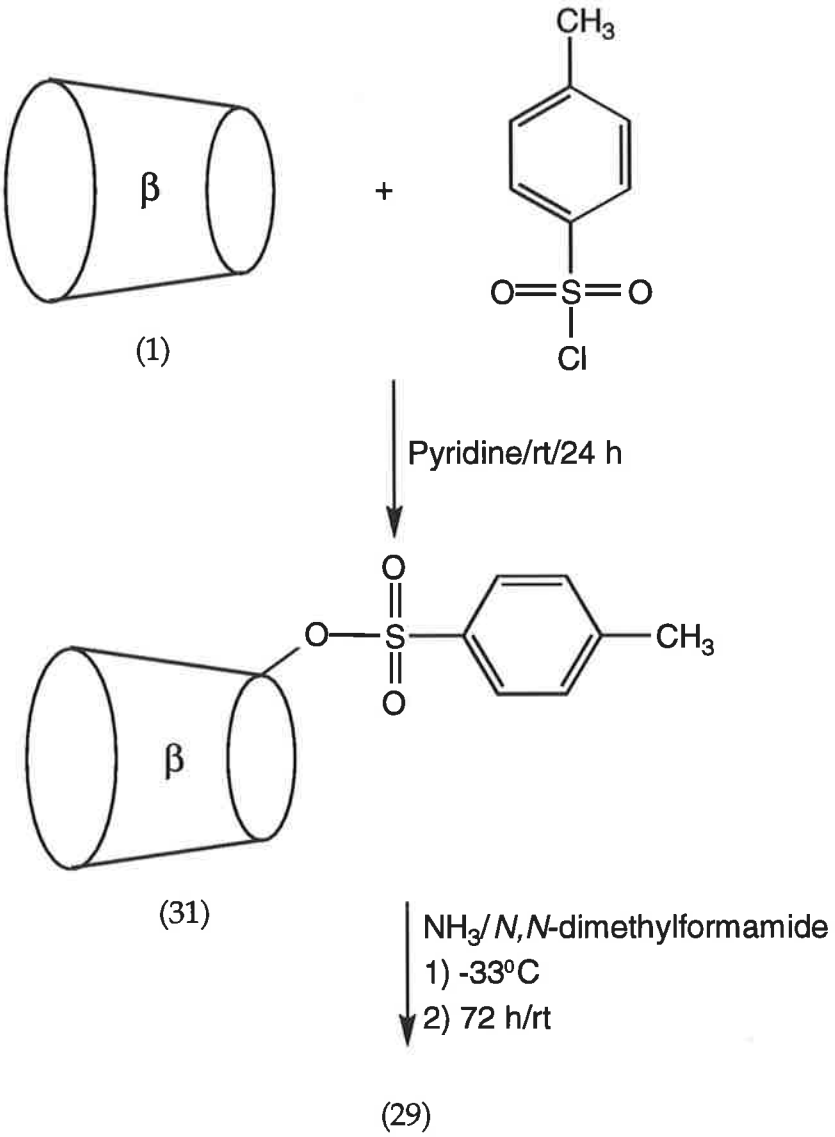
(30)

group in the former case and one C-3 hydroxy substituent of β-CD (1) is replaced by an amino group in the latter case, are of interest. The aqueous solubilities of the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) are approximately 70 g/100 ml in the former case and greater than 70 g/100 ml in the latter case.[74] The pK_as[75] of the conjugate acids of the cyclodextrins (29) and (30) in aqueous media are 8.7 and 7.5 respectively, therefore under physiological conditions, the amino substituents are protonated.

As mentioned previously the alkylated cyclodextrins, DMCD and TMCD, tend to attract lipids due to their lipophilic nature, making them unsuitable for use as complexation agents in the pharmaceutical industry. It is reasonable to expect that the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) will be considerably less lipophilic than DMCD and TMCD, however if they are to be of general utility, they must retain the ability to form inclusion complexes. Therefore the aim of the work described in Chapter One of this thesis was to examine the influence of the amino groups of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) on the complexation of guests.

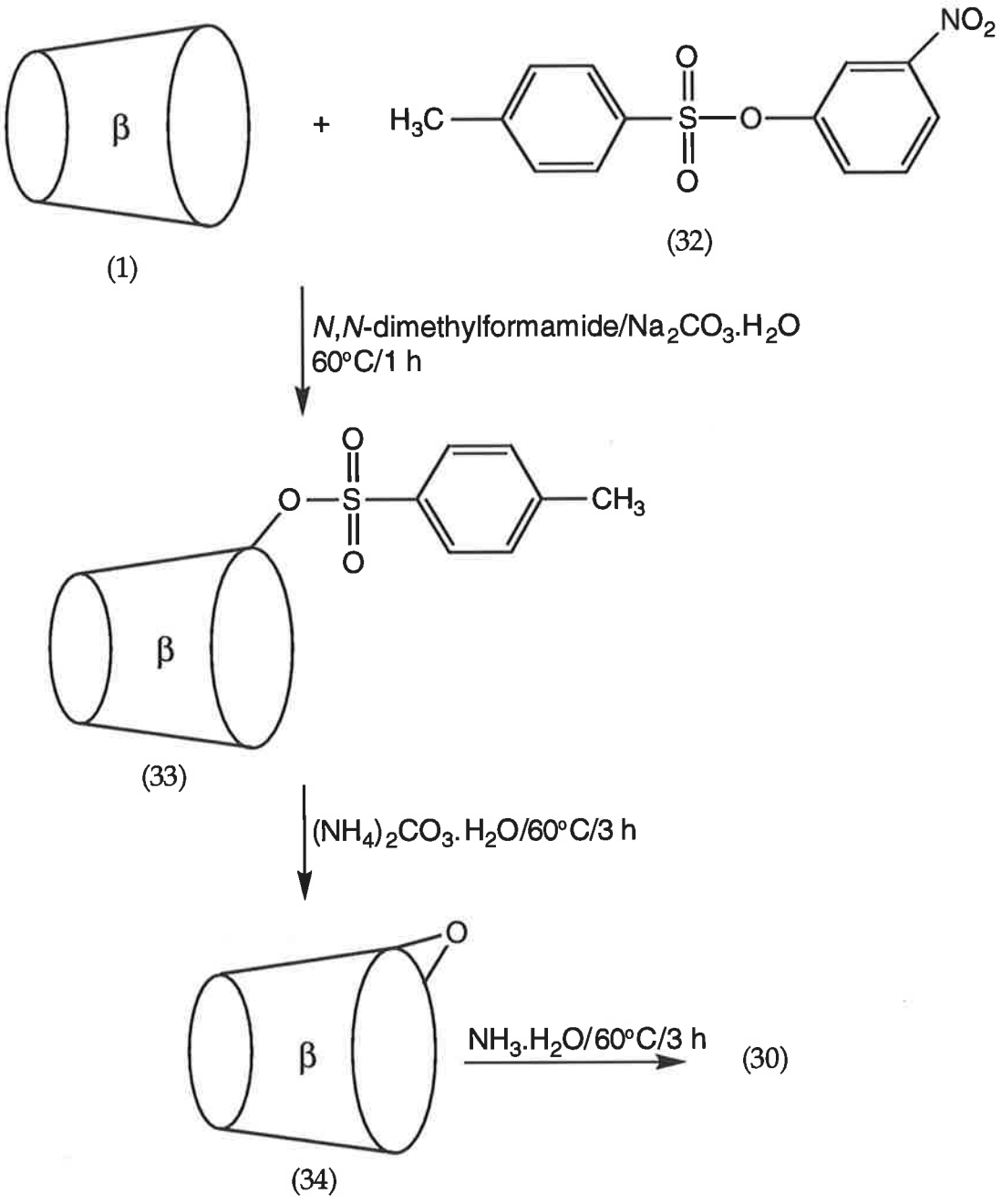
β-CD-6-NH_2 (29) has been synthesized in two steps from β-CD (1) (Scheme 11).[76] One C-6 hydroxy substituent of β-CD (1) has been tosylated by reaction of β-CD (1) and one equivalent of 4-toluenesulfonylchloride in pyridine at room temperature.[76] Reaction of the tosylate (31) and anhydrous ammonia in *N,N*-dimethylformamide afforded β-CD-6-NH_2 (29).[76] In the early 1980's, Breslow *et al.*,[77] reported the functionalization of one C-2 hydroxy group of β-CD (1) (Scheme 12). Tosylation of one C-2 hydroxy group was accomplished by reaction of β-CD (1) and one equivalent of 3-nitrophenyltosylate (32) in *N,N*-dimethylformamide, buffered at pH 9.9. The reaction was buffered at pH 9.9 so that the secondary hydroxy substituents of β-CD (1) would be selectively deprotonated. It has been stated that the reagent (32) includes in the cavity of β-CD (1) and as result the C-2 hydroxy groups of β-CD (1) are nearest to the electropositive sulfur of the reagent (32). Murakami *et al.*,[78] developed an

alternative synthesis of the tosylate (33). Reaction of β-CD (1) and dibutyltin oxide produces the stannyl intermediate (35). It was reported that the C-2^A oxygen of the cyclodextrin (35) was more nucleophilic than the unmodified hydroxy groups of the cyclodextrin (35). In addition the stannylated C-2^A oxygen of the cyclodextrin (35) was more nucleophilic than the corresponding C-3^A stannylated oxygen. Therefore reaction of the cyclodextrin derivative (35) and 4-toluenesulfonylchloride produced the tosylate (33).[78] β-CD-3-NH_2 (30) has been



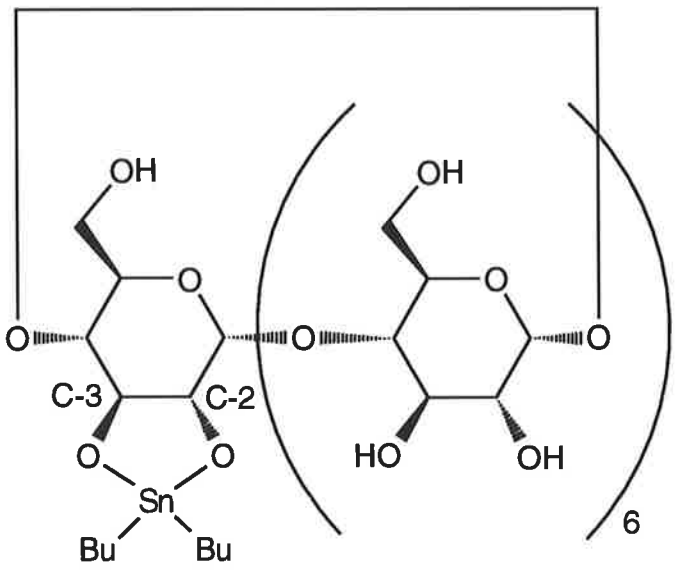
Scheme 11

synthesized by reaction of the tosylate (33) and aqueous ammonium carbonate to firstly produce the mannoepoxide (34) which then reacted with aqueous ammonia (Scheme 12).[79] It should be noted that the stereochemistry at the C-3



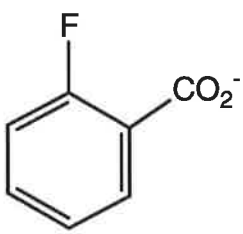
Scheme 12

and C-2 centres of the modified glucopyranose residue of β-CD-3-NH_2 (30) is inverted compared to that of the unmodified residues.[79]



(35)

The guests (36)-(39) were chosen for the complexation study with β-CD (1) and the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) in the first place so as to monitor their inclusion using ^{19}F nmr spectroscopy. The



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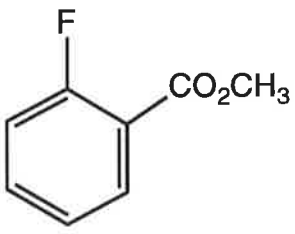

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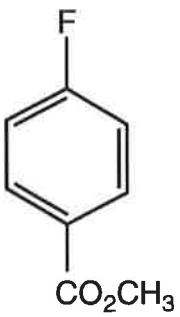
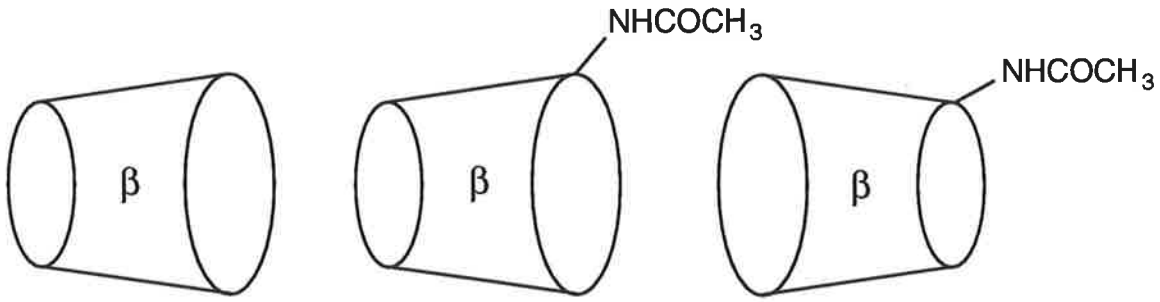


(39)

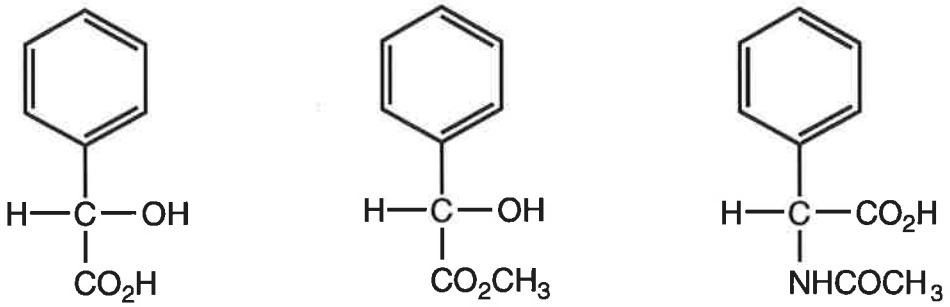
investigation was carried out in order to determine what effect factors such as cyclodextrin and guest charge and hydration have on complexation. To do so, complexation of β-CD (1) and the cyclodextrins (29) and (30) with the guests (36)-(39) was studied at pH 6.0 so as to limit the formation of the conjugate acids of the guests (36) and (37) and the free amines of the hosts, β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30). It is well known that the pK_as of carboxylic acids can increase by as much as one unit upon complexation with α-CD or β-CD (1),[24] however such

changes would have only a small effect on the concentration of the anions (36) and (37) at pH 6.0, since the pK_as of the conjugate acids of the anions (36) and (37) are 3.52 and 3.85, respectively. The *ortho* and *para*-anions (36) and (37) are similar in size, as are the esters (38) and (39), however the dipole moments of the *ortho*-isomers (36) and (38) are larger than those of the corresponding *para*-isomers (37) and (39). These compounds were ultimately selected so as to probe the effect of the dipole moment of the guest on complexation. Complex formation was monitored through observation of the variations of the ^{19}F chemical shifts of the fluoro substituents of the guests (36)-(39). Stability constants of the inclusion complexes of β-CD (1) and the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) with the guests (36)-(39) were determined by firstly measuring the ^{19}F chemical shifts of the fluoro substituents of the guests (36)-(39) in a range of solutions consisting of a standard guest concentration but varying cyclodextrin concentration and then employing a non-linear regression analysis to give the derived stability constants. The results and discussion of this work is reported in Chapter One of this thesis.

Cyclodextrins are chiral molecules and as a result they form diastereomeric inclusion complexes with enantiomers of chiral guests.[79-83] Recently Murakami *et al.*,[79] studied the interaction of β-CD (1), 3^A-acetamido-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (40) and 6^A-acetamido-6^A-deoxy-β-cyclodextrin (41) and the chiral guests, mandelic acid (42), methyl mandelate (43) and *N*-acetyl-α-phenylglycine (44). ^1H nmr spectroscopy was used to monitor complex formation. The methine proton signals of the guests (42) and (44) in the presence of β-CD (1) and the amides (40) and (41) were split into two peaks. The splitting was described to be a result of the difference in the induced chemical shifts of the *R* and *S*-enantiomers from the formation of diastereomeric host-guest complexes.[79] The difference between the two peaks when the amide (40) was used as the host was considerably greater than when the amide (41) or β-CD (1) was employed. The methine proton signal of the ester (43) was also split



(1) (40) (41)

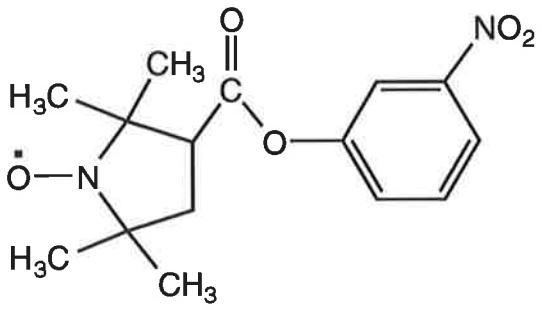


(42) (43) (44)

into two peaks in the presence of the amide (40) but no such splitting was observed in the presence of the amide (41) or β-CD (1).[79] In general the results indicated that the cyclodextrin (40) formed diastereomeric inclusion complexes with the enantiomers of the guests (42)-(44) and that the included enantiomers were in a substantially more different chemical environment as compared to that seen in the corresponding β-CD (1) and the amide (41) complexes. The large difference between the chemical environments of the included enantiomers of the guests (42)-(44) in the presence of the cyclodextrin (40) is probably due to the amide functionality of the cyclodextrin (40) pointing toward the interior of the cavity hence making the cavity of the cyclodextrin (40) less symmetrical than that of β-CD (1) and the cyclodextrin (41). The amide substituent of the cyclodextrin (40) points in to the cavity as a result of the inversion of stereochemistry at the C-2 and C-3 centres of the modified glucopyranose residue, which occurs during the synthesis of it's precursor (30).[79]

Enantioselectivity has also been observed in reactions catalysed by cyclodextrins.[25,26,28,34,43,44,80-89] Such a process usually involves the initial formation of diastereomeric complexes. Subsequent reaction of the more reactive diastereomeric complex affords a product with high enantioselectivity.

Flohr *et al.*,[26,28] noted chiral discrimination by α-CD in the hydrolysis of the ester (45). The authors found that α-CD was acylated by the (+)-enantiomer of the ester (45) seven fold more rapidly than by the (-)-enantiomer.

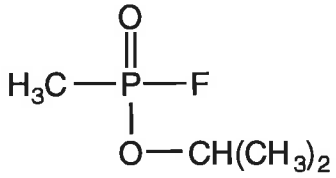


(45)

It was determined that the α-CD-ester (45) diastereomeric complexes were formed at the same rate but the α-CD-(+)-enantiomer complex was the more reactive. Further studies showed that there was a negligible difference in the rates of deacylation of the covalent diastereomeric intermediates. However, because the (+)-enantiomer covalent intermediate was formed more rapidly and hence present in higher concentration, the corresponding hydrolysis product possessing the (+)-stereochemistry was formed selectively. In direct contrast, the ester (45) did not undergo hydrolysis stereoselectively in the presence of β-CD (1).[26,28] Chiral discrimination was noted in the hydrolysis of the *meta*-nitrophenyl ester of *N*-acetylphenylalanine in the presence of β-CD (1). The *S*-enantiomer was hydrolysed two fold faster than the *R*-enantiomer.[84]

Vanhooidonk *et al.*,[43] observed chiral discrimination by α-CD in the hydrolysis of the chiral organophosphate (46). It was noted that a secondary hydroxy group of α-CD was phosphonylated by the (-)-enantiomer of the fluoro

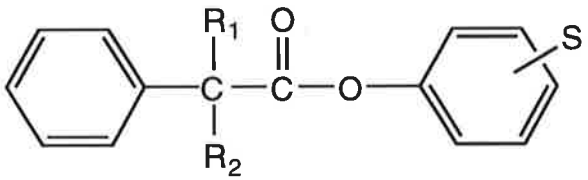
compound (46), thirty six fold faster than the rate observed for the acylation of α-CD by the (+)-enantiomer of the fluoro compound (46). Cleavage of the diastereomeric phosphonyl-α-CD intermediates also occurred at different rates.



(46)

There was a seven fold enhancement for the hydrolysis of the (-)-phosphonyl-α-CD intermediate as compared to that of the corresponding (+)-phosphonyl-α-CD intermediate. Further studies indicated that the (+)-enantiomer actually formed a more stable inclusion complex with α-CD, however, the (-)-phosphonyl-α-CD complex was the more reactive one.[43,85] This may be attributed to the possibility that the secondary hydroxy substituents of α-CD are near the phosphorus in the (-)-phosphonyl-α-CD complex.

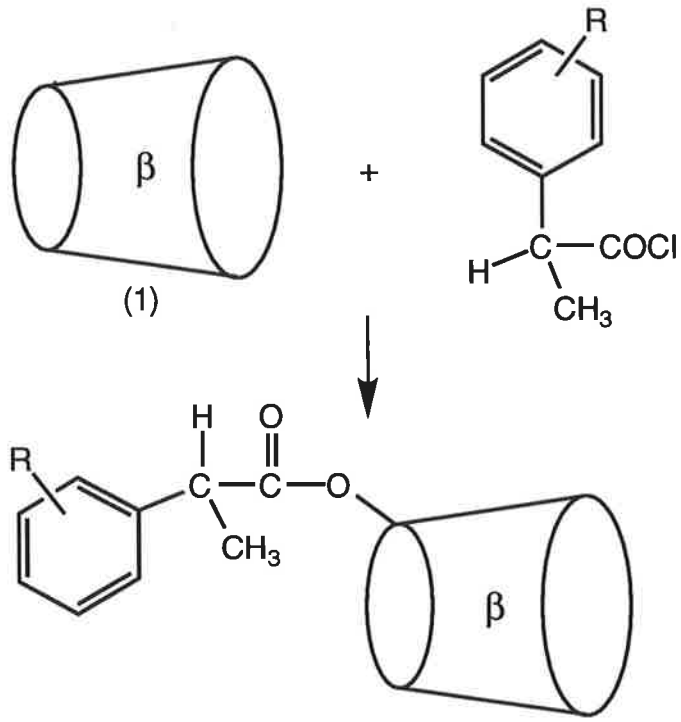
In the late 1980's Fornasier *et al.*,[87] investigated the hydrolysis of the chiral nitrophenyl esters (47)-(52) in the presence of β-CD (1) and α-CD. The greatest extent of chiral discrimination reported was for the hydrolysis of the ester (48) in the presence of β-CD (1), where the *R*-enantiomer of the ester (48) was hydrolysed approximately sixteen fold more rapidly than the corresponding



	R_1	R_2	S
(47)	H	CH_3	*p*-NO_2
(48)	H	CH_3	*m*-NO_2
(49)	H	OCH_3	*p*-NO_2
(50)	H	OCH_3	*m*-NO_2
(51)	CF_3	OCH_3	*p*-NO_2
(52)	CF_3	OCH_3	*m*-NO_2

S-isomer. In general β-CD (1) was found to exhibit a larger discriminatory effect than that observed in the presence of α-CD. Easton *et al.*,[88] witnessed chiral

discrimination in the acylation of β-CD (1) with the racemic acid chlorides of the arylpropanoic acids (53)-(55) in aqueous media (Scheme 13). The corresponding cyclodextrin esters (56)-(58) were isolated and analysed.[88] The greatest extent of chiral discrimination observed was in the reaction of the acid chloride (53) and



R
(53) *p*-$CH_2CH(CH_3)_2$
(54) H
(55) *o*-FC_6H_4

R
(56) *p*-$CH_2CH(CH_3)_2$
(57) H
(58) *o*-FC_6H_4

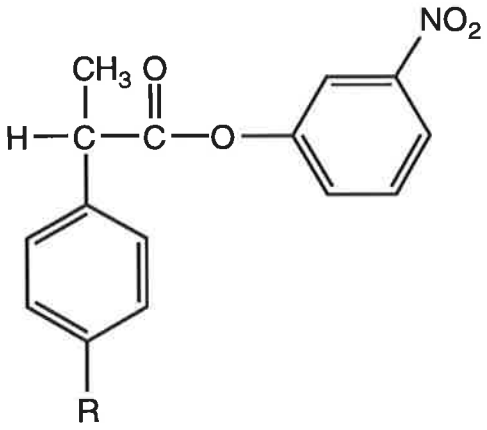
Scheme 13

β-CD (1). The diastereomers of the ester (56) were produced in a ratio of 4.5:1 in favour of the *R*-derived isomer (Table 3).[88] Easton *et al.*,[89] also studied the deacylation of the cyclodextrin esters (56)-(58) (Table 3). Once again, chiral discrimination was noted. The greatest degree of chiral discrimination observed was for the hydrolysis of the ester (56), where the *R*-derived diastereomer was hydrolysed ten fold more rapidly than the *S*-derived diastereomer.[89] The diastereoselectivity in the formation of the ester (56) was found to be complementary to that of the hydrolysis of the ester (56). Similarly the diastereoselectivity in the deacylation of the esters (57) and (58) was found to be complementary to that seen in their formation. [88,89]

Table 3 Diastereoselectivity of the synthesis and hydrolysis of the esters (56)-(58).

Ester	Synthesis	Hydrolysis
(56)	4.5 : 1	10 : 1
(57)	1.3 : 1	2 : 1
(58)	1.7 : 1	7 : 1

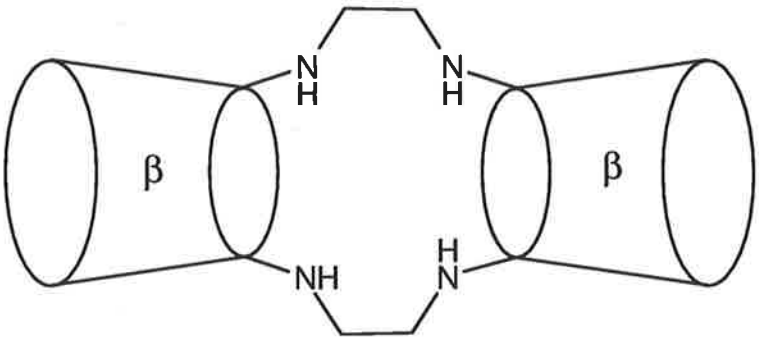
Murakami *et al.*,[79] observed greater spectroscopic discrimination in the formation of diastereomeric inclusion complexes between enantiomers of chiral guests and the C-3 substituted β-cyclodextrin (40) as compared to that seen when the corresponding C-6 substituted β-cyclodextrin (41) or β-CD (1) was present as the host, and it is probable that such a process correlates to thermodynamic discrimination of the same order. The ability of β-CD (1) to catalyse reactions of chiral guests stereoselectively, as demonstrated by Fornasier *et al.*,[87] and Easton *et al.*,[88] prompted the investigation of similar reactions of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) with the chiral esters (48) and (59) in the present work. In a similar system the hydrolysis and subsequent decarboxylation of two malonate substituted cyclodextrin derivatives was also investigated. It was anticipated that the higher enantioselectivity of complexation by the amide (40), observed by Murakami *et al.*,[79] would correlate with higher diastereoselectivity in the reactions of the modified cyclodextrin (30). As mentioned previously it is thought that the cavity of β-CD-3-NH_2 (30) is less symmetrical than that of β-CD-6-NH_2 (29), which is in turn less symmetrical than that of β-CD (1). Therefore β-CD-3-NH_2 (30) and, to a lower degree, β-CD-6-NH_2 (29) would be expected to show greater enantioselectivity in the formation of diastereomeric inclusion complexes with the enantiomers of the esters (48) and (59). Reactions of diastereomeric complexes would then give rise to products with high diastereoselectivities. The results and discussion of this work is outlined in Chapter Two of this thesis.



R
(48) H
(59) $CH_2CH(CH_3)_2$

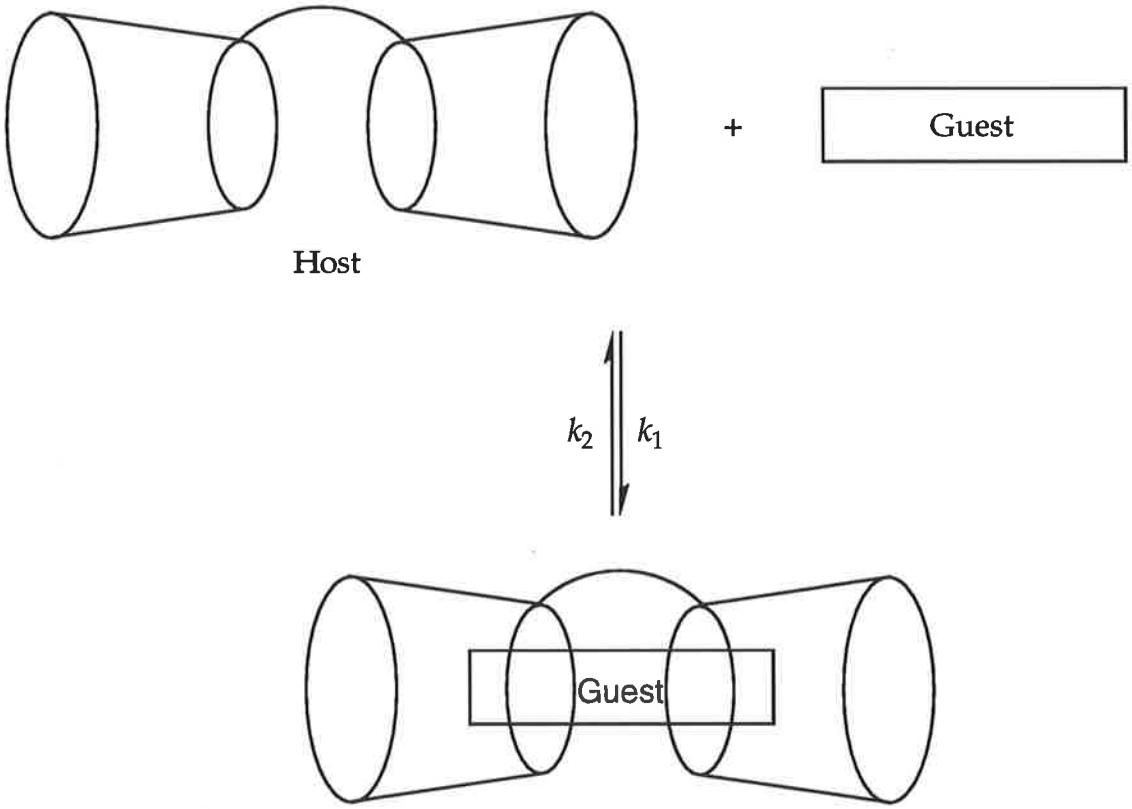
It has been established that cyclodextrins exhibit enzyme-like recognition and catalytic properties, however the guest-binding ability of cyclodextrins is weaker than that exhibited by enzymes. In order to enhance binding, additional binding sites have been introduced on to cyclodextrins.[90-93]

Tabushi *et al.*,[90] first witnessed the increase in binding of substrates to linked cyclodextrins as compared to parent cyclodextrin in the late 1970's. The authors found that TNS (4) bound to the tetramine (60) to form a 1:1 complex of



(60)

greater stability than the corresponding parent β-CD (1)-TNS (4) complex.[90] It was suggested that the enhancement in binding shown for the tetramine (60) TNS (4) 1:1 complex was due to the second hydrophobic cavity of the dimer (60) participating in binding (Scheme 14). Breslow *et al.*,[91] reported the synthesis and studies of various linked cyclodextrins. These authors studied the interaction

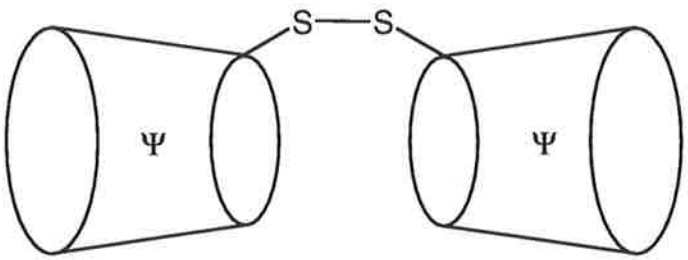


1:1 Inclusion complex

Stability constant of inclusion complex = $K = k_1/k_2$

Scheme 14

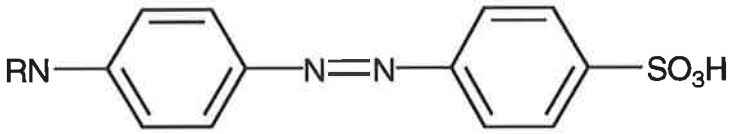
of various guests with the disulphide (61). Binding constants of several guest molecules with the disulphide (61) were determined at 25°C in water.[91]



Ψ
(61) β-CD
(64) α-CD

The results indicated that guests bearing two aromatic moieties of a favourable geometry bound very strongly to the disulphide (61) to form the corresponding 1:1 inclusion complexes.[91] In addition to the van der Waals interactions

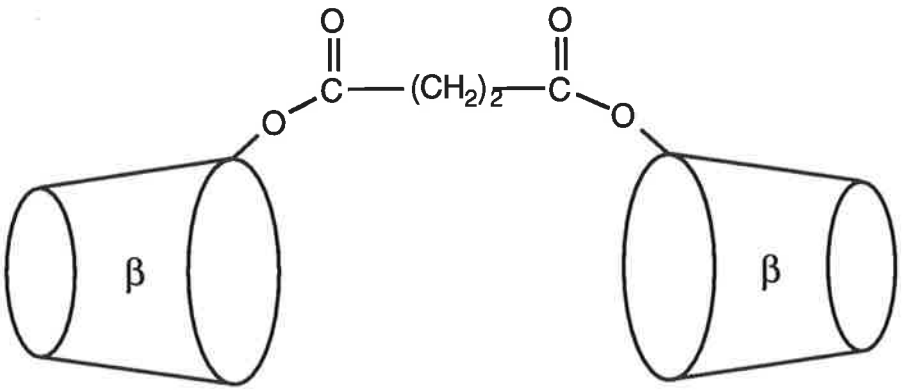
occurring between the guests and the disulphide (61), secondary forces such as hydrogen bonding were thought to contribute to binding. Fujita *et al.*,.[92,93] studied the complexation of the disulphides (61) and (64) with the aromatic guests, ethyl orange (62) and methyl orange (63). The association constant of the disulphide (61)-ethyl orange (62) 1:1 inclusion complex was approximately



	R
(62)	$(CH_2CH_3)_2$
(63)	$(CH_3)_2$

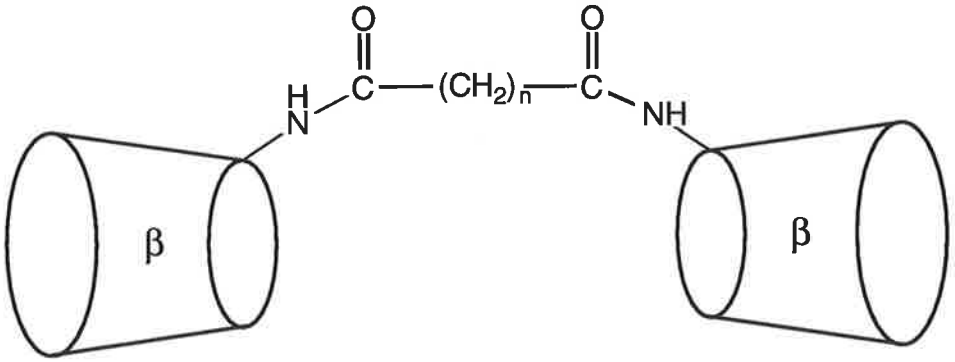
two hundred and twenty fold larger than the association constant derived from the corresponding β-CD (1)-ethyl orange (62) complex.[92] Similarly the association constant of the dimer (61)-methyl orange (63) 1:1 complex was significantly larger (196 fold) than the corresponding association constant of the β-CD (1)-methyl orange (63) complex. The high association constants observed for the disulphide (61)-ethyl orange (62) and methyl orange (63) complexes were not observed when the disulphide (64) was subjected to the analogous complexation studies. The association constant of the disulphide (64)-ethyl orange (62) complex was only marginally larger (1.2 fold) than the corresponding association constant derived for the α-CD-ethyl orange (62) complex. Similarly, the association constant of the disulphide (64)-methyl orange (63) complex was only approximately four fold larger than that of the α-CD-methyl orange (63) complex.[92] It was thought that both of the β-cyclodextrin cavities of the disulphide (61) were participating in binding of the guests (62) and (63). In another example of cooperative binding by linked cyclodextrins, Harada *et al.*,[94] found that the stability constant of the diester (65)-TNS (4) 1:1 inclusion complex was ten fold higher than the corresponding β-CD (1)-TNS (4) complex. When using other guests only a maximum four fold enhancement in binding was noted indicating that the additional cyclodextrin was less effective as a

complementary binding site.[94] Easton *et al.*,[95,96] reported the synthesis of the



(65)

C-6-C-6 linked β-cyclodextrins (66)-(68). The stability constants of their 1:1 inclusion complexes with TNS (4) were determined (Table 4).[96] The results



(66) n = 3
(67) n = 2
(68) n = 0

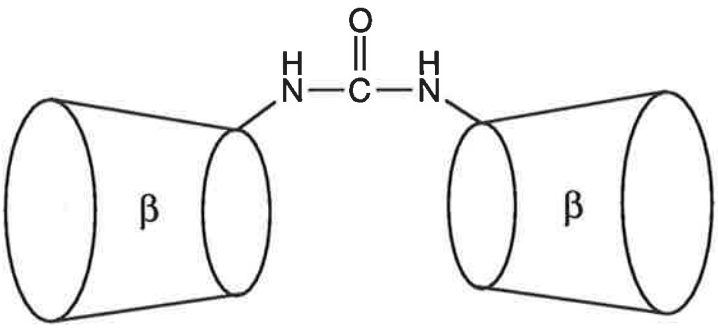
indicated that the dimers (66)-(68) formed more stable 1:1 inclusion complexes with the guest (4) than the corresponding β-CD (1)-guest (4) complex.[96] In addition the stability of the diamide (66)-(68)-guest (4) complexes increased as the length of the bridge between the cyclodextrin cavities was shortened. In order to extend this study a dimer possessing even a shorter link than the oxalamide tether of the dimer (68) was synthesized and studied in the present work. The urea linked β-cyclodextrin (69) was prepared and a complexation study with the guest (4) was carried out. The dimer (70) was also prepared and studied in order to complete the series of C-6-C-6 linked diamides. To complete

a systematic complexation study of diamide linked cyclodextrins with the guest

Table 4 Complexation of TNS (4) and the cyclodextrins (66)-(68).

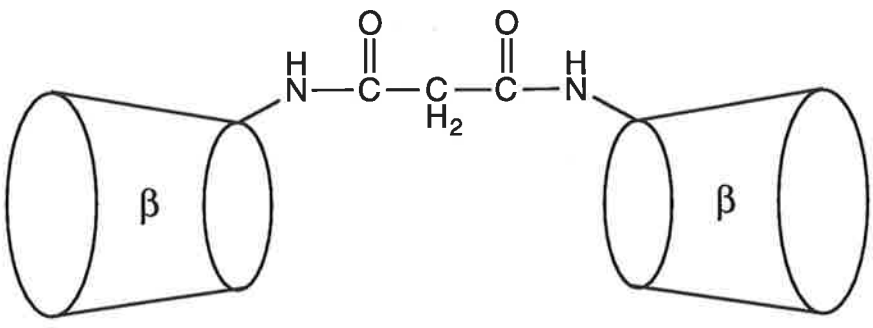
Cyclodextrin	Stability constant K (mol^{-1} dm^{-3})[a]
β-CD (1)	2,800
(66)	7,500
(67)	15,700
(68)	29,000

[a] Represents the stability constants of the TNS (4)-cyclodextrin (1), (66)-(68) 1:1 complexes.



(69)

(4), C-3-C-3 and C-6-C-3 substituted linked cyclodextrins were also prepared and studied. The results and discussion of this work is outlined in Chapter Three of this thesis.

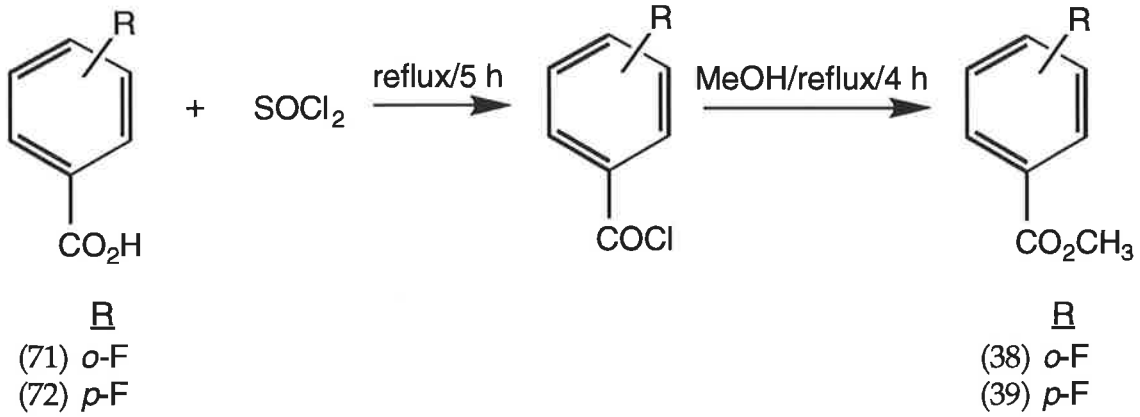


(70)

CHAPTER 1

In order to carry out the work described in this Chapter, the guests (38) and (39) and the cyclodextrin (30) were firstly synthesized.

The methyl ester (38) was prepared by initially converting the free acid (71) to the acid chloride which was then treated with methanol to give the crude ester (38) as a yellow oil. The ester (38) was separated from any unreacted starting material by a base wash and was then distilled to give the ester (38) as a colourless clear liquid in 81% yield (Scheme 15). Thin layer chromatographic



R
(71) *o*-F
(72) *p*-F

R
(38) *o*-F
(39) *p*-F

Scheme 15

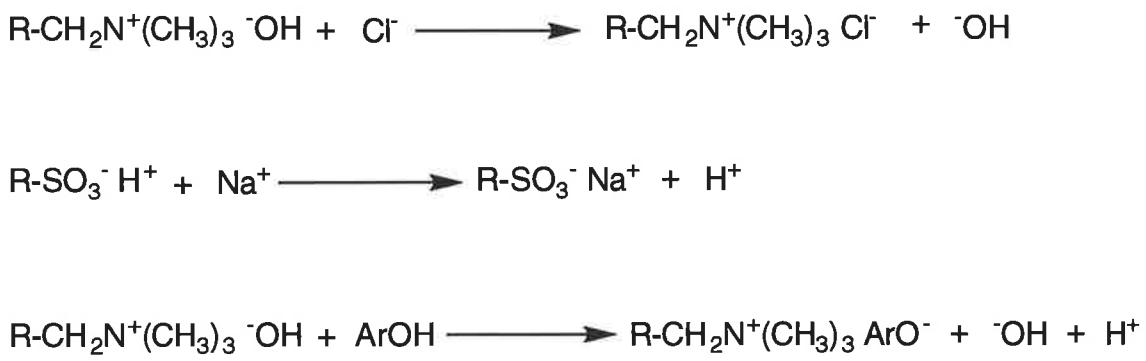
analysis (tlc) of the ester (38) gave only one spot which was less polar than that of the free acid (71) indicating that the ester (38) was pure. The ^{1}H nmr spectrum of the ester (38) showed complicated multiplets present from 7.3 to 7.8 ppm, which were assigned to the hydrogens of the aromatic ring. The intense splitting seen in the aromatic region of the spectrum was due to the coupling of the aromatic hydrogens to the fluoro substituent of the aromatic ring. A singlet present at approximately 3.81 ppm was assigned to the hydrogens of the methyl group. The ^{19}F nmr spectrum of the ester (38) showed one peak at -34.83 ppm for the fluoro substituent. It should be noted that the ^{19}F chemical shift of the

fluoro substituent of the free acid (71) was -38.87 ppm however this peak was not seen in the ^{19}F nmr spectrum of the ester (38). The *para*-ester (39) was prepared and purified by the method outlined for the ester (38) (Scheme 15). The pure ester (39) was obtained as a colourless clear liquid in 89% yield. Once again tlc analysis of the ester (39) showed only one spot which was less polar than that of the acid (72). The ^{1}H nmr spectrum of the ester (38) also showed a series of complicated multiplets present from 7.2 to 8.1 ppm which were assigned to the hydrogens of the aromatic ring. The extensive splitting observed for the aromatic hydrogens of the *para*-ester (39) was once more due to the coupling of the aryl hydrogens to the fluoro substituent of the aromatic ring. A singlet found at 3.92 ppm was assigned to the hydrogens of the methyl group. The ^{19}F nmr spectrum of the *para*-ester (39) showed one peak at -28.51 ppm for the fluoro substituent. The ^{19}F chemical shift of the fluoro substituent of the acid (72) was -32.97 ppm, however such a peak was not present in the ^{19}F nmr spectrum of the *para*-ester (39).

β-CD-6-NH_2 (29) was presented as a gift,[97] which had been synthesized by using a modification of the procedure developed by Slessor *et al.*,[76] as described in the Introduction of this thesis. Normal phase HPLC analysis of the cyclodextrin (29) gave one peak which was more polar than that of β-CD (1). The proton coupled ^{13}C nmr spectrum of β-CD-6-NH_2 (29) gave the expected information. A triplet present at 41.3 ppm was assigned to the C-6^{A} carbon of the modified glucopyranose unit, where as the unmodified C-6^{B-G} carbons were assigned to the multiplet seen at 61.3 ppm.

β-CD-3-NH_2 (30) was synthesized in three steps from β-CD (1) (Scheme 12). The tosylate (33) was prepared from β-CD (1) using the procedure developed by Breslow *et al.*[77] Breslow *et al.*[77] separated the tosylate (33) from sodium chloride and 3-nitrophenol by the use of Sephadex size exclusion chromatography to give the pure tosylate (33) as a white solid in 10% yield. In direct contrast the tosylate (33) in the present work was separated from sodium

chloride and 3-nitrophenol by the application of mixed bed ion exchange chromatography to afford the pure tosylate (33) again as a white solid in 10% yield. Purification of the tosylate (33) by this method was found to be considerably more rapid as compared to purification by the use of size exclusion chromatography. The mixed bed ion exchange resin adopted comprised of equivalent amounts of anion and cation exchange resins in their hydroxide and hydrogen forms. The removal of sodium, chloride and 3-nitrophenolate ions occurred simultaneously to liberate equivalent amounts of hydroxide anions and hydrogen cations which combined to give water (Equations 1-3). Attempts to prepare the tosylate (33) using the alternative procedure developed



Equations 1-3

by Murakami *et al.*,[78] were unsuccessful. Normal phase HPLC analysis of the tosylate (33) gave one peak which was less polar than that of β-CD (1) indicating that the tosylate (33) was pure. Fast atom bombardment mass spectroscopic analysis (FAB-MS) of the tosylate (33) gave a peak at 1289 for the protonated molecular ion (M+H). The ^{1}H nmr spectrum of the tosylate (33) showed two doublets present at 7.43 and 7.84 ppm which were assigned to the four hydrogens of the aromatic ring. A singlet found at 2.40 ppm was assigned to the hydrogens of the methyl substituent. Peaks present from 3.30 to 5.90 ppm were assigned to the hydrogens of the cyclodextrin moiety. Integration of the spectrum gave the required 4:3 ratio for the aryl hydrogens to the methyl hydrogens of the aryl

substituent. Adopting the method reported by Murakami *et al.*,[79] the tosylate (33) was reacted with aqueous ammonium carbonate and gave the epoxide (34) which was then reacted with aqueous ammonia to afford β-CD-3-NH_2 (30). The cyclodextrin (30) was separated from β-CD (1), the tosylate (33) and the epoxide (34) by the application of Sephadex cation exchange chromatography to give pure β-CD-3-NH_2 (30) as a pale yellow crystalline solid in 78% yield based on the tosylate (33) and in 7.8% yield based on β-CD (1). Normal phase HPLC analysis of β-CD-3-NH_2 (30) showed one peak which was more polar than that of β-CD (1). The FAB-MS spectrum of β-CD-3-NH_2 (30) showed a peak at 1135, for the protonated molecular ion. The purity and molecular formula of β-CD-3-NH_2 (30) was confirmed by microanalysis. Microanalytical data showed that the amine (30) was complexed with six water molecules. It should be mentioned that water molecules included in the cavities of cyclodextrins cannot be removed by conventional drying methods. In order to confirm the substitution of the amino group at the C-3 position, a proton coupled ^{13}C nmr spectrum of β-CD-3-NH_2 (30) was recorded. A doublet present at 54.2 ppm was assigned to the C-3^A carbon of the modified glucopyranose unit and was consistent with that of the literature value.[79]

As described in the Introduction of this thesis, the stability constants of the inclusion complexes of the guests (36)-(39) and β-CD (1) and the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) were determined with the aid of ^{19}F nmr spectroscopy. The ^{19}F chemical shifts (δ_F) of the fluoro substituents of the guests (36)-(39) in the absence of cyclodextrin in pH 6.0 phosphate buffer containing 10% deuterium oxide were measured and recorded (Table 5). Deuterium oxide was present in all solutions so that the spectrometer could be locked on it's deuterium frequency. Solutions were prepared using 0.2 mol dm^{-3} phosphate buffer (pH 6.0) so as to ensure that the guests (36) and (37) exist as their conjugate bases. The ^{19}F chemical shifts (δ_{obs}) of the fluoro substituents of the guests (36)-(39) in pH 6.0 phosphate buffer were also

measured for a range of solutions which were constant in concentration of the guests (36)-(39) (*ca.* 1 mmol dm^{-3}) and increasing in concentration of the hosts (1), (29) and (30) (1-13 mmol dm^{-3}) containing 10% deuterium oxide. Solutions containing the cyclodextrins (1), (29) and (30) were also made up in pH 6.0 phosphate buffer so as to ensure the formation of the conjugate acids of the cyclodextrins (29) and (30). It is expected that the guests (36)-(39) in the presence of the cyclodextrins (1), (29) and (30) in solution are rapidly interconverting between their non-complexed and complexed states. Therefore the ^{19}F chemical shifts (δ_{obs}) recorded for each of the solutions are timed averaged chemical shifts of the fluoro substituents of the guests (36)-(39) in their free (δ_F) and complexed (δ_C) states. Plots (Fig. 1-4) of ^{19}F chemical shift versus concentration of the cyclodextrin for each case were subjected to a non-linear regression analysis, and by the application of equations 4 and 5, the stability constant K for each complex was derived (Table 5). In addition the ^{19}F chemical shifts of the fluoro substituents of the guests (36)-(39) in their fully complexed states (δ_C) were also derived. From this the magnitude of change ($\Delta\delta$) in chemical shift of the fluoro substituents of the guests (36)-(39) from their non-complexed (δ_F) to fully complexed (δ_C) states was calculated (Table 5).

$$K = [COMPLEX]\,([SUBSTRATE]\,.\,[HOST])^{-1} \qquad \textbf{Equation 4}$$

$$(\delta_{obs}) = (\delta_{guest}\,[guest] + \delta_{complex}\,[complex])/([guest] + [complex] \qquad \textbf{Equation 5}$$

The stability constants of the complexes formed with β-CD (1) vary considerably with the identity of the guest. It was clearly seen that the β-CD (1)-ester (38) and (39) complexes were more stable than the corresponding β-CD (1)-anion (36) and (37) complexes. The complexes of the esters (38) and (39) were found to be greater than four fold more stable than those of the corresponding benzoate anions (36) and (37). This suggests that although van der Waals

Figure 1 Plots of ^{19}F chemical shift (ppm) of the fluoro substituent of the guest (36) against the concentration of the host's (1), (β-CD-6-NH_3^+) and (β-CD-3-NH_3^+).

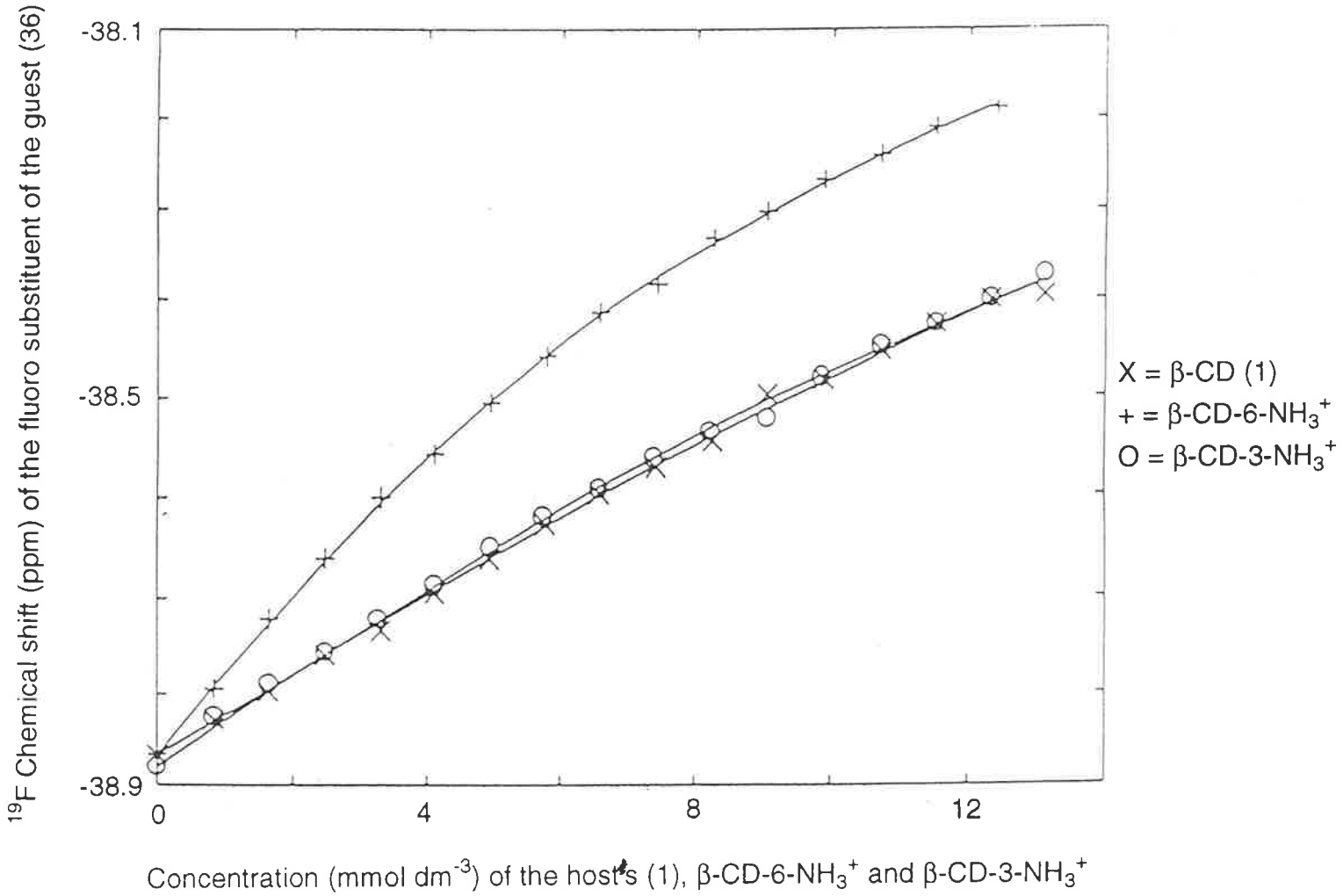


Figure 2 Plots of ^{19}F chemical shift (ppm) of the fluoro substituent of the guest (37) against the concentration of the host's (1), (β-CD-6-NH_3^+) and (β-CD-3-NH_3^+).

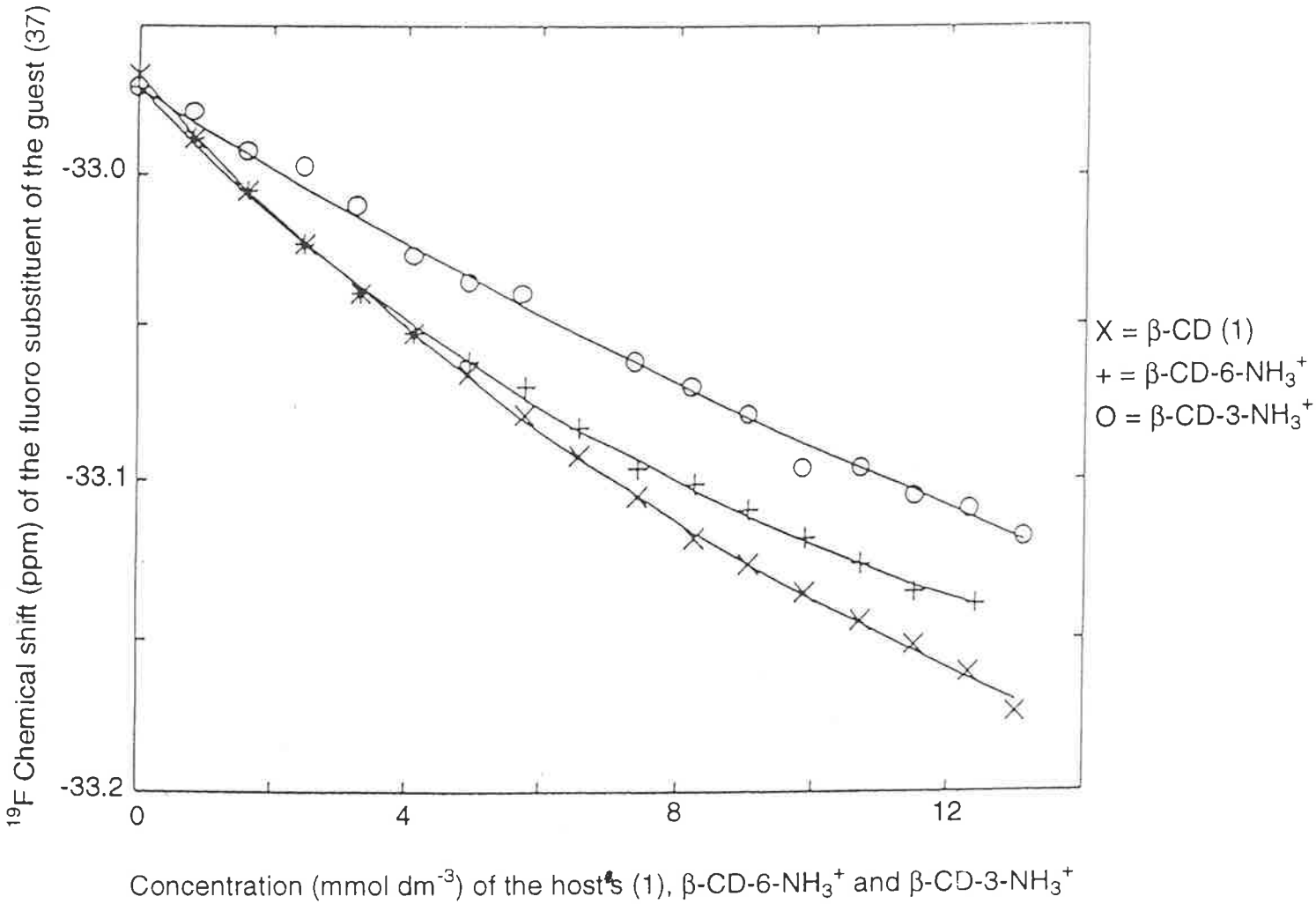


Figure 3 Plots of ^{19}F chemical shift (ppm) of the fluoro substituent of the guest (38) against the concentration of the host's (1), (β-CD-6-NH_3^+) and (β-CD-3-NH_3^+).

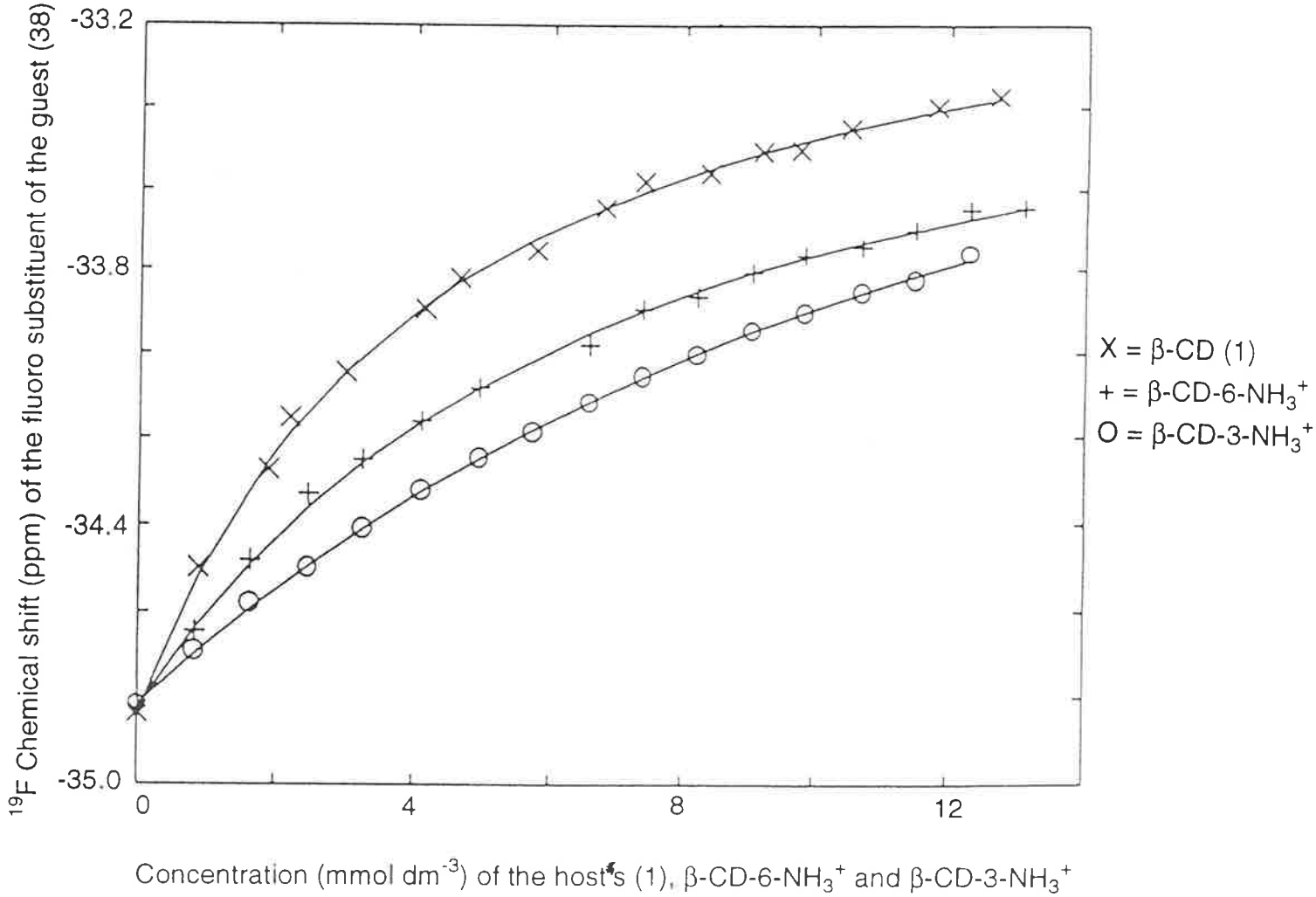
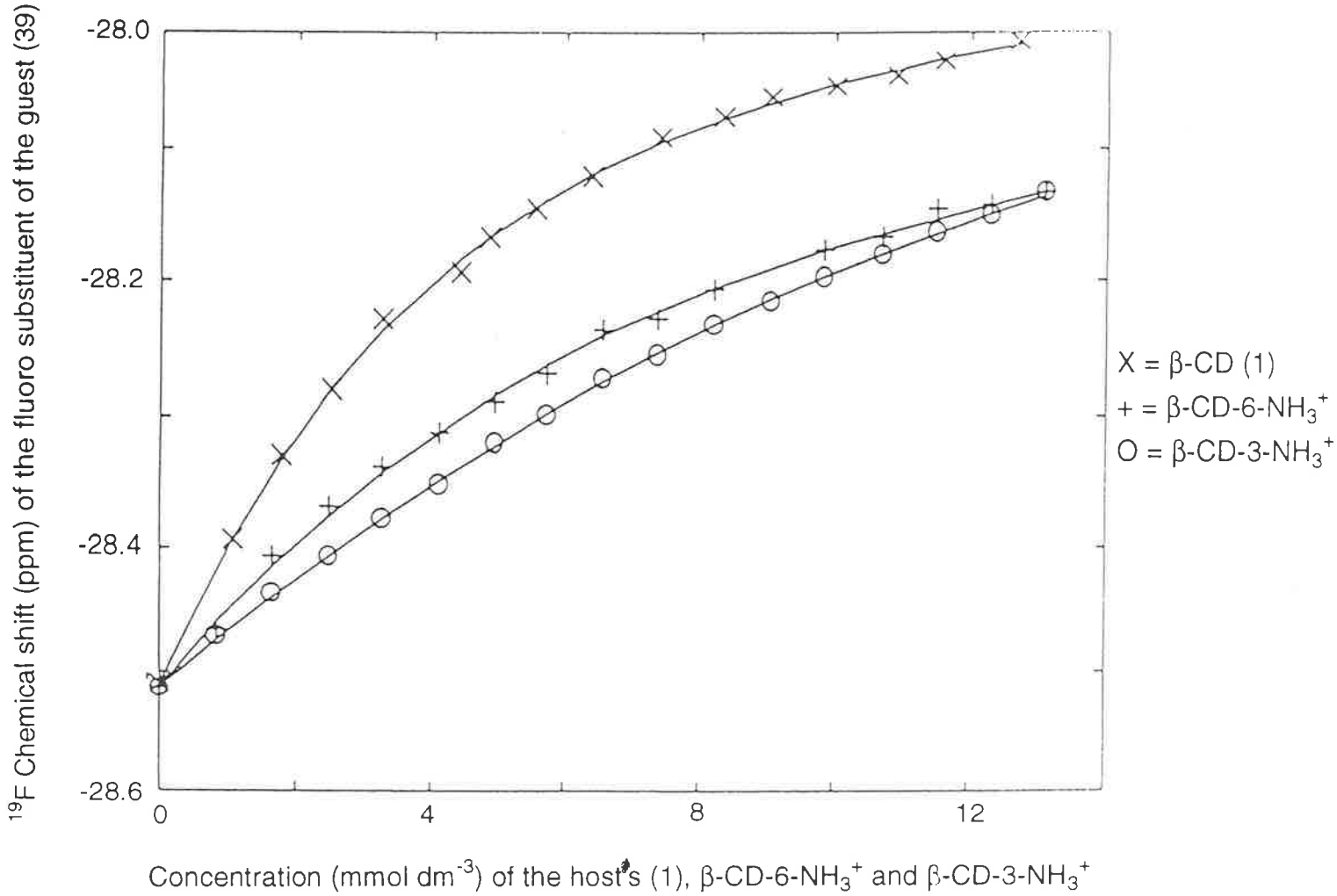


Figure 4 Plots of ^{19}F chemical shift (ppm) of the fluoro substituent of the guest (39) against the concentration of the host's (1), (β-CD-6-NH_3^+) and (β-CD-3-NH_3^+).



interactions between the aromatic moieties of each of the guests (36)-(39) and the hydrophobic interior of the cyclodextrin annulus result in complexation, the stronger solvation of the carboxylates (36) and (37) by the water molecules present destabilises their inclusion complexes. The stability constant of the β-CD (1)-*ortho*-ester (38) complex is greater than that of the complex of the *para*-isomer (39). This may be attributed to the effect of the complementary dipole moments of β-CD (1) and the guests (38) and (39) on the inclusion complexes.

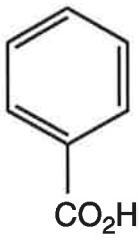
Table 5 Stability constants and ^{19}F chemical shifts of cyclodextrin-fluorinated guest inclusion complexes, in 10% aqueous D_2O at 295.5 K and I = 0.40 mol dm^{-3}.

Cyclodextrin	Guest	K (mol^{-1} dm^3)	δ_{free} or $\delta_{complex}$ (ppm)	$\Delta\delta$ (ppm)
-	(36)	-	-38.87	
-	(37)	-	-32.97	
-	(38)	-	-34.83	
-	(39)	-	-28.51	
(1)	(36)	19 ± 3	- 36.44 ± 0.30	+ 2.43
(1)	(37)	50 ± 2	- 33.49 ± 0.02	- 0.52
(1)	(38)	253 ± 11	- 32.91 ± 0.03	+ 1.92
(1)	(39)	228 ± 7	- 27.83 ± 0.01	+ 0.68
(29)	(36)	65 ± 2	- 37.29 ± 0.03	+ 1.58
(29)	(37)	69 ± 4	- 33.35 ± 0.02	- 0.38
(29)	(38)	152 ± 7	- 33.04 ± 0.04	+ 1.79
(29)	(39)	128 ± 7	- 27.90 ± 0.02	+ 0.61
(30)	(36)	32 ± 3	- 37.20 ± 0.10	+ 1.67
(30)	(37)	19 ± 5	- 33.72 ± 0.20	- 0.75
(30)	(38)	69 ± 2	- 32.52 ± 0.05	+ 2.31
(30)	(39)	59 ± 2	- 27.64 ± 0.01	+ 0.87

The dipole moment of α-CD has been found to be 13.5 D, (1 D = 3.33564 x 10^{-30} C m) with the positive end of the dipole adjacent to the ring of the primary hydroxy groups and the negative end adjacent to the ring of the secondary hydroxy groups, delineating the narrow and wide ends of the α-CD annulus respectively.[98,99] The negative end of the dipole moment of α-CD resides adjacent to the ring of the twelve C-2 and C-3 hydroxy groups of α-CD since there are six more hydroxy groups present at that end of the molecule as compared to the opposite end which consists of six primary C-6 hydroxy groups. It has been observed that the dipole moments of *para*-nitrophenol (73), *para*-hydroxybenzoic acid (74) and benzoic acid (75) are antiparallel to the direction of

(73)

(74)



(75)

the dipole moment of α-CD in their inclusion complexes, such that the nitro and carboxylic acid substituents are in the vicinity of the primary C-6 hydroxy groups of α-CD.[98] The orientation of the dipole moment of β-CD (1) is likely to be analogous to that of α-CD and it is probable that the fluorinated esters (38) and (39) employed in this investigation align dipoles antiparallel to that of β-CD (1), with this alignment contributing to the stability of the corresponding complexes. As the dipole moment of the *ortho*-ester (38) is greater than that of the corresponding *para*-substituted isomer (39), the complex of the former with β-CD (1) has the highest stability.

The contribution of the dipole moment of the guest to the stability of the inclusion complexes is also evident in the stability constants of the

complexes of the esters (38) and (39) with the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30). The *ortho*-ester (38), with the larger dipole moment, forms the more stable complex with each of the modified cyclodextrins (29) and (30). The greater dipole moment of the *ortho*-benzoate (36) compared to that of the *para*-isomer (37) is not reflected as a general trend in the stability constants of the complexes with β-CD (1) and the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30), so other factors that are not easily discerned must affect the relative stability of these complexes. It is interesting to note the greater stability of the complexes of the anions (36) and (37) with the conjugate acid of β-CD-6-NH_2 (29) compared to that of the corresponding complexes with β-CD (1) and the conjugate acid of β-CD-3-NH_2 (30). The additional stabilisation may be attributed to ionic interactions between the host and guests, which are significant only for the complexes of the conjugate acid of β-CD-6-NH_2 (29), where interaction between charged groups of the host and guests is compatible with the antiparallel alignment of the dipole moments of the host and guests in the inclusion complexes.

The complexes of the esters (38) and (39) with the conjugate acid of β-CD-6-NH_2 (29) are each less stable than those with β-CD (1). This is probably a reflection of the decreased hydrophobicity of the annulus of the modified cyclodextrin (29), resulting from the effect of hydration of the protonated amino substituent to impinge on the character of the cyclodextrin cavity. The stability constants of the complexes of the esters (38) and (39) with the conjugate acid of β-CD-3-NH_2 (30) are even lower than those with the conjugate acid of β-CD-6-NH_2 (29). The synthesis of β-CD-3-NH_2 (30) occurs with inversion of stereochemistry at C-2 and C-3 of the modified glucopyranose unit,[79] with the result that the amino substituent intrudes into the cavity of the cyclodextrin. The consequent hydration of the protonated substituent will decrease the hydrophobicity of the cyclodextrin annulus, to an even greater extent than for the conjugate acid of β-CD-6-NH_2 (29), and decreased stability of the inclusion

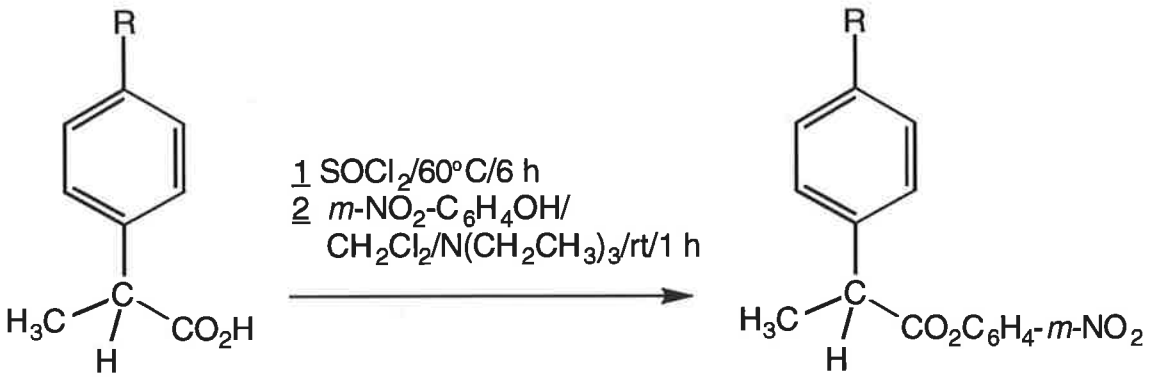
complexes of the esters (38) and (39) follows due to a decrease in the degree of van der Waals interactions between the aromatic rings of the guests (38) and (39) and the cavity of the conjugate acid of β-CD-3-NH_2 (30) as compared to that seen when the conjugate acid of β-CD-6-NH_2 (29) is present as the host. The complexes of the anions (36) and (37) with the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) are each less stable than the corresponding complexes of the esters (38) and (39). This may be attributed to the effect of solvation of the anions (36) and (37), as discussed above for the complexes of the guests (36)-(39) with β-CD (1).

The change in the ^{19}F chemical shift of the fluoro substituent on complexation of each of the guests (36)-(39) is remarkably independent of the cyclodextrin or the stability of the complex (Table 5). This indicates that the mode of complexation by the modified cyclodextrins (29) and (30) is similar to that by β-CD (1). With each cyclodextrin, complexation results in the signals of the *ortho*-substituted anion (36) and the corresponding ester (38) moving downfield by 1.5 - 2.5 ppm, the signal of the *para*-substituted ester (39) moving downfield by 0.6 - 0.9 ppm, and that of the *para*-substituted anion (37) moving upfield by 0.4 - 0.7 ppm. The downfield shifts of the signals for the *ortho*-substituted anion (36) and the esters (38) and (39) indicate more extensive hydrogen bonding of the fluoro substituents consistent with their being in close proximity to cyclodextrin hydroxy groups in their inclusion complexes.[100-103] This is likely to involve cyclodextrin primary hydroxyl groups in the case of the *ortho*-isomers (36), and (38) and cyclodextrin secondary hydroxyl groups in the case of the *para*-ester (39), based on the antiparallel alignment of host and guest dipole moments in the inclusion complexes. The upfield shift of the signal encountered for the *para*-substituted benzoate (37) indicates that in the complexes of this species the fluoro substituent is imbedded in the hydrophobic cyclodextrin annulus.[100-103] Presumably the depth of penetration of the anions (36) and (37) into the cyclodextrin cavities is less than that of the esters (38) and

(39), due to more extensive hydration of the anions (36) and (37). This partial inclusion of the *ortho*-substituted anion (36) will maintain the fluoro substituent of that species in a hydrophilic environment near cyclodextrin hydroxyl groups, while the effect of partial inclusion of the *para*-isomer (37) will be to place the fluoro substituent of that species within the hydrophobic region of the cyclodextrin.

CHAPTER 2

The racemic esters (48) and (59) were synthesized (Scheme 16) in order to investigate chiral discrimination in their reactions with β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30). The ester (48) was prepared by converting the acid (76) to the corresponding acid chloride which was then treated with *meta*-nitrophenol. The ester (48) was purified by silica gel chromatography and



R	
(76)	H
(77)	$CH_2CH(CH_3)_2$

R	
(48)	H
(59)	$CH_2CH(CH_3)_2$

Scheme 16

distillation, and obtained as a yellow oil in 72% yield. Thin layer chromatography on the ester (48) showed only one spot, which was less polar than those of *meta*-nitrophenol and the acid (76), indicating that the ester (48) was pure. The ^1H nmr spectrum of the ester (48) showed the required information for such a system. Multiplets present at 7.4 to 8.2 ppm were assigned to the hydrogens of the two aromatic rings. A quartet present at 4.04 ppm and a doublet observed at 1.66 ppm were assigned to the benzylic methine and methyl hydrogens respectively. Proton decoupled ^{13}C nmr analysis of the ester (48) also exhibited the required information. Signals present at 45.6 and 18.4 ppm were assigned to the benzylic methine and methyl carbons

respectively. Signals at 117.2 to 151.2 ppm were assigned to the aromatic carbons and a signal at 172.5 ppm corresponded to the carbonyl carbon of the ester moiety. The racemic ester (59) was synthesized and purified by an analogous procedure to that described for the ester (48), and obtained as a viscous yellow-orange coloured oil in 80% yield. Once more, tlc analysis of the ester (59) exhibited only one spot, which was less polar than those of *meta*-nitrophenol and the acid (77). The ^{1}H nmr spectrum of the nitrophenyl ester (59) showed an AB quartet present at 7.16 and 7.36 ppm which was assigned to the hydrogens of the *para*-substituted aromatic ring. Multiplets present at 7.3 to 8.1 ppm were assigned to the hydrogens of the *meta*-substituted aromatic ring. A quartet present at 3.97 ppm was ascribed to the propanoate methine hydrogen and a doublet observed at 1.61 ppm was assigned to the hydrogens of the propanoate methyl group. A doublet and a multiplet present at 0.90 and 1.9 ppm respectively corresponded to the methyl and methine hydrogens of the isobutyl side chain, and finally a doublet at 2.47 ppm was assigned to the methylene hydrogens of the isobutyl side chain. Signals at 45.0, 44.7, 30.1, 22.2 and 18.4 ppm in the proton decoupled ^{13}C nmr spectrum of the ester (59) were assigned to the methylene, methine and methyl carbons of the isobutyl side chain, and to the methyl and methine carbons of the propanoate moiety. Signals observed at 116.8 to 151.2 ppm were assigned to the aromatic carbons, and a signal at 172.5 ppm was assigned to the carbonyl carbon of the ester moiety.

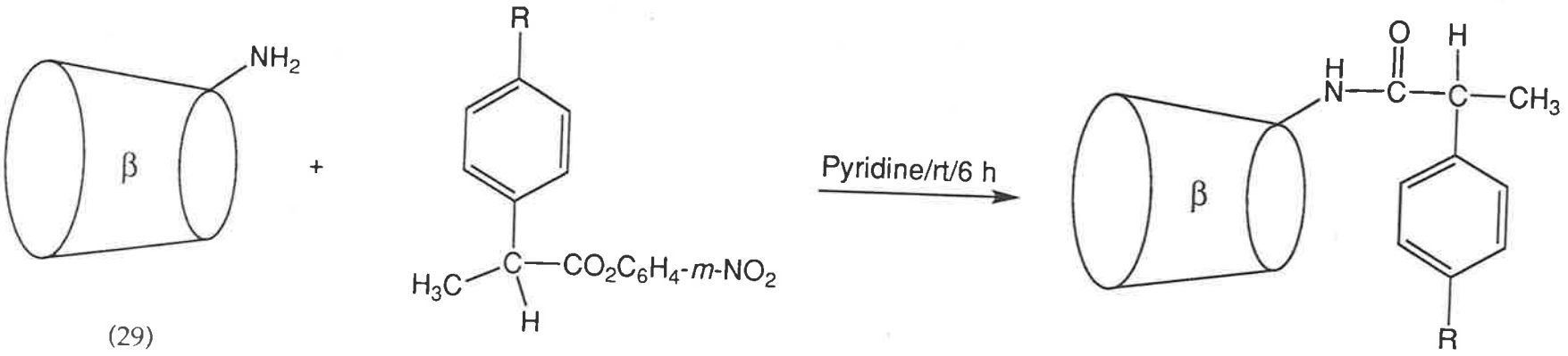
The diastereoselectivity of the reaction of β-CD-6-NH_2 (29) and the ester (48) was determined by the use of ^{1}H and ^{13}C nmr spectroscopy. The reaction was performed in pyridine and at room temperature (Scheme 17). Eight mole equivalents of the ester (48) were used in order to ensure that it was present in excess and therefore the apparent enantioselectivity would not be reduced due to a significant depletion of one enantiomer. The cyclodextrin (78) was isolated and purified by the application of Sephadex cation exchange chromatography, and obtained as a white crystalline solid in 60% yield. Normal

phase HPLC analysis of the cyclodextrin (78) showed only one peak which was less polar than those of β-CD-6-NH_2 (29) and β-CD (1). A peak present at 1267 in the FAB-MS of the cyclodextrin (78) corresponded to the protonated molecular ion (M+H). The infrared spectrum of the cyclodextrin (78) showed a peak at 1652 cm^{-1} which was assigned to the carbonyl of the amide functionality. Microanalytical data showed that the amide (78) was present in the hexahydrate form. The proton decoupled ^{13}C nmr spectrum of the amide (78) showed peaks at 178.5 and 178.2 ppm in a ratio of 2:1, and these were assigned to carbonyl carbons of the diastereomers. Signals at 144.0 to 129.2 ppm were assigned to the carbons of the aromatic ring. Signals observed at 20.9 and 20.3 ppm in a ratio of 2:1 and at 48.2 and 47.8 ppm in a ratio of 2:1 corresponded to the methyl and benzylic methine carbons of the diastereomers. A signal at 42.6 ppm was assigned to the C-6^A carbon of the modified glucopyranose unit of each diastereomer. ^1H nmr spectroscopy of the cyclodextrin (78) showed doublets at 1.30 and 1.24 ppm in a ratio of 2:1, and these were assigned to the methyl hydrogens of the diastereomers. An unresolved multiplet observed at 7.3 to 7.2 ppm was assigned to the hydrogens of the aromatic ring. A signal corresponding to the benzylic hydrogen was not seen due to it being obscured by resonances resulting from the hydrogens of the cyclodextrin moiety. The ^1H and ^{13}C nmr spectral data of the cyclodextrin (78) showed that the reaction of the ester (48) with β-CD-6-NH_2 (29) proceeded with a 2:1 diastereoselectivity. As a comparison to this reaction, the diastereoselectivity of the analogous reaction of β-CD-3-NH_2 (30) was also investigated (Scheme 18). Sephadex cation exchange chromatography of the crude product furnished the amide (80) as a colourless clear glassy solid in 48% yield. HPLC analysis of the amide (80) gave rise to one peak, which was less polar than those of β-CD-3-NH_2 (30) and β-CD (1). The infrared spectrum of the cyclodextrin (80) showed a peak at 1650 cm^{-1} which was ascribed to the carbonyl of the amide functionality. FAB-MS analysis of the cyclodextrin (80) showed a peak at 1267 for M+H. Once again the purity and

molecular formula of the cyclodextrin (80) was confirmed by elemental analysis. Microanalytical data showed that the amide (80) was present in the heptahydrate form. Proton decoupled ^{13}C nmr spectroscopic analysis of the cyclodextrin (80) showed peaks at 180.4 and 179.3 ppm in a ratio of 2:1 and these were assigned to the amide carbonyl carbons of the diastereomers. Signals evident from 144.7 to 129.2 ppm were ascribed to the aromatic carbons. Signals at 20.4 and 18.9 ppm in a ratio of 2:1 were assigned to the methyl carbons of the diastereomers, while peaks seen at 48.4 and 49.7 ppm in a ratio of 2:1 corresponded to the benzylic methine carbons of the diastereomers. Signals at 53.2 and 52.9 ppm in a ratio of 2:1 were assigned to the C-3^A carbons attached to the amide nitrogens of the diastereomers. ^1H nmr spectroscopy of the amide (80) at room temperature produced a poorly resolved spectrum. A broad signal present at approximately 1.35 ppm was assigned to the methyl hydrogens of both diastereomers. When the ^1H nmr experiment was conducted at an elevated temperature (343 K), the resolution of the spectrum was greatly improved, and two distinct doublets corresponding to the methyl hydrogens of the diastereomers were clearly seen at 1.37 and 1.42 ppm, in an approximate ratio of 2:1. A multiplet observed at 7.3 ppm was assigned to the aromatic hydrogens. The signal corresponding to the benzylic methine hydrogen was obscured by the signals for the cyclodextrin hydrogens. The enhancement in resolution of the ^1H nmr spectrum of the amide (80) at 343 K can be attributed to an increase in tumbling of the cyclodextrin moiety resulting from the increase in energy. The nmr spectral data of the cyclodextrin (80) showed that the reaction of the ester (48) with β-CD-3-NH_2 (30) proceeded with a diastereoselectivity of 2:1, which was identical to that seen for the analogous reaction of β-CD-6-NH_2 (30) described above.

The reaction of the ester (59) with β-CD-6-NH_2 (29) was also investigated in order to study the effect of the isobutyl side chain of the ester (59) on the diastereoselectivity of the reaction (Scheme 17). Once again the reaction conditions employed were identical to those of the reactions of β-CD-6-NH_2 (29)

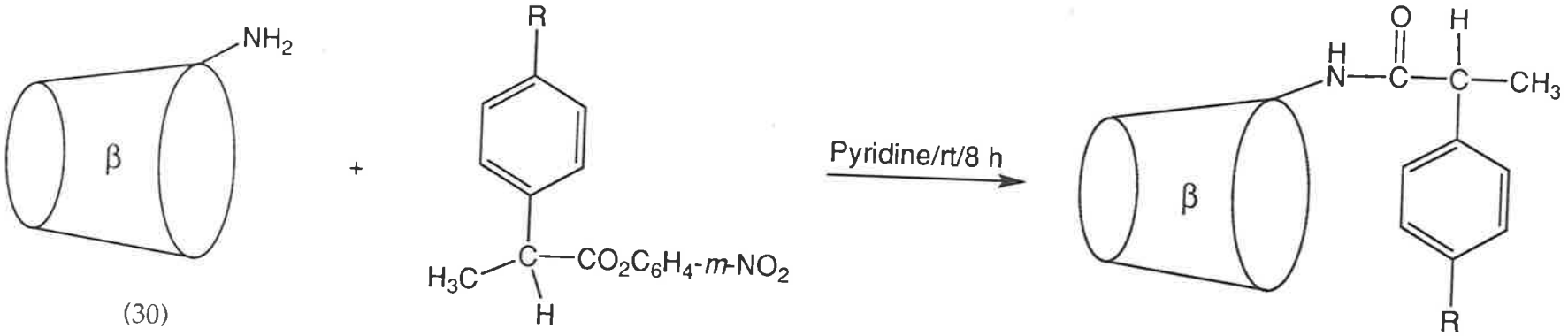
and β-CD-3-NH_2 (30) with the ester (48). After cation exchange chromatography of the crude product, the amide (79) was isolated in 76% yield as a white powder. Normal phase HPLC analysis of the cyclodextrin (79) showed only one peak, which was less polar than those of β-CD-6-NH_2 (29) and β-CD (1). A peak at 1650 cm^{-1} in the infrared spectrum of the cyclodextrin (79) was assigned to the carbonyl of the amide functionality. A peak at 1323 in the FAB-MS corresponded to M+H. Microanalytical data showed that the amide (79) was present in the hexahydrate form. ^1H nmr analysis of the amide (79) at 347 K showed doublets at 1.30 and 1.23 ppm in a ratio of 2:1, and these were assigned to the propanoate methyl hydrogens of the diastereomers. Once again the signal corresponding to the benzylic methine hydrogen was obscured by the signals related to the cyclodextrin hydrogens. Two sets of AB quartets at 7.19 and 7.04 ppm and at 7.21 and 7.06 ppm in a ratio of 2:1 were assigned to the aromatic hydrogens of the diastereomers. Doublets observed at 0.86 and 2.41 ppm and a multiplet present at 1.8 ppm were assigned to the methyl, methylene and methine hydrogens of the isobutyl substituent. Proton decoupled ^{13}C nmr analysis of the cyclodextrin (79) also confirmed the existence of two diastereomers in a ratio of 2:1. Signals present at 175.1 and 174.9 ppm in a ratio of 2:1 were assigned to the carbonyl carbons of the diastereomers. Signals at 140.7 to 128.0 ppm were ascribed to the aromatic carbons. A peak at 45.3 ppm corresponded to the benzylic methine carbon and peaks at 19.9 and 19.6 ppm were assigned to the propanoate methyl carbons of the diastereomers. Signals seen at 23.3 and 30.8 ppm were assigned to the methyl and methine carbons of the isobutyl side chain. The signal corresponding to the methylene carbon of the isobutyl side chain was obscured by the signals of the solvent (d^6-DMSO). The signal corresponding to the C-6^A carbon attached to the nitrogen of the amide functionality was also obscured by the signals of d^6-DMSO. Nmr spectral data of the cyclodextrin (79) showed that the reaction of β-CD-6-NH_2 (29) with



R
(48) H
(59) $CH_2CH(CH_3)_2$

R
(78) H
(79) $CH_2CH(CH_3)_2$

Scheme 17



R
(48) H
(59) $CH_2CH(CH_3)_2$

R
(80) H
(81) $CH_2CH(CH_3)_2$

Scheme 18

the racemic ester (59) proceeded with a 2:1 diastereoselectivity, identical to those of the reactions described above.

To determine the stereochemistry of the diastereomers of the amide (79) produced from the reaction of β-CD-6-NH_2 (29) with the racemic ester (59), an authentic sample of one diastereomer was required. Reaction of β-CD-6-NH_2 (29) with the *S*-enantiomer of the ester (59) gave the corresponding diastereomer of the amide (79). ^1H nmr spectroscopy on a mixture of the products of these reactions showed an enhancement in the intensity of the more downfield doublet corresponding to the propanoate methyl hydrogens of one diastereomer. It was therefore concluded that the major diastereomer formed from the reaction of β-CD-6-NH_2 (29) with the racemic ester (59) possessed the *S*-stereochemistry.

To further the investigation of chiral discrimination, the reaction of β-CD-3-NH_2 (30) with the ester (59) was also studied (Scheme 18). The amide (81) was prepared by the usual procedure and isolated as a cream coloured solid in 52% yield, after Sephadex cation exchange chromatography. Normal phase HPLC analysis on the amide (81) gave rise to one peak, which was less polar than those of β-CD-3-NH_2 (30) and β-CD (1). The FAB-MS of the cyclodextrin (81) possessed a signal at 1323 for M+H. Infrared spectroscopy again revealed the presence of an amide functionality at 1650 cm^{-1}. Microanalysis showed that the amide (81) was complexed with three and a half water molecules. The ^1H nmr spectrum of the cyclodextrin (81) recorded at 377 K exhibited a sharp doublet at 1.42 ppm and this was assigned to the hydrogens of the propanoate methyl groups of the diastereomers. Two sets of overlapping AB quartets at 7.04 and 7.21 ppm and at 7.08 and 7.25 ppm, in an approximate ratio of 1:1, were assigned to the aromatic hydrogens of the diastereomers. The signal corresponding to the benzylic methine hydrogen was obscured by the presence of signals assigned to the cyclodextrin hydrogens. The methylene and methine hydrogens of the isobutyl side chain were assigned to the doublet and multiplet present at 2.44

and 1.8 ppm respectively. A multiplet at 0.8 ppm was assigned to the methyl hydrogens of the isobutyl side chain of both diastereomers. The proton decoupled ^{13}C nmr spectrum of the cyclodextrin (81) showed a peak at 178.9 ppm and this was assigned to the carbonyl carbons of both diastereomers. Signals at 142.7 to 129.6 ppm were ascribed to the aromatic carbons. A peak at 47.2 ppm was assigned to the benzylic methine carbon, and a signal at 18.1 ppm was assigned to the propanoate methyl carbons of both diastereomers. Signals at 23.7 and 32.7 ppm corresponded to the methyl and methine carbons of the isobutyl side chain. The signal corresponding to the benzylic methylene carbon of the isobutyl substituent was obscured by the signals of the solvent (d^6-DMSO). A peak at 53.1 ppm was assigned to the C-3^A carbon of the modified glucopyranose unit. From the nmr spectral data of the cyclodextrin (81), it was evident that β-CD-3-NH_2 (30) underwent reaction with the ester (59) without any diastereoselectivity.

Since the diastereoselectivity of the reaction of β-CD-6-NH_2 (29) and the ester (48), and of the reactions of β-CD-3-NH_2 (30) with the esters (48) and (59) was of low order, it was deemed not important to establish the absolute stereochemistry of the products of these reactions. The low chiral discrimination observed in the reactions of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) with the esters (48) and (59), summarised in Table 6 was unexpected. As

Table 6 Diastereoselectivity in the synthesis of the cyclodextrins (78)-(81).

Product	Diastereoselectivity
(78)	2:1
(79)	2:1
(80)	2:1
(81)	1:1

described in the Introduction of this thesis, chiral discrimination in the

reactions of cyclodextrins and racemic substrates is thought to occur due to the selective formation of one diastereomeric complex. Reaction of the dominant complex furnishes a product with high diastereoselectivity. Because the cyclodextrins (29) and (30) were thought to possess cavities less symmetrical than that of β-CD (1), they were expected to exhibit a greater degree of chiral discrimination in the formation of diastereomeric inclusion complexes. This was then expected to lead to products with greater diastereoselectivity than that reported by Fornasier *et al.*,[87] for the reaction of β-CD (1) and the ester (48). However from the results obtained in the present work this was not the case (Table 6).

It was thought that a possible reason for the differences between the diastereoselectivity of the reaction of β-CD (1) and the ester (48) described by Fornasier *et al.*,[87] and that encountered for the analogous reactions of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30) described in the present work may be linked to the reaction solvents. In the work of Fornasier *et al.*,[87] the reaction was carried out in aqueous media however in the present work the reactions were performed in pyridine. It is well known that guests bind to cyclodextrins more strongly in aqueous media than in organic solvents. Since pyridine is an aromatic solvent, it probably includes in the cavities of the cyclodextrins (29) and (30), and as a result reduces the extent of complexation of the enantiomers of the esters (48) and (59) as compared to that seen for the complexation of β-CD (1) and the ester (48) in aqueous media. In order to make a closer comparison between the work described by Fornasier *et al.*,[87] and the present work, the reactions of the cyclodextrins (29) and (30) with the ester (48) were also performed in aqueous media. The products from these reactions were again subjected to nmr spectroscopy.

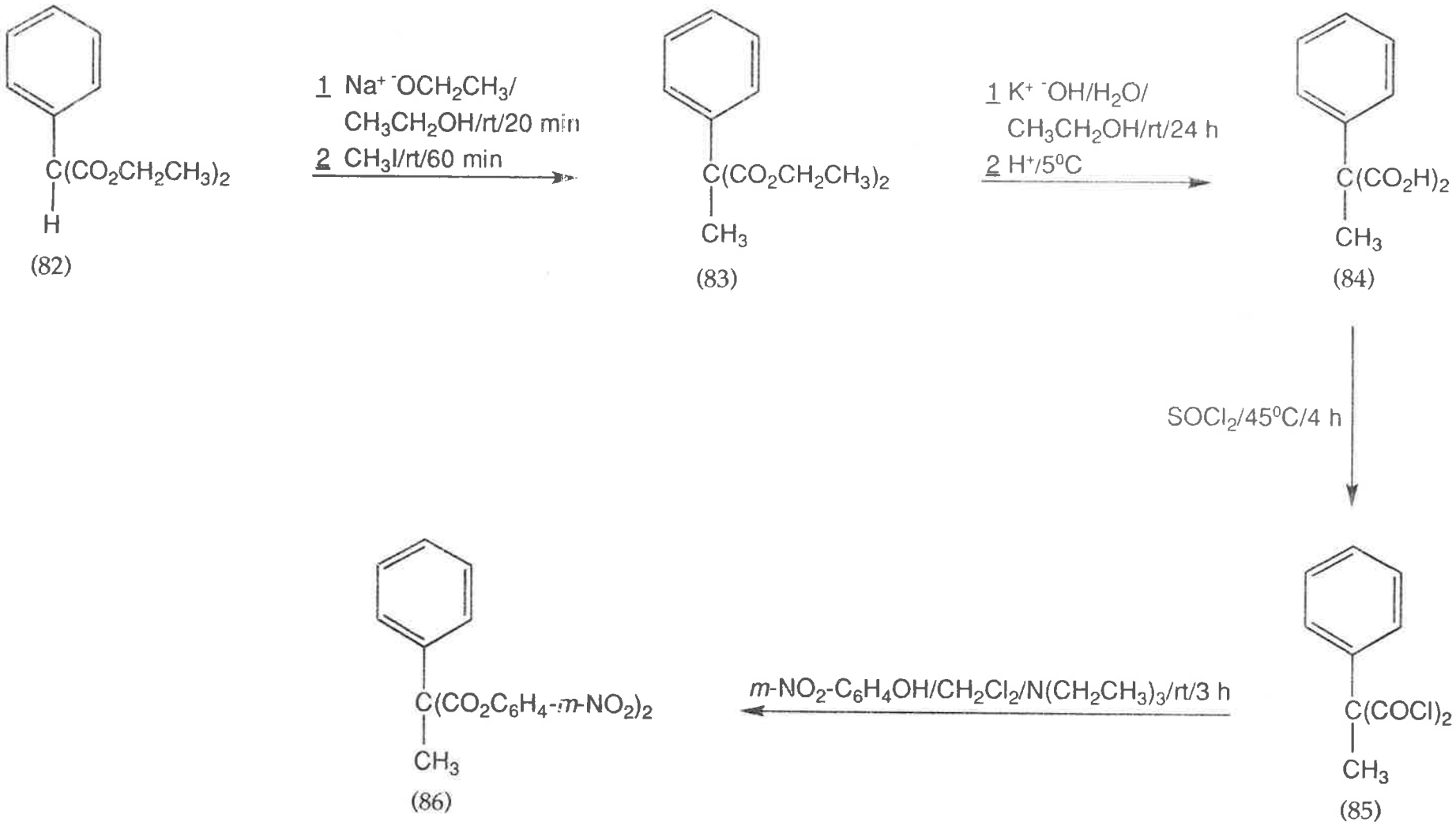
The reaction of β-CD-6-NH_2 (29) with eighty equivalents of the ester (48) was performed at room temperature in aqueous sodium borate buffer, at pH 9.0 so as to ensure that the amino group and hydroxyl groups of the

cyclodextrin (29) were predominantly nonionised. It was established that the product formed from this reaction was not the amide (78). The ^{1}H nmr spectrum of the product showed doublets at 1.70 and 1.65 ppm in a ratio of 4:1 in favour of the more downfield doublet. ^{1}H nmr spectroscopic analysis of a mixture of this product and that of the amide (78) prepared from the reaction of β-CD-6-NH_2 (29) and the ester (48) in pyridine confirmed that the product formed from the reaction performed in aqueous media was not the amide (78) but probably ester derivatives of β-CD-6-NH_2 (29). Similarly the reaction of β-CD-3-NH_2 (30) and the ester (48) was performed in aqueous sodium bicarbonate, at pH 8.0 so as to ensure that the amino and hydroxyl substituents of β-CD-3-NH_2 (30) were not ionised. The product formed from this reaction was found to be the amide (80). The proton decoupled ^{13}C nmr spectrum was nearly identical to that of a sample of the amide (80) produced from the analogous reaction performed in pyridine. Signals at 48.4 and 49.5 ppm in the ratio of 2:1 were assigned to the benzylic methine hydrogens of the diastereomers of the amide (80). Signals at 20.5 and 19.0 ppm in a ratio of 2:1 corresponded to the methyl hydrogens of the diastereomers, and peaks at 53.0 and 52.7 ppm in a ratio of 2:1 were ascribed to the $C\text{-}3^{A}$ carbons attached to the amide nitrogens of the diastereomers. Infrared spectroscopic analysis of the product showed a peak at 1650 cm^{-1}, characteristic of a carbonyl of an amide functionality.

The results obtained from the reactions of the cyclodextrin (29) and the ester (48) in aqueous media and in pyridine indicate that complexation probably took place prior to reaction in the former case however the extent of complexation in the latter case is likely to have been minor. The fact that no amide (78) formation was detected as a result of reaction of β-CD-6-NH_2 (29) and the ester (48) in aqueous media, is consistent with the ester (48) being included in the cavity of β-CD-6-NH_2 (29) prior to reaction, with the carbonyl group of the ester (48) located near the C-2 and C-3 hydroxyl groups. Since the diastereoselectivity of the reaction of β-CD-3-NH_2 (30) and the ester (48)

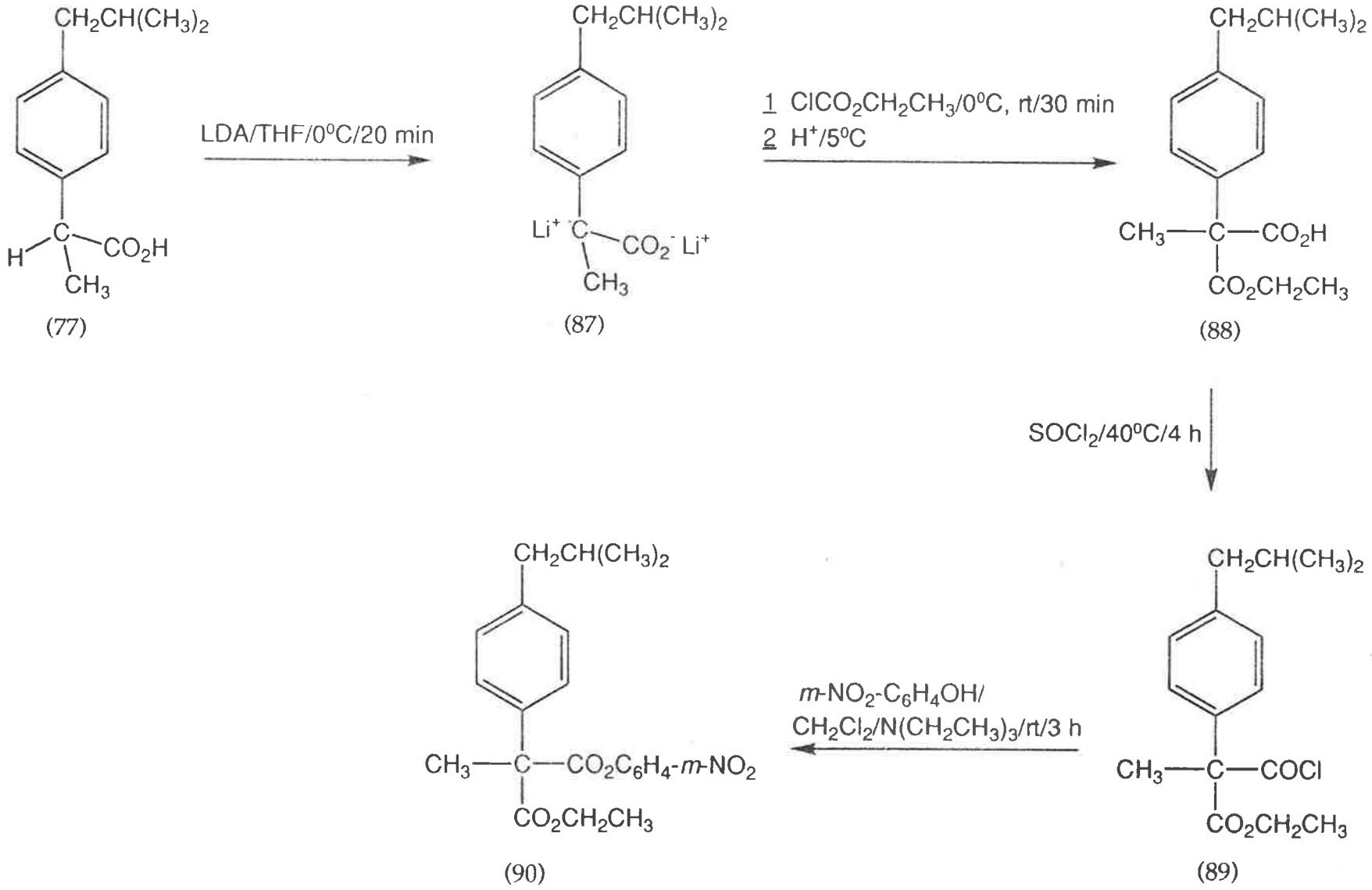
performed in aqueous media is the same as that of the analogous reaction conducted in pyridine, complexation probably did occur prior to reaction in both of these cases. The poor diastereoselectivity obtained in the reaction of the cyclodextrin (30) and the ester (48) is possibly a reflection of the effect of the amino group of β-CD-3-NH_2 (30). It has been established that the modified glucopyranose residue of β-CD-3-NH_2 (30) is distorted and this may reduce the ability of the cavity of the cyclodextrin (30) to discriminate between enantiomers of the ester (48).

As mentioned in the Introduction of this thesis, the investigation of chiral discrimination in the reactions of two malonate substituted β-cyclodextrins was also carried out. In the first instance, the diesters (86) and (90) were prepared. The nitrophenyl diester (86) was prepared in four steps (Scheme 19). Diethyl phenylmalonate (82) was methylated following a modification of the procedure described by Vogel *et al.*,[104] to give diethyl methylphenylmalonate (83) as a yellow oil. Subsequent hydrolysis of the diester (83) followed by acidification gave methylphenylmalonic acid (84) as a white solid. Methylphenylmalonic acid (84) was converted to the acid chloride (85) and then treated with *meta*-nitrophenol to produce the diester (86). The diester (86) was then purified by silica gel chromatography and isolated as a viscous yellow-olive coloured oil in an overall yield of 31% based on diethyl phenylmalonate (82). Thin layer chromatographic analysis of the diester (86) exhibited only one spot, which was less polar than those of *meta*-nitrophenol and methylphenylmalonic acid (84). Infrared spectroscopy of the diester (86) showed a peak at 1762 cm^{-1} characteristic of a carbonyl of a phenolic ester. FAB-MS analysis of the diester (86) gave a peak at 436 for M+H. Microanalysis confirmed the molecular formula and purity of the diester (86). The ^1H nmr spectrum of the diester (86), showed a singlet at 2.27 ppm and this was assigned to the hydrogens of the methyl group. Multiplets observed at 7.5 to 8.2 ppm were assigned to the hydrogens of the three aromatic rings. The proton decoupled ^{13}C nmr spectrum


C(CO2CH2CH3)2
H
(82)
1 Na+ -OCH2CH3/
CH3CH2OH/rt/20 min
2 CH3I/rt/60 min
C(CO2CH2CH3)2
CH3
(83)
1 K+ -OH/H2O/
CH3CH2OH/rt/24 h
2 H+/5°C
C(CO2H)2
CH3
(84)
SOCl2/45°C/4 h
C(COCl)2
CH3
(85)
m-NO2-C6H4OH/CH2Cl2/N(CH2CH3)3/rt/3 h
C(CO2C6H4-m-NO2)2
CH3
(86)

Scheme 19

of the diester (86) exposed a peak at 21.8 ppm which was assigned to the carbon of the methyl group. A peak at 59.1 ppm was assigned to the quaternary benzylic carbon. Peaks at 117.0 to 150.7 ppm were assigned to the aromatic carbons and a peak at 169.0 ppm corresponded to the carbonyl carbons of the equivalent ester moieties. The diester (90) was synthesized in several steps from the racemic acid (77) (Scheme 20). Following the procedure described by Krapcho *et al.*,[105] the acid (77) was deprotonated with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) to give the corresponding dianion (87). Reaction of the dianion (87) with ethylchloroformate, followed by acidification, afforded the mixed ester acid (88) as an orange coloured oil after work up. The crude acid (88) was converted to the corresponding acid chloride (89), and then treated with *meta*-nitrophenol to give the diester (90). The crude product was purified by silica gel chromatography to give the diester (90) as a viscous yellow-orange oil in 23% yield based on the acid (77). Once more tlc analysis on the diester (90) gave only one spot, which was less polar than those of the acid (77) and 3-nitrophenol. Infrared spectroscopy of the diester (90) showed peaks at 1740 and 1765 cm^{-1} which were assigned to the carbonyls of the ester functionalities. FAB-MS analysis of the diester (90) gave a peak at 400 for M+H. The molecular formula and purity of the diester (90) was confirmed by microanalysis. The ^{1}H nmr spectrum of the diester (90) showed an AB quartet splitting pattern at 7.17 and 7.36 ppm which was allocated to the hydrogens of the *para*-substituted aromatic ring, and multiplets at 7.4 to 8.1 ppm were assigned to the hydrogens of the *meta*-substituted aromatic ring. Two doublets and a multiplet observed at 0.90, 2.48 and 1.6 ppm respectively, corresponded to the methyl, methylene and methine hydrogens of the isobutyl side chain respectively, and a triplet and a quartet at 1.33 and 4.33 ppm respectively were assigned to the methyl and methylene hydrogens of the ethoxy group. An intense singlet at 2.01 ppm was assigned to the hydrogens of the methyl group attached to the quaternary benzylic carbon. The proton decoupled ^{13}C nmr spectrum of the diester (90)



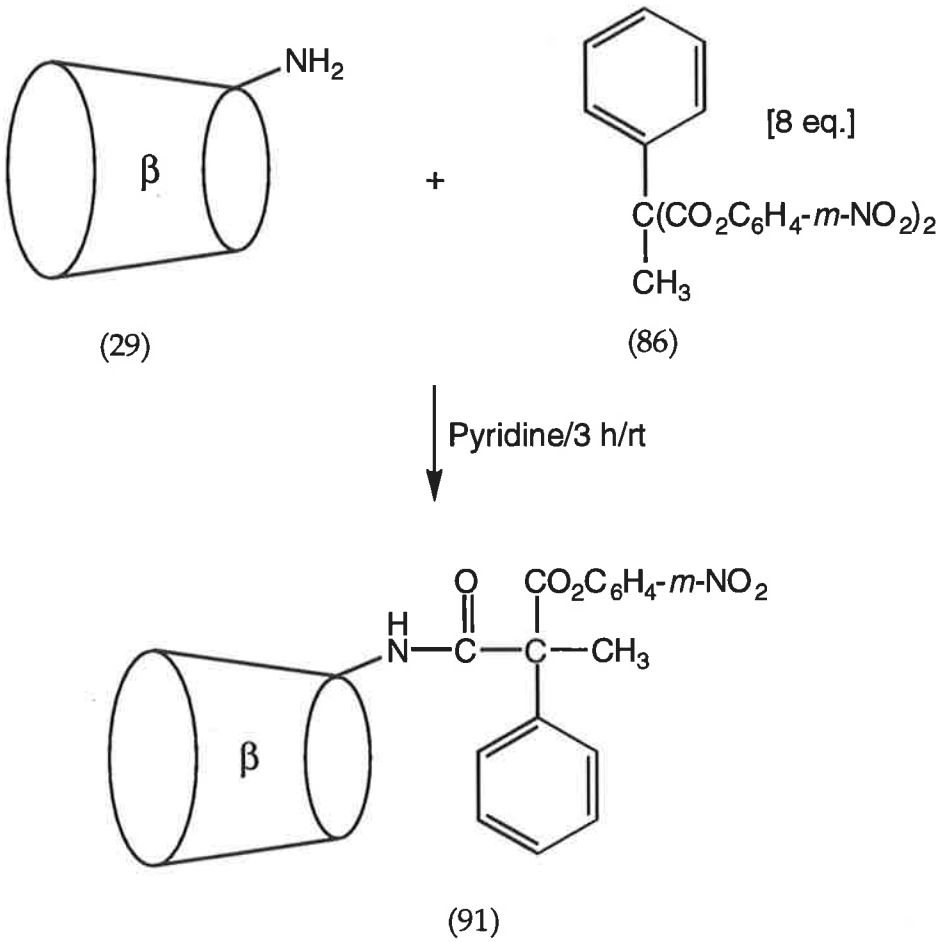
(77) (87) (88) (89) (90)

Scheme 20

exhibited peaks which were consistent for such a system. Signals at 62.2, 58.7, 45.0, 30.1, 22.4, 21.2, and 14.1 ppm corresponded to the seven non-cyclodextrin aliphatic carbons. Peaks observed from 117.1 to 151.1 ppm were assigned to the carbons of both aromatic rings and signals at 169.9 and 171.0 ppm corresponded to the carbonyl carbons of the ester moieties.

The reaction of β-CD-6-NH_2 (29) with the diesters (86) and (90) produced the cyclodextrins (91) and (92) which were then hydrolysed and subsequently decarboxylated to give the amides (78) and (79). The diastereoselectivity of the reactions was again determined by ^{1}H and ^{13}C nmr spectroscopy. The malonate substituted cyclodextrin (91) was prepared from the reaction of β-CD-6-NH_2 (29) with eight equivalents of the diester (86) in pyridine at room temperature and was isolated as a white powder in 74% yield (Scheme 21). Excess diester (86) was used in the preparation of the cyclodextrin (91) in order to ensure that only monosubstitution of the diester (86) occurred. The FAB-MS of the amide-ester (91) contained a peak at 1432 for M+H. The infrared spectrum of the cyclodextrin (91) showed peaks at 1658 and 1712 cm^{-1} characteristic of amide and ester carbonyls. Microanalytical data showed that the cyclodextrin (91) was present in the trihydrate form. ^{1}H nmr spectroscopic analysis of the malonate (91) at 298 K, showed a singlet at 1.97 ppm which was assigned to the hydrogens of the methyl group. The hydrogens of the aromatic rings were assigned to the multiplets at 7.3 to 8.4 ppm. Proton decoupled ^{13}C nmr spectroscopy of the cyclodextrin (91) showed peaks at 171.8 and 171.1 ppm which were assigned to the ester and amide carbonyl carbons. Peaks at 117.0 to 151.4 ppm were assigned to the aromatic carbons of both rings and a signal at 22.0 ppm was assigned to the methyl carbon. The signal corresponding to the quaternary benzylic carbon could not be seen presumably due to it being embedded under the signals assigned to the C-6^{B-G} cyclodextrin carbons. Similarly the signal corresponding to the C-6^{A} carbon was not observed due to it being coincidental with that of d^{6}-DMSO. The diastereoselectivity of the

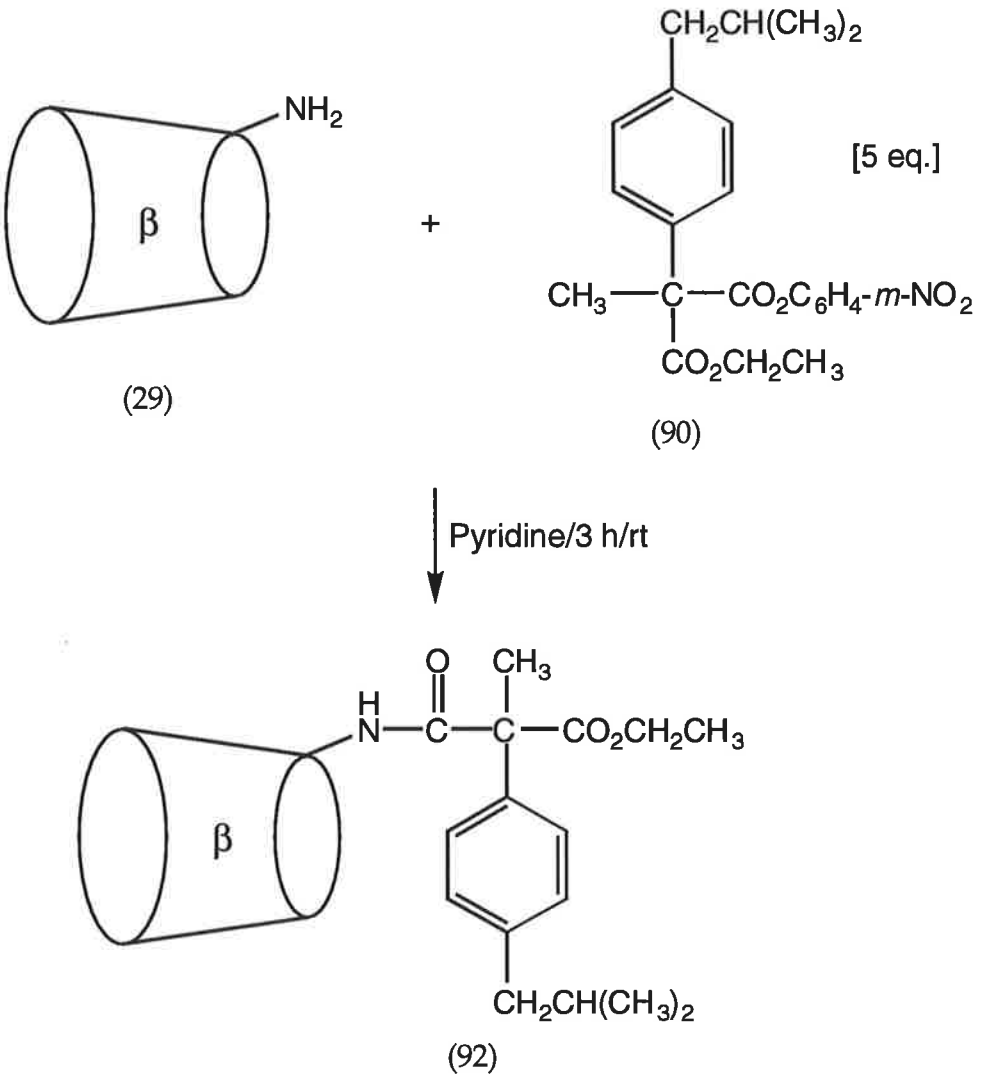
reaction of β-CD-6-NH_2 (29) and the diester (86) could not be determined, however this was not further pursued since any diastereoselectivity would be



Scheme 21

lost in the following hydrolysis and subsequent decarboxylation steps. The malonate substituted cyclodextrin (92) was synthesized in one step from the reaction of β-CD-6-NH_2 (29) with five equivalents of the diester (90) in pyridine at room temperature (Scheme 22). The cyclodextrin (92) was purified by the application of cation exchange chromatography and obtained as a white crystalline solid in 62% yield. FAB-MS of the malonate substituted cyclodextrin (92) showed a signal at 1417 for the molecular ion plus sodium (M+Na). Infrared spectroscopy revealed the presence of amide and ester functionalities with peaks at 1656 and 1712 cm^{-1}. Microanalytical data showed that the

cyclodextrin (92) was complexed with three water molecules. ^1H nmr spectroscopy of the cyclodextrin (92) at 343 K showed singlets at 1.72 and 1.75



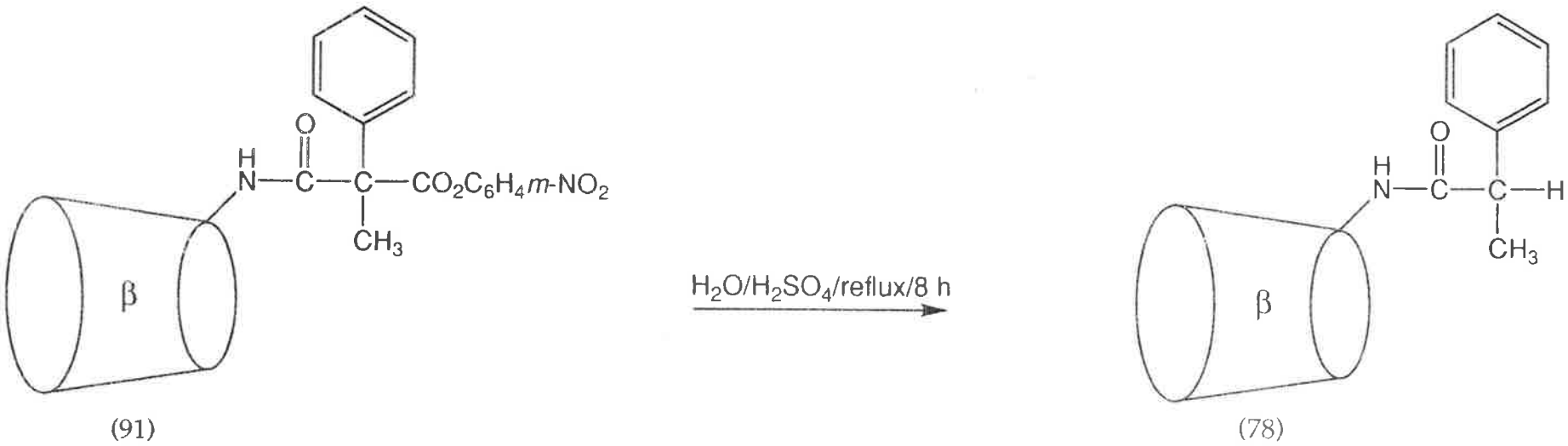
Scheme 22

ppm in a ratio of 1:1 which were assigned to the hydrogens of the methyl groups attached to the quaternary benzylic carbons of the diastereomers. Unresolved multiplets at 0.8 and 2.5 ppm were assigned to the methyl and methylene hydrogens of the isobutyl side chain of the diastereomers. A multiplet at 2.0 ppm was ascribed to the methine hydrogen of the isobutyl side chain. An unresolved triplet and an unresolved quartet at 1.21 and 4.20 ppm respectively corresponded to the methyl and methylene hydrogens of the ethoxy group. Two

overlapping AB quartets at 7.13 and 7.18 and at 7.16 and 7.21 ppm were assigned to the hydrogens of the aromatic rings of the diastereomers. The proton decoupled ^{13}C nmr spectrum of the cyclodextrin (92) exhibited signals at 171.2 and 171.9 ppm for carbonyl carbons. Signals at 126.7 to 140.2 ppm were assigned to the aromatic carbons. Six signals at 21.4, 22.0, 29.4, 44.0, 58.6 and 59.6 ppm arose from the seven aliphatic non-cyclodextrin carbons. Presumably only two signals, at 21.4 and 22.0 ppm, arose from the aliphatic methyl carbons. The peak corresponding to the C-6^A carbon of the modified glucopyranose unit was not observed presumably due to it being coincidental with that of d^6-DMSO. The nmr spectral data of the cyclodextrin (92) showed that the reaction of β-CD-6-NH_2 (29) and the diester (90) proceeded without any diastereoselectivity.

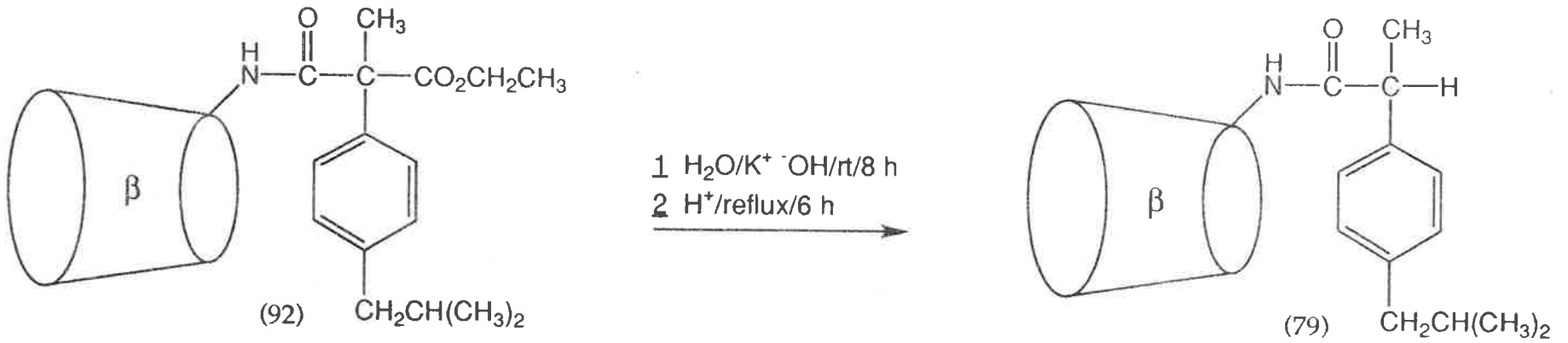
The hydrolysis and subsequent decarboxylation of the cyclodextrin (91) afforded the amide (78) in 53% yield after work up (Scheme 23). The cyclodextrin (92) was similarly converted to the amide (79) in 61% yield after work up (Scheme 24). FAB-MS and ^1H nmr spectra of these products were identical to those of the amides (78) and (79) prepared as shown in Scheme 17. In particular doublets at 1.30 and 1.24 ppm in the ^1H nmr spectrum, in a ratio of 2:1, showed that the amide (78) was produced from the cyclodextrin (91) as a mixture of diastereomers in a ratio of 2:1. Similarly doublets at 1.30 and 1.23 ppm in the ^1H nmr spectrum, in a ratio of 2:1, indicated that the amide (79) was produced from the amide-ester (92) as a mixture of diastereomers in a ratio of 2:1. The diastereoselectivity of formation of the cyclodextrins (78) and (79) from the amide-esters (91) and (92), respectively (Table 7), is identical to that for the amides (78) and (79) produced from the reactions of β-CD-6-NH_2 (29) with the esters (48) and (59), respectively.

The hydrolysis and subsequent decarboxylation of the malonate substituted cyclodextrins (91) and (92) initially affords the enol tautomers (95) and (96). Since the enolic moieties of the tautomers (95) and (96) consist of sp^2 hybridised carbons, any diastereoselectivity obtained in the formation of the



(91) (78)

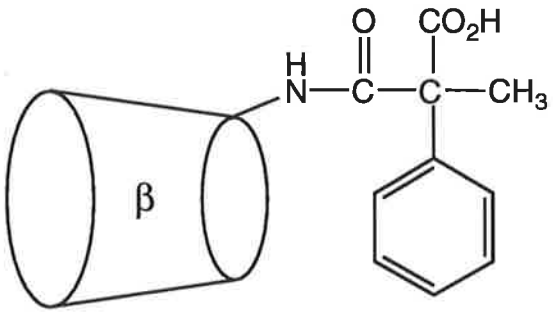
Scheme 23



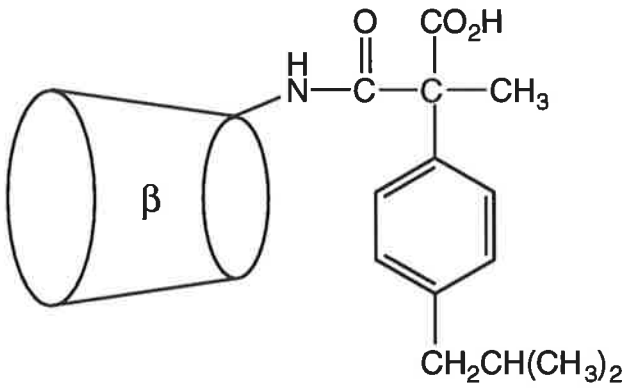
(92) (79)

Scheme 24

cyclodextrins (91) and (92) would thus be lost after decarboxylation of the acid intermediates (93) and (94). The diastereoselectivity in the production of the



(93)



(94)

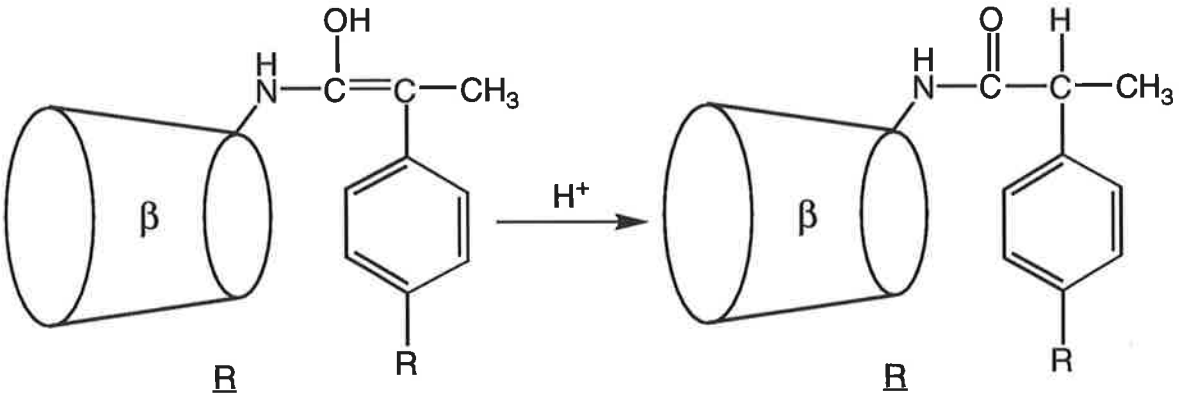
cyclodextrins (78) and (79) is generated in the tautomerisation from the enols (95) and (96) to the keto tautomers (78) and (79) (Scheme 25). It is envisaged

Table 7 Diastereoselectivity in the formation of the cyclodextrins (78) and (79) *via* decarboxylation of the acids (93) and (94).

Cyclodextrin	Ratio
(78)	2 : 1
(79)	2 : 1

that the enols (95) and (96) exist in conformations where their aryl groups are

included in the cavities of their cyclodextrin moieties. However the low diastereoselectivity of formation of the amides (78) and (79) indicates that such conformations do not enhance the diastereoselectivity of tautomerisation, presumably due to the small size of the proton being transfered to the pro-chiral centre.



R	R
(95) H	(78) H
(96) $CH_2CH(CH_3)_2$	(79) $CH_2CH(CH_3)_2$

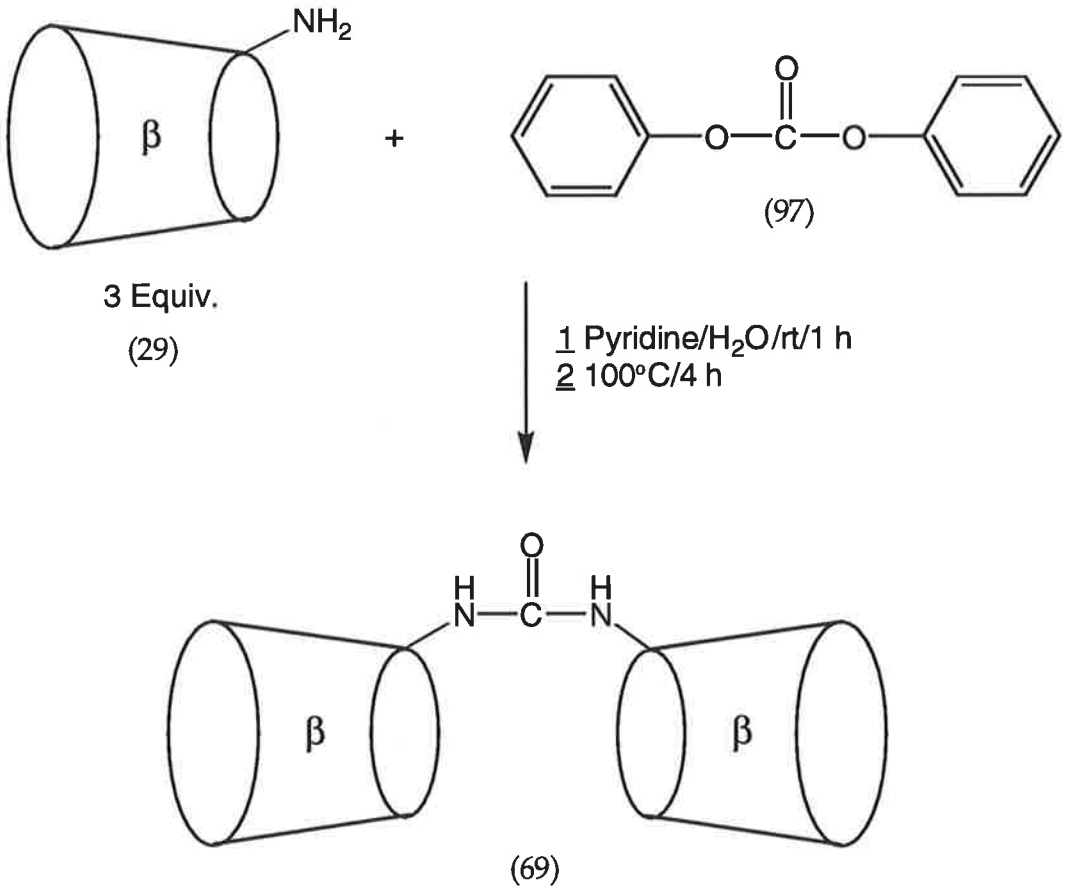
Scheme 25

In conclusion the formation of cyclodextrin amide derivatives of aryl-substituted propanoic acids namely, 2-phenylpropanoic acid (76) and the non-steroidal anti-inflammatory drug Ibuprofen (77), can be accomplished by two different methodologies each with some degree of diastereoselectivity. Unfortunately the application of a diastereoselective synthesis of such compounds *via* the routes shown in Schemes 23 and 24 seems ineffective due to the low stereoselectivity of this method. Likewise the complete resolution of these compounds from reaction of the cyclodextrins (29) and (30) with the esters (48) and (59) is also limited, since the diastereoselectivity of this procedure is also of low order.

CHAPTER 3

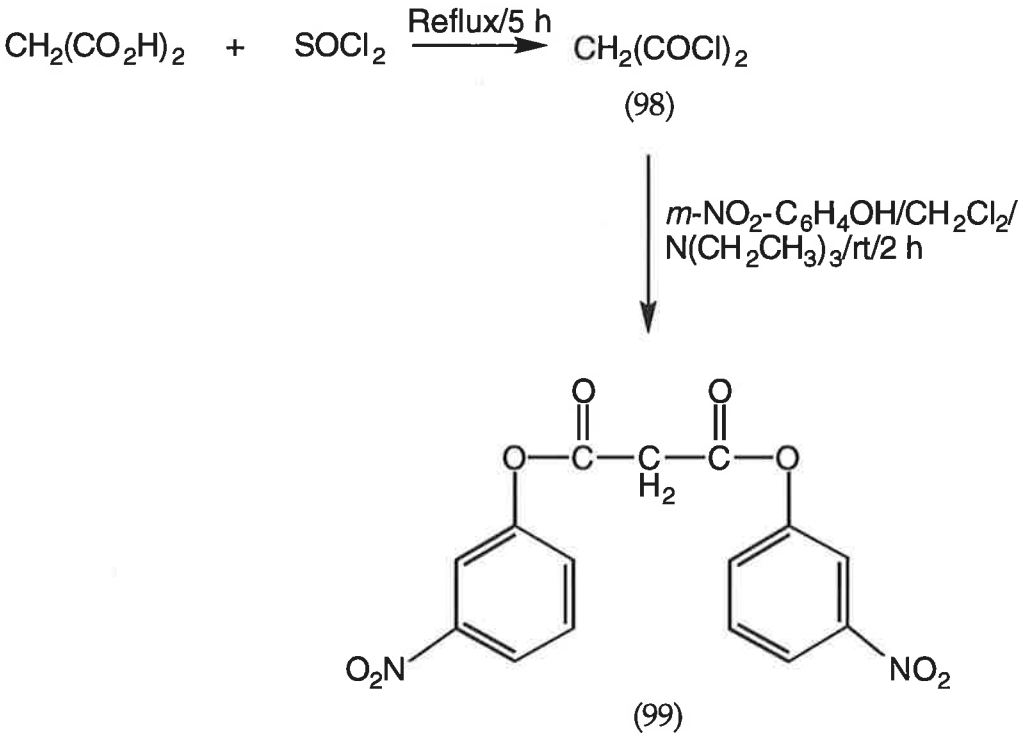
To further the investigation of cooperative binding by C-6-C-6 linked β-cyclodextrins with the guest (4), the urea and malonamide linked β-cyclodextrins (69) and (70) were synthesized.

The urea (69) was prepared by treatment of three equivalents of β-CD-6-NH_2 (29) with one equivalent of diphenyl carbonate (97) in a pyridine-water solvent system (Scheme 26). The crude dimer (69) was separated from unreacted β-CD-6-NH_2 (29) by the application of Sephadex cation exchange chromatography to furnish the urea (69) as a white solid in 53% yield. HPLC analysis of the dimer (69) gave one peak which was more polar than those of



Scheme 26

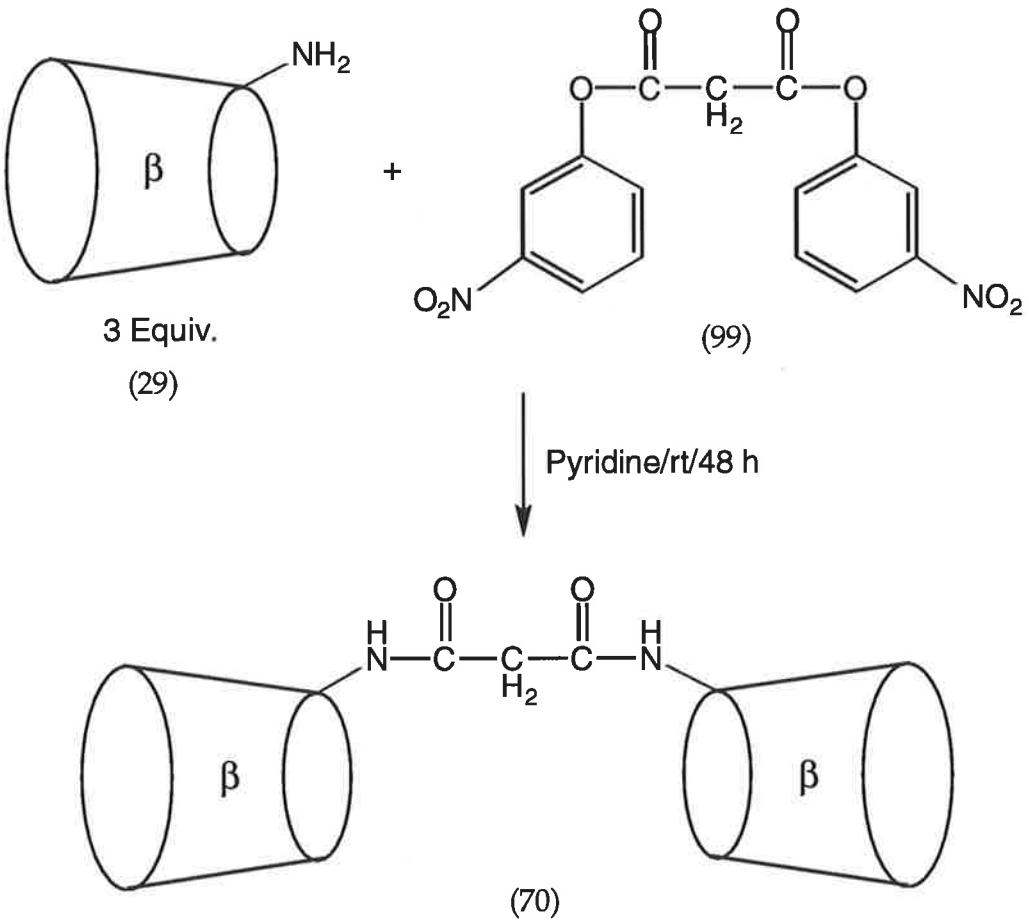
β-CD (1) and β-CD-6-NH_2 (29). FAB-MS analysis of the dimer (69) showed a peak at 2293 for M+H. Infrared spectroscopy confirmed the presence of the urea functionality, with an absorption at 1658 cm^{-1} for the carbonyl. Microanalytical data showed that the urea (69) was present in the hexahydrate form. Proton decoupled ^{13}C nmr spectroscopic analysis of the dimer (69) exhibited the required information for such a system. A signal at 162.3 ppm was assigned to the carbonyl carbon of the urea functionality and a signal at 42.3 ppm was ascribed to the equivalent C-6^A carbons of the modified glucopyranose units. To synthesize the malonamide (70), the diester (99) was firstly prepared (Scheme 27). The reaction of malonyl chloride (98) and *meta*-nitrophenol in the presence



Scheme 27

of triethylamine gave the diester (99) as a brown solid. The product was purified by silica gel chromatography to afford the diester (99) as a white powder in 69% yield. Thin layer chromatography on the ester (99) showed only one spot which was less polar than that of *meta*-nitrophenol. The melting point of the diester

(99) was found to be consistent with the literature value,[106] hence confirming the purity. A singlet present at 4.10 ppm and a multiplet present at 7.6 to 8.3 ppm in the ^1H nmr spectrum of the diester (99) corresponded to the methylene and aromatic hydrogens respectively. The malonamide (70) was prepared by reaction of β-CD-6-NH_2 (29) and the diester (99) in pyridine at room temperature for 48h (Scheme 28). Sephadex cation exchange chromatography of the crude product gave the dimer (70) as a colourless clear glassy solid in 78% yield. HPLC analysis of the malonamide (70) exposed only one peak, which was more polar



Scheme 28

than those of β-CD (1) and β-CD-6-NH_2 (29). The infrared spectrum of the dimer (70) showed an absorption at 1658 cm^{-1} which was assigned to the carbonyls of the amide functionalities. FAB-MS of the dimer (70) showed a peak at 2336 for M+H. Microanalytical data showed that the dimer (70) was complexed with six

water molecules. The proton decoupled ^{13}C nmr spectrum of the dimer (70) showed a signal at 171.3 ppm which was assigned to the equivalent carbonyl carbons of the malonamide functionality and a signal at 42.5 ppm corresponded to the equivalent C-6^A carbons of the modified glucopyranose units.

The inclusion of TNS (4) in the linked cyclodextrins (69) and (70) in aqueous phosphate buffer at pH 6.9 and ionic strength 0.1 mol dm^{-3} was studied by monitoring the increase in fluorescence of solutions containing 1.0 μmol dm^{-3} TNS (4) with increasing concentrations of the dimers (69) and (70) over the range (0 - 1.0 mmol dm^{-3}). Plots (Figures 5 and 6) of intensity of fluorescence versus concentration of cyclodextrin for each case were subjected to a non-linear least-squares regression analysis which then gave the stability constants of the 1:1 inclusion complexes (Table 8 see page 80). The stability constant (K) of the 1:1 urea (69)-TNS (4) complex was 55,000 mol^{-1} dm^3, and the stability constant (K) of the analogous malonamide (70) complex was found to be 12,000 mol^{-1} dm^3. The stability constant (K) of the β-CD (1)-TNS (4) 1:1 complex measured under analogous conditions was 2,800 mol^{-1} dm^3.[96]

To complete a systematic study on the complexation of TNS (4) with diamide linked β-cyclodextrins, C-3-C-3 and C-3-C-6 substituted dimers were synthesized. The C-3-C-3 substituted succinamide (104) and oxalamide (105) and the C-3-C-6 substituted succinamide (107) were prepared by similar procedures to those employed for the synthesis of the urea (69) and the malonamide (70). In the first instance the diesters (102) and (103) were prepared (Scheme 29) by an analogous procedure to that used for the synthesis of the diester (99). The acid chlorides (100) and (101) were available commercially. The diester (102) was purified by recrystallization to give the pure material in 54% yield as a cream coloured solid. Tlc analysis of the diester (102) showed only one spot, which was less polar than that of *meta*-nitrophenol. The melting point of the ester (102) was found to be consistent with the literature value,[94] hence confirming the purity. The diester (103) was also purified by recrystallization to

Figure 5 Plot of intensity of fluorescence of TNS (4) against the concentration (mmol dm^{-3}) of the urea linked cyclodextrin (69).

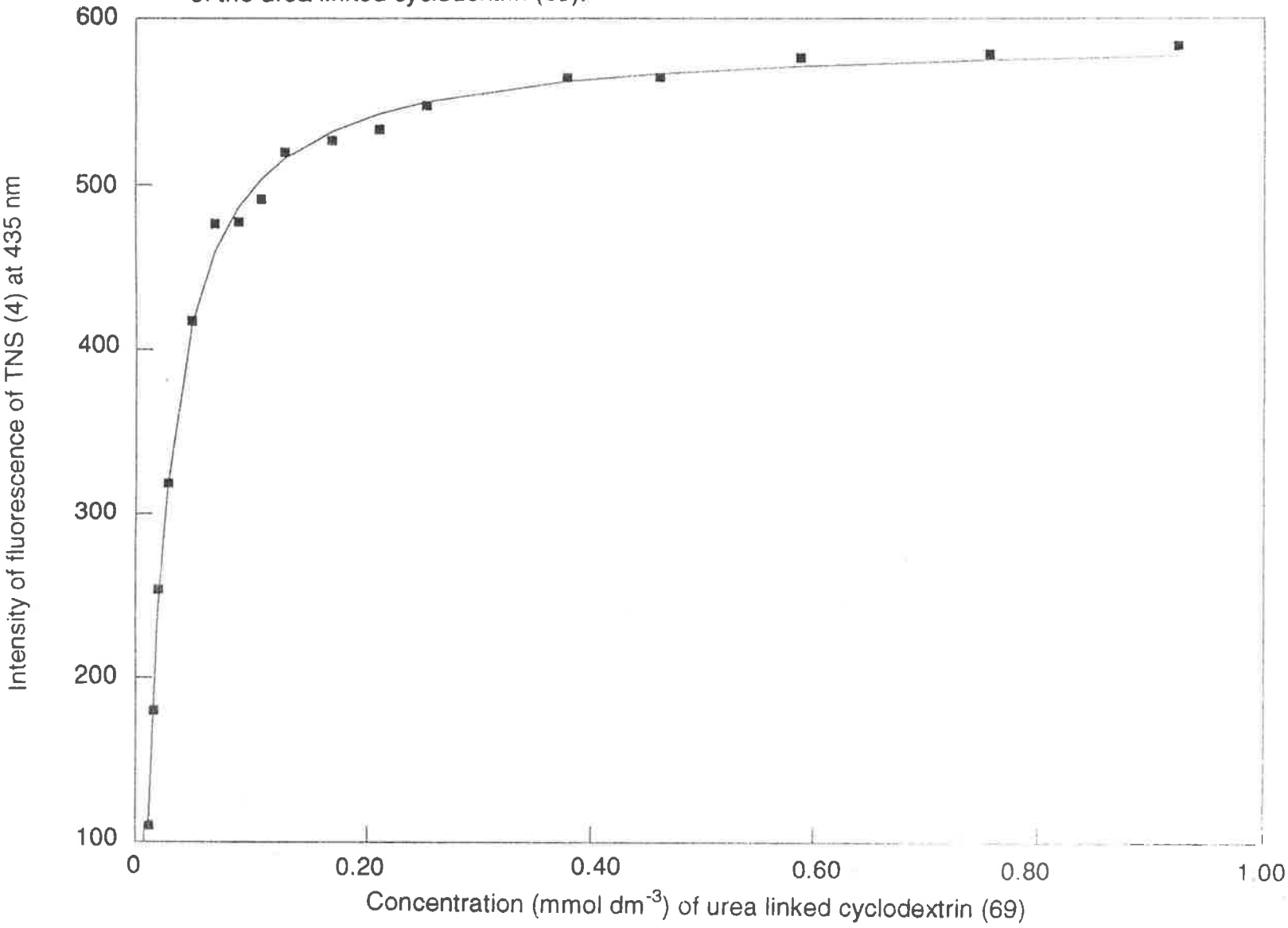
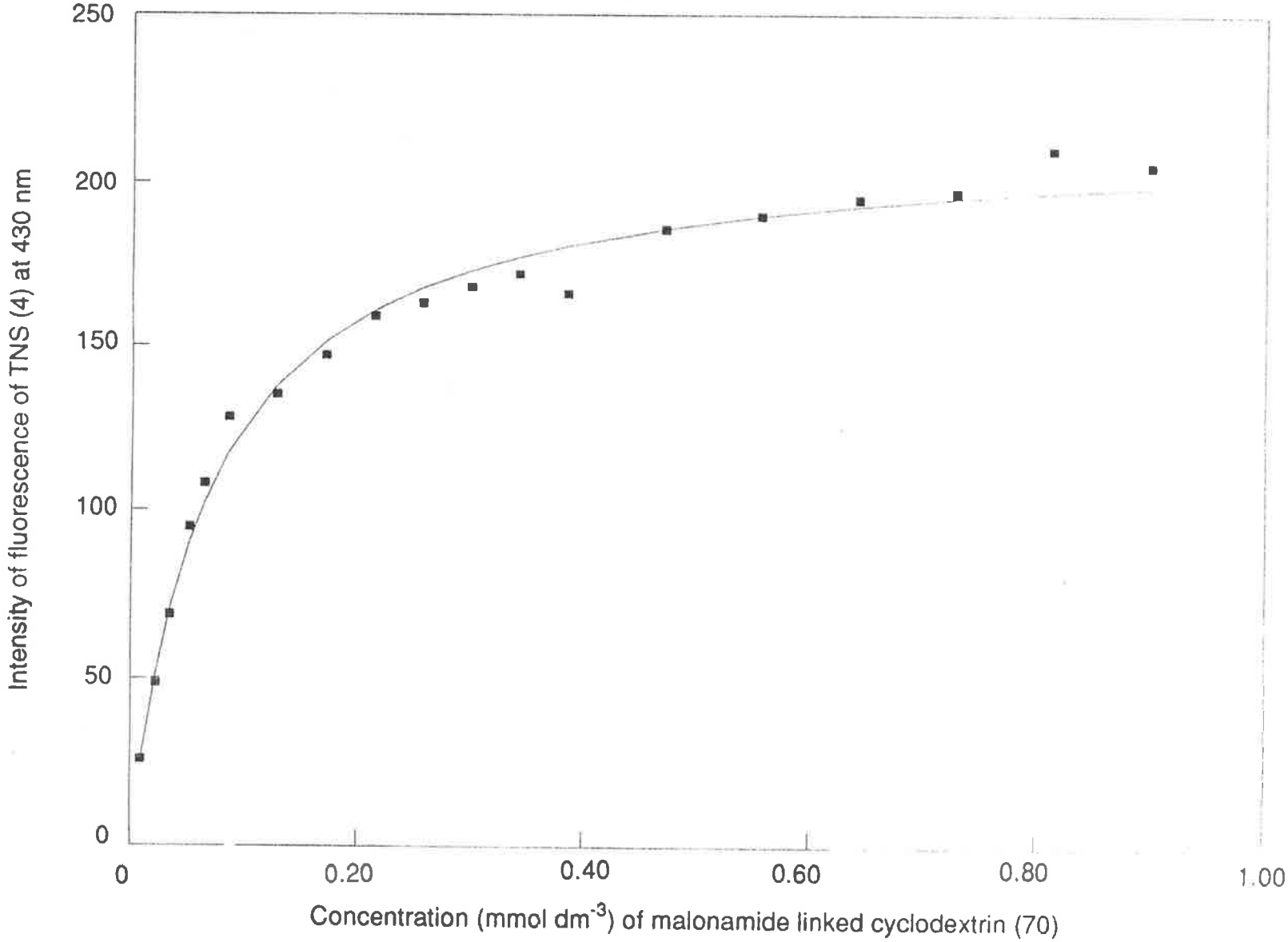
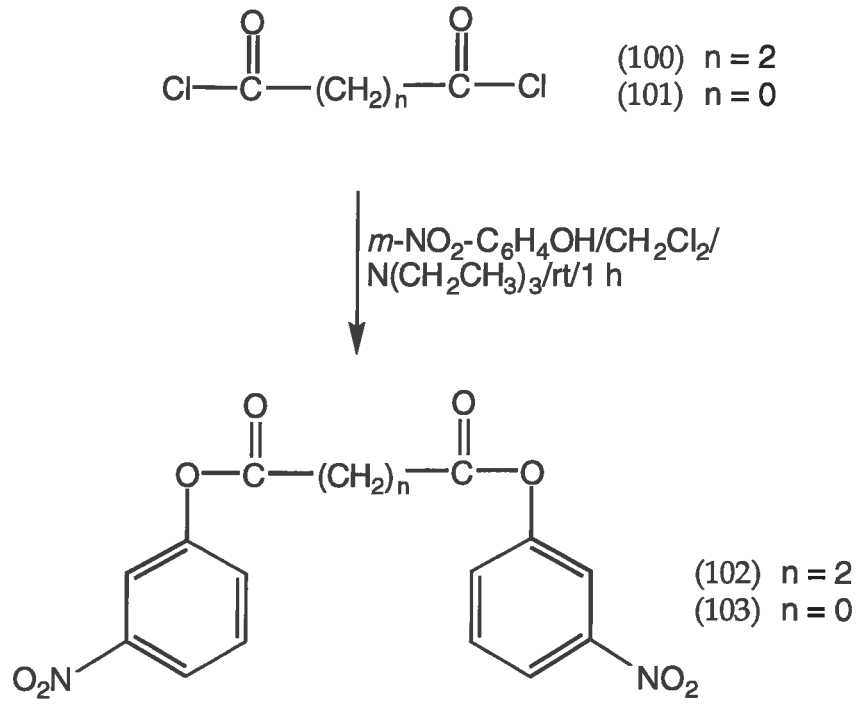


Figure 6 Plot of intensity of fluorescence of TNS (4) against the concentration (mmol dm^{-3}) of the malonamide linked cyclodextrin (70).



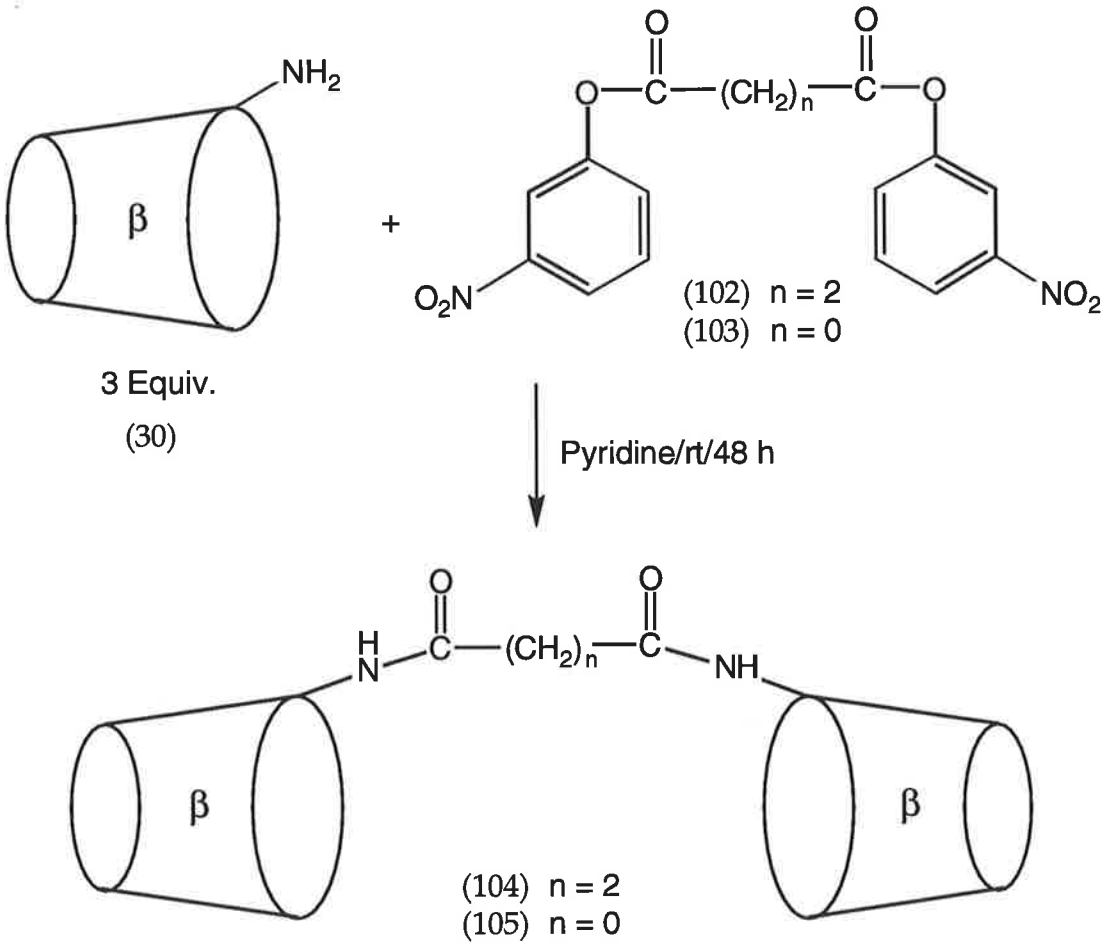
give the material as a white powder in 41% yield. Similarly tlc on the ester (103) showed only one spot, which was less polar than that of *meta*-nitrophenol. Once again the melting point of the diester (103) was found to be consistent with the literature value,[107] therefore confirming the purity. The succinamide (104)



Scheme 29

was prepared from the reaction of β-CD-3-NH_2 (30) and the diester (102) (Scheme 30). The reaction conditions used were analogous to those employed for the preparation of the malonamide (70). Sephadex cation exchange chromatography on the product gave the succinamide (104) in 47% yield as a cream coloured solid. HPLC on the succinamide (104) gave one peak, which was more polar than those of β-CD (1) and β-CD-3-NH_2 (30). FAB-MS on the succinamide (104) showed a peak at 2350 for M+H, and infrared spectroscopy showed an absorption at 1662 cm^{-1} which was assigned to the carbonyls of the succinamide group. The proton decoupled ^{13}C nmr spectrum of the succinamide (104) showed a peak at 176.9 ppm which was assigned to the carbonyl carbons of the amide groups. A signal at 53.0 ppm was assigned to the equivalent C-3^A carbons of the modified

glucopyranose units and a signal at 32.3 ppm corresponded to the equivalent methylene carbons of the succinamide functionality. The oxalamide (105) was similarly prepared from the reaction of β-CD-3-NH_2 (30) and the diester (104) (Scheme 30). Once again, Sephadex cation exchange chromatography on the

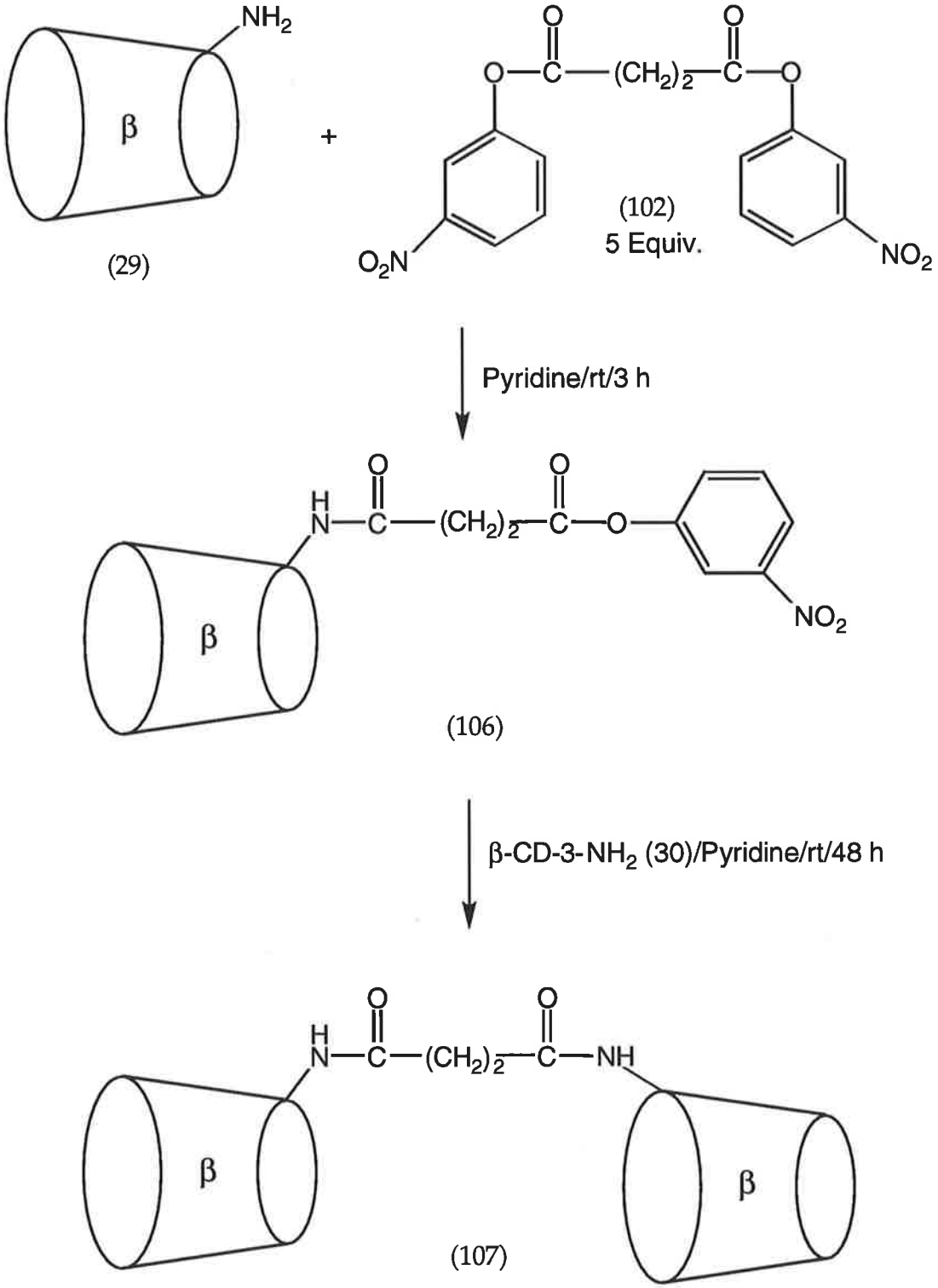


Scheme 30

crude product furnished the oxalamide (105) in 29% yield as a colourless clear glassy solid. HPLC on the oxalamide (105) gave one peak, which was more polar than those of β-CD (1) and β-CD-3-NH_2 (30). FAB-MS analysis of the dimer (105) showed a peak at 2322 for M+H, and infrared spectroscopy showed a peak at 1660 cm^{-1} which was assigned to the carbonyls of the amide groups. Microanalytical data showed that the oxalamide (105) was present in the decahydrate form. Proton decoupled ^{13}C nmr spectroscopy on the oxalamide (105) showed a signal

at 162.6 ppm which was assigned to the carbonyl carbons of the oxalamide functionality. A signal at 54.0 ppm corresponded to the C-3^A carbons of the modified glucopyranose units.

The synthesis of the C-3-C-6 substituted succinamide (107) firstly required the preparation of the amide-ester (106). The cyclodextrin (106) was prepared from the reaction of β-CD-6-NH_2 (29) and five equivalents of the diester (102) in pyridine at room temperature for 3h (Scheme 31). Excess diester (102) was used in order to ensure that it only underwent monosubstitution. The product of this reaction was washed with diethyl ether and acetone and then dried to afford the amide-ester (106) in 76% yield as a cream coloured powder. The cyclodextrin (106) was found to be quite unstable in the presence of water and hence was not subjected to HPLC. Tlc on the cyclodextrin (106) gave one spot, which was less polar than those of β-CD (1) and β-CD-6-NH_2 (29). FAB-MS analysis of the amide-ester (106) gave a peak at 1356 for M+H. Infrared spectroscopy on the cyclodextrin (106) gave signals at 1712 and 1660 cm^{-1} which are characteristic of ester and amide carbonyls. Proton decoupled ^{13}C nmr spectroscopy on the amide-ester (106) showed signals at 175.6 and 176.0 ppm for the carbonyl carbons. Signals at 121.7 to 155.6 ppm corresponded to the aromatic carbons and signals at 34.0 and 34.4 ppm were assigned to the non-equivalent methylene carbons of the succinamide group. The signal corresponding to the C-6^A carbon of the modified glucopyranose unit could not be seen, presumably due to it being coincidental with those of the solvent (DMSO). The succinamide (107) was synthesized by reaction of the amide-ester (106) and two equivalents of β-CD-3-NH_2 (30) in pyridine for 48h at room temperature (Scheme 31). Two equivalents of the amine were employed in the reaction so as to ensure the amide-ester (106) underwent complete substitution. Sephadex cation exchange chromatography on the crude product afforded the succinamide (107) as a clear colourless glassy solid in 32% yield based on the cyclodextrin (106). HPLC on the



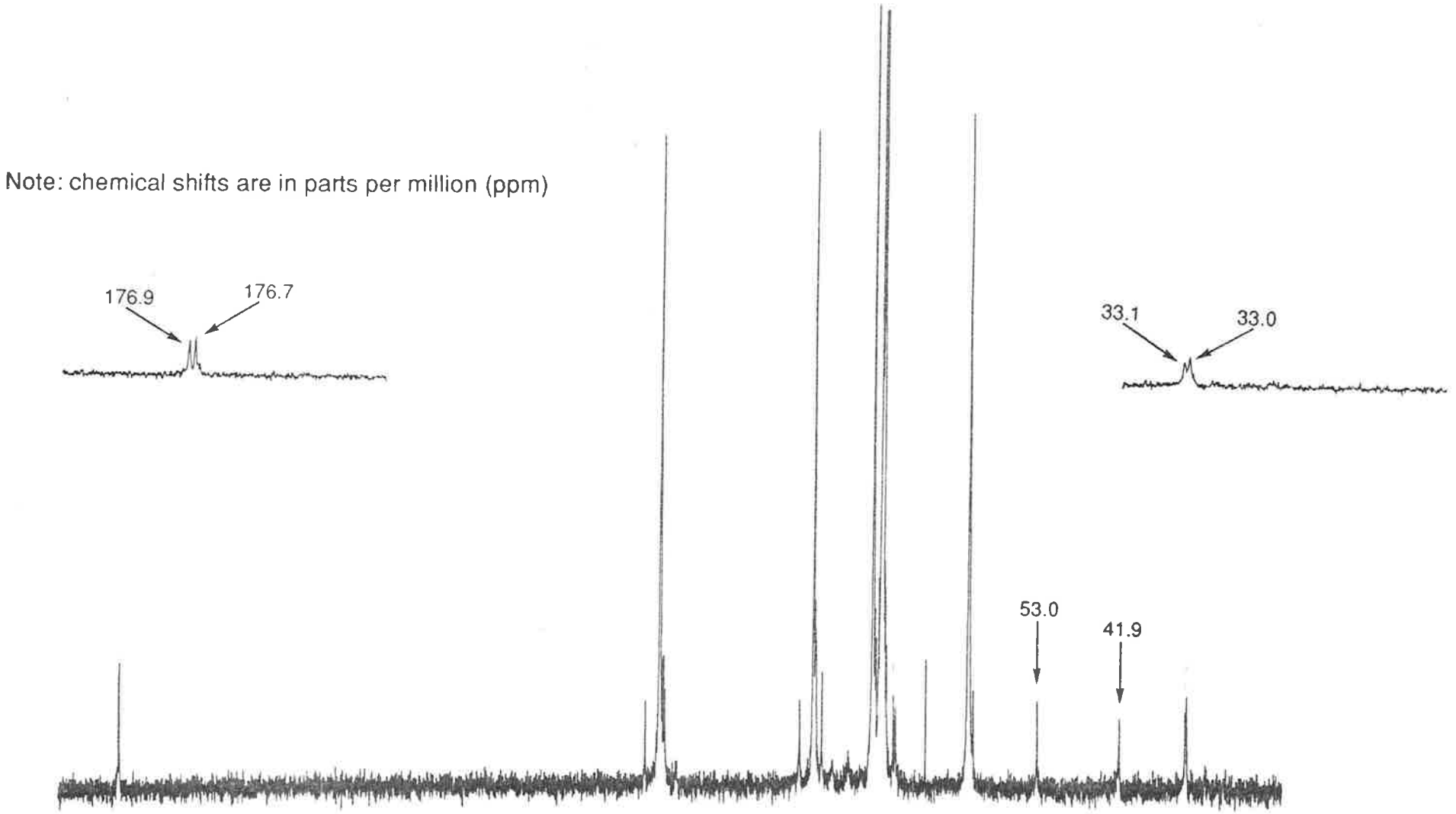
Scheme 31

dimer (107) gave only one peak, which was more polar than those of β-CD (1) and β-CD-3-NH_2 (30). Infrared spectroscopic analysis of the succinamide (107) showed an absorption at 1650 cm^{-1} which was assigned to the carbonyls of the

amide groups. FAB-MS on the dimer (107) exhibited a signal at 2350 for M+H. Microanalytical data showed that the dimer (107) was present in the dodecahydrate form. The proton decoupled ^{13}C nmr spectrum of the succinamide (107) once again exhibited the required information for such a system (Figure 7). Signals at 176.7 and 176.9 ppm corresponded to the non-equivalent carbonyl carbons of the succinamide group. Similarly signals at 33.0 and 33.1 ppm corresponded to the non-equivalent methylene carbons of the succinamide functionality. The C-3^A carbon of the modified glucopyranose unit of the C-3 substituted cyclodextrin moiety was assigned to the signal at 53.0 ppm and the C-6^A carbon of the modified glucopyranose unit of the other cyclodextrin moiety was assigned to the signal at 41.9 ppm. A DEPT ^{13}C nmr spectrum of the dimer (107) was also recorded in order to confirm the assignment of these signals. This type of experiment shows methylene carbons as negative peaks and methine carbons as positive peaks. Positive and negative signals at 53.0 ppm and at 41.9 ppm, respectively, confirmed the assignment of their related signals in the normal ^{13}C nmr spectrum of the dimer (107) described above. It should be noted that negative peaks at 33.0 and 33.1 ppm also confirmed the assignment of the non-equivalent methylene carbons of the succinamide group.

As described for the urea (69) and the malonamide (70), the inclusion of TNS (4) in the linked cyclodextrins (104), (105) and (107) in aqueous phosphate buffer, at pH 6.9 and ionic strength 0.1 mol dm^{-3}, was studied by monitoring the increase in fluorescence of solutions containing 1.0 μmol dm^{-3} TNS (4) with increasing concentrations of the dimers (104), (105) and (107) over the range (0 - 1.0 mmol dm^{-3}). Plots (Figures 8-10) of intensity of fluorescence versus concentration of the dimers (104), (105) and (107) were once more subjected to non-linear least-squares regression analysis which afforded the stability constants of the 1:1 inclusion complexes (Table 8). The stability constants (K) of the TNS (4)-linked cyclodextrin (104), (105) and (107) inclusion

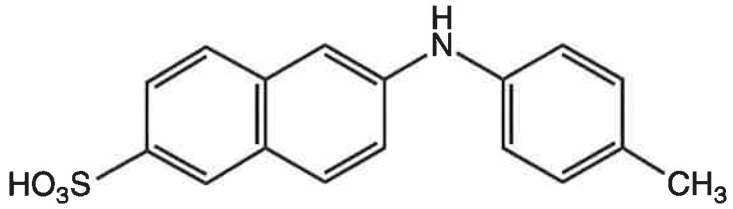
Figure 7 ^{13}C nmr spectrum of the succinamide (107).



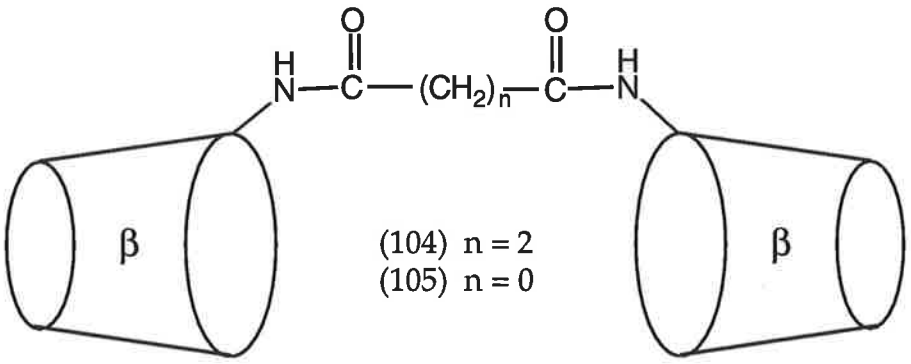
complexes were found to be 8,800, 5,500 and 11,050 mol^{-1} dm^3 respectively.

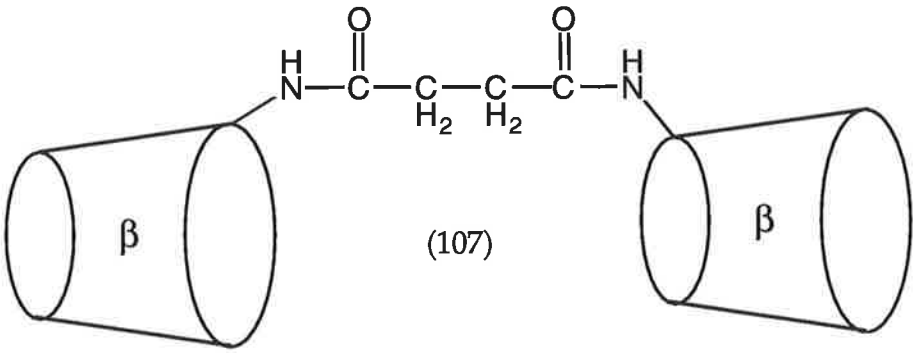
Table 8 Stability constants (K) of 1:1 cyclodextrin-TNS (4) inclusion complexes.

Cyclodextrin	Stability constant (K) (mol^{-1} dm^3)
(1)	2,800[96]
(105)	5,500
(104)	8,800
(107)	11,050
(67)	15,700[96]
(70)	12,000
(68)	29,000[96]
(69)	55,000

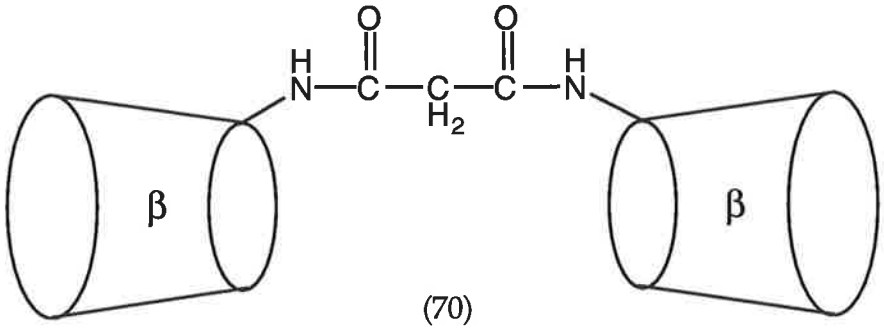


(4)

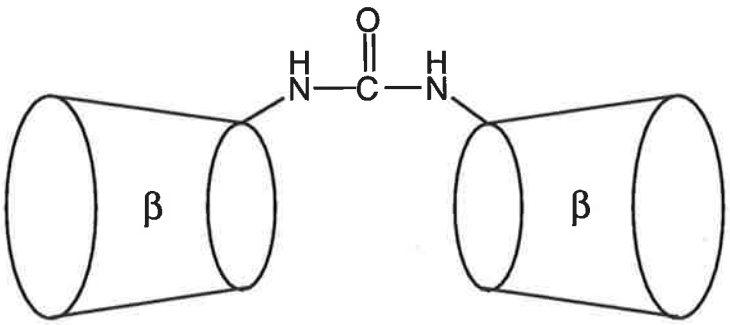




(107)



(70)



(69)

The difference between the stability constant of the 1:1 succinamide (104)-TNS (4) inclusion complex and that of the corresponding β-CD (1)-TNS (4) complex indicates that there is minor cooperative binding by the cyclodextrin annuli of the dimer (104). The difference between the stability constant of the oxalamide (105)-TNS (4) complex and that of the corresponding β-CD (1) complex represents only the statistical increase anticipated for the two cyclodextrin annuli of this species. On the other hand the stability constant of the succinamide (107)-TNS (4) complex was found to be approximately two fold

Figure 8 Plot of intensity of fluorescence of TNS (4) against the concentration (mmol dm^{-3}) of the succinamide linked cyclodextrin (104).

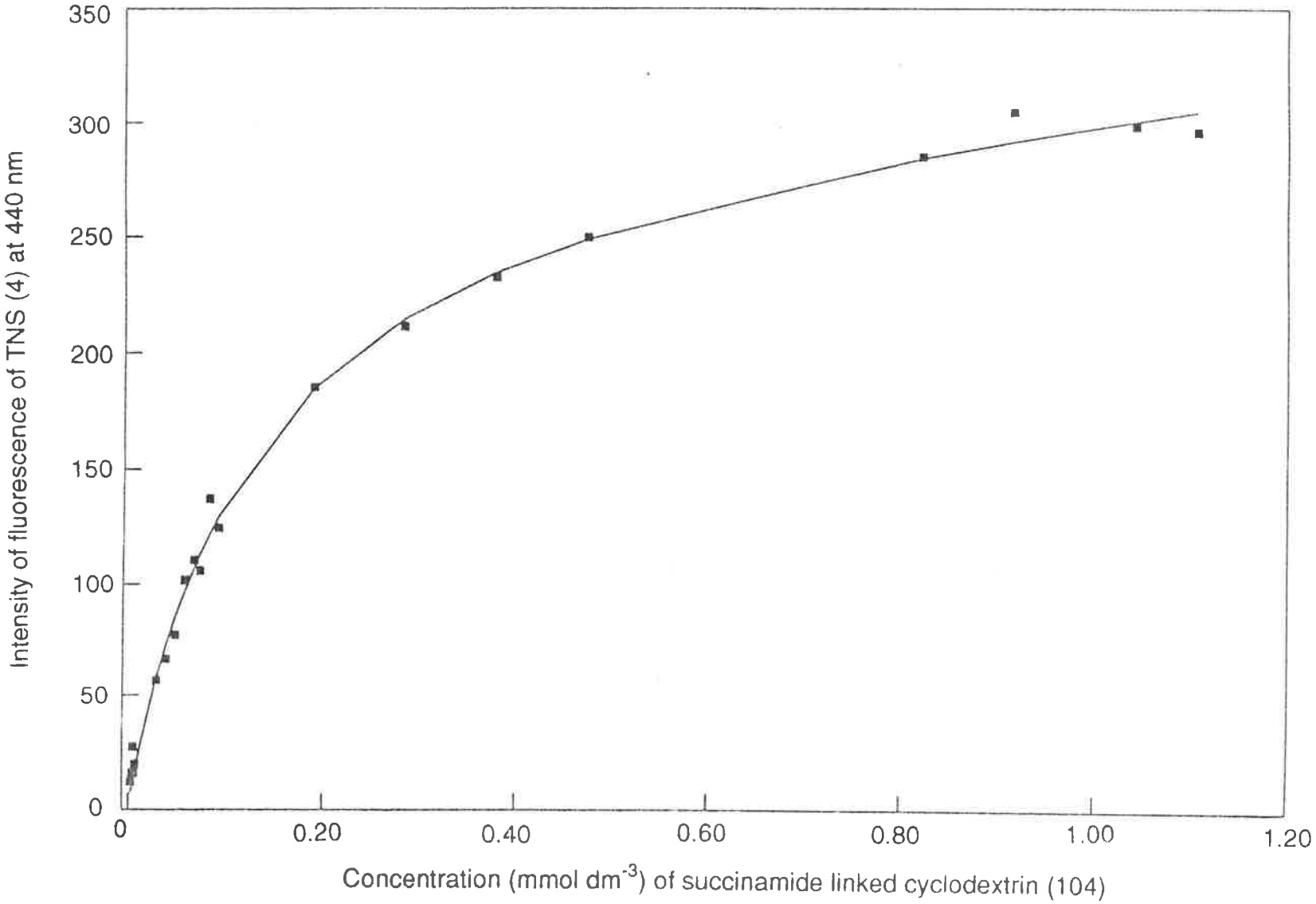


Figure 9 Plot of intensity of fluorescence of TNS (4) against the concentration (mmol dm^{-3}) of the oxalamide linked cyclodextrin (105).

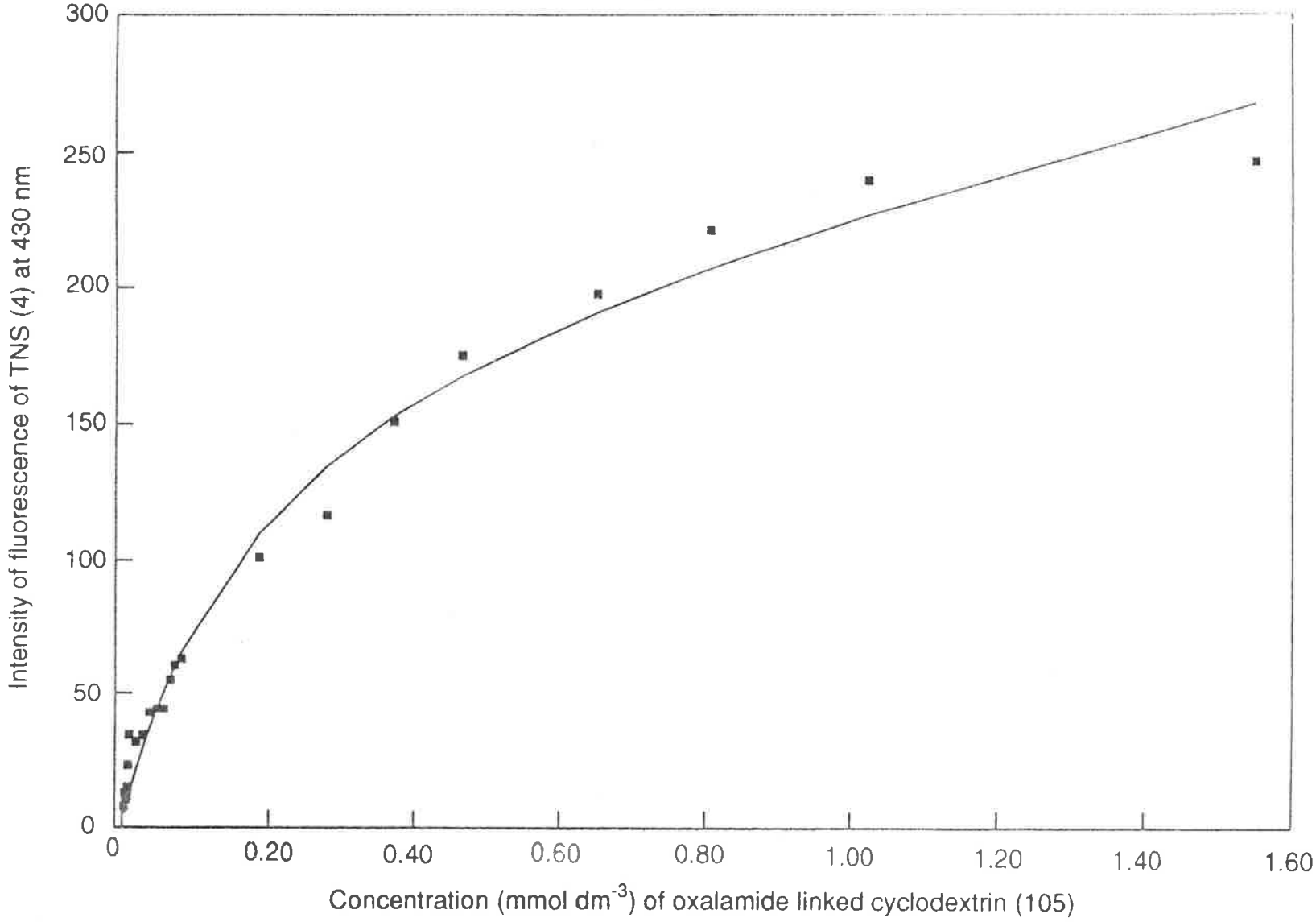
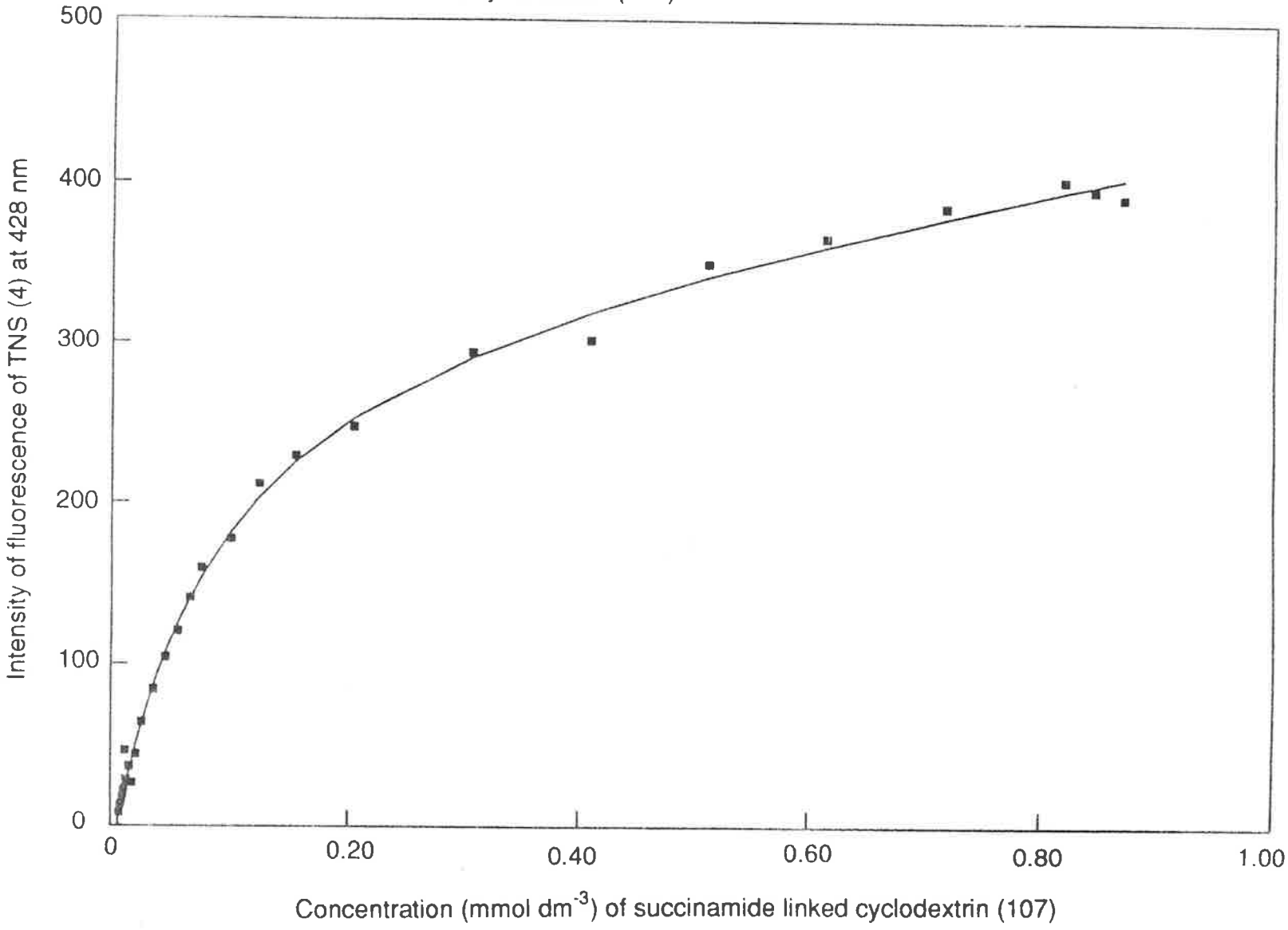


Figure 10 Plot of intensity of fluorescence of TNS (4) against the concentration (mmol dm^{-3}) of the succinamide linked cyclodextrin (107).



greater than the statistical increase anticipated for the presence of two cyclodextrin annuli. The results of this work show that the extent of cooperative binding by the cyclodextrin annuli of C-3-C-3 and C-6-C-3 diamide linked β-cyclodextrins is only modest, and the extent of cooperative binding shown is generally of a lower order than that exhibited by the corresponding C-6-C-6 diamide linked β-cyclodextrins (Table 8).[96] In addition the extent of cooperative binding by the cyclodextrin annuli of the C-3-C-3 diamide linked β-cyclodextrins (104) and (105) decreases as the length of the bridge connecting the annuli is shortened, however the opposite effect is seen with the C-6-C-6 diamide linked β-cyclodextrins (67) and (68).[96] Presumably the conformations of C-3-C-3 substituted diamide linked β-cyclodextrin-TNS (4) complexes are more strained than those of the corresponding C-6-C-6 substituted diamide linked cyclodextrin complexes. The strain can be attributed to the stereochemistry of the cyclodextrin substitution, where the substituents are thought to point toward the interior of the cyclodextrin annuli analogous to the stereochemistry of the amino group of their precursor (30) (see page 44). In direct contrast, the substituents of C-6-C-6 substituted diamide linked cyclodextrins are attached to sterically non-hindered methylene carbons which are free to point away from the cyclodextrin cavity, resulting in less strained systems.

As mentioned above and in the Introduction of this thesis, the extent of cooperative binding by the cyclodextrin annuli in the 1:1 complexes of the TNS (4)-C-6-C-6 substituted diamide linked cyclodextrins (67) and (68) seems to increase as the length of the bridge connecting the cyclodextrin moieties is shortened. On this basis the stability constant of the TNS (4)-malonamide (70) complex is marginally lower than expected. Nevertheless the malonamide (70) still shows cooperative binding, with the magnitude being lower than that of the oxalamide (68)-TNS (4) complex.

The most notable result of the present work was the magnitude of the stability constant of the urea (69)-TNS (4) 1:1 complex (55,000 mol^{-1} dm^3).

The stability constant of this complex is approximately two fold larger than that of the corresponding oxalamide (68) complex. The extent of cooperative binding by the urea linked cyclodextrin (69) is all the more remarkable because the included TNS (4) must pass through the narrow end of each cyclodextrin annulus and presumably the complexation involves the unfavourable alignment of the opposing dipole moments of the cyclodextrin annuli. Since the urea (69) possesses a tether which is even shorter than that of the oxalamide (68), this confirms the hypothesis stated above, that the C-6-C-6 substituted diamide linked β-cyclodextrin possessing the shortest tether forms the most stable 1:1 complex with TNS (4).

During the course of this work, Nolte *et al.*,[108,109] reported a related study of the synthesis and complexation of C-3-C-3 diamide linked cyclodextrins with TNS (4). They reported[108] the stability constant of 10,500 mol^{-1} dm^3 for the 1:1 succinamide (104)-TNS (4) complex which is similar to the value obtained in the present work. The extent of cooperative binding seen with their C-3-C-3 substituted diamide linked cyclodextrins was much less than that seen for C-6-C-6 substituted diamide linked cyclodextrins in the present work. Of late Petter *et al.*,[110] reported the synthesis and a systematic complexation study of C-6-C-6 substituted disulphide and dithioether linked cyclodextrins. Cooperative binding by these dimers was demonstrated and in common with the present work the extent of binding was found to increase as the length of the bridge connecting the cyclodextrin annuli was shortened.[110]

The synthesis and complexation studies of a variety of other linked cyclodextrins have also been reported.[111-113] Deschenaux *et al.*,[111] reported the preparation and physico-chemical properties of a C-6-C-6 substituted bipyridine-coupled permethylated β-cyclodextrin and of it's Re(I) transition metal complex. Toda *et al.*,[112] reported the synthesis of and complexation by a C-6-C-6 substituted cyclodextrin heterodimer possessing α- and β-cyclodextrin moieties, and Lawrence *et al.*,[113] reported the preparation and complexation characteristics of a

C-3-C-3 substituted pyridine-linked cyclodextrin. In 1993 Breslow[114] studied the thermodynamic aspects of cooperative binding by linked cyclodextrins. Specifically they examined enthalpy and entropy effects associated with complexation. Breslow *et al.*,[115,116] have also investigated the hydrolysis of various substrates in the presence of cyclodextrin dimers. It has been reported that a bipyridyl linked cyclodextrin acts as a cocatalyst and enhances the metal catalysed hydrolysis of various esters.[115,116] Most recently Breslow *et al.*,[117] have published a review focussing on their work with cyclodextrin dimers. Together with the work discussed in this Thesis, an overall picture of cooperative binding by linked cyclodextrins is being elucidated.

CONCLUSION

The complexation studies described in Chapter One of this Thesis indicate that β-CD (1) generally forms more stable inclusion complexes with aromatic guests as compared to those of the conjugate acids of the modified cyclodextrins (29) (β-CD-6-NH_3^+) and (30) (β-CD-3-NH_3^+). It is also evident that inclusion complexes of β-CD-6-NH_3^+ are generally more stable than the analogous β-CD-3-NH_3^+ complexes. It is obvious that the protonated amino groups of β-CD-6-NH_3^+ and β-CD-3-NH_3^+ adversely affect the binding ability, presumably because the protonated amino groups reduce the hydrophobicity of the cyclodextrin cavities. In the case of β-CD-3-NH_3^+ this is probably a consequence of the inverted stereochemistry of the C-3 and C-2 centres of the modified glucopyranose residue, where the protonated amino group points directly in to the cavity of the cyclodextrin. The aqueous solubilities of β-CD-6-NH_3^+ and β-CD-3-NH_3^+ are many fold greater than that of β-CD (1), but the results from these complexation studies indicate that the advantages of this greater solubility, e.g., in the pharmaceutical industry as complexing agents, may be off-set, particularly in the case of β-CD-3-NH_3^+ due to its diminished complexation ability.

The work described in Chapter Two of this Thesis shows that reactions of the cyclodextrins (29) and (30) with *meta*-nitrophenyl esters of 2-phenylpropanoic acid (48) and Ibuprofen (59) in pyridine produce the corresponding amides (78)-(81) with modest diastereoselectivity of the order of 2:1. Similarly formation of the amides (78) and (79) was accomplished from the hydrolysis and subsequent decarboxylation of the malonate substituted cyclodextrins (91) and (92). The diastereoselectivity of these reactions was also 2:1. In concluding, the results of the work indicate that enantiomers of aryl-substituted propanoic acids such as 2-phenylpropanoic acid (76) and the non-steroidal anti-

inflammatory drug Ibuprofen (77) cannot be efficiently resolved or synthesized stereoselectively using these procedures.

Two C-3-C-3, one C-3-C-6 and two C-6-C-6 substituted diamide linked cyclodextrins have been prepared. In general stability constants of their 1:1 inclusion complexes with 6-(*para*-toluidino)-2-naphthalenesulfonic acid indicated that both cyclodextrin moieties of the dimers participate in binding. The results showed that the extent of cooperative binding by the cyclodextrin annuli of the C-3-C-3 and C-6-C-3 diamide linked β-cyclodextrins is only modest as compared to that of the corresponding C-6-C-6 diamide linked cyclodextrins. The extent of cooperative binding by the cyclodextrin annuli of the C-3-C-3 substituted linked β-cyclodextrins decreases as the length of the bridge connecting the annuli is shortened, however the opposite effect is observed with C-6-C-6 substituted diamide linked cyclodextrins. Presumably the inclusion complex conformations of the C-3-C-3 and C-3-C-6 substituted diamide linked β-cyclodextrins are more strained than those of the corresponding C-6-C-6 substituted diamide linked cyclodextrins especially when the tether joining the annuli of these dimers is shortened. The strain may be attributed to the stereochemistry of the cyclodextrin substitution, where the substituents of the C-3 modified cyclodextrins point toward the interior of the cyclodextrin annuli, analogous to the stereochemistry of the amino group of their precursor, 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin. In direct contrast, the substituents of C-6-C-6 substituted diamide linked cyclodextrins are attached to sterically non-hindered methylene carbons which are free to point away from the cyclodextrin cavity, resulting in less strained systems.

In concluding a particularly significant result of the work was the magnitude of the stability constant of the 1:1 C-6-C-6 substituted urea linked cyclodextrin-6-(*para*-toluidino)-2-naphthalenesulfonic acid complex (55,000 mol^{-1} dm^3). This stability constant is approximately two fold larger than that of the corresponding C-6-C-6 substituted oxalamide linked cyclodextrin complex and twenty fold larger than that of the corresponding β-CD (1) complex.[96] This result

firmly supports the concept of cooperative binding by linked cyclodextrins and the hypothesis, that as the length of the tether connecting the cyclodextrin annuli of C-6-C-6 diamide linked cyclodextrins is shortened, the extent of cooperative binding increases.

EXPERIMENTAL

Nomenclature

Viewing the secondary hydroxyl end of β-cyclodextrin each glucopyranosyl residue is labelled clockwise from A to G. The A residue is determined by the substitution which takes priority. Thus each substituent is assigned a prefix indicating the number of the carbon to which the substituent is attached and the letter of the glucopyranose residue. For example, 6^{A}-*O*-4-toluenesulfonyl-β-cyclodextrin indicates that a 4-toluenesulfonyl group is attached to the oxygen on the C-6 carbon of the A-glucopyranose unit.

General

Melting points were determined on a Kofler hot-stage apparatus under a Reichert microscope and are uncorrected. Melting points of cyclodextrins were not recorded since they undergo decomposition upon heating.

Elemental analyses were performed by the Chemistry Department of the University of Otago in Dunedin, New Zealand or by Victorian Chemical & Micro Analytical Services Pty. Ltd. Elemental analyses of cyclodextrin based compounds were performed on the hydrated species since removal of water from the cavity of a cyclodextrin is not possible using conventional drying methods.

Thin layer chromatography (tlc) was performed using Kieselgel 60 F_{254} (Merck) on aluminium backing plates. The running solvent used for cyclodextrin based

compounds was 1:8:1 chloroform/acetic acid/water. Visualisation was achieved by dipping the plate either in a solution of diphenylamine (0.1 g), aniline (0.5 ml) and 85% phosphoric acid (1 ml) in acetone (10 ml), or a solution of 10:1 acetone/15% sulphuric acid, and heating to char the spots. Flash chromatography was performed using Merck Kieselgel 60 (230-400 mesh ASTM) silica. Solvent systems employed are mentioned individually with the text.

Column cation exchange chromatography was performed using Sephadex SP-C 25 cation exchange resin (Pharmacia). Elution was achieved under a positive pressure of nitrogen.

Desalting was carried out by the use of AG 501 X8 mixed bed ion-exchange resin (Bio-rad Chemicals).

High performance liquid chromatography (HPLC) was carried out using a Waters model 510 solvent delivery system coupled to a Waters Model 410 differential refractometer in conjunction with an I.C.I. DP-700 data station. The column used was a Waters 3.9 x 300 mm Carbohydrate analysis column and was run at 1.5 ml min^{-1}. Solvent system (A): acetonitrile/water, 70:30, v/v; Solvent system (B): acetonitrile/water, 60:40, v/v.

Infrared spectra were recorded on a Hitachi 270-30 spectrometer. Samples were prepared as either Nujol mulls or neat between sodium chloride plates.

Mass spectra were recorded on a VG ZAB 2HF mass spectrometer using the FAB technique with Xe or Ar as the collision gas. The samples were dissolved in water (H_2O) or *N*,*N*-dimethylformamide (DMF) and introduced in to the spectrometer in a glycerol matrix.

Proton decoupled carbon nuclear magnetic resonance (^{13}C nmr) spectra were recorded on a Bruker ACP-300 spectrometer operating at 75.8 MHz. Spectra of cyclodextrin based compounds were recorded as solutions in deuterium oxide (D_2O) or deuteriated dimethyl sulphoxide (DMSO), using a 10% solution of sodium 3-(trimethylsilyl)-1-propane sulphonate as an internal standard. The chemical shifts are quoted as δ in parts per million (ppm). Spectra of non-cyclodextrin compounds were recorded as solutions in deuteriated chloroform ($CDCl_3$) using tetramethylsilane as an internal standard. The chemical shifts are quoted as δ in parts per million (ppm) downfield from the internal standard.

Proton nuclear magnetic resonance (^1H nmr) spectra were recorded on a Varian T60 spectrometer operating at 60 MHz, or a Bruker ACP-300 spectrometer operating at 300 MHz. ^1H nmr spectra of cyclodextrin based compounds were exclusively recorded on the Bruker ACP-300 spectrometer as solutions in D_2O or DMSO. The chemical shifts are recorded as δ in parts per million relative to appropriate standards. Sample temperature was controlled by a Bruker B-VT 1000 variable temperature unit to within ± 0.3 K. ^1H nmr spectra of non-cyclodextrin based compounds were recorded as solutions in $CDCl_3$ using tetramethylsilane as an internal standard. The multiplicity of the resonances in all spectra are abbreviated as follows: q, quartet; t, triplet; d, doublet; s, singlet; m, multiplet.

^1H-broad-band decoupled fluorine nuclear magnetic resonance spectra (^{19}F nmr) spectra were recorded on a Bruker CXP-300 spectrometer operating at 282.35 MHz, locked on the deuterium frequency of D_2O. Chemical shifts were measured from a 2% CF_3CO_2Na-D_2O external reference. An average of 1000 transients was accumulated in a 8192 point data base for each solution, and the solution temperature (295.5 K) was controlled to within ± 0.3 K using a Bruker B-VT 1000 variable temperature unit.

Fluorescence measurements were recorded on a Perkin Elmer model LE 50 or a Perkin Elmer model 3000 spectrometer. The sample cuvette, 1 cm^3, was kept at 25°C. The excitation wavelength was 366 nm and a cut off filter at 390 nm was installed in the emission light path. The fluorescence of samples was measured between 400-500 nm.

All solvents were purified by distillation before use. Anhydrous pyridine was obtained by distillation from solid potassium hydroxide and was stored over 4Å molecular sieves.[118] Anhydrous *N*,*N*-dimethylformamide (DMF) was obtained by distillation from solid calcium sulphate and was stored over 4Å molecular sieves.[118] Water used in all cases was of HPLC grade obtained from purification using a Waters Milli-Q™ system.

β-Cyclodextrin (1), 6^A-amino-6^A-deoxy-β-cyclodextrin (29) and 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (30) were each stored in a vacuum desiccator over phosphorus pentoxide for at least 48 h before use.

β-Cyclodextrin was supplied by Nihon Shokuhin Kako Co. and contained 10% water. 2-Fluorobenzoic acid (71) and the corresponding *para*-isomer (72) were obtained from Aldrich. Sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS) (4) was also obtained from Aldrich. Diphenyl carbonate was obtained from Aldrich and recrystallized from ethanol.

Synthesis

3-Nitrophenyl 4-toluenesulfonate (32).

To a mixture of 4-toluenesulfonyl chloride (35.0 g, 184 mmol), 3-nitrophenol (28.0 g, 201 mmol) and chloroform (500 ml), triethylamine (20.0 g, 198 mmol) was added dropwise over a period of 20 min. The mixture was allowed to stir for a further 1 h at room temperature. The mixture was then washed with water (2 x 200 ml) and the organic layer was separated and dried over $MgSO_4$. Evaporation of the solvent under vacuum afforded an orange solid. The crude solid was recrystallized from ethyl acetate/hexane to give 35.5 g (66%) of the *title compound* as tan-cream coloured needles, m.p. 112-113°C (lit.[119] 113-114°C).

^1H nmr (300 MHz, $CDCl_3$) δ_H 2.49, s, 3H, Ar-C<u>H</u>$_3$; 7.38 and 7.75, ABq, J=8.4 Hz, 4H, Ar-<u>H</u>; 7.3-8.2, m, 4H, Ar-<u>H</u>.

2^A-*O*-4-Toluenesulfonyl-β-cyclodextrin (33).[77]

To a solution of 3-nitrophenyl 4-toluenesulfonate (32) (20.0 g, 68.3 mmol) in DMF (800 ml), dry β-CD (1) (80 g, 70.5 mmol) was added in one portion. To the resultant solution, 0.2 mol dm^{-3} sodium carbonate (480 ml) solution was added and the resultant suspension was left to stir at 60°C for 1 h. The reaction mixture was then cooled to room temperature and acidified using concentrated HCl. To the acidified solution, acetone (6 L) was added which produced a precipitate. The suspension was filtered and the filtrate was concentrated to a yellow oil (20 ml), which was subsequently added to acetone (6 L) with vigorous stirring. The white precipitate which formed was isolated and washed with

acetone (2 L). The crude product was dissolved in milli-Q water (1 L) and the solution was filtered. Mixed bed ion exchange resin (200 g) was added in one portion to the filtrate and the resultant suspension was left to stir to remove sodium chloride.# The resin was then removed by filtration. Evaporation of the filtrate to dryness under reduced pressure and then further drying of the remaining solid under high vacuum for 24 h yielded 8.8 g (9.7%) of the *title compound* as a white flaky solid.

Removal of sodium chloride was monitored by taking aliquots (1 ml) at 5 min. intervals during the desalting process and analysing them for the presence of chloride using the silver nitrate standard test.[120]

HPLC (Solvent system A) t_R 0.52 relative to β-CD (1) (12.5 min). FAB-MS M+H 1289. ^{1}H nmr (300 MHz, DMSO) δ_H 2.40, s, 3H, Ar-C<u>H</u>$_3$; 3.3-4.1, 4.5-4.8, 5.6-5.9, m, 69H; 7.43 and 7.84, ABq, J=8.1 Hz, 4H, Ar-<u>H</u>.

3^A-Amino-3^A-deoxy-(2^A*S*,3^A*S*)-β-cyclodextrin (30).[79]

To a solution of 2^A-*O*-4-toluenesulfonyl-β-cyclodextrin (33) (8.0 g, 6.2 mmol) in water (200 ml), ammonium carbonate (10% w/v, 250 ml) was added and the resultant solution was left to stir for 3 h at 60°C. The reaction contents were then concentrated to dryness under vacuum to give a cream coloured solid which was then redissolved in aqueous ammonia (25% v/v, 100 ml). The solution was then allowed to stir at 60°C for a further 3 h. Evaporation of the solvent under reduced pressure gave the crude product as a yellow solid. The crude material was redissolved in water (50 ml) and the solution was evaporated to dryness under reduced pressure. This process was then repeated. The crude product was then redissolved in water (10 ml) and loaded on to a

column containing Sephadex cation exchange resin. Impurities were then removed by eluting with water (300 ml) and then methanol/water (10% methanol in water, 200 ml). Aqueous ammonia (5% v/v) was then passed through the column until all of the product was collected. The ammonia eluant fractions containing the product were combined and evaporated to dryness under reduced pressure. The remaining solid was then redissolved in water (200 ml) and the solution was subsequently evaporated to dryness under reduced pressure. This process was repeated twice. The remaining solid was further dried over phosphorus pentoxide under vacuum to give 5.5 g (78%) of the *title compound* as a pale yellow solid.

HPLC (Solvent system B) t_R 1.3 relative to β-(CD) (1) (4.6 min). FAB-MS M+H 1135. Anal. calcd. for $C_{42}H_{71}NO_{34}.6H_2O$: C, 40.55; H, 6.68; N, 1.13. Found: C, 40.72; H, 6.65; N, 1.27%. ^1H nmr (300 MHz, D_2O) δ_H 3.0, 3.6-4.2, 4.8, 4.9-5.1, m, 71H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 105.5-102.5, C-1^{A-G}; 83.0-80.9, C-4^{A-G}; 77.9-73.1, C-2^{A-G}, C-3^{B-G}, C-5^{A-G}; 62.3-61.6, C-6^{A-G}; 54.2, C-3^{A}.

Methyl 2-fluorobenzoate (38).

A mixture of 2-fluorobenzoic acid (71) (4.0 g, 28.6 mmol) and thionyl chloride (20.0 g, 202 mmol) was left to stir at reflux for 4 h. The resulting solution was then cooled to room temperature and concentrated under vacuum leaving a yellow residue. The residue was then added to dry methanol (100 ml) dropwise and the resulting solution was left to stir at reflux for a further 4 h. Removal of the solvent under vacuum afforded the crude ester (38) which was then redissolved in dichloromethane (100 ml) and washed with water (2 x 100 ml) and aqueous sodium bicarbonate (2 x 100 ml). The organic layer was dried over $MgSO_4$ and then evaporated under vacuum to give a clear yellow oil.

Distillation of the oil yielded 3.4 g (77%) of the *title compound* as a colourless clear liquid, b.p. 137-139°C/28 mm (Lit.[121] 209-212°C).

^{1}H nmr (300 MHz, $CDCl_3$) δ_H 3.81, s, 3H, -OCH_3; 7.3-7.8, m, 4H, Ar-H. ^{19}F nmr (282.35 MHz, D_2O) δ_F -34.83.

Methyl 4-fluorobenzoate (39).

The procedure used was identical to that employed for the preparation of methyl 2-fluorobenzoate (38). Reaction of 4-fluorobenzoic acid (72) (4.0 g, 28.6 mmol) with thionyl chloride (20 g, 202 mmol) and then methanol (100 ml) gave 3.6 g (82%) of the *title compound* as a colourless clear liquid after distillation, b.p. 135-137°C/28 mm (Lit.[121] 198-200°C).

^{1}H nmr (300 MHz, $CDCl_3$) δ_H 3.92, s, 3H, -OCH_3; 7.2-8.1, m, 4H, Ar-H. ^{19}F nmr (282.35 MHz, D_2O) δ_F -28.51.

3-Nitrophenyl 2-phenylpropionate (48).[87]

To thionyl chloride (5.0 g, 50.5 mmol), 2-phenylpropionic acid (76) (1.0 g, 6.7 mmol) was added in one portion. The resultant solution was left to stir at room temperature for 24 h. Unreacted thionyl chloride was removed under vacuum leaving a golden coloured residue. To a solution of the residue in dichloromethane (50 ml), 3-nitrophenol (1.85 g, 13.4 mmol) was added in one portion followed by triethylamine (1.35 g, 13.4 mmol) dropwise over a period of 10 min. The mixture was left to stir at room temperature for 30 min. Tlc (10% hexane in dichloromethane) on the reaction mixture showed the presence of

the *title compound* (R_f 0.85) and 3-nitrophenol (R_f 0.4). The solvent was then removed under reduced pressure leaving the crude product as a tan coloured oil. The residue was then chromatographed (dichloromethane/hexane, 9:1) leaving an oil which was subsequently distilled to yield 1.31 g (72%) of the *title compound* as a clear light yellow oil, b.p. 190-193°C/0.4 mm.

ν_{max} (neat) 1736 cm^{-1} (ester C=O). ^1H nmr (300 MHz, $CDCl_3$) δ_H 1.66, d, J=7.2 Hz, 3H, Ar-CH-C$\underline{H}_3$; 4.04, q, J=7.2 Hz, 1H, Ar-C$\underline{H}$-CH_3; 7.4-8.2, m, 9H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, $CDCl_3$) δ_C 18.4, Ar-CH-$\underline{C}H_3$; 45.6, Ar-$\underline{C}$H-CH_3; 117.2, 120.8, 127.5, 127.7, 127.9, 129.0, 130.0, 139.4, 151.2, Ar; 172.5, ester C=O.

No comparative literature data has been reported.

3-Nitrophenyl 2-(4-(2-methylpropyl)-phenyl)-propionate (59).[96]

To thionyl chloride (25.0 g, 253 mmol), dry 2-(4-(2-methylpropyl)-phenyl)-propionic acid (77) (5.0 g, 24.3 mmol) was added in one portion. The resultant mixture was allowed to stir at reflux for 5 h. Excess thionyl chloride was removed under reduced pressure leaving a dark brown residue. To a solution of the residue in chloroform (50 ml), 3-nitrophenol (5.0 g, 36 mmol) was added in one portion and then triethylamine (3.6 g, 35.6 mmol) dropwise over a period of 5 min. The resultant mixture was then left to stir for 1 h at room temperature after which time the ester (59) had formed. The solvent was subsequently removed under vacuum leaving an oily residue which was redissolved in dichloromethane (10 ml) and filtered. Tlc (20% hexane in dichloromethane) on the filtrate showed the presence of the *title compound* (R_f 0.90) and 3-nitrophenol (R_f 0.3). The filtrate was then chromatographed

(dichloromethane/hexane, 8:2) giving an oil which was distilled to yield 6.35 g (80%) of the *title compound* as a viscous golden oil, b.p. 185-188°C/0.2 mm.

ν_{max} (neat) 1745 cm^{-1} (ester C=O). ^1H nmr (300 MHz, $CDCl_3$) δ_H 0.90, d, J=6.6 Hz, 6H, $-CH_2-CH(C\underline{H}_3)_2$; 1.61, d, J=7.2 Hz, 3H, Ar-CH-$C\underline{H}_3$; 1.9, m, 1H, $-CH_2-C\underline{H}(CH_3)_2$; 2.47, d, J=7.2 Hz, 2H, $-C\underline{H}_2CH(CH_3)_2$; 3.97, q, J=7.2 Hz, 1H, Ar-$C\underline{H}$-CH_3; 7.16 and 7.36, ABq, J=7.8 Hz, 4H, Ar-$\underline{H}$; 7.3-8.1, m, 4H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, $CDCl_3$) δ_C 22.2, 18.4, $-CH_2-CH(\underline{C}H_3)_2$, Ar-CH-$\underline{C}H_3$; 30.1, $-CH_2-\underline{C}H(CH_3)_2$; 44.7, 45.0, $\underline{C}H_2CH(CH_3)_2$, Ar-$\underline{C}$H-$CH_3$; 116.8, 120.6, 126.8, 127.5, 129.0, 129.9, 136.8, 141.1, 148.7, 151.2, Ar; 172.5, ester C=O.

6^A-Deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78).

Method A: 6^A-Deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78) was prepared from 6^A-amino-6^A-deoxy-β-cyclodextrin (29).

To a solution of 3-nitrophenyl 2-phenylpropionate (48) (1.0 g, 3.69 mmol) in dry pyridine (7 ml), 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (500 mg, 0.44 mmol) was added in one portion. The reaction mixture was then left to stir for 6 h at room temperature. The reaction contents were then added to diethyl ether (40 ml) with vigorous stirring. The resultant off-white precipitate was isolated and washed with diethyl ether (50 ml) and acetone (50 ml). The crude material was redissolved in water (5 ml) and added dropwise to acetone (30 ml) with vigorous stirring. The resultant precipitate was again isolated and washed with acetone (50 ml). The product was then redissolved in water (50 ml) and loaded on to a column containing Sephadex cation exchange resin. The column was eluted with water until all of the product was collected. Evaporation to dryness of the combined fractions under vacuum and further drying of the remaining solid

over phosphorus pentoxide under vacuum for 3 days yielded 335 mg (60%) of the *title compound* as a white flaky solid.

HPLC (Solvent system A) t_R 0.60 relative to β-(CD) (1) (15.4 min). ν_{max} (nujol mull) 1652 cm^{-1} (amide C=O). FAB-MS M+H 1267. Anal. calcd. for $C_{51}H_{79}NO_{35}.6H_2O$: C, 44.54; H, 6.77; N, 1.10. Found: C, 44.57; H, 6.63; N, 0.86%. ^1H nmr (300 MHz, DMSO) δ_H 1.24, d, J=6.6 Hz, 3H, Ar-CH-C$\underline{H}_3$, minor diastereomer, 1.30, d, J=7.2 Hz, 3H, Ar-CH-C$\underline{H}_3$, major diastereomer, diastereomer ratio, 2:1; 3.2-3.6, 4.5, 4.8, 5.6-5.8, m, 70H; 7.2-7.3, m, 5H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, $CDCl_3$) δ_C 20.9, Ar-CH-$\underline{C}H_3$, major diastereomer, 20.3, Ar-CH-$\underline{C}H_3$, minor diastereomer, diastereomer ratio, 2:1; 48.2, Ar-$\underline{C}$H-CH_3, major diastereomer, 47.8, Ar-$\underline{C}$H-CH_3, minor diastereomer, diastereomer ratio, 2:1; 104.3-103.6, C-1^{A-G}; 85.8-82.6, C-4^{A-G}; 75.4-73.1, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 62.2, C-6^{B-G}; 42.6, C-6^A; 129.2 129.3 129.5, 130.9, 131.1, 143.9, 144.0, Ar; 178.5, amide C=O, major diastereomer, 178.2, amide C=O, minor diastereomer, diastereomer ratio, 2:1.

Method B: 6^A-Deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78) was prepared from 6^A-Deoxy-6^A-(2-(3-nitrophenoxycarbonyl)-2-phenylpropion-amido)-β-cyclodextrin (91).

To a suspension of the amide-ester (91) (300 mg, 0.21 mmol) in water (8 ml), concentrated sulphuric acid (3 drops) was added. The reaction mixture was left to stir at reflux for 8 h. The solution was then cooled to room temperature and concentrated to approximately 2 ml under vacuum. The concentrate was then added dropwise to acetone (10 ml) with vigorous stirring. The precipitate which formed was isolated and washed with acetone (10 ml) and diethyl ether (10 ml). The product was redissolved in water (10 ml) and then evaporated to dryness under reduced pressure. The remaining solid was further dried over phosphorus pentoxide under vacuum for 48 h to give 144 mg (53%) of the *title*

compound as a white solid, identical in all respects to the sample obtained as described in *Method A*.

6^A-Deoxy-6^A-(2-(4-(2-methylpropyl)-phenyl)-propionamido)-β-cyclodextrin (79).

Method A: 6^A-Deoxy-6^A-(2-(4-(2-methylpropyl)-phenyl)-propionamido)-β-cyclodextrin (79) was prepared from 6^A-amino-6^A-deoxy-β-cyclodextrin (29).

The procedure used was identical to that employed for the preparation of 6^A-deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78). Reaction of 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (500 mg, 0.44 mmol) with 3-nitrophenyl 2-(4-(2-methylpropyl)-phenyl)-propionate (59) (1.2 g, 3.69 mmol) in pyridine (7 ml) for 6 h at room temperature gave 443 mg (76%) of the *title compound* as a white powder after Sephadex chromatography.#

The crude material was dissolved in methanol/water (50% methanol in water, 30 ml) and loaded on to a column containing Sephadex cation exchange resin. The column was eluted with the same solvent system until all of the product was collected. Evaporation under vacuum to dryness of the combined fractions containing the product and further drying of the remaining solid over phosphorus pentoxide for two days gave the *title compound* in the yield stated above.

HPLC (Solvent system A) t_R 0.54 relative to β-(CD) (1) (13.8 min). ν_{max} (nujol mull) 1650 cm^{-1} (amide C=O). FAB-MS M+H 1323. Anal. calcd. for $C_{55}H_{87}NO_{35}.6H_2O$: C, 46.15; H, 6.92; N, 1.05. Found: C, 46.04; H, 7.01; N, 0.98%. ^1H nmr (300 MHz, DMSO) at 347 K δ_H 0.86, d, J=6.6 Hz, 6H, $-CH_2CH(C\underline{H}_3)_2$; 1.23, d, J=7.2 Hz, 3H, Ar-CH-$C\underline{H}_3$, minor diastereomer, 1.30, d, J=7.2 Hz, 3H, Ar-CH-

C<u>H</u>$_3$, major diastereomer, diastereomer ratio, 2:1; 1.8, m, 1H, -CH$_2$C<u>H</u>(CH$_3$)$_2$; 2.41, d, J=7.2 Hz, 2H, -C<u>H</u>$_2$CH(CH$_3$)$_2$; 3.2-3.7, 4.2-4.8, m, 70H; 7.19 and 7.04, ABq, J=8.2 Hz, 4H, Ar-<u>H</u>, major diastereomer, 7.21 and 7.06, ABq, J=8.0 Hz, 4H, Ar-<u>H</u>, minor diastereomer, diastereomer ratio, 2:1. ^{13}C nmr (75.8 MHz, DMSO) δ_C 19.6, Ar-CH-<u>C</u>H$_3$, minor diastereomer, 19.9, Ar-CH-<u>C</u>H$_3$, major diastereomer, diastereomer ratio, 2:1; 23.3, -CH$_2$CH(<u>C</u>H$_3$)$_2$; 30.8, -CH$_2$<u>C</u>H(CH$_3$)$_2$; 45.3, Ar-<u>C</u>H-CH$_3$ 103.1, C-1^{A-G}; 82.6, C-4^{A-G}; 73.2-74.1, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 60.9, C-6^{B-G}; 128.0, 129.6, 133.0, 140.2, 140.3, 140.5, 140.7, Ar; 174.9, amide C=O, minor diastereomer, 175.1, amide C=O, major diastereomer, diastereomer ratio, 2:1.

Method B: 6^A-Deoxy-6^A-(2-(4-(2-methylpropyl)-phenyl)-propionamido)-β-cyclodextrin (79) was prepared from 6^A-deoxy-6^A-(2-ethoxycarbonyl-2-(4-(2-methylpropyl)-phenyl)-propionamido)-β-cyclodextrin (92).

A solution of the amide-ester (92) (310 mg, 0.22 mmol) in aqueous sodium hydroxide (5 ml, 2 mol dm^{-3}) was left to stir at room temperature for 8 h. The mixture was then acidified to approximately pH 1.0 by adding concentrated sulfuric acid dropwise. The acidified solution was left to stir for a further 6 h at reflux. The reaction mixture was then cooled to room temperature and let stand overnight. The precipitate which formed was isolated and washed with cold water (2 ml) and then dried over phosphorus pentoxide under vacuum for 48 h to give 178 mg (61%) of the *title compound* as a white powder, identical in all respects to the sample obtained as described in *Method A*.

3^A-Deoxy-3^A-(2-phenylpropionamido)-(2^A*S*,3^A*S*)-β-cyclodextrin (80).

The *title compound* was prepared and purified by the procedures employed for 6^A-deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78). Reaction of 3^A-amino-

3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (30) (500 mg, 0.44 mmol) with 3-nitrophenyl 2-phenylpropionate (48) (1.0 g, 3.69 mmol) in pyridine (10 ml) for 8 h at room temperature yielded 265 mg (48%) of the *title compound* as a clear glassy solid after Sephadex chromatography.

HPLC (Solvent system A) t_R 0.81 relative to β-(CD) (1) (12.9 min). ν_{max} (nujol mull) 1650 cm^{-1} (amide C=O). FAB-MS M+H 1267. Anal. calcd. for $C_{51}H_{79}NO_{35}.7H_2O$: C, 43.97; H, 6.68; N, 1.01. Found: C, 44.06; H, 6.75; N, 1.01%. ^1H nmr (300 MHz, DMSO) at 343 K δ_H 1.37, d, J=6.3 Hz, 3H, Ar-CH-C$\underline{H}_3$, major diastereomer, 1.42, d, J=7.5 Hz, 3H, Ar-CH-C$\underline{H}_3$, minor diastereomer, diastereomer ratio, 2:1; 3.3-4.1, 4.6-4.9, m, 70H; 7.3, m, 5H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, D_2O) δ_C 18.9, Ar-CH-$\underline{C}H_3$, minor diastereomer, 20.4, Ar-CH-$\underline{C}H_3$, major diastereomer, diastereomer ratio, 2:1; 48.4, Ar-$\underline{C}$H-CH_3, major diastereomer, 49.7, Ar-$\underline{C}$H-CH_3, minor diastereomer, diastereomer ratio 2:1; 105.2-102.9, C-1^{A-G}; 83.0-82.2, C-4^{A-G}; 77.0-70.4, C-2^{A-G}, C-3^{B-G}, C-5^{A-G}; 62, C-6^{A-G}; 52.9, C-3^A, minor diastereomer, 53.2, C-3^A, major diastereomer, diastereomer ratio, 2:1; 129.2, 129.4, 129.8, 131, 141.7, 144.7, Ar; 179.3, amide C=O, minor diastereomer, 180.4, amide C=O, major diastereomer, diastereomer ratio, 2:1.

3^A-Deoxy-3^A-(2-(4-(2-methylpropyl)-phenyl)-propionamido)-(2^AS,3^AS)-β-cyclodextrin (81).

The *title compound* was prepared by the procedure employed for 6^A-deoxy-6^A-(2-phenylpropionamido)-β-cyclodextrin (78). Reaction of 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (30) (500 mg, 0.44 mmol) with 3-nitrophenyl 2-(4-(2-methylpropyl)-phenyl)-propionate (59) (1.2 g, 3.69 mmol) in pyridine (10 ml) for 8 h at room temperature yielded 303 mg (52%) of the *title compound* as a creamy white coloured powder after Sephadex chromatography.#

The crude material was dissolved in methanol/water (30% methanol in water, 30 ml) and loaded on to a column containing Sephadex cation exchange resin. The column was eluted with the same solvent system until all of the product was collected. Evaporation under vacuum to dryness of the combined fractions containing the product and further drying of the remaining solid over phosphorus pentoxide for two days gave the *title compound* in the yield stated above.

HPLC (Solvent system A) t_R 0.78 relative to β-(CD) (1) (12.9 min). ν_{max} (nujol mull) 1650 cm^{-1} (amide C=O). FAB-MS M+H 1323. Anal. calcd. for $C_{55}H_{87}NO_{35}.3.5H_2O$: C, 47.69; H, 6.79; N, 1.02. Found: C, 48.0; H, 7.39; N, 0.94%. ^1H nmr (300 MHz, DMSO) at 387 K δ_H 0.8, m, 6H, $-CH_2CH(C\underline{H}_3)_2$, diastereomers; 1.42, d, J=7.2 Hz, 3H, Ar-CH-$C\underline{H}_3$; 1.8, m, 1H, $-CH_2C\underline{H}(CH_3)_2$; 2.44, d, J=6.3 Hz, 2H, $-C\underline{H}_2CH(CH_3)_2$; 3.2-3.7, 4.6-4.9, m, 70H; 7.04 and 7.21, ABq, J=7.8 Hz, 4H, Ar-$\underline{H}$, diastereomer (i), 7.08 and 7.25, ABq, J=7.2 Hz, 4H, Ar-$\underline{H}$, diastereomer (ii), diastereomer ratio, 1:1. ^{13}C nmr (75.8 MHz, DMSO) δ_C 18.1, Ar-CH-$\underline{C}H_3$; 23.7, $-CH_2CH(\underline{C}H_3)_2$; 32.7, $-CH_2\underline{C}H(CH_3)_2$; 47.2, Ar-$\underline{C}$H-$CH_3$; 105.5-104.1, $C\text{-}1^{A\text{-}G}$; 83.3-83, $C\text{-}4^{A\text{-}G}$; 75.7-70.5, $C\text{-}2^{A\text{-}G}$, $C\text{-}3^{B\text{-}G}$, $C\text{-}5^{A\text{-}G}$; 61.9, $C\text{-}6^{A\text{-}G}$; 53.1, $C\text{-}3^{A}$; 129.6, 131.4, 142.5, 142.7, Ar; 178.9, amide C=O.

Diethyl methylphenylmalonate (83).[104]

To dry ethanol (200 ml) under nitrogen at room temperature, sodium metal (2 g, 87 mmol) was added in two portions over 15 min. Diethyl phenylmalonate (82) (15 g, 64 mmol) was added in one portion and the reaction mixture was then left to stir at room temperature for 20 min. To the solution methyl iodide (12.5 g, 87 mmol) was added and the reaction mixture was then left to stir at room temperature for 1 h. Upon completion of the reaction, the solvent was removed

under reduced pressure to afford a tan/gold residue. The residue was redissolved in dichloromethane (150 ml) and washed with water (2 x 150 ml). The combined organic extracts were dried over $MgSO_4$ and then evaporated to dryness under vacuum to give the crude product as a deep yellow oil. The crude product was distilled under reduced pressure to give 11.5 g (70%) of the *title compound* as a clear light yellow liquid, b.p. 195-198°C/28 mm (Lit.[122] 156-158°C/10 mm.

^1H nmr (60 MHz, $CDCl_3$) δ_H 1.30, t, J=7.5 Hz, 6H, $-OCH_2C\underline{H}_3$; 2.10, s, 3H, $-C-C\underline{H}_3$; 4.40, q, J=7.0 Hz, 4H, $-OC\underline{H}_2CH_3$; 7.6, m, 5H, Ar-<u>H</u>.

Methylphenylmalonic acid (84).

To a mixture of sodium hydroxide (1.2 mol dm^{-3}, 100 ml) and diethyl methylphenylmalonate (83) (10 g, 40 mmol), ethanol (400 ml) was added. The solution was then left to stir at room temperature for one day. The solvent was removed under vacuum to give a yellow/green residue which was redissolved in water (50 ml.) The solution was cooled to 0°C and acidified carefully, not allowing the temperature to rise above 10°C (add ice if necessary) with concentrated sulphuric acid to pH 1. The acidified solution was then extracted three times with diethyl ether (100 ml). The ethereal extracts were combined and dried over $MgSO_4$ and then concentrated to dryness leaving a white solid. The solid was then further dried under high vacuum to give 4.0 g (52%) of the *title compound* as a white solid, m.p. 154-156°C (lit.[122] 156-157°C).#

#The crude product was of sufficient purity and hence was not recrystallized

^1H nmr (60 MHz, $CDCl_3$) δ_H 1.75, s, 3H, $-C\underline{H}_3$; 7.4, m, 5H, Ar-<u>H</u>.

Bis-3-nitrophenyl methylphenylmalonate (86).

A mixture of methylphenylmalonic acid (86) (3.0 g, 15.5 mmol) and thionyl chloride (10.0 g, excess) was left to stir at 45°C for 5 h. Unreacted thionyl chloride was then evaporated under reduced pressure to afford the crude diacid chloride (85). To a solution of the diacid chloride (85) in dichloromethane (20 ml), 3-nitrophenol (6.5 g, 46.5 mmol) was added in one portion and triethylamine (3.1 g, 31 mmol) was then added dropwise over 10 min. The resultant solution was left to stir at room temperature for 3 h after which time the diester (86) had formed. The solvent was removed under reduced pressure and the remaining residue redissolved in dichloromethane (10 ml). Tlc (55% hexane in dichloromethane) of the filtrate showed the presence of the *title compound* (R_f 0.45), 3-nitrophenol (R_f 0.05) and 3-nitrophenyl 2-phenylpropionate (R_f 0.65). Silica gel chromatography (dichloromethane/hexane 6:4) of the filtrate, yielded 2.1 g (31%) of the *title compound*, as a yellow/olive green coloured oil.

ν_{max} (neat) 1762 cm^{-1} (ester C=O). FAB-MS M+H 436. Anal. calcd. for $C_{22}H_{16}N_2O_8$: C, 60.55; H, 3.67; N, 6.42 Found: C, 60.42; H, 3.74; N, 6.22%. ^1H nmr (300 MHz, $CDCl_3$) δ_H 2.27, s, 3H, -C-$\underline{C}H_3$; 7.5-8.2, m, 13H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, $CDCl_3$) δ_C 21.8, -C-$\underline{C}H_3$; 59.1, -$\underline{C}$-CH_3; 117.0, 121.4, 127.2, 127.6, 128.8, 128.9, 130.3, 138, 139.9, 150.7, Ar; 169.0, ester C=O.

Ethyl 3-nitrophenyl methyl-(4-(2-methylpropyl)-phenyl)-malonate (90).

To a solution of dry 2-(4-(2-methylpropyl)-phenyl)-propionic acid (77) (5.0 g, 24.3 mmol) in dry tetrahydrofuran (THF) (250 ml) under nitrogen at 0°C, a solution of lithium diisopropylamide (LDA) (53.5 mmol) in THF (50 ml) was added dropwise over 15 min at 0°C. The resultant solution was left to stir for 20 min.

Ethyl chloroformate (2.8 g, 25.9 mmol) was added and the mixture was then allowed to stir for a further 30 min at room temperature. The solvent was then removed under reduced pressure to afford an orange residue. Water (100 ml) was added to the residue and the mixture was acidified with concentrated hydrochloric acid at 0°C. That solution was extracted with dichloromethane (3 x 150 ml) and the combined organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to give the crude mixed ester-acid (88) as a yellow oil. The crude ester-acid (88) was added to thionyl chloride (20 g, 202 mmol) dropwise over 10 min. The solution was then left to stir at 40°C for 3 h. Unreacted thionyl chloride was removed under vacuum to give the crude acid chloride (89) as a brown-golden coloured oil. To a solution of the acid chloride (89) in dichloromethane (50 ml), 3-nitrophenol (5.0 g, 35.9 mmol) was added in one portion. Triethylamine (3.6 g, 35.6 mmol) was then added dropwise over a period of 15 min. The resultant solution was left to stir for 3 h at room temperature. The solvent was then removed under vacuum leaving a dark brown-golden coloured residue which was redissolved in dichloromethane (10 ml) and filtered. Tlc (40% hexane in dichloromethane) on the filtrate showed the presence of the *title compound* (R_f 0.6), 3-nitrophenol (R_f 0.1) and 3-nitrophenyl 2-(4-(2-methylpropyl)-phenyl)-propionate (59) (R_f 0.75). The filtrate was then subjected to silica gel chromatography (dichloromethane/hexane, 6:4, v/v) to yield 2.2 g (23%) of the *title compound*, as a yellow oil.

ν_{max} (neat) 1740 (ester C=O), 1765 (ester C=O) cm^{-1}. FAB-MS M+H 400. Anal. calcd. for $C_{22}H_{16}N_2O_8$: C, 66.17; H, 6.27; N, 3.51. Found: C, 66.13; H, 6.23; N, 3.46%. ^1H nmr (300 MHz, $CDCl_3$) δ_H 0.90, d, J=6.5 Hz, 6H, -CH($C\underline{H}_3$)$_2$; 1.33, t, J=7.2 Hz, 3H, -$OCH_2C\underline{H}_3$; 1.6, m, 1H, -$C\underline{H}(CH_3)_2$; 2.01, s, 3H, -C-$C\underline{H}_3$; 2.48, d, J=7.2 Hz, 2H, -$C\underline{H}_2CH(CH_3)_2$; 4.33, q, J=6.9 Hz, 2H, -$OC\underline{H}_2CH_3$; 7.17 and 7.36, ABq, J=8.4 Hz, 4H, Ar-$\underline{H}$; 7.4-8.1, m, 4H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, $CDCl_3$) δ_C 21.2, 14.1, 22.4, -CH_2-CH($\underline{C}H_3$)$_2$, -$OCH_2\underline{C}H_3$, -C-$\underline{C}H_3$; 30.1, CH_2-$\underline{C}H(CH_3)_2$; 45.0, $\underline{C}H_2CH(CH_3)_2$;

58.7, 62.2, -<u>C</u>-CH_3, -O<u>C</u>H_2CH_3; 117.1, 121, 127.1, 127.8, 129.2, 130.1, 134.4, 141.7, 148.7, 151.1, Ar; 169.9, ester C=O; 171.0, ester C=O.

6^A-Deoxy-6^A-(2-(3-nitrophenoxycarbonyl)-2-phenylpropionamido)-β-cyclodextrin (91).

To a solution of the diester (86) (2.18 g, 5 mmol) in pyridine (8 ml), 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (700 mg, 0.62 mmol) was added in three portions over a period of 1 h. The resultant solution was left to stir for a further 3 h at room temperature. The reaction contents were then added dropwise to diethyl ether (30 ml) with vigorous stirring. The resultant precipitate was isolated and washed with diethyl ether (50 ml) and then dried under high vacuum for 24 h to give 660 mg (74%) of the *title compound* as a white powder.

ν_{max} (nujol mull) 1658 (amide C=O), 1712 (ester C=O) cm^{-1}. FAB-MS M+H 1432. Anal. calcd. for $C_{58}H_{82}N_2O_{39}.3H_2O$: C, 46.90; H, 5.93; N, 1.89. Found: C, 47.28; H, 6.23; N, 2.00%. ^{1}H nmr (300 MHz, DMSO) δ_H 1.97, s, 3H, -C-C<u>H</u>$_3$; 3.2-3.6, 4.4-4.5, 4.8-4.9, 5.6-5.8, m, 70H; 7.3-8.4, m, 9H, Ar-<u>H</u>. ^{13}C nmr (75.8 MHz, DMSO) δ_C 22.0, -C-<u>C</u>H_3; 102.5, C-1^{A-G}; 81.8, C-4^{A-G}; 73.5-72.6, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 60.3, C-6^{B-G}; 117.0-151.4, Ar; 171.1, C=O; 171.8, C=O.

6^A-Deoxy-6^A-(2-ethoxycarbonyl-2-(4-(2-methylpropyl)-phenyl)-propionamido)-β-cyclodextrin (92).

To a solution of the diester (90) (1.24 g, 3.1 mmol) in pyridine (7 ml), 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (700 mg, 0.62 mmol) was added. The resultant solution was left to stir for a further 3 h at room temperature. The reaction

contents were added dropwise to diethyl ether (35 ml) with vigorous stirring. The resultant precipitate was isolated and washed with diethylether (50 ml) and acetone (50 ml). The solid was then redissolved in water (15 ml) and loaded on to a column containing a Sephadex cation exchange resin. The column was eluted with water untill all of the product was collected. The combined fractions containing the product was evaporated under vacuum to dryness and the remaining solid was further dried over phosphorus pentoxide under vacuum for 48 h to give 534 mg (62%) of the *title compound* as a white crystalline solid.

HPLC (Solvent system A) t_R 0.59 relative to β-CD-6-NH_2 (29) (21.8 min). ν_{max} (nujol mull) 1656 (amide C=O), 1712 (ester C=O) cm^{-1}. FAB-MS M+Na 1417. Anal. calcd. for $C_{58}H_{91}NO_{37}.3H_2O$: C, 48.07; H, 6.70; N, 1.04. Found: C, 48.04; H, 7.04; N, 0.89%. ^1H nmr (300 MHz, DMSO) δ_H 0.8, m, 6H, $-CH_2CH(C\underline{H}_3)_2$, diastereomers; 1.21, t, J=7.3 Hz, 3H, $-OCH_2C\underline{H}_3$; 1.72, s, 3H, $-C-C\underline{H}_3$, diastereomer (1), 1.75, s, 3H, $-C-C\underline{H}_3$, diastereomer (2), diastereomer ratio, 1:1; 2.0, m, 1H, $-CH_2C\underline{H}(CH_3)_2$; 2.5, m, 2H, $-C\underline{H}_2CH(CH_3)_2$, diastereomers; 3.2-3.7, 4.8-5.0, m, 70H; 4.20, q, J=7.0 Hz, 2H, $-OC\underline{H}_2CH_3$; 7.13 and 7.18, ABq, J=7.9 Hz, 4H, Ar-H, diastereomer (1); 7.16 and 7.21, ABq, J=8.5 Hz, 4H, Ar-H, diastereomer (2), diastereomer ratio, 1:1; ^{13}C nmr (75.8 MHz, DMSO) δ_C 21.4, 22.0, 44.0, 29.4, 58.6, 59.6, $-C-\underline{C}H_3$, $-\underline{C}-CH_3$, $-CH_2CH(\underline{C}H_3)_2$, $-\underline{C}H_2CH(CH_3)_2$, $-CH_2\underline{C}H(CH_3)_2$, $-O\underline{C}H_2CH_3$, $-OCH_2\underline{C}H_3$, 101.9, C-1^{A-G}; 81.3, C-4^{A-G}; 72.9-71.8, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 60.8, C-6^{B-G}; 126.7, 128.5, 136.6, 140.2, Ar; 171.2, C=O, diastereomer (1), 171.9, C=O, diastereomer (2), diastereomer ratio, 1:1.

_N,N'_-Bis-(6^A-deoxy-6^A-β-cyclodextrinyl)-urea (69).

To a stirring solution of 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (500 mg, 0.44 mmol) in pyridine/water (10 ml, 6:4) diphenyl carbonate (97) (30 mg, 0.14 mmol)

was added in two portions over a period of 1 h at room temperature. The mixture was then left to stir for an additional 4 h at 100°C. The resultant solution was evaporated to dryness under reduced pressure. The remaining solid was redissolved in pyridine (5 ml) and the solution was added dropwise to diethyl ether (30 ml) with vigorous stirring. The precipitate which formed was isolated and washed with diethyl ether (30 ml) and acetone (30 ml). The solid was then dissolved in water/methanol (10% methanol in water, 15 ml) and the resultant solution was loaded on to a column containing Sephadex cation exchange resin. The column was eluted using the same solvent system until all the product was collected. Evaporation under vacuum to dryness of the eluant containing the product and further drying of the remaining solid over phosphorus pentoxide under vacuum for 24 h afforded 170 mg (53%) of the *title compound* as a white solid.

HPLC (Solvent system B) t_R 1.77 relative to β-(CD) (1) (4.6 min). ν_{max} (nujol mull) 1658 cm^{-1} (amide C=O). FAB-MS M+H 2293. Anal. calcd. for $C_{85}H_{140}N_2O_{69}.6H_2O$: C, 42.46; H, 6.33; N, 1.17. Found: C, 42.35; H, 6.35; N, 1.04%. ^1H nmr (300 MHz, D_2O) δ_H 3.4-3.6, 3.8-3.9, 5.0, m, 140H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 103.8, C-1^{A-G}; 87.6-83.0, C-4^{A-G}; 75.0-72.7, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 62.2, C-6^{B-G}; 42.3, C-6^A; 162.3, amide C=O.

Bis-3-nitrophenyl malonate (99).

An anhydrous mixture of malonic acid (1.0 g, 9.62 mmol) and thionyl chloride (10.0 g, 101 mmol) was left to stir at reflux for 5 h. Unreacted thionyl chloride was removed by evaporation under reduced pressure to give a tarry black residue. Distillation of the residue yielded 0.89 g (66%) of malonyl dichloride (98) as a golden coloured liquid. To a solution of malonyl dichloride (98) (0.89 g,

6.36 mmol) in dichloromethane (50 ml), 3-nitrophenol (3.0 g, 21.7 mmol) was added in one portion and then triethylamine (2.2 g, 21.8 mmol) dropwise over a period of 10 min. The reaction mixture was left to stir for an additional 2 h at room temperature. Tlc (5% hexane in dichloromethane) on the reaction mixture showed the presence of the *title compound* (R_f 0.95) and 3-nitrophenol (R_f 0.45). Evaporation of the solvent under vacuum gave a tan-golden coloured solid which was redissolved in dichloromethane (10 ml). The solution was filtered and the filtrate was subsequently chromatographed (dichloromethane/hexane, 19:1) to yield 2.30 g (69%) of the *title compound* as a white powder, m.p. 126-128°C (lit.[106] 128-129°C).

^{1}H nmr (60 MHz, $CDCl_3$) δ_H 4.10, s, 2H, -C<u>H</u>$_2$-; 7.6-8.3, m, 8H, Ar-<u>H</u>.

N,N'-Bis-(6^A-deoxy-6^A-β-cyclodextrinyl)-malonamide (70).

To a stirred solution of 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (500 mg, 0.44 mmol) in pyridine (10 ml), bis-3-nitrophenyl malonate (99) (48 mg, 0.14 mmol) was added in two portions over a period of 1 h at room temperature. The mixture was then left to stir for an additional 48 h at room temperature. The solution was then added dropwise to a mixture of diethyl ether and acetone (1:1, 100 ml) with vigorous stirring. The precipitate which formed was isolated and washed with acetone (100 ml) and diethyl ether (100 ml) and then redissolved in water (5 ml). The solution was added dropwise to acetone (40 ml) with vigorous stirring which gave a white precipitate, which was then isolated and once again washed with acetone (50 ml) and diethyl ether (50 ml). The remaining solid was redissolved in water (20 ml) and loaded on to a column Sephadex cation exchange resin. The column was eluted with water until all the product was collected. The eluant containing the product was evaporated to dryness under

reduced pressure and the remaining solid was then further dried over phosphorus pentoxide under vacuum for 48 h to give (255 mg (78%) of the *title compound* as a colourless clear glassy solid.

HPLC (Solvent system B) t_R 1.63 relative to β-CD (1) (4.6 min). ν_{max} (nujol mull) 1658 cm^{-1} (amide C=O). FAB-MS M+H 2336. Anal. calcd. for $C_{87}H_{142}N_2O_{70}.6H_2O$: C, 42.73; H, 6.30; N, 1.15. Found: C, 42.55; H, 6.34; N, 1.40%. ^1H nmr (300 MHz, D_2O) δ_H 3.3-3.6, 3.8-4.0, 5.0, m, 140H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 103.8, C-1^{A-G}; 87.5-83.0, C-4^{A-G}; 75.0-72.0, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 62.2, C-6^{B-G}; 42.5, C-6^A; 171.3, amide C=O.

Bis-3-nitrophenyl succinate (102).

To a stirred solution of succinyl dichloride (100) (5.0 g, 32.5 mmol) in dichloromethane (100 ml), 3-nitrophenol (10.0 g, 72.5 mmol) was added in one portion and then triethylamine (7.32 g, 72.5 mmol) dropwise over a period of 15 min. The resultant mixture was left to stir at room temperature for a further 1 h. The reaction contents were then washed with water (2 x 100 ml) and the organic layer was separated and dried over $MgSO_4$. Tlc (10% hexane in dichloromethane) on the organic extract showed the presence of the *title compound* (R_f 0.9) and 3-nitrophenol (R_f 0.4). Evaporation of the solvent under reduced pressure gave the crude diester (102) as a tan-golden coloured solid which was purified by recrystallization from ethyl acetate to give 6.35 g (54%) of the *title compound* as a cream coloured flaky powder, m.p. 154-155°C (lit.[94] 153-154°C).

N,N'-Bis-(3^A-deoxy-3^A-($2^AS,3^AS$)-β-cyclodextrinyl)-succinamide (104).

The *title compound* was prepared and purified using the procedures employed for the synthesis of N,N'-bis-(6^A-deoxy-6^A-β-cyclodextrinyl)-malonamide (70). Reaction of 3^A-amino-3^A-deoxy-($2^AS,3^AS$)-β-cyclodextrin (30) (500 mg, 0.44 mmol) with bis-3-nitrophenyl succinate (102) (46.8 mg, 0.13 mmol) in pyridine (10 ml) for 48 h at room temperature yielded 145 mg (47%) of the *title compound* as an off white glassy solid after Sephadex chromatography.

HPLC (Solvent system B) t_R 1.83 relative to β-CD (1) (4.6 min). ν_{max} (nujol mull) 1662 cm^{-1} (amide C=O). FAB-MS M+H 2350. ^1H nmr (300 MHz, D_2O) δ_H 2.6, m, 4H, -($C\underline{H}_2$)$_2$-; 3.5-3.8, 4.1, 4.8-5.1, m, 140H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 105.8-103.2, C-1^{A-G}; 83.1-82.0, C-4^{A-G}; 75.2-71.9, C-2^{A-G}, C-3^{B-G}, C-5^{A-G}; 62.3-61.7, C-6^{A-G}; 53.0, C-3^A; 32.3, -($\underline{C}H_2$)$_2$; 176.9, amide C=O.

Bis-3-nitrophenyl oxalate (103).

To a solution of oxalyl chloride (101) (1.0 g, 7.94 mmol) in dichloromethane (20 ml), 3-nitrophenol (2.5 g, 18.1 mmol) was added in one portion, followed by triethylamine (1.83 g, 18.1 mmol) dropwise over 20 min. The resultant suspension was left to stir at room temperature for 30 min. The precipitate which formed was isolated and washed with ethyl acetate (15 ml). The crude product was then recrystallized from nitrobenzene to give 1.1 g (41%) of the *title compound* as a white powder, m.p. 224-226°C (lit.[107] 226-227°C).

N,N'-Bis-(3^A-deoxy-3^A-(2^A*S*,3^A*S*)-β-cyclodextrinyl)-oxalamide (105).

The *title compound* was prepared and purified using the procedures employed for the synthesis of N,N'-bis-(6^A-deoxy-6^A-β-cyclodextrinyl)-malonamide (70). Reaction of 3^A-amino-3^A-deoxy-(2^A*S*,3^A*S*)-β-cyclodextrin (30) (600 mg, 0.53 mmol) with bis-3-nitrophenyl oxalate (103) (59.8 mg, 0.18 mmol) in pyridine (10 ml) for 48 h at room temperature yielded 120 mg (29%) of the *title compound* as a colourless clear glassy solid after Sephadex chromatography.

HPLC (Solvent system B) t_R 1.85 relative to β-CD (1) (4.6 min). ν_{max} (nujol mull) 1660 cm^{-1} (amide C=O). FAB-MS M+H 2322. Anal. calcd. for $C_{86}H_{140}N_2O_{70}.10H_2O$: C, 41.26; H, 6.40; N, 1.12. Found: C, 41.40; H, 6.33; N, 1.16%. ^{1}H nmr (300 MHz, D_2O) δ_H 3.6-3.9, 5.0, m, 140H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 105.2-103.4, C-1^{A-G}; 83.1-82.9, C-4^{A-G}; 75.1-73.5, C-2^{A-G}, C-3^{B-G}, C-5^{A-G}; 62.3, C-6^{A-G}; 54.0, C-3^A; 162.6, amide C=O.

6^A-Deoxy-6^A-(3-(3-nitrophenoxycarbonyl)-propionamido)-β-cyclodextrin (106).[96]

To a solution of bis-3-nitrophenyl succinate (102) (800 mg, 2.2 mmol) in pyridine (10 ml), 6^A-amino-6^A-deoxy-β-cyclodextrin (29) (500 mg, 0.44 mmol) was added in three portions over a period of 45 min. The resultant solution was then left to stir for a further 3 h at room temperature. The reaction mixture was then added dropwise to diethyl ether (100 ml) with vigorous stirring. The precipitate which formed was isolated and washed with acetone (50 ml) and then diethyl ether (100 ml). The solid was further dried under high vacuum for 72 h to afford 455 mg (76.3%) of the *title compound* as a cream coloured powder.

Tlc R_f 1.55 relative to β-CD (1) (0.5). ν_{max} (nujol mull) 1660 (amide C=O), 1712 (ester C=O) cm^{-1}. FAB-MS M+H 1356. ^1H nmr (300 MHz, DMSO) δ_H 2.8, m, 4H, -($C\underline{H}_2$)$_2$; 3.4-3.6, 4.5, 4.8, 5.7, m, 78H; 7.7-8.4, m, 4H, Ar-$\underline{H}$. ^{13}C nmr (75.8 MHz, DMSO) δ_C 106.7, C-1^{A-G}; 88.3-86.2, C-4^{A-G}; 77.8-74.6, C-2^{A-G}, C-3^{A-G}, C-5^{A-G}; 64.7, C-6^{B-G}; 34.4, 34.0, -(CH_2)$_2$; 121.7, 122.3, 125.3, 125.7, 133.5, 135.8, 153, 155.6, Ar; 175.6, C=O; 176.0, C=O.

N-(3^A-Deoxy-3^A-(2^AS,3^AS)-β-cyclodextrinyl)-N'-(6^A-deoxy-6^A-β-cyclodextrinyl)-succinamide (107).

To a solution of 3^A-amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin (30) (500 mg, 0.44 mmol) in pyridine (8 ml), 6^A-deoxy-6^A-(3-(3-nitrophenoxycarbonyl)-propionamido)-β-cyclodextrin (106) (365 mg, 0.27 mmol) was added in two portions over a period of 1 h. The resultant solution was left to stir for a further 48 h at room temperature. The mixture was then added dropwise to diethyl ether (80 ml) with vigorous stirring. The precipitate which formed was isolated and washed with acetone (100 ml) and diethyl ether (80 ml). The crude material was purified and dried as described for N,N'-bis-(6^A-deoxy-6^A-β-cyclodextrinyl)-malonamide (70) to yield 205 mg (32.3%) of the *title compound* as a colourless clear glassy solid.

HPLC t_R 1.96 relative to β-CD (1) (4.6 min). ν_{max} (nujol mull) 1650 cm^{-1} (amide C=O). FAB-MS M+H 2350. Anal. calcd. for $C_{88}H_{144}N_2O_{70}.12H_2O$: C, 41.17; H, 6.55; N, 1.09. Found: C, 41.45; H, 6.67; N, 1.13%. ^1H nmr (300 MHz, D_2O) δ_H 2.60, m, 4H, -($C\underline{H}_2$)$_2$; 3.6-3.8, 5.0, m, 140H. ^{13}C nmr (75.8 MHz, D_2O) δ_C 105.8-103.1, C'-1^{A-G}, C-1^{A-G}; 84.9-81.9, C'-4^{A-G}, C-4^{A-G}; 75.2-71.9, C'-2^{A-G}, C-2^{A-G}, C'-3^{B-G}, C-3^{A-G}, C'-5^{A-G}, C-5^{A-G}; 62.3-61.7, C'-6^{A-G}, C-6^{B-G}; 53.0, C'-3^A; 41.9, C-6^A; 33.1, 33.0, -($C\dot{H}_2$)$_2$; 176.7, 176.9, amide C=O.

Complexation of the guests (36)-(39) with β-CD (1) and the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (29).

^{19}F nmr samples

Solutions for complexation studies were prepared in 5 mm tubes, using 0.2 mol dm^{-3} phosphate buffer (pH 6.0) in 10% aqueous D_2O, and contained one of the fluorinated compounds (36)-(39) (*ca.* 1 mmol dm^{-3}) and either β-CD (1), β-CD-6-NH_2 (29) or β-CD-3-NH_2 (30), (*ca.* 1-12 mmol dm^{-3}).

Phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2)

Stock A; Potassium dihydrogenphosphate (KH_2PO_4) (68.046 g) was dissolved in water, and the total volume was made up to 1 L (0.5 mol dm^{-3}) with water.

Stock B; Disodium hydrogenphosphate (Na_2HPO_4) (35.492 g) was dissolved in water, and the total volume was made up to 500 ml (0.5 mol dm^{-3}) with water.

Phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2); To stock A (265 ml), stock B (44.8 ml) was added, the total volume was then made up to 1 L with water.

Preparation of stock solutions of the guests (36)-(39).

Stock (Guest 36); *Ortho*-fluorobenzoic acid (71) (31 mg) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 100 ml (2.21 mmol dm^{-3}) with the above buffer.

Stock (Guest 37); *Para*-fluorobenzoic acid (72) (29.2 mg) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 100 ml (2.09 mmol dm^{-3}) with the above buffer.

Stock (Guest 38); Methyl *ortho*-fluorobenzoate (18.8 mg) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 100 ml (1.22 mmol dm^{-3}) with the above buffer.

Stock (Guest 39); Methyl *para*-fluorobenzoate (23.2 mg) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 100 ml (1.51 mmol dm^{-3}) with the above buffer.

Preparation of stock solutions of the conjugate acids of β-CD-6-NH_2 (29) and β-CD-3-NH_2 (30).

Stock (β-CD-6-NH_3^+); β-CD-6-NH_2 (29) (0.371 g) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 10 ml (32.69 mmol dm^{-3}) with the above buffer.

Stock (β-CD-3-NH_3^+); β-CD-3-NH_2 (30) (0.375 g) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 10 ml (33.04 mmol dm^{-3}) with the above buffer.

Stock (β-CD); β-CD (1) (0.933 g) was dissolved in phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2), the total volume was then made up to 25 ml (32.9 mmol dm^{-3}) with the above buffer. Note; Due to the poor aqueous solubility of β-CD (1), Individual samples containing β-CD (1) and the guests (37)-(39) were prepared by weighing out the appropriate mass of β-CD (1).

Sample preparation

All samples were prepared by adding the appropriate guest stock solution (1 ml) to 2 ml volumetric flasks containing D_2O (0.2 ml), and then adding the required volume of the appropriate host stock solution. A total volume of 2 ml in each case was achieved by adding the necessary amount of phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2). Samples containing β-CD (1) were prepared by weighing out the appropriate mass of β-CD (1) in 2 ml volumetric flasks containing the appropriate guest stock solution (1 ml) and D_2O. A total volume of 2 ml was achieved by adding the necessary amount of phosphate buffer 0.2 mol dm^{-3} pH 6.0 (I = 0.2).

Table 9 ^{19}F Chemical shifts of the *ortho*-substituted anion (36) in the presence of increasing concentrations of β-CD (1).

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-38.867
1.865	0.001	-38.828
3.731	0.002	-38.798
5.596	0.002	-38.759
7.462	0.003	-38.733
9.327	0.004	-38.694
11.193	0.005	-38.660
13.058	0.006	-38.625
14.923	0.007	-38.595
16.789	0.007	-38.569
18.654	0.008	-38.543
20.520	0.009	-38.496
22.385	0.010	-38.481
24.251	0.011	-38.452
26.116	0.012	-38.426
27.981	0.012	-38.400
29.847	0.013	-38.396

Table 10 ^{19}F Chemical shifts of the *para*-substituted anion (37) in the presence of increasing concentrations of β-CD (1).

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-32.967
1.866	0.001	-32.988
3.732	0.002	-33.005
5.598	0.002	-33.023
7.464	0.003	-33.040
9.330	0.004	-33.053
11.196	0.005	-33.066
13.062	0.006	-33.079
14.928	0.007	-33.092
16.793	0.007	-33.105
18.659	0.008	-33.118
20.525	0.009	-33.126
22.391	0.010	-33.135
24.257	0.011	-33.144
26.123	0.012	-33.152
27.989	0.012	-33.161
29.855	0.013	-33.174

Table 11 ^{19}F Chemical shifts of the *ortho*-substituted ester (38) in the presence of increasing concentrations of β-CD (1).

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-34.834
2.000	0.001	-34.497
4.300	0.002	-34.272
5.000	0.002	-34.151
6.800	0.003	-34.043
9.400	0.004	-33.896
10.500	0.005	-33.822
13.100	0.006	-33.753
15.400	0.007	-33.645
16.700	0.007	-33.580
19.000	0.008	-33.559
20.800	0.009	-33.507
22.100	0.010	-33.503
23.800	0.010	-33.451
26.800	0.012	-33.399
28.900	0.013	-33.373

Table 12 ^{19}F Chemical shifts of the *para*-substituted ester (39) in the presence of increasing concentrations of β-CD (1).

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-28.510
2.400	0.001	-28.393
4.000	0.002	-28.328
5.700	0.003	-28.280
7.400	0.003	-28.229
10.000	0.004	-28.194
11.000	0.005	-28.168
12.600	0.006	-28.146
14.500	0.006	-28.121
16.800	0.007	-28.090
18.900	0.008	-28.073
20.500	0.009	-28.056
22.800	0.010	-28.047
24.700	0.011	-28.038
26.400	0.012	-28.025
28.800	0.013	-28.008

Table 13 ^{19}F Chemical shifts of the *ortho*-substituted anion (36) in the presence of increasing concentrations of β-CD-6-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-38.867
1.865	0.001	-38.794
3.731	0.002	-38.720
5.596	0.002	-38.660
7.462	0.003	-38.599
9.327	0.004	-38.556
11.193	0.005	-38.504
13.058	0.006	-38.457
14.923	0.007	-38.413
16.789	0.007	-38.383
18.654	0.008	-38.331
20.520	0.009	-38.301
22.385	0.010	-38.266
24.251	0.011	-38.240
26.116	0.012	-38.210
27.981	0.012	-38.189

Table 14 ^{19}F Chemical shifts of the *para*-substituted anion (37) in the presence of increasing concentrations of β-CD-6-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-32.971
1.865	0.001	-32.988
3.731	0.002	-33.005
5.596	0.002	-33.023
7.462	0.003	-33.040
9.327	0.004	-33.053
11.193	0.005	-33.062
13.058	0.006	-33.070
14.923	0.007	-33.083
16.789	0.007	-33.096
18.654	0.008	-33.101
20.520	0.009	-33.109
22.385	0.010	-33.118
24.251	0.011	-33.126
26.116	0.012	-33.135
27.981	0.012	-33.139

Table 15 ^{19}F Chemical shifts of the *ortho*-substituted ester (38) in the presence of increasing concentrations of β-CD-6-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-34.825
1.860	0.001	-34.644
3.720	0.002	-34.480
5.579	0.002	-34.328
7.439	0.003	-34.250
9.299	0.004	-34.160
11.159	0.005	-34.082
14.878	0.007	-33.982
16.738	0.007	-33.896
18.598	0.008	-33.866
20.457	0.009	-33.809
22.317	0.010	-33.766
24.177	0.011	-33.745
26.037	0.011	-33.701
27.896	0.012	-33.650
29.800	0.013	-33.645

Table 16 ^{19}F Chemical shifts of the *para*-substituted ester (39) in the presence of increasing concentrations of β-CD-6-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-28.514
1.860	0.001	-28.466
3.720	0.002	-28.406
5.579	0.002	-28.367
7.439	0.003	-28.337
9.299	0.004	-28.311
11.159	0.005	-28.289
13.018	0.006	-28.268
14.878	0.007	-28.237
16.738	0.007	-28.229
18.598	0.008	-28.207
22.317	0.010	-28.177
24.177	0.011	-28.168
26.037	0.011	-28.146
27.896	0.012	-28.142
29.756	0.013	-28.133

Table 17 ^{19}F Chemical shifts of the *ortho*-substituted anion (36) in the presence of increasing concentrations of β-CD-3-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-38.880
1.860	0.001	-38.824
3.720	0.002	-38.789
5.579	0.002	-38.755
7.439	0.003	-38.720
9.299	0.004	-38.686
11.159	0.005	-38.647
13.018	0.006	-38.617
14.878	0.007	-38.591
16.738	0.007	-38.560
18.598	0.008	-38.534
20.457	0.009	-38.521
22.317	0.010	-38.478
24.177	0.011	-38.448
26.037	0.011	-38.426
27.896	0.012	-38.400
29.756	0.013	-38.374

Table 18 ^{19}F Chemical shifts of the *para*-substituted anion (37) in the presence of increasing concentrations of β-CD-3-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-32.971
1.866	0.001	-32.979
3.720	0.002	-32.992
5.579	0.002	-32.997
7.439	0.003	-33.010
9.299	0.004	-33.027
11.159	0.005	-33.036
13.018	0.006	-33.040
16.738	0.007	-33.062
18.598	0.008	-33.070
20.457	0.009	-33.079
22.317	0.010	-33.096
24.177	0.011	-33.096
26.037	0.011	-33.105
27.896	0.012	-33.109
29.756	0.013	-33.118

Table 19 ^{19}F Chemical shifts of the *ortho*-substituted ester (38) in the presence of increasing concentrations of β-CD-3-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-34.817
1.860	0.001	-34.691
3.720	0.002	-34.579
5.579	0.002	-34.497
7.439	0.003	-34.406
9.299	0.004	-34.320
11.159	0.005	-34.246
13.018	0.006	-34.186
14.878	0.007	-34.116
16.738	0.007	-34.056
18.598	0.008	-34.004
20.457	0.009	-33.948
22.317	0.010	-33.905
24.177	0.011	-33.857
26.037	0.011	-33.827
27.896	0.012	-33.762

Table 20 ^{19}F Chemical shifts of the *para*-substituted ester (39) in the presence of increasing concentrations of β-CD-3-NH_3^+.

Mass of host (mg)	Concentration of host (mol dm^{-3})	Chemical shift δ_{obs} (ppm)
0.000	0.000	-28.514
1.860	0.001	-28.471
3.720	0.002	-28.436
5.579	0.002	-28.406
7.439	0.003	-28.376
9.299	0.004	-28.350
11.159	0.005	-28.319
13.018	0.006	-28.298
14.878	0.007	-28.272
16.738	0.007	-28.255
18.598	0.008	-28.233
20.457	0.009	-28.216
22.317	0.010	-28.198
24.177	0.011	-28.181
26.037	0.011	-28.164
27.896	0.012	-28.151
29.756	0.013	-28.133

Complexation of the linked cyclodextrins (69), (70), (104), (105) and (107) with TNS (4).

Phosphate buffer pH 6.9 (I = 0.1).

Di-sodium hydrogenphosphate (Na_2HPO_4) (3.525 g) and potassium dihydrogenphospate (KH_2PO_4) (3.412 g) were dissolved in water. The total volume was then made up to 1 L by further addition of water.

TNS (4) stock solution (5×10^{-5} mol dm^{-3}).

Sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (8.40 mg) was dissolved in water. A total volume of 500 ml (5.01×10^{-5} mol dm^{-3}) was achieved by further addition of water.

Host stock solutions.

Stock urea (69); *N,N'*-Bis-(6^{A}-deoxy-6^{A}-β-cyclodextrinyl)-urea (95 mg) was dissolved in phosphate buffer pH 6.9 (I = 0.1). A total volume of 20 ml (2.07×10^{-3} mol dm^{-3}) was then achieved by further addition of the above buffer.

Stock malonamide (70); *N,N'*-Bis-(6^{A}-deoxy-6^{A}-β-cyclodextrinyl)-malonamide (100 mg) was dissolved in phosphate buffer pH 6.9 (I = 0.1). A total volume of 20 ml (2.14×10^{-3} mol dm^{-3}) was then achieved by further addition of the above buffer.

Stock succinamide (104) #1; *N,N'*-bis-(3^{A}-deoxy-3^{A}-(2^{A}S,3^{A}S)-β-cyclodextrinyl)-succinamide (93 mg) was dissolved in phosphate buffer pH 6.9 (I = 0.1). A total volume of 25 ml (1.58 x 10^{-3} mol dm^{-3}) was then achieved by further addition of the above buffer.

Stock succinamide (104) #2; Stock succinamide (104) #1 (1 ml) was made up to 10 ml (1.58 x 10^{-4} mol dm^{-3}) by the addition of phosphate buffer pH 6.9 (I = 0.1).

Stock oxalamide (105) #1; *N, N'*-bis-(3^{A}-deoxy-3^{A}-(2^{A}S,3^{A}S)-β-cyclodextrinyl)-oxalamide (90 mg) was dissolved in phosphate buffer pH 6.9 (I = 0.1). A total volume of 25 ml (1.55 x 10^{-3} mol dm^{-3}) was then achieved by further addition of the above buffer.

Stock oxalamide (105) #2; Stock oxalamide (105) #1 (1 ml) was made up to 10 ml (1.55 x 10^{-4} mol dm^{-3}) by the addition of phosphate buffer pH 6.9 (I = 0.1).

Stock succinamide (107) #1; *N'*-(6^{A}-deoxy-6^{A}-β-cyclodextrinyl)-*N*-(3^{A}-deoxy-3^{A}-(2^{A}S,3^{A}S)-β-cyclodextrinyl)-succinamide (150 mg) was dissolved in phosphate buffer pH 6.9 (I = 0.1). A total volume of 50 ml (1.28 x 10^{-3} mol dm^{-3}) was then achieved by further addition of the above buffer.

Stock succinamide (107) #2; Stock succinamide (107) #1 (2 ml) was made up to 20 ml (1.28 x 10^{-4} mol dm^{-3}) by the addition of phosphate buffer pH 6.9 (I = 0.1).

The inclusion of TNS (4) and sample preparation.

The complexation of TNS (4) with the linked cyclodextrins (69), (70), (104), (105) and (107) in aqueous phosphate buffer at pH 6.9 and ionic strength 0.1 mol dm^{-3}

was studied by monitoring the increase in fluorescence of solutions containing a standard TNS (4) concentration (1×10^{-6} mol dm^{-3}) and with increasing concentrations of cyclodextrin over the range ($0 - 1.0 \times 10^{-3}$ mol dm^{-3}). Each sample solution was prepared by adding the guest (4) stock solution (0.1 ml) to a 5 ml volumetric flask containing the required volume of the appropriate cyclodextrin stock solution. A total volume of 5 ml in each case was achieved by adding the necessary amount of phosphate buffer pH 6.9 ($I = 0.1$).

Table 21 The complexation of the urea linked cyclodextrin (69) and TNS (4).

Volume of stock (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 435 nm
0.01	4.14×10^{-6}	89
0.02	8.28×10^{-6}	159
0.03	1.24×10^{-5}	232
0.05	2.07×10^{-5}	298
0.1	4.14×10^{-5}	396
0.15	6.21×10^{-5}	455
0.20	8.28×10^{-5}	456
0.25	1.04×10^{-4}	470
0.30	1.24×10^{-4}	499
0.40	1.66×10^{-4}	506
0.50	2.07×10^{-4}	513
0.60	2.48×10^{-4}	527
0.90	3.73×10^{-4}	544
1.10	4.55×10^{-4}	544
1.40	5.80×10^{-4}	556
1.70	7.45×10^{-4}	558
2.20	9.11×10^{-4}	563
2.30	9.52×10^{-4}	575

Table 22 The complexation of the malonamide linked cyclodextrin (70) and TNS (4).

Volume of stock (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 430 nm
0.02	8.56×10^{-6}	26
0.05	2.14×10^{-5}	49
0.08	3.42×10^{-5}	69
0.12	5.14×10^{-5}	95
0.15	6.42×10^{-5}	108
0.20	8.56×10^{-5}	128
0.30	1.28×10^{-4}	135
0.40	1.71×10^{-4}	147
0.50	2.14×10^{-4}	159
0.60	2.57×10^{-4}	163
0.70	3.00×10^{-4}	168
0.80	3.42×10^{-4}	172
0.90	3.85×10^{-4}	166
1.10	4.71×10^{-4}	186
1.30	5.56×10^{-4}	190
1.50	6.42×10^{-4}	195
1.70	7.28×10^{-4}	197
1.90	8.13×10^{-4}	210
2.10	8.99×10^{-4}	205

Table 23 The complexation of the succinamide linked cyclodextrin (104) with TNS (4)

Volume of stock #2 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 428 nm
0.08	2.53×10^{-6}	8
0.12	3.79×10^{-6}	15
0.15	4.74×10^{-6}	20
0.18	5.69×10^{-6}	23
0.23	7.27×10^{-6}	23
0.26	8.22×10^{-6}	29
Volume of stock #1 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 428 nm
0.06	1.90×10^{-5}	61
0.09	2.84×10^{-5}	69
0.12	3.79×10^{-5}	79
0.15	4.74×10^{-5}	101
0.18	5.69×10^{-5}	110
0.21	6.64×10^{-5}	105
0.23	7.27×10^{-5}	134
0.26	8.22×10^{-5}	122
0.59	1.83×10^{-4}	178
0.89	2.81×10^{-4}	202
1.17	3.70×10^{-4}	221
1.47	4.65×10^{-4}	238
2.64	8.34×10^{-4}	271
2.94	9.29×10^{-4}	287
3.30	1.04×10^{-3}	283
3.50	1.11×10^{-3}	281

Table 24 The complexation of the oxalamide linked cyclodextrin (105) and TNS (4).

Volume of stock #2 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 430 nm
0.08	2.48×10^{-6}	5
0.12	3.72×10^{-6}	12
0.15	4.65×10^{-6}	12
0.18	5.58×10^{-6}	17
0.21	6.51×10^{-6}	15
0.23	7.13×10^{-6}	17
0.26	8.06×10^{-6}	16
Volume of stock #1 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 430 nm
0.06	1.86×10^{-5}	19
0.09	2.79×10^{-5}	27
0.12	3.72×10^{-5}	38
0.15	4.65×10^{-5}	36
0.18	5.58×10^{-5}	38
0.21	6.51×10^{-5}	47
0.23	7.13×10^{-5}	49
0.26	8.06×10^{-5}	59
0.29	8.99×10^{-5}	65
0.60	1.86×10^{-4}	106
0.90	2.79×10^{-4}	122
1.20	3.72×10^{-4}	156
1.5	4.65×10^{-4}	180
2.10	6.51×10^{-4}	202
2.60	8.06×10^{-4}	226
3.30	1.02×10^{-3}	244

Table 25 The complexation of the succinamide linked cyclodextrin (107) and TNS (4).

Volume of stock #2 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 428 nm
0.04	1.02×10^{-6}	6
0.08	2.05×10^{-6}	17
0.12	3.07×10^{-6}	15
0.16	4.10×10^{-6}	19
0.20	5.12×10^{-6}	20
0.24	6.14×10^{-6}	24
0.28	7.17×10^{-5}	28
0.38	9.73×10^{-5}	52
0.48	1.23×10^{-5}	33
0.60	1.54×10^{-5}	41
0.80	2.05×10^{-5}	31
Volume of stock #1 (ml)	Cyclodextrin (mol dm^{-3})	Fluorescence at 428 nm
0.12	3.07×10^{-5}	46
0.16	4.10×10^{-5}	65
0.20	5.12×10^{-5}	83
0.24	6.14×10^{-5}	101
0.28	7.17×10^{-5}	115
0.38	9.73×10^{-5}	151
0.48	1.23×10^{-4}	167
0.60	1.54×10^{-4}	200
0.80	2.05×10^{-4}	220
1.20	3.07×10^{-4}	233
1.60	4.10×10^{-4}	277
2.00	5.12×10^{-4}	283
2.40	6.14×10^{-4}	329

2.80	7.17×10^{-4}	348
3.20	8.19×10^{-4}	365
3.30	8.45×10^{-4}	383
3.40	8.70×10^{-4}	376
3.50	8.96×10^{-4}	374

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