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Cyclodextrin Assisted Enantiomeric Recognition of Amino Acid Imides and Toward Synthesis of Dolabellane Diterpenoid B

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CYCLODEXTRIN ASSISTED ENANTIOMERIC RECOGNITION OF AMINO ACID IMIDES AND TOWARD SYNTHESIS OF DOLABELLANE DITERPENOID B

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by

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Dedicated to:
My parents, Keshavlal and Indiraben

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

ABSTRACT

There is a strong market demand for enantiomerically pure drugs. One solution to this problem is to develop a simple methodology for transferring synthetically designed racemic drugs into optically pure ones. Many synthetic drugs are by nature amides, therefore, amino acid based models for transformation of racemates into optically pure compounds were selected for this study. Formation of self-assembly molecular aggregates of properly modified amino acids was observed with and without the presence of cyclodextrins. Cyclodextrin assisted formation of polymer-like self-assemblies and enantiomeric resolution of these amino acids were studied using 1D and 2D NMR spectroscopy, EleacroSpray Ionization Mass Spectroscopy (ESIMS), Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF-MS). The role of π-π stacking interaction between aromatic moieties in enantiomeric resolution was demonstrated by calculating association constants of this host-guest system.

Dolabellane diterpenoids share the unique feature of a *trans*-bicyclo[9.3.0]tetradecane and most of them express antimicrobial, antitumor and antiviral activities. They are primarily obtained from marine resources. Dolabellane diterpenoid **B** was isolated from the Okinawan soft coral of the genus *Clavularia* by Iguchi and co-workers. Current efforts toward the synthesis of dolabellane diterpenoid **B** is discussed along with the plans for completion of its synthesis.

I. Introduction

1.1 Cyclodextrins

The cyclodextrins (CDs), also called cycloamyloses and Schrödinger dextrins, are cyclic oligosaccharides formed from starch by the action of certain bacteria, such as *Bacillus macerans*, *Klebsiella oxytoca*, *Bacillus circulans*.[1,2] Three readily available cyclodextrins, α-CD, β-CD and γ-CD, are commonly referred to as the natural CDs. They have six, seven and eight *D*-glucopyranosyl units, respectively, connected through α–(1→4)-glycosidic bonds. In 1891 Villier[3] isolated approximately 3.0 g of a crystalline substance from ~1000 g starch and named it "cellulosine". This substance later proved to be α-cyclodextrin. Subsequently, Freudenberg and co-workers described the first purification scheme for the isolation of homogeneous and pure crystalline α-and β-CDs and postulated the cyclic structure of these CDs in 1936.[4] In 1948, γ-CD was discovered, also by the same group.[5] Subsequent structural analysis of CDs revealed that CDs with five or less glucopyranosyl units are too strained, while on the other hand, CDs with more than eight residues (for example δ-CD with nine glucose units) are structurally too flexible and therefore, prone to hydrolysis.[6] The hydrolysis rate of CDs increases in the order of α-CD <β-CD < γ-CD< δ-CD.

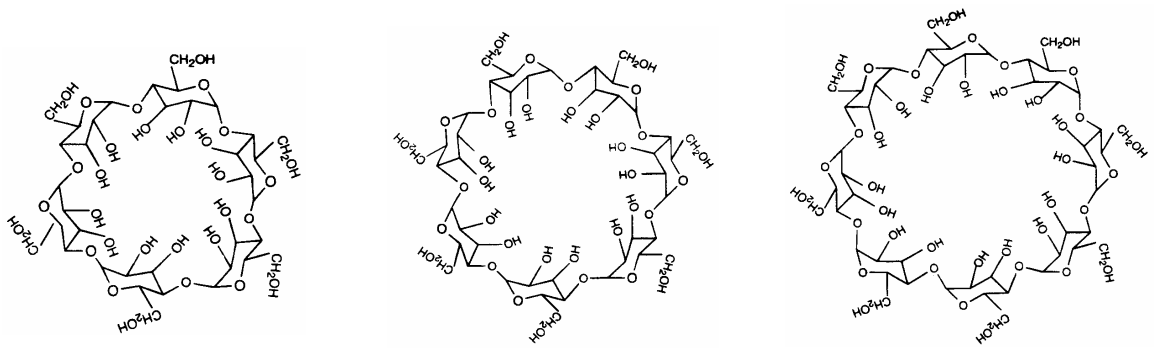


Figure 1.1 The structures of α, β and γ cyclodextrins[6]

The $^4C_1$ chair conformation of the glucose unit makes the cyclodextrin shape appear as a conical cylinder or wreath-shaped truncated cone, in which all secondary hydroxyl groups (the O(2)-H and O(3)-H)) are on one side of the ring, and the primary hydroxyl groups (O(6)-H) are situated on the other side of the ring. The inner surface of the cavity is dominated by C-3 CH groups, C-5 CH groups and glycosidic oxygens atoms and are relatively hydrophobic[7]. The primary hydroxyl rim of the cavity opening has a somewhat reduced diameter compared to the secondary hydroxyl rim, since free rotation of primary hydroxyl groups reduce the effective diameter of the cavity.[2]

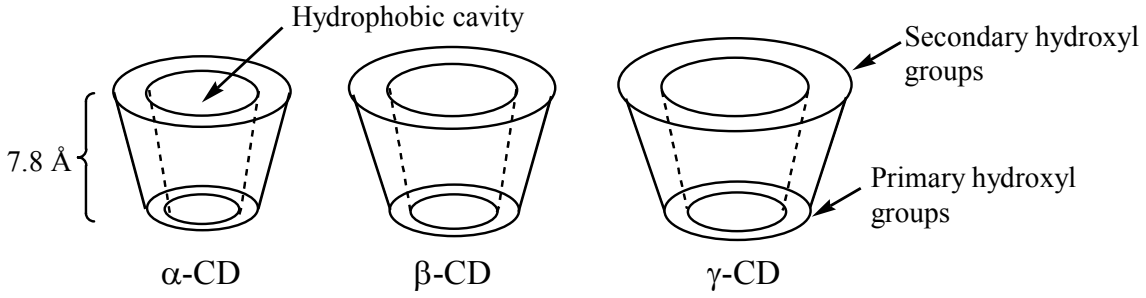


Figure 1.2 Schematic representations of α, β and γ cyclodextrins

Table 1.1 Characteristics of α, β and γ–CD

	α– CD	β–CD	γ–CD
no. of glucose units	6	7	8
mol. wt	972	1135	1297
solubility in water, g 100 mL^{-1} at room temp.	14.5	1.85	23.2
cavity diameter, Å	4.7-5.3	6.0-6.5	7.5-8.3
approximate volume of cavity, $Å^3$	174	262	427

Some of the most important characteristics of CDs are summarized in Table 1.1. β-CD is the least soluble in water compared to α- and γ-CD due to a perfect arrangement of hydrogen bonding on the CD ring and a few interactions with surrounding water molecules. During the complex formation with other molecules, cavity size plays a very important role. The cavity size of CDs increase in the order of α-CD <β-CD < γ-CD. Surprisingly, CDs with more than eight glucose units (bigger than γ-CD) have an even smaller cavity size compared to γ-CD due to the fact that their shape is not anymore conical but rather collapsed.[2]

1.2 Cyclodextrin Inclusion Complexes

The inner surface of the CD cavity is slightly hydrophobic because it is occupied by the C-3 and C-5 hydrogens. On the other hand, the major interactions in aqueous media between the surrounding water molecules and CDs occur through the primary hydroxyl group from the CD ring. In this way a hydrophobic cavity (host) is formed that can bind a substrate (guest) of the proper shape and polarity (Figure 1.3) [2]. These CD complexes containing the appropriate "guest" molecule are usually referred to as an inclusion complex.

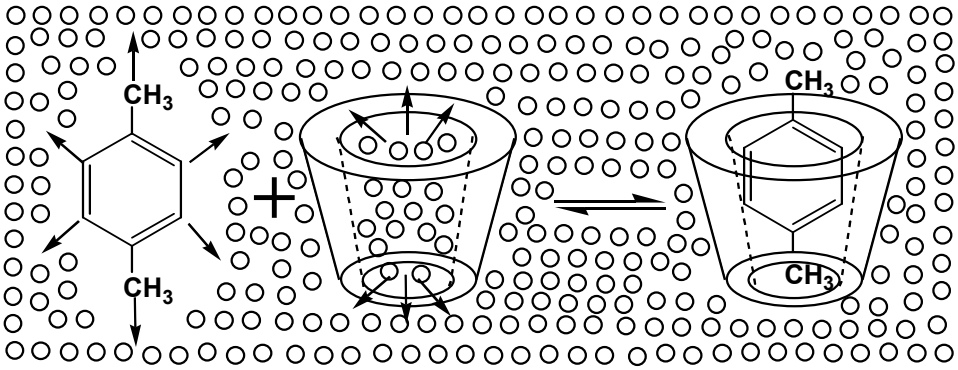


Figure 1.3 Schematic representation of a CD inclusion complex formation. *p*-Xylene is the guest molecule; the small circles represent water molecules.

CDs can form inclusion complexes with a wide variety of different guest compounds[8] including phenols,[9-11] aliphatic alcohols,[12-19] diols,[17-19] amino acids,[20-21] hydrocarbons,[12-22] aromatic amines,[23] amines and acids, [24]oligopeptides,[25] sugars, [26] azo compounds,[27-30] cyclohexanes,[31] naphthalene derivatives and other aromatic compounds, [32-35] as well as different pharmaceutical compounds.[36-37]. During the binding of these guest molecules with native or modified cyclodextrins, the hydrophobic part of the guest molecule enters the core of the cyclodextrin cavity, while the hydrophilic part, which is often charged, stays on the periphery of the primary or secondary rim of the cavity. [38] Literature reports have indicated that the most frequent the host:guest ratio observed is 1:1; however 2:1, 1:2, 2:2, or even higher order complicated associations also exist.[39-54] In fact, a ternary complex consisting of a single guest molecule complexed with two different cyclodextrins was recently reported by Giorgi and Tee. [55]

The stability of these cyclodextrin inclusion complexes can be determined by the calculation of the complex stability, or binding constant, K_a. In aqueous solution equilibrium is established between the dissociation and association of complexes, and is governed by the

thermodynamic equilibrium shown below. In this equation, (L) is *ligand* or host cyclodextrin and (S) is *substrate* or guest molecule.[6]

$$S + L \rightleftharpoons SL$$

$$SL + L \rightleftharpoons SL_2$$

$$S + SL \rightleftharpoons S_2L$$

The stepwise binding constant for these equilibriums, denoted K_{11}, K_{12} and K_{21}, are defined here.

$$K_{11} = \frac{[SL]}{[S][L]}$$

$$K_{12} = \frac{[SL_2]}{[SL][L]}$$

$$K_{21} = \frac{[S_2L]}{[S][SL]}$$

The cyclodextrin inclusion complexes studied so far have been performed using the native CDs (α-CD, β-CD and γ-CD) and many covalently modified CDs prepared from native forms.[56-61] Larger cyclodextrins having more than eight glucose units are not practical host molecules, due to the weaker driving force of the substitution of the high enthalpy water molecules with guest molecules in the CD cavity.[2]

1.3 Spectroscopic Methods used for Studying CD Inclusion Complexes

There are a number of interactions responsible for the formation of inclusion complexes between CDs and the guest molecule. These interactions include hydrogen bonding,[62,63] Van der Waals' forces,[64,65] and π–π interactions.[66,67] There are a number of different methods employed by researchers studying the formation of inclusion complexes.[68] Among them are electron-spin resonance (ESR) spectroscopy,[69,70] proton nuclear magnetic resonance (NMR) spectroscopy,[71-73]

ultraviolet (UV) and visible spectroscopy,[74-76] circular dichroism (CD) spectroscopy or optical rotatory dispersion (ORD),[77-80] Raman spectroscopy,[81] fluorescence spectroscopy,[82,83] X-ray analysis,[84-87] potentiometry,[88] positron annihilation,[89] and thermoanalytical methods,[90] electro-spray ionization mass spectroscopy (ESIMS).[91-101]

1.3.1 NMR Spectroscopy for the Study of CD Inclusion Complexes

The first report of CD inclusion complexes studied used ^{1}H NMR spectroscopy, was performed in an aqueous media, and appeared in the literature thirty years ago.[98-100] However, from that point forward,[101-103] new 2D NMR methods and high-field instruments have completely changed the way research has been applied to the study of the formation of cyclodextrin complexes. [104] For instance, during the complex formation between cyclodextrin and the guest molecule, chemical shift changes in NMR spectra of both the host and the guest molecule protons occur due to weak nonbonding interactions. [105-116] Binding constants can be calculated using these changes in chemical shifts, which ultimately gives a researcher useful information regarding the stability of the formed complex, as well as unique structural information.[105-108] Given the fact that each glucose unit of the host CD molecule has five chiral centers, CD cavities have a chiral environment. This ensures that the formation of an inclusion complex with a racemic mixture of a chiral guest molecule will ultimately form two diastereomeric inclusion complexes.[61] Again, in such instances, NMR spectroscopy has been a useful tool in the investigation of chiral recognition properties of CD cavities.[109-114] For example, from the change in chemical shift, it is possible to determine from which side of the CD cavity (the primary hydroxyl side or the wider secondary hydroxyl side) the guest insertion occurs, as well as the depth of penetration of the guest molecule insertion into the CD cavity.[106,107,114,115]

1. 4 Chirality and Cyclodextrin Recognition

A chiral molecule, defined as a molecule that is not superimposable on its mirror image, exists as a pair of stereoisomers. These two stereoisomers, or enantiomers, are structurally identical and have the same physical properties. They do differ, however, in their three-dimensional spatial arrangement, and have no planes of symmetry. The accepted designation for these sterioisomers is commonly known as *R* and *S*.

When a plane of polarized light is passed through a sample of each enantiomer, one will rotate the light to the left ((-)-enantiomer), and the other to the right ((+)-enantiomer). If the light was passed through a 50:50 mixture, no rotation would be observed and this mixture would be considered racemic and optically inactive. Classically defined, a racemic mixture is a mixture of the two enantiomers in equal proportions.

Diastereomers are stereoisomers that are not mirror images. Two diastereomers are different compounds, and have different relative stereochemistry and different physical properties.

1.5 Optical Activation

There are two general approaches used in the production of nonracemic chiral organic compounds (optical activation): [117] (i) resolution of enantiomers from the racemic solution; and (ii) asymmetric syntheses, involving chemical or enzymatic procedures using either chiral or achiral reagents. Both methods used in the preparation of enantiomerically pure compounds are very expensive and time consuming.

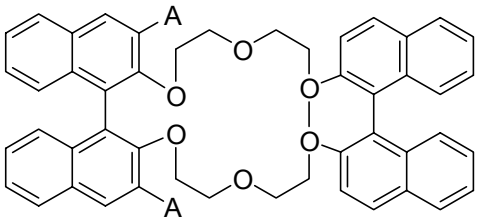
1.6 Resolution of Enantiomers

Enantiomeric resolution from racemic mixtures can be done in two ways: they include (1) the crystallization of enantiomers from racemic solutions without using resolving agents and (2) resolutions by diastereomeric complex formation.[117]

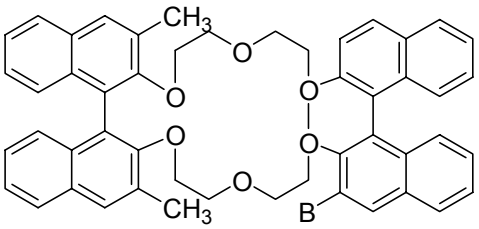
1.6.1 Diastereomeric Inclusion Complex Formation

Separation of enantiomers from racemate solution is called resolution. Enantiomers can not be resolved easily from solution because they have the same physical and chemical properties. Difference in properties can be achieved by derivatisation with some optically active compounds like cyclodextrins and form diastereomeric complexes. Separation of a racemate into a mixture of diastereomers through the formation of inclusion complexes is a very well known approach used in the resolution of compounds from a mixture. Schlenk[118] first used the inclusion method for resolution in 1965. Inclusion compounds can be divided into two broad classes: (a) the *cavitates*[119] in which the guest molecule is partially or entirely enclosed within a host molecule (e.g., cyclodextrins, crown ethers **1**-**4**) (b) *clathrates,* in which the guest molecules are surrounded by host molecules.[120] In *cavitates*, inclusion of one enantiomer of amino acid derivatives (e.g. **5**-**11**) in the cavity of racemic cyclic crown ethers[121] (e.g. **1**-**4**) leads to crystallization of only the more stable diastereomeric complex in enantiomerically pure form.[122-124] Different stability and solubility of diastereomeric complexes could lead one of the diastereomeric complexes to crystallize out selectively from the racemate solution more rapidly than others and as a result, this approach can be used for the enantiomeric resolution. The desired enantiopure guest compound can be recovered using dissolution, or extraction of the diastereomeric complex.[117]

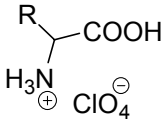
The first chiral macrocyclic compounds were reported by Cram and co-workers in 1973.[125] Subsequently, many chiral macrocyclic compounds have been synthesized and tested with respect to their ability to function as hosts of inclusion complexes with many different chiral amino acid guest compounds. Along with enantiomeric resolutions of guests, enantiomeric resolution of hosts using the chiral guest have also been observed in liquid-liquid and liquid-solid chromatography.[126-130] Chiral recognition of amino ester salts from aqueous solution to organic solvent by various macrocyclic hosts has been attempted.[131,132]



1, A= H
2, A= CH_3



3, B= H
4, B= CH_3

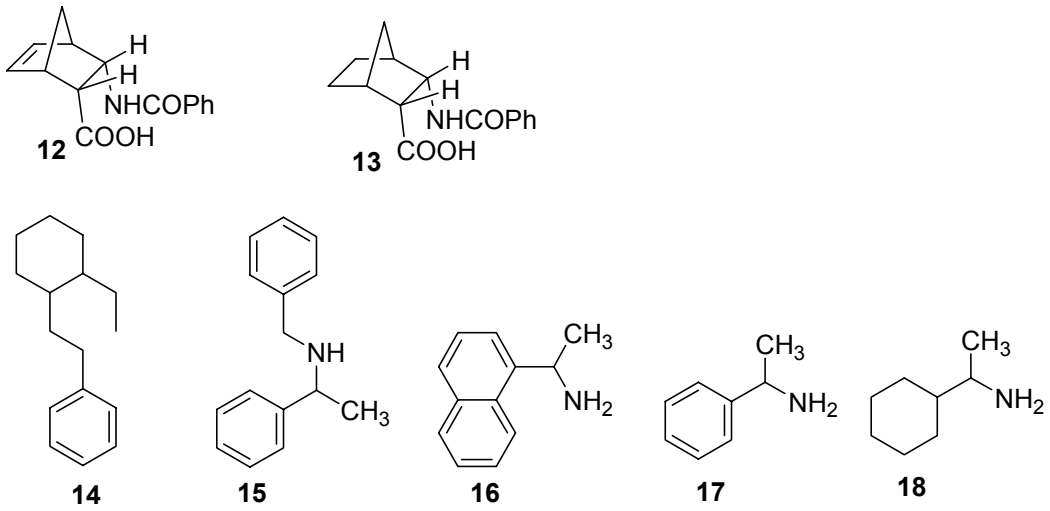


5, R= C_6H_5
6, R= *p*-HOC_6H_5
7, R= $C_6H_5CH_2$
8, R= $(CH_3)_2CH$
9, R= $C_8H_6NCH_2$
10, R= $CH_3S(CH_2)_2$
11, R= CH_3

The extent of chiral recognition has been determined for the complexation between seven chiral amino acid perchlorates (**5-11**) and four macrocyclic polyether hosts (**1-4**) containing chiral elements by Cram and coworkers.[122] It was found that host **1** formed a more stable diastereomeric complex (*RR*)(D) with the D-enantiomer of all seven amino acid perchlorate guests while hosts **2**, **3**, and **4** preferentially form stronger diastereomeric inclusion complexes

(*SS*)(L) with the L-enantiomer. Hosts such as **2**, which contain two chiral elements, provided the highest chiral recognition in complexation. The two methyl groups of **2** extended the chiral barriers of the naphthalene rings and provided greater binding with the guest. Many other researchers have modified multi-heteromacrocycles by adding different functionality such as saccharides,[133-135], tartaric acid[136-138], 9,9'-spirobifluorene[123] to improve their ability as hosts for chiral recognition.

Recently Nohira[139] and co-workers have studied chiral recognition ability of optically pure molecules **12** and **13** with different chiral amines **14-18** in solution using ^{1}H NMR titration method.

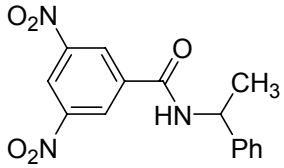


It was proposed that *endo*-3-benzamidonorborn-5-ene-2-carboxylic acid **12** can be used to resolve **14**, while chiral resolution of chiral amines **14-18** was unsuccessful using optical pure **13**. It was demonstrated that the additional CH-π interactions between the vinylic hydrogen atom of **12** and aromatic ring of **14** provided extra stability to the formed structure which resulted in optical resolution. Both optically active **12** and **13** showed chiral recognition with chiral amines

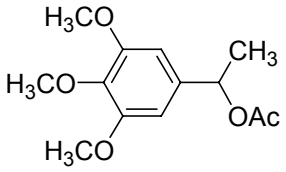
14-18. The chiral recognition ability also increases as the aromatic feature of the amines increases, which indicates that the π-π interaction should be the major factor.

1.6.2 π–π Interaction in Complex Formation

The non-bonding interactions between electron rich and electron poor aromatic compounds play a very important role in chiral recognition through diastereomeric complex formation.[140-142] Aromatic interactions are important contributors in protein folding and structure. [143-144] Recently, the role of aromatic π–π interactions has been investigated in α-helix and β-hairpin structures by Waters.[145] Chiral discrimination through nonbonding interactions between π-electron poor aromatic compound **19*S*** and π–electron rich aromatic compound **20** has been studied[146] using ^{1}H NMR and IR spectroscopy.



19*S*

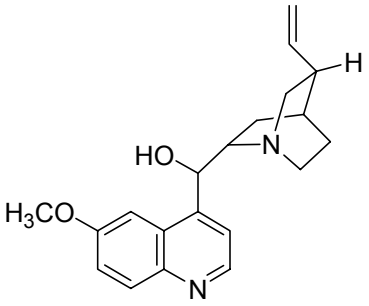


20

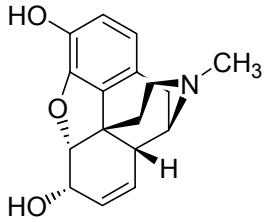
For example, the ^{1}H NMR spectra of racemate **20** showed only one set of signals for both enantiomers. In the presence of template **19*S***, two sets of signals were obtained, each from different enantiomer and in ratio according to the enantiomeric composition. This clearly supports the formation of two diastereomeric complexes of **19*S*** with each enantiomer of **20,** which have different properties. The IR spectra of the complex also supports the presence of diastereomeric nonbonding π-π interactions between the electron rich and the electron poor compounds.

1.7 The Emergence of Chiral Drugs in Pharmaceutical Industries

Drugs such as *quinine* (**21**) and *morphine* (**22**) are isolated from natural resources as single enantiomers. But the synthesis of these natural products in a laboratory setting has yielded racemic mixtures of these important pharmaceuticals. Obtaining the desired enantiomerically pure compound from the racemate is challenging both in the development of new stereoselective drug manufacturing and the affordability of the production of such compounds. Until recently, many commonly used therapeutic agents marketed by pharmaceutical companies have been synthesized and utilized as racemic mixtures. However, with the emergence of new technology, as well as the discoveries of potentially disastrous side effects from the unwanted enantiomer, there has been a scientific drive towards the development of enantiomerically pure drugs.[147]



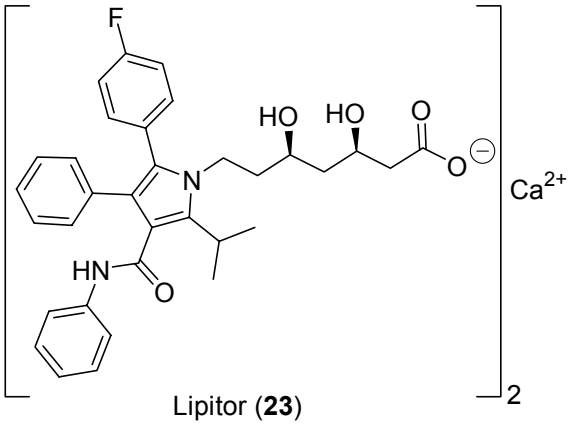
Quinine (**21**)



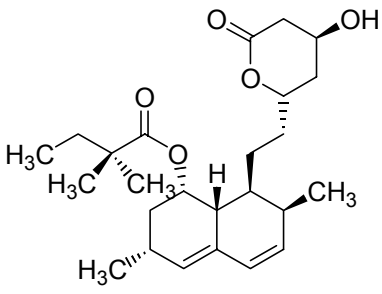
morphine (**22**)

When therapeutic agents accumulate in the body, they react with specific binding sites and these binding sites have specific physical shapes. If a chiral racemic mixture of a drug has been used, then both enantiomers of the drug compete with each other to fit into the active site of the receptor. There are several drawbacks to this. Usually, one isomer binds preferentially while the other has little or no activity and many times it causes some serious side effects. Furthermore, since the biological messenger molecules and cell surface receptors that medicinal chemists

target are chiral, ideally synthetically designed drugs should match this asymmetry. Pharmaceutical industries are now switching from currently marketed racemic drugs into more active single isomer drugs, or more preferably, they are targeting the development of safe drug delivery systems that will be capable of carrying only the biologically active enantiomer of the racemic drug to the targeted biomolecule. The 1999/2000 annual sales of chiral drugs were reported being close to one-third of all drug sales worldwide.[148] The top two best selling drugs, Lipitor (**23**) and Zocor (**24**), with combined sales of almost $14 billion in 2002 are single-enantiomer drugs. Lipitor is the most popular drug ever sold in history. In 2002, worldwide sales of single-enantiomer drugs reached more than $159 billion.



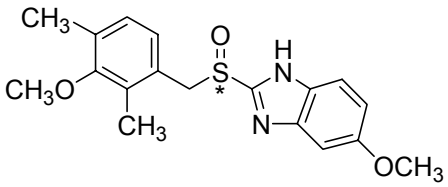
Lipitor (**23**)



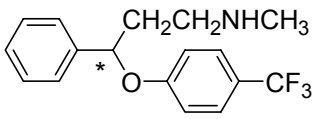
Zocor (**24**)

Many of the pharmaceutical companies are switching from marketing racemic compounds to optically pure drugs. For instance, AstraZeneca and Wayne's racemate proton pump inhibitor *omeprazole* (**25**) (brand name: Prilosec/Losec). Clinical trials show that the biological activity comes only from the (*S*)-enantiomer.[149] This company has patented the (*S*)-isomer separately[150] as *esomeprazol* (brand name: Nexium). It is prescribed for healing ulcers by preventing secretion of gastric acid. Clinical trial results show that more patients with erosive esophagitis and heartburn were healed with *esomeprazole* than with *omeprozole*. Economically, it is more

feasible to develop a general procedure that can "extract" only one enantiomer of the racemic drug and later on deliver it to the targeted biomolecule.



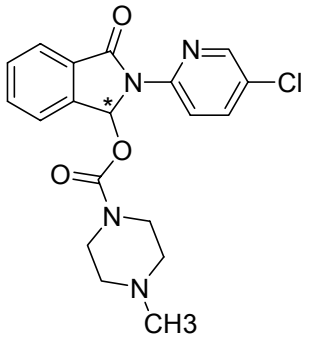
Omeprazole (**25**)



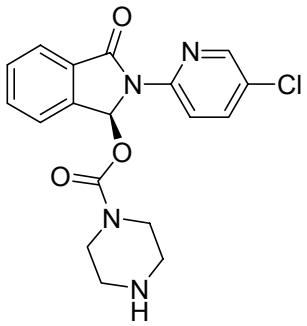
Prozac (**26**)

Racemic drug *Prozac* (**26**) (generic name: *Fluoxetine*), is one of the best selling drugs marketed by Eli Lilly. Its both enantiomers are nearly equipotent as inhibitors of serotonin uptake but the *(S)*-isomer has a longer washout period. Furthermore, its *nor* (*N*-demethyl) metabolite is much more potent than *(R)-norprozac* and accumulates on long-term treatment.[151] *(R)-prozac* is essentially a single active moiety and has the potential to provide a shorter washout period and, hence, greater flexibility for treating depression. Recently this company has received clearance from FDA to market (*R*)-*prozac* for the treatment of bulimia nervosa, an eating disorder that afflicts more than 1 million Americans each year.

Another very important racemic drug is *zopiclone* (**27**), originally developed and marketed by Rhone-Poulec Rorer. The biological activity of *zopiclone* (**27**) come from its metabolite, (*S*)-*desmethylzopiclone* (**28**), in which an *N*-methyl piperazine has been demethylated. [152] Due to the fact that the prodrugs can compete with one another for the same enzyme site which metabolizes them, all marketed prodrugs should be used as one pure enantiomer. Such competition can lead to unfavorable drug interactions if one drug ties up the available enzyme and the other drug builds up to excess levels in the blood.

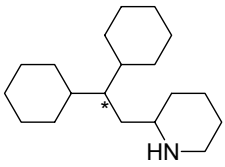


Zopiclone (**27**)

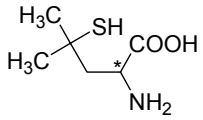


(*S*)-Desmethylzopiclone (**28**)

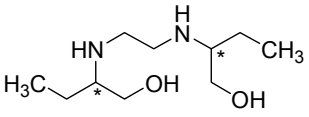
Other disaterous incidences occurring from the use of racemic mixtures of drugs include *perhexiline* (**29**). This racemic drug was prescribed to control abnormal heart rhythms. In the 1980's, many people died because of accumulating this racemic drug. After thorough research on the metabolism of this drug, it was discovered that the *R* enantiomer of *perhexiline*, which had a much longer half-life and more slowly metabolized, was responsible. According to Caldwell,[147] it is true that racemic drugs can cause problems because of the different biological activity of both enantiomers, but they can also cause serious problems due to difference in the pharmacokinetics as well. Many lives might have been saved if the enantiomerically pure drug had been given to patients instead of the racemic form of *perhexiline* (**29***)*.



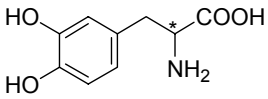
Perhexiline (**29**)



Penicillamine (**30**)



Ethambutol (**31**)



Dopa (**32**)

Other examples include the (*S*)-enantiomer of the anti-arthritic compound *penicillamine* (**30**), which is responsible for the biological activity, while the (*R*)-enantiomer of this drug is

extremely toxic. The (*S*,*S*)-form of *ethambutol* (**31**) is a tuberculostatic agent but the (*R*,*R*)-form can cause blindness. The Parkinson's disease drug *dopa* (**32**) is marketed in an enantiomerically pure (L-*dopa*) form because the D-form causes serious side-effects such as granulocytopenia (a loss of white blood cells that leaves patients prone to infections).

New single stereoisomer drugs have proven to be safer, exhibit fewer side effects, and are more potent than their racemic counterparts. For instance, enantiomerically pure drugs are able to affect the desired process as does the racemic mixture of the same coumpound, yet typically, lower doses of enantiopure compounds are administered to patients due to the fact that the absorption and uptake of desired enantiomers is not hindered by the competitive binding of the unwanted enantiomer. Additionally, potential life threatening side effects elicited by the administration of the unwanted enantiomer through the racemate are also eliminated by using enantiopure compounds. According to the FDA (Food and Drug Administration, USA), new optically active drugs should be prepared for marketing in their enantiomerically pure form.[153]

There are two approaches commonly used to prepare single stereoisomer compounds. These approaches include (i) synthesis of a desired enantiomerically pure product using either chiral intermediates or chiral catalysts and (ii) separation of enantiomers from racemic mixtures using chiral separation techniques. While relatively successful, both approaches tend to be very expensive and often cause added difficulties in both preparation and separation methods. To sidestep each of these drawbacks, researchers in this field are beginning to address the issue of the production of enantiopure compounds by developing new chemical techniques, namely chiral drug delivery systems. These drug delivery systems can address each of these problems and allow either inexpensive preparation procedures for the production of racemic products or use already developed synthetic procedures for the preparation of racemic mixtures of active

therapeutics. By using simple and inexpensive methods of purification the desired enantiomerically pure targeted compound can be isolated in high yield and high optical purity from the mixture.

1.8 Cyclodextrins as Drug Carrier

The ideal drug delivery systems for such a task have to fulfill some requirements. The system has to deliver the necessary amount of compound to the receptor or the targeted binding site in an efficient and precise manner.[154-156] Suitable carrier molecules (hosts) must be used to overcome undesirable properties commonly associated with biologically active compounds, such as stability and solubility in aqueous media. Many pharmaceutical drugs on the market today, as well as potential drugs undergoing clinical trials, have relatively low water solubility; as a result, these are usually administered in substantially higher doses than required with the common idea being that the excess of the drug will be secreted from the body without any side effects. This, however, is not always the case and in many instances the excess drug is responsible for adverse side effects. In many cases co-solvents (usually alcohol) are used to increase drug uptake. However, there are examples throughout the literature that indicate that cyclodextrins, one example of a chiral host system, can form inclusion complexes with a variety of compounds, provided the guest is able to fit inside the cyclodextrin cavity.[157] There are two benefits for using cyclodextrins as chiral drug carriers. The first is a decrease in toxicity[158] and an increase in the water solubility, stability, and bioavailability of the drug. The second is the selective chiral delivery of only one enantiomer of the starting racemic mixtures. Considering these favorable properties, cyclodextrins can be used as an additive in the marketed drug. Additional advantages include the fact that CDs can be chemically modified, which in turn can increase guest-host

interactions and enantioselectivity. For example, chemically modified cyclodextrin derivatives have been prepared to enhance the physiochemical properties and inclusion capacity of natural cyclodextrins as drug carriers.[159-166] Cyclodextrins can also form stable complexes with a variety of drugs including prostaglandins, barbiturates, steroids, and nonsteroid anti-inflammatory drugs.[167]

1.9 Chiral Recognition by Native Cyclodextrins

All reactions that take place in living cells are catalyzed by enzymes. Enzyme mediated chemical transformations are stereospecific. Therefore, there is no need for nature to develop methods of enantiomeric separation. Enzymes preferentially bind one enantiomer over other. Examples include enzymes that synthesize most proteins preferentially bind only L amino acids, while enzymes that metabolize sugars bind only D sugars.[168] Racemic substrates can be used in enzyme catalyzed reactions, and in such cases only one enantiomer will be incorporated into the product. Unfortunately, enzymes cannot be used as resolving agents of enantiomers from racemates, because they are pH, temperature and solvent dependant and are not easy to handle in laboratory settings. It is also difficult to isolate enzymes from nature in an amount that can be used in organic synthesis. Thus, synthetic organic chemists are drawn to small organic compounds which can mimic some of the properties of enzymes and can be used as resolving agents. One of these compounds is cyclodextrin.

CD inclusion complexes made with a variety of guest compounds can be used as a method of chiral resolution, but the exact nature of the interaction between the CD and the guest molecule, which leads to enantiomeric discrimination, is still not known in detail. It is widely accepted that various forces,[169-172]such as hydrogen bonding, Van der Waals' forces,

hydrophobic interactions, and π–π interactions[173,174] contribute to the complex formation. Since cyclodextrins are capable of forming stable inclusion complexes in aqueous solution with a variety of aromatic compounds,[175-178] it is reasonable to propose that cyclodextrins can form diasteriomeric inclusion complexes with racemic aromatic compounds. Based on the knowledge that diasteriomers have different physical properties, it is reasonable to expect that one of the diasteriomeric inclusion complexes will crystallize out from the racemic aqueous cyclodextrin solution more readily than the other. It has been well demonstrated throughout the literature that in the case of conglomerates, once one of the enantiomers starts to crystallize from the solution, it is possible to obtain high optical purity of this enantiomer.[179] It is also well demonstrated that better chiral discrimination is obtained with molecular systems, such as cyclodextrins, that have many chiral centers.

Armstrong[180-182] summarized the requirements for chiral recognition and stereoselective binding with β-CD, and these requirements also hold true for α- and γ-CD.

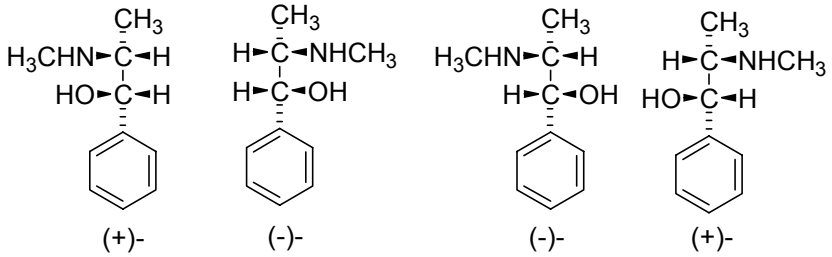
(a) An inclusion complex must be formed.

(b) There must be a tight fit of the included moiety within CD.

(c) The chiral center of the guest or a substituent of the chiral center (e.g., a carboxylic acid group) must be able to form at least one strong interaction with the hydroxy groups on the surface of the CD cavity.

Cramer and Dietsche first proposed the use of cyclodextrin inclusion complexes with a variety of guests with β-CD for resolution of guest enantiomers from racemates.[183] Mularz and coworkers have reported the importance of hydrogen bonding between the guest and cyclodextrin during the complex formation.[184] They proposed that all four isomers of adrenergic drug *ephedrine* (**33**) and *pseudoephedrine* (**34**) form inclusion complex with β-cyclodextrin.The

ammonium group of these drugs form hydrogen bonds with secondary hydroxyl group of β-CD while the hydroxyl groups form hydrogen bonds with either ether oxygen or primary hydroxyl oxygen atoms. The (*S*,*S*)-form of *pseudoephedrine* was resolved from mixture of diastereomers by using β-CD mobile phase.



ephedrine (**33**) pseudoephedrine (**34**)

Li and co-worker Purdy[185] studied stability and structure of the inclusion complexes of β-cyclodextrin with dinitro phenyl (DNP) amino acids (DNP-valine, DNP-leucine, and DNP-methionine) (Figure 1.4). They proposed the necessity of side chain interactions between β-CD and amino acids to form inclusion complexes, which leads to chiral discrimination.

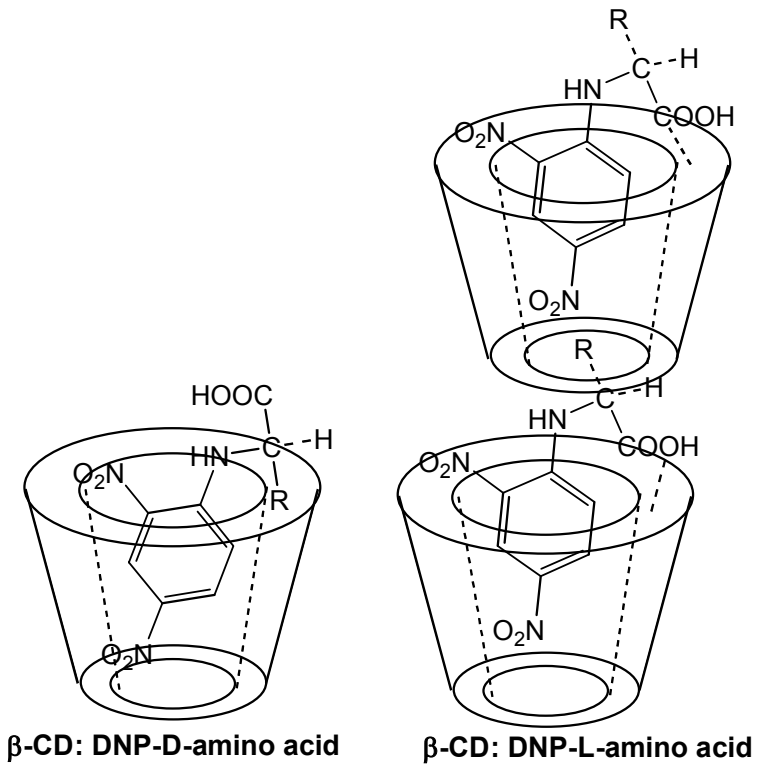


Figure 1.4 Structures of inclusion complexes of β-cyclodextrin with DNP-D- valine and with DNP-L-valine.

Figure 1.4 clearly shows the chiral discrimination of the two enantiomers of DNP-valine amino acid in forming inclusion complexes with β-CD. It was found that in all three DNP-amino acid's inclusion complexes with β-CD, the dissociation constant for L- enantiomers has always lower than that of D-enantiomers. The dinitrophenyl group, which is the hydrophobic part of the amino acid studied, enters the β-CD cavity from the wider secondary hydroxyl rim and forms a stable inclusion complex. The large chemical shift change (Δδ) of H-3 and H-5 protons of β-CD (figure 1.5) during complexation indicates that the DNP group of amino acid is inside β–CD cavity. Also, the larger Δδ value for the 5′ proton in the presence of DNP-D-valine compared to DNP-L-valine indicated the deeper penetration of DNP group inside β-CD cavity.[185] The alkyl group of DNP-amino acid plays a very important role in chiral recognition between two

enantiomers. The alkyl groups of DNP-L-amino acids form 1:2 inclusion complexes with the β-CD cavity, whereas in the case of DNP-D-amino acids a 1:1 complex is formed due to steric repulsion of alkyl groups with the CD hydroxyl group. The shallower insertion of the DNP-L-amino acid leaves enough space in the β-CD cavity to host the alkyl group from another DNP-L-amino acid.

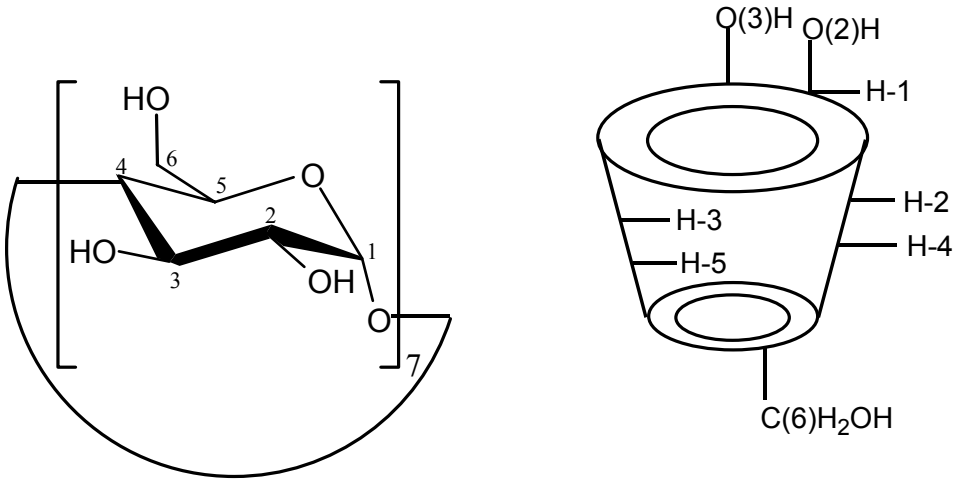


Figure 1.5 Localization of the "external" and "internal" protons in β-CD

The crystal structures of the complexes of β-CD with the anti-inflammatory,[167] antipyretic, and analgesic agent fenoprofen (FP), determined by Hamilton and Chen, also provide a good example of cyclodextrins acting as a resolving agent in racemic mixtures. Fenoprofen, a racemic drug developed at Eli Lilly, has two optical isomers, (*R*)-(-) and (*S*)-(+). On the basis of 50% inhibition of the fatty acid cyclooxygenase system, the *S* stereoisomer was found to be 2 times more potent than the racemate and ~35 times more active than the *R* stereoisomer. [186] This provides another very good example of the importance of enantiomerically pure drugs.

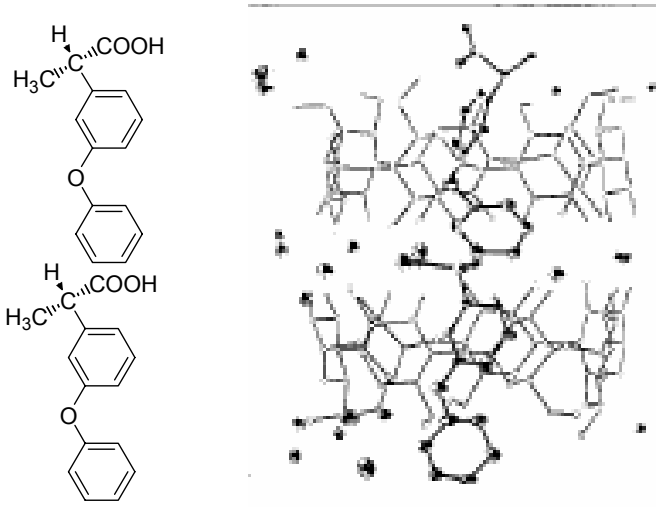


Figure 1.6 ORTEP plot of dimer β-CD-(*S*)-fenoprofen complex (head to tail packing arrangement)

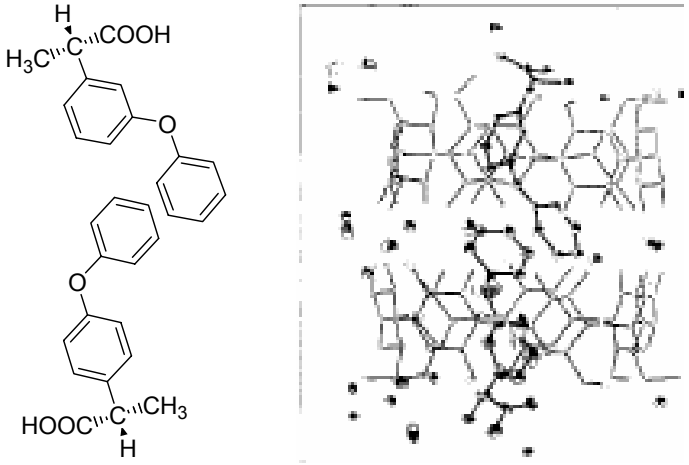


Figure 1.7 ORTEP plot of dimer β-CD-(*R*)-fenoprofen complex (head to head packing arrangement)

In the crystal, the two FP-β-CD complexes exist in such a way that the two β-CD molecules face their wider opening side (which contains secondary hydroxyl groups) in a head to head manner. One fenoprophen guest molecule is inserted in the cavity of each β-CD monomer forming a 1:1 complex.[187] In the crystal structures of FP-β-CD complexes, the packing arrangement of each isomer of fenoprophen is different. For example the two independent

fenoprophen guest molecules R1 and R2 of the *R* complex are oriented in the β-CD cavities with their phenoxy groups pointing toward the wider rim end of the CD, giving an antiparallel or head-to-head arrangement, while those of the *S* complex pack in a parallel, or head-to-tail, manner. This is unexpected considering the hydrophobic cavity of cyclodextrin. It is proposed that steric factors are responsible for the unusual packing arrangement of *S*-complex. Both carboxylic acid groups of the (*S*)-fenoprofen form strong H-bonds with hydroxyl groups of β-CDs in (*S*)-FP-β-CD complex, while in (*R*)-FP-β-CD complex, both carboxylic acid groups form hydrogen bonds with water alone. This explains 3 times more binding affinity of (*S*)-FP with β-CD compared to (*R*)-FP.[158]

Rekharsky and co-workers have studied the complexation and chiral recognition behavior of γ-cyclodextrin with various enantiomeric and diastereomeric dipeptide pairs.[188] They proposed that better chiral recognition was found with diastereomeric dipeptides compared to enantiomeric peptide pairs.

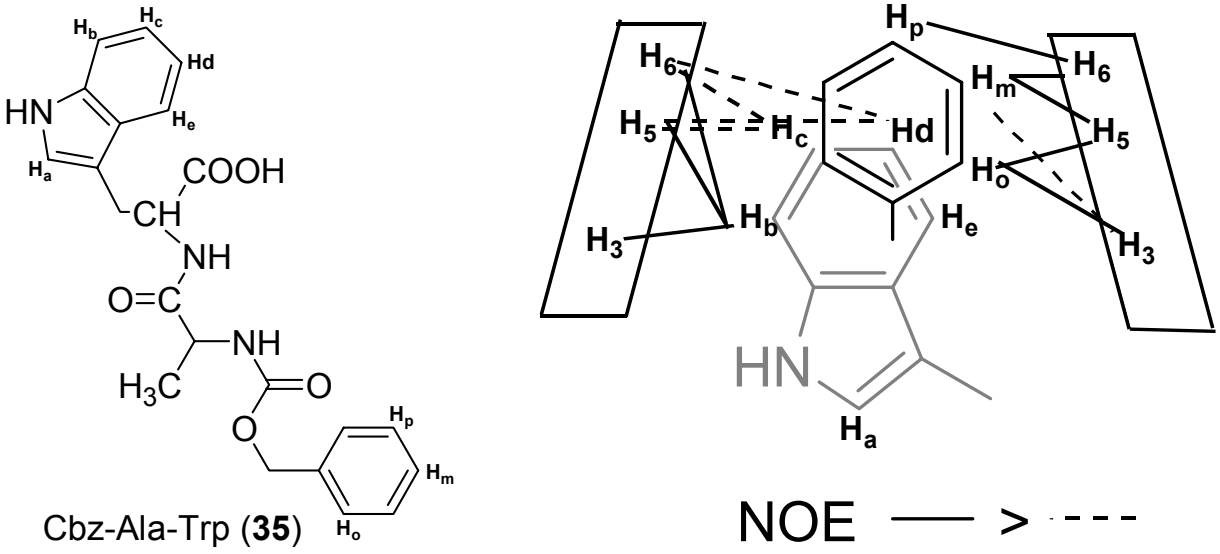
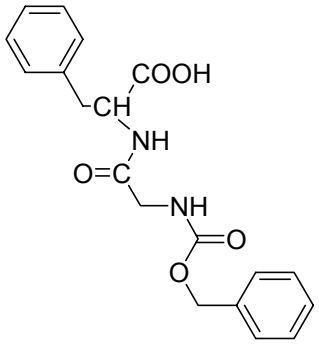
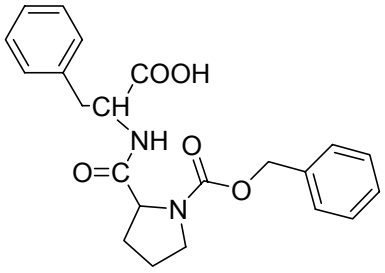


Figure 1.8 Plausible complex structure Cbz-D-Ala-L-Trp with γ-CD

During their complaxation study they proposed that γ-cyclodextrin showed an excellent chiral recognition behavior with Cbz-D/L-Ala-L-Trp (**35**) diastereomer guest pair (Figure 1.8). For instance, the D,L-isomer pair of this dipeptide showed more than seven times higher affinity toward γ-CD compared to L,L-isomer. This affinity enhancement for D,L-isomer is entropy driven. From NOE cross-peak results (Figure 1.8), it was proposed that upon complexation, the large size of indole ring prevents it from rotation and thus it change the depth of penetration by sliding parallel to the γ-CD cavity axis. NOE three space coupling interaction from ROESY spectra indicates interaction between indole ring hydrogens (Hb-He) and Cbz's Ho, Hm and Hp with γ-CD cavity walls. This is also indicates the stacking of indole/Cbz rings inside cavity.



Cbz-Gly-Phe (**36**)



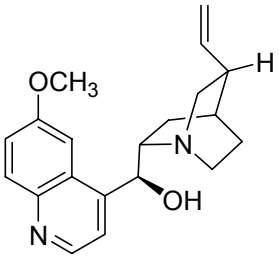
Cbz-Pro-Phe (**37**)

There is a small difference in association constants of two isomers of Cbz-Gly-Phe (**36**), bacause the long flexible tether is conformationally restricted upon stacking.[188] When Gly is replaced with rigid Pro residue, which provides less conformation of the tether, gives higher chiral discrimination. The D,L-isomer of Cbz-Pro-Phe (**37**) provides more than five times higher affinity compared to the L,L-isomer, because only D,L-isomer perfectly fits into the CD cavity,

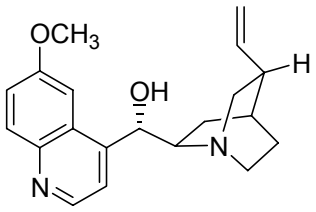
giving higher chiral discrimination. The large negative entropy and large negative enthalpy of complaxation also indicated the improper fitting of L,L-isomer into γ-CD cavity.

1.10 Chiral Recognition by Modified Cyclodextrins

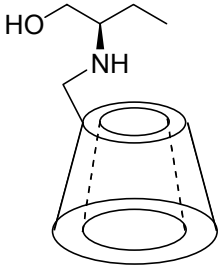
In native cyclodextrins (α-, β- and γ-CDs), the glucopyranose units are symmetrically arranged. This higer symmetriy of native cyclodextrins is often responsible for their poor chiral recognition. There are many examples in literatures indicate the improvement of chiral recogniton by using functionalized cyclodextrins which are prepared by chemical modifications of native cyclodextrins.[159-166] It is proposed that mono and diaminated β-cyclodextrins can provide better enantiomeric recognition compared to native β-CD.[189-191] The higher chiral recognition ability of these modified cyclodextrins is attributes to the three-point interaction.[192] The three-point interaction was originally proposed by Easson[193] to explain chiral recognition of two enantiomers of a drug. Enantiospecific conversion of a prochiral reactant to chiral product by enzymes was explained by this three-point interaction by Ogston. [194] Recently, Liu and co-workers proposed that binding of Quinine (**38**) and Quinidine (**39**) can be enhanced in great extent by using mono(6-deoxy-6-{[(*R)*-1-(hydroxymrthyl)propyl]amino})-β-cyclodextrin (**40**) in contras to native β-CD. [195]



Quinine (**38**)



Quinidine (**39**)



(**40**)

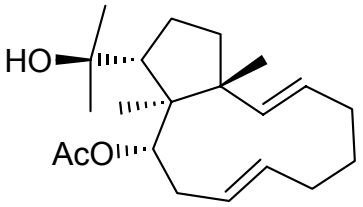
In this chirally-modified β-CD host molecule (**40**), one of the primary hydroxyl group in 6-position is replaced by chiral [1-(hydroxymethyl)propyl]amino substituent. The enhanced diastereoselective recognition ability of (**40)** is attributed to this modification, which provides stronger *Van der Waals*, hydrophobic and additional hydrogen bonding interactions between host (**40**) and the guest molecules Quinine (**38**) and Quinidine (**39**). The K_a values for the complaxation of (**38**) and (**39**) with (**40**) were determined to be 84200 and 27300 Mol^{-1}, respectively, which corresponds to a diastereoselectivity of *ca*. 3:1.

1.11 Terpenoids

For the past century, scientists have been using isolation techniques to extract many organic compounds commonly considered "natural products" from plants, mainly using steam distillation and stepwise extraction methods. These odoriferous or flavoring volatile oily plant extracts are called *essential oils*, and have been used in medicines, spices and perfumes. Such plant essential oils consist of a mixture of lipids, known as terpenes. Terpenes were originally named after turpentine, the volatile oil extracted from pine trees. Terpenes containing oxygen atoms are usually referred as terpenoids. Terpenes are classified according to the number of isoprene units they contain. Most terpenes have skeletons of 10, 15, 20 or 30 carbons. Terpenoids with 20 carbons are referred as diterpenoids.

1.12 Dolabellane Diterpenoids

Dolabellane diterpenoids, obtained primarily from marine sources, share the unique feature of a *trans*-bicyclo[9.3.0]tetradecane nucleus and in many cases, exhibit antimicrobial, antitumor and antiviral activities.[196-198] The remarkable biological activity of most dolabellane diterpenoids prompted research for the total synthesis of various dolabellane diterpenoids. There are many literature available on synthetic studies[199-208] of dolabellane diterpenoids as well as their total synthesis.[198,209-213] Dolabellane diterpenoid **41** (figure 1.9) from sea hare was initially isolated by Faulker et al. in 1976 from the opistobranch mollusk *Dolabella californica* at Isla Partida, Gulf of California.[214]

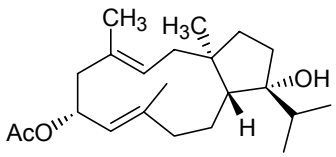


41

Figure 1.9 First isolated dolabellane diterpenoid

Okinawa sea waters have been recognized as rich sources for marine natural products, including the soft corals of genus *Clavularia*. Most of these natural products show remarkable biological activities. It was found that *Clavularia viridis*, a marine plant, has a rich source of prostanoids[215-221] and steroids[222-226]. Many other terpenoids[227-232] were also found from other species of *Clavularia,* such as *C. koellikeri.*[233]

Many Dolabellanes exhibit significant antitumor activity, and *in vivo* potency against the influenza virus and adenovirus and nearly all dolabellanes possess antimicrobial activity against bacteria.[208] Dolabellanes have also been discovered in the brown seaweeds of *Dictyotaceae*,[234-235] the sea whips of *Eunicea*,[236-237] the liverwort *Odonttoschisma denudatum*,[238] the mollusk *Aplysia dactylomela*,[239] and soft corals of *Clavularia*.[240-241] Dolabellane diterpenoids are chemical precursors of fusicoccanes (a group of diterpenoids known for significant biosynthetic plant growth regulators[242]). Dolabellanes (such as **42** Figure 1.10) also serve as biogeneric precursors of 5-7-6 tricyclic terpenes of the dolastanes (a class of marine natural products).[198,208,243,244]



29

Figure 1.10 Dolabellane diterpenoid **42**

1.13 Isolation, Purification and Biological Activity of Dolabellane Diterpenoids

Two new diterpenoids, **B** and **D,** as well as two known diterpenoids **A** (dolabellatrienone) and **C** (claenone), having a dolabellane skeleton (*trans*-bicyclo[9.3.0]tetradecan) were recently isolated from the Okinawan soft coral of the genus *Clavularia* by Iguchi and co-workers (Figure 1.11) using the following methodology:[233]

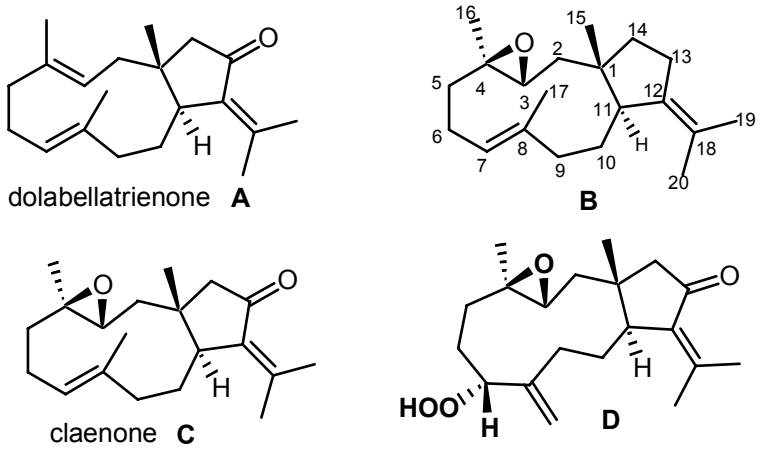


Figure 1.11 Isolated dolabellane diterpenoids **A**, **B**, **C** and **D**

The MeOH extract of wet specimens (1.1 kg) was partitioned between ethyl acetate and water. An EtOAc-soluble portion (18.9 g) was obtained by concentrating the organic layer under reduced pressure, from which 10 g of crude material was chromatographed on a silica-gel column. Stepwise elution with solvents of increasing polarity (hexane, hexane- EtOAc (4:1), EtOAc, and MeOH) was used to collect four different fractions from the crude sample. Stepwise further purification of fraction 2 (3.5 g) with silica-gel column using hexane- EtOAc in 10:1 ratio gave seven samples, among which only samples 2 and 3 were saved. Reversed-phase HPLC (eluted with MeOH-H_2O = 9:1) of sample 3 (142 mg) gave compound **A** (30 mg). Compound **B** was obtained by purification of sample 2 (340 mg) in two steps (i) flash chromatography (eluted

with hexane-2-propanol = 20:1) and then (ii) normal phase HPLC (eluted with hexane-2-propanol = 30:1) gave 48 mg of compound **B**.[233]

Fraction 3 (6.0 g) was chromatographed on a silica-gel column using EtOAc as the mobile phase. 1.0 g of fraction 3 was again chromatographed with hexane-EtOAc (4:1), which gave five samples. Further repeated purification of sample 1 (599 mg) with flash chromatography using hexane-EtOAc (5:1) gave compound **C** (273 mg) and **D** (96 mg).[233]

Compound **A** was identified as dolabellatrienone,[245] which was previously isolated from the Caribbean gorgonian octocorals *Eunicea calyculata* and *Eunicea laciniata*.[246] The absolute stereochemistry of dolabellatrienone was specified by previously reported[247] synthesis. Dolabellatrienone **A** has been shown to express weak cytotoxic activity toward human colon (HCT 116) cell line (IC_{50} 10 μM).[248] Compound **C** was identified as claenone,[206,239] found from the Okinawan soft coral of the genus *Clavularia.* Claenone **C** has been shown to express ichthyotoxic activity toward killifish *Oryzias latipes* (minimum lethal concentration: 10 μM/mL)[231] and potent cytotoxic activity toward human prostate cancer WMF (GI_{50} $2.42x10^{-7}$M) and RB cells (GI_{50} $3.06x10^{-7}$M).[246] Claenone **C** was found to gradually oxidize to **D** at room temperature in an ethyl acetate solution. This change is due to oxidation of the double bond at C-7 of Claenone **C**. If the ethyl acetate solution of **C** was stirred at room temperature under an atmosphere of O_2, 37% of **C** converted to compound **D**. This suggests that Compound **D** is not actually extracted from the soft coral of the genus *Clavularia*, but rather formed from **C** during the isolation process.[233] Compound **D** showed moderate cytotoxic activity against ovarian cancer, colon cancer, lung cancer, and stomach cancer cells.[249]

The molecular formula of $C_{20}H_{32}O$ for **B** was determined[233] by elemental analysis and the ^{13}C spectrum. DEPT experiments and ^{13}C NMR spectrum indicated the presence of five methyls,

seven SP^3 methylenes, two SP^3 methines, two SP^3 quarternary carbons, one SP^2 methine and three SP^2 quarternary carbons. With the help of ^1H and ^{13}C NMR spectra, the presence of a trisubstituted epoxide, a trisubstituted double bond, a tetrasubstituted double bond and three olefinic methyl groups was identified.[233]

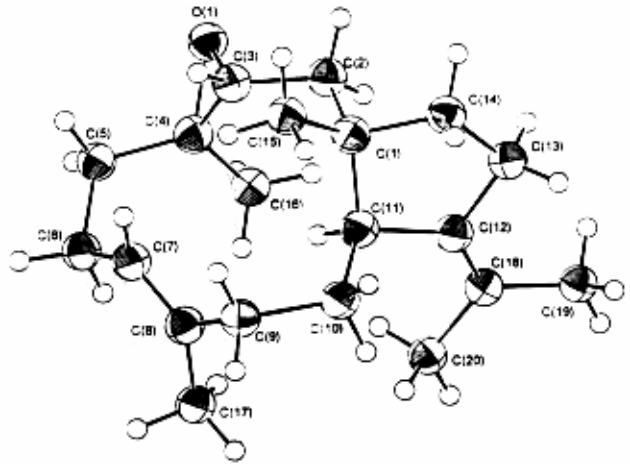


Figure 1.12 Perspective view (ORTEP) of **B**

The single crystal of **B** was grown and X-ray crystallographic analysis was carried out to obtain the relative stereochemistry of the four chiral centers (C-1,-3,-4 and -11) as well as the stereochemistry of the trisubstituted double bond.[233] The results of the X-ray analysis suggest the 1S*, 3R*, 4R* and 11S* relative configurations for the four chiral centers as well as the *E* configuration for the trisubstituted double bond (C_7-C_8).

II. Cyclodextrin Assisted Chiral Recognition of Amino Acid Imides

Resolution of enantiomers can be accomplished by using stereoselective cyclodextrin co-crystallization or precipitation of one enantiomer from a water solution of racemic compounds. It has been projected in the literature that stronger enantiomeric recognition can be accomplished if two enantiomers compete for binding into multi-chiral resolving molecules, such as chiral crown ethers or cyclodextrins.[250] Cyclodextrins can form inclusion complexes with a variety of amino acids that fit inside the cavity of cyclodextrin. Nonbonding interactions, such as hydrogen bonding, electrostatic interactions, hydrophobic interactions, π-π-aromatic stacking, dipole-dipole interactions between cyclodextrins and amino acids are responsible for the complex formation, as well as for the formation of molecular associates.[251,252] Aromatic π-π stacking interactions between electron poor and electron rich moieties are crucial for the formation of molecular associates, interactions between substrate and enzymes, biomolecular associates, organic molecule conformation, etc.[253-257] Multi-point interactions, which are interactions between diastereomeric complexes and ligands with many chiral centers such as cyclodextrins, are very important for enhanced enantiomeric recognition.[258]

2.1 Amino Acid Imide Guest Compounds with One Chiral Center

2.1.1 Cyclodextrin Assisted Formation of Homochiral Polymer-like Associate in One Chirality System

The proposed formation of self-assembly[259] and cyclodextrin assisted molecular associates are presented in Figure 2.1.[260] Molecule **M** has four moieties (a polar carboxylic group, a chiral center, electron poor and electron rich aromatic moieties) that should be essential for the formation of homochiral polymer-like molecular associates. The two aromatic moieties (electron poor and electron rich) of two different M molecules enable them to form self assembly molecular associates **2M** through π-π stacking. The π-π stacking of aromatic complexes are very weak, and therefore these molecular associates should be stabilized by binding into the cyclodextrin cavity thus forming the ternary inclusion complex **2M-CD** (Figure 2.1). In this **2M-CD** complex, two aromatic moieties, each from different **M** molecules, can now form a new cyclodextrin inclusion complex and molecular associate with a new **M** molecule. In such a way the polymer-like molecular associate would be formed.

The polar groups of these associates, such as carboxylates, interact with water molecules by forming hydrogen bonds. On the other hand, the chirality of **M** makes the molecular associate diasteriomeric and stereoselective for incorporating new enantiomer of **M** into the homochiral molecular associate.

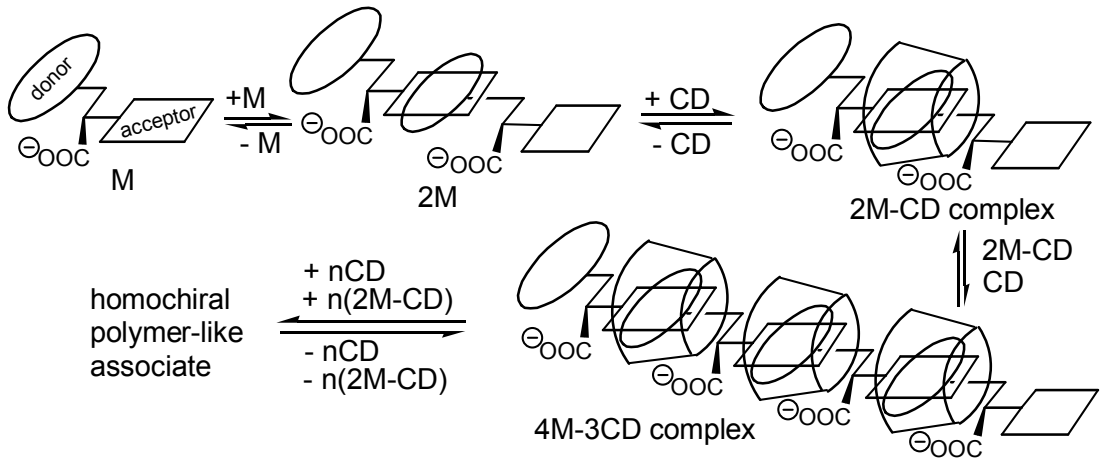


Figure 2.1 Possible pathway to cyclodextrin assisted formation of homochiral polymer-like associate.

2.1.2 Benzo[*de*]isoquinoline-1,3-dione Derived Amino Acids

The proper molecular system must be selected to test the validity of the proposed cyclodextrin assisted self-assembly approach to enantiomeric recognition. Amino acids are the best molecular choice for this study, because they are an abundant source of chiral molecular systems in organic chemistry. Since the majority of them do not form strong inclusion complexes with cyclodextrins, introducing both electron-rich and electron-poor aromatic moieties through simple chemical modifications of natural amino acids seemed appropriate to perform the proposed studies. For instance, three aromatic compounds, with noticeable difference in aromatic electronic properties, are methylindole, toluene, and methylbenzoisoquinoline-1,3-dione (Figure 2.2). The AM1[261] computed frontier molecular orbital (HOMO-LUMO) energies suggest that of these three molecules, toluene is electronically neutral, indole is electron rich and, benzoisoquinoline-1,3-dione is electron poor (Figure 2.2). These moieties are incorporated into amino acids to accomplish the requirements to study the proposed formation of molecular associates.

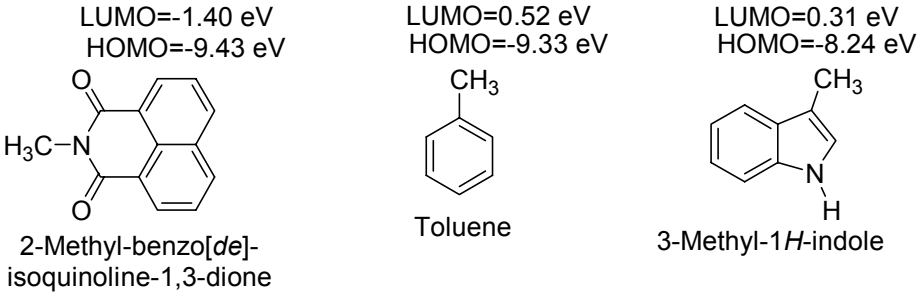


Figure 2.2 The AM1 estimated HOMO-LUMO energies for the aromatic moieties

To show the formation of molecular associates discussed in Figure 2.1, three amino acids were selected: alanine (no aromatic moiety), phenylalanine (aromatic electronic properties), and trypthophan (electron-rich aromatic electronic properties). Through amino group replacement with benzo[*de*]isoquinoline-1,3-dione (Figure 2.2), new molecular systems **30a**, **30b**, and **30c** were generated (Figure 2.3). These three molecules have the same electron poor aromatic moiety; therefore the LUMO orbital energy for all is similar while big differences exist in the HOMO energies. The AM1 estimated LUMO-HOMO energy difference, as measured for π-π aromatic stacking capabilities, are 8.00 eV, 7.85 eV, and 7.69 eV for **30a**, **30b**, and **30c,** respectively. Since guest compound **30c** has the lowest LUMO-HOMO energy gap, it is expected to form the most stable π-π molecular stacking that can be also complexed with cyclodextrins.

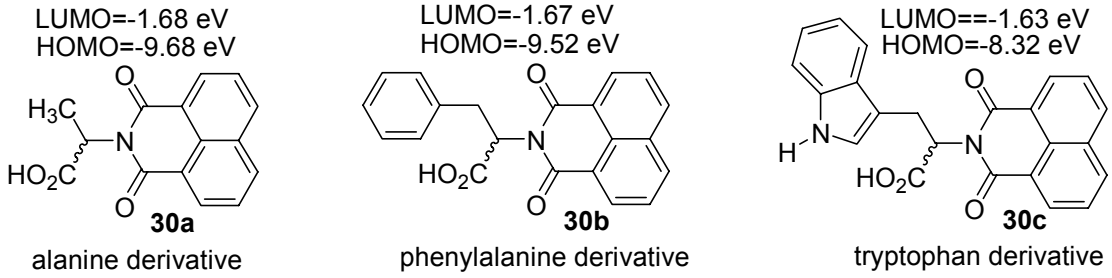
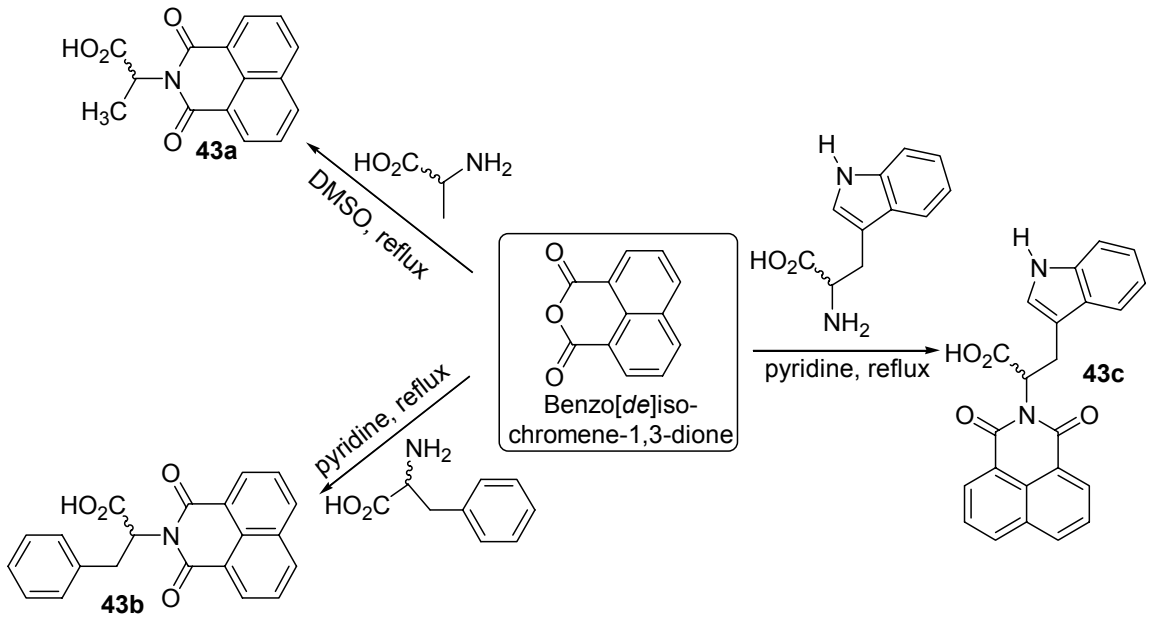


Figure 2.3 The AM1 estimated HOMO-LUMO energies for three modified amino acids

The synthetic conditions for the preparation of these amino acid derivatives are presented in Scheme 2.1. Reactions were performed with the amino acid and benzo[*de*]isochromene-1,3-dione in DMSO as a solvent for the alanine derivative and in pyridine as a solvent for phenylalanine and tryptophan derivatives (Scheme 2.1). The isolated yields range between 80-95%.



Scheme 2.1 Preparation of guest molecules 4**3a**, 4**3b** and 4**3c**

It is well demonstrated in literature that NMR non-equivalency of enantiomers can be observed if one of the enantiomers is present in excess.[262,263] To demonstrate the formation of self-assembly molecular associates through weak nonbonding interactions between two **M** molecules, spectroscopic studies of racemic and non-racemic highly concentrated solutions of 4**3a**, 4**3b**, and 4**3c** were studied. ^{1}H NMR spectra of the *S* and *R* enantiomers of all three compounds at different concentration (0.0001 to 0.3 M) and different molar ratio (1:10, 1:5, 1:1,

5:1, and 10:1) were recorded in DMSO, but we were not able to observe any NMR evidence for enantiomeric recognition.

The formation of molecular complexes is well studied in water media. The NMR study of compounds 4**3a**, 4**3b,** and 4**3c** can not be performed in water because of poor water solubilities of these compounds. Therefore, sodium salts of 4**3a**, 4**3b,** and 4**3c** were used for the NMR spectroscopic studies. Neither 4**3a** nor 4**3b** non-equivalence mixtures at any concentration range up to 0.1 M and any enantiomeric ratio show spectroscopic difference. Contrary to this, there is NMR enantiomeric nonequivalence of non-racemic 4**3c** (*R*:*S*, 1:4 and 4:1) (Figure 2.4). The spectroscopic recognition is not due to the formation of molecular associates through hydrogen bonding, nor through electrostatic interactions between the polar carboxylate group and sodium, because these interactions are also present in 4**3a** and 4**3b,** and would be observed in the spectra of these compounds. The only reasonable explanation is that molecule 4**3c** can form molecular associates through π–π stacking between the electronic rich indole and electronic poor benzo[*de*]isochromene-1,3-dione aromatic moieties with the excess enantiomer acting as a resolving agent.

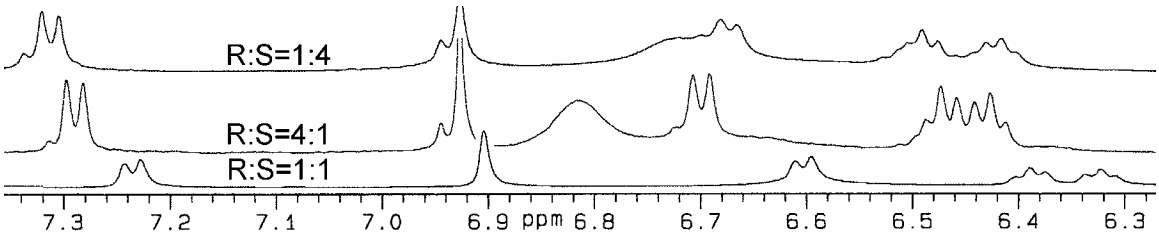


Figure 2.4 The NMR spectra of aromatic portion of 4**3c** (0.1M) in aqueous $NaHCO_3$ (0.003 M) with different enantiomer ratios

As proposed in figure 2.1, if the π-π stacking interaction of aromatic moieties is responsible for enantiomeric recognition, then the NMR spectroscopic recognition can be

enhanced by cyclodextrin encapsulation (binding). Both aromatic moieties tend to bind into the cyclodextrin cavity, and then orient themselves in the proper way for aromatic stacking and the formation of strong homochiral molecular associates. This is perfectly demonstrated in Figure 2.5 for cyclodextrin assisted enantiomeric recognition of racemic 4**3c**.

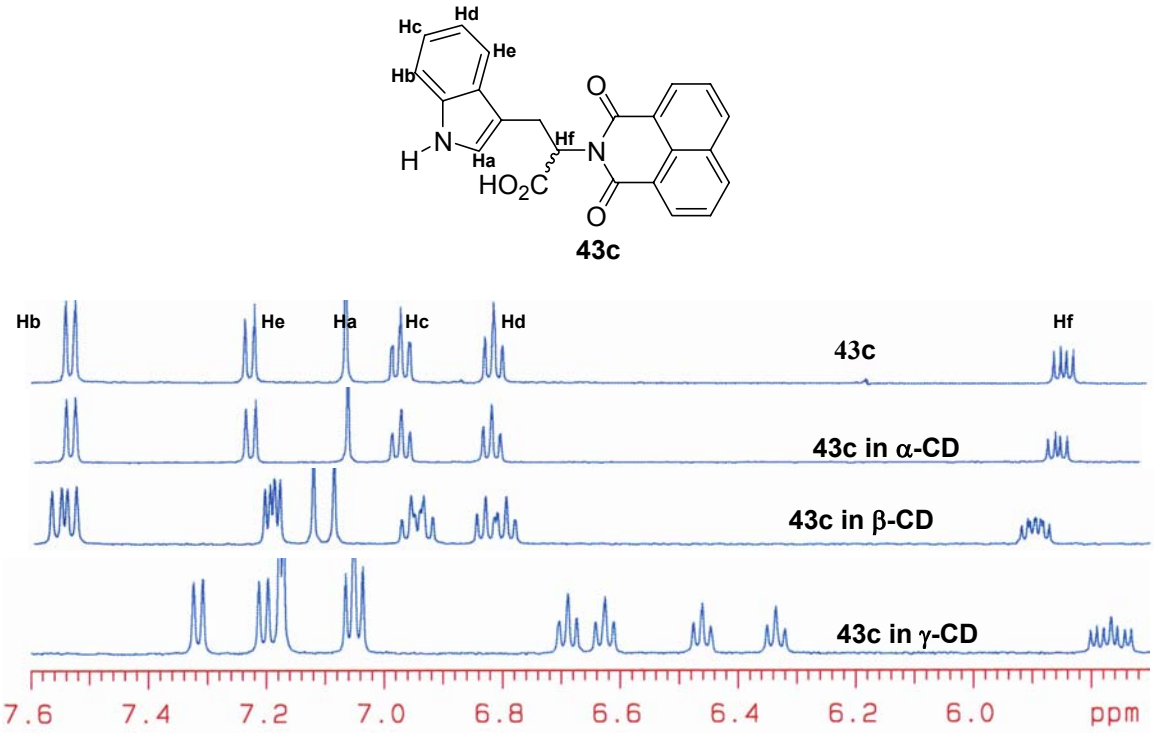


Figure 2.5 A portion of NMR spectra of racemic **43c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) with α, β, and γ-CD (0.01 M), respectively.

The best enantiomeric discrimination for compound **43c** was obtained in γ-cyclodextrin by comparison to β-cyclodextrin, while no enantiomeric discrimination was observed in α-cyclodextrin. These results are not surprising considering that the cyclodextrin cavity must be large enough to accommodate two aromatic rings. Although there are some chemical shift changes indicating an interaction between α-CD and racemic **43c**, it is unlikely that α-CD can form a ternary (two molecules and one cyclodextrin) or higher degree cyclodextrin complex with the **30c** dimer, as shown in Figure 2.6. Enantiomeric discrimination was observed in the NMR of

racemic **43c** with β-CD, but was not strong enough to elicit CD enhanced enantiomeric separation. We believe this is due to the formation of a "normal" 1:1 complex (Figure 2.6) between racemic **43c** and β-cyclodextrin. The NMR of racemic **43c** in γ-CD clearly shows the NMR enantiomer nonequivalence of **43c** yielding two sets of peaks, well resolved for both enantiomers. γ -CD has the largest cavity of the three studied cyclodextrins; therefore, it can form ternary as well as higher order diastereomeric inclusion complexes (Figure 2.6), which can provide sufficient spectroscopic differences resulting in NMR discrimination.

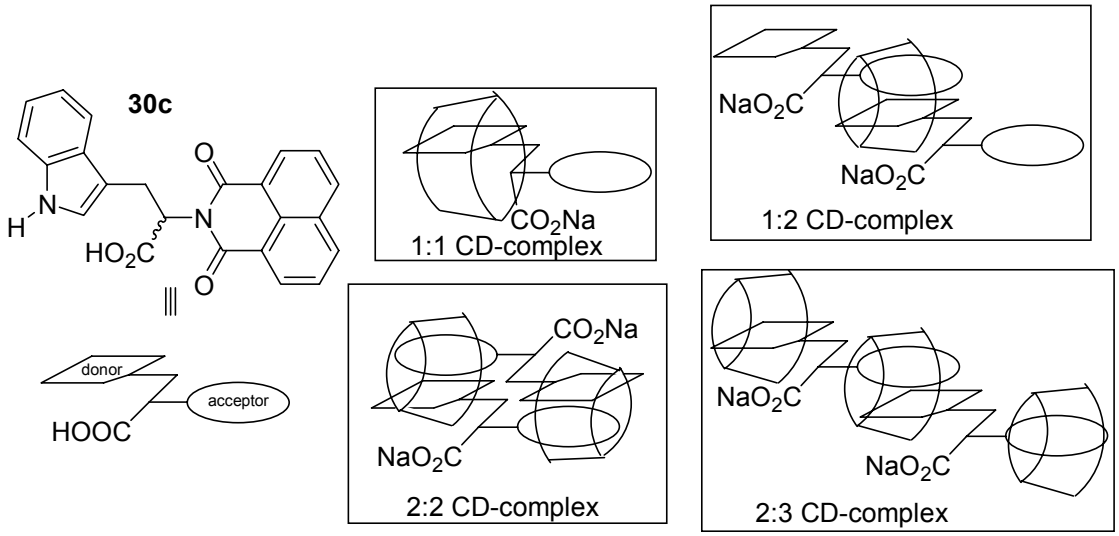


Figure 2.6. Possible π-π aromatic molecular complexes between CD and **43c**

A few of the many possibilities in the formation of molecular complexes between amino acid imide derivative **43c** and cyclodextrins are shown in Figure 2.6. All of these molecular complexes are in dynamic equilibrium in aqueous media. NMR spectroscopic study shows the average chemical shifts for all of these molecular aggregates.

There are two sets of peaks in the NMR of racemate **43c** in the presence of γ-CD. It was also possible to easily recognize each enantiomer peak, as well as determine the enantiomer composition of individual enantiomers by NMR in γ-CD when compared with the racemate

NMR in γ-CD (Figure 2.7). If the NMR of racemate **43c** is compared with the NMR of **43c*R*** and **43c*S*** in γ -CD, it can be seen that in the presence of γ-CD, proton signals of enantiomer **43c*S*** are more affected compared to that of **43c*R***. For example, the two triplets are shifted more upfield and the singlet is shifted more downfield in enantiomer **43c*S*** compared to enantiomer **43c*R*** in the presence of γ-CD. Based on this observation, it might be suggested that γ-CD forms a more stable diastereomeric complex with **43c*S*** compared to **43c*R***.

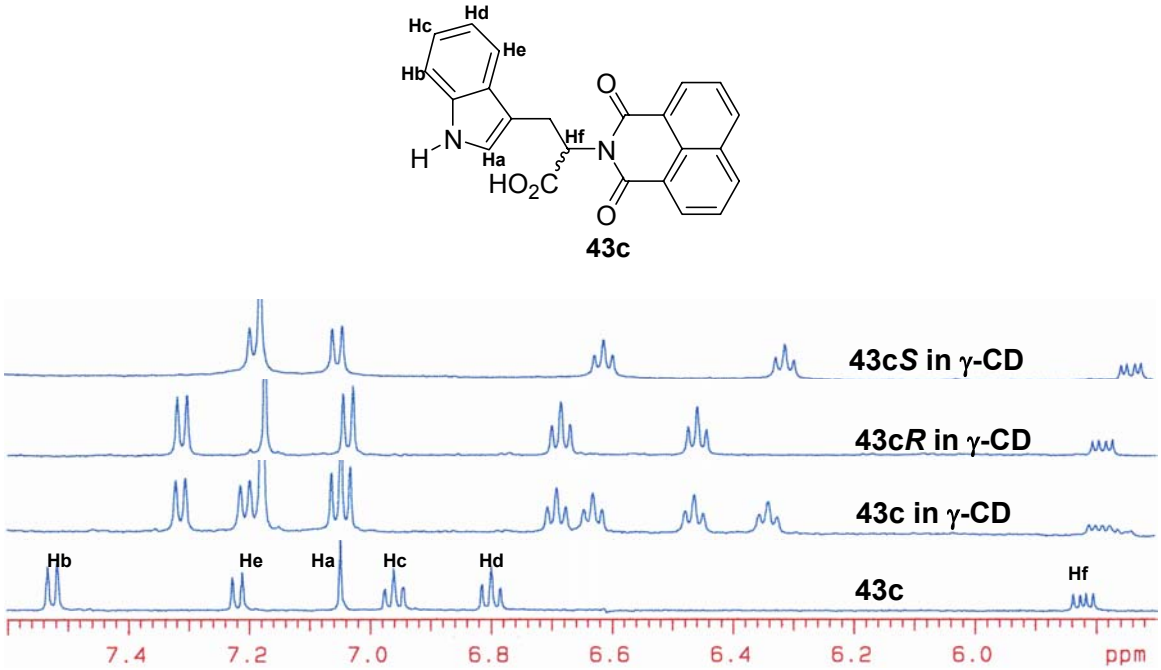


Figure 2.7 A portion of NMR spectra of **43c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and γ-CD (0.01 M)

The NMR spectroscopic recognition was not observed in α-cyclodextrin due to the fact that neither the indole nor benzo[*de*]isochromene-1,3-dione aromatic moieties can bind into the α-cyclodextrin cavity. The β-cyclodextrin cavity is sufficiently large to accommodate the indole ring. If there is formation of molecular associates with an order higher than the 1:1 cyclodextrin complex with respect to **43c**, the electrospray ionization mass spectroscopy (ESIMS) should show a molecular peak for each complex. ESIMS spectra of **43c*R*** in presence of β-CD (Figure

2.8) indicates the formation of 1:1 cyclodextrin inclusion complex formation (1517.4 m/z) but no higher order (1:2 or 2:1) complex between β-CD and **43c*R*** was observed.

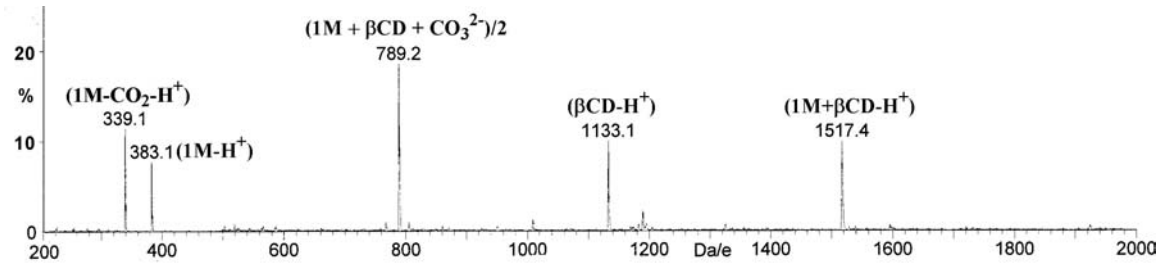


Figure 2.8 Negative Electrospray mass spectra of **43c*R*** (0.001 mol) in aqueous $NaHCO_3$ (0.003 M) and β-cyclodextrin (β–CD, 0.01 M)

The best NMR enantiomeric recognition has been seen with guest compound **30c** in γ-CD **(**Figure 2.5). The electrospray ionization mass spectra of **43c** with γ-CD shows the formation of 2:1 and 1:2 γ-CD complexes (signals at 1488 and 1032, Figure 2.9). All the other molecular complexes are present as well.

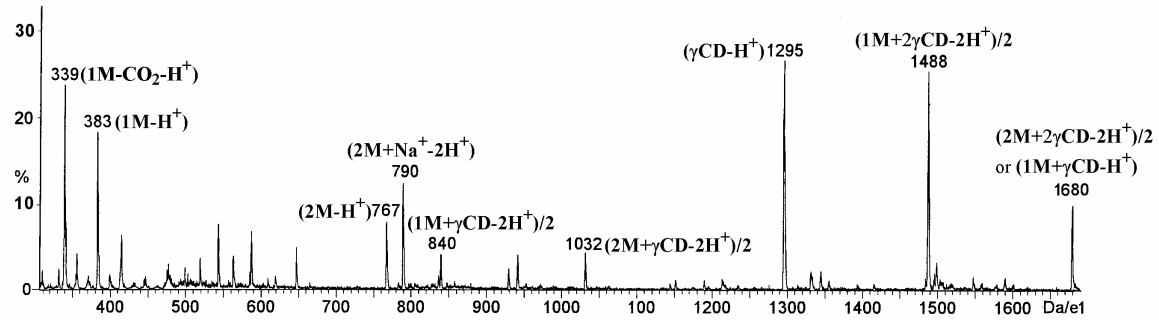


Figure 2.9 Negative ESIMS of **43cS** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

Even though ^{1}H NMR and ESIMS spectra show enantiomeric recognition of compound **30c** with β- and γ-cyclodextrin through the formation of molecular associates, they do not

provide experimental evidence for the formation of the polymer-like cyclodextrin assisted molecular assemblies presented in Figure 2.1. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectroscopy (MALDI-TOF-MS) spectra analysis of the aqueous γ-cyclodextrin solution of **43c** was performed to demonstrate the formation of the polymer-like molecular associates. The MALDI MS spectra (Figure 2.10) shows typical fragmentation similar to the fragmentation of polymers which indicates the structural patterns presented in Figure 2.1. All possible fragments were detected with lower intensity for larger molecular fragments (Figure 2.10).

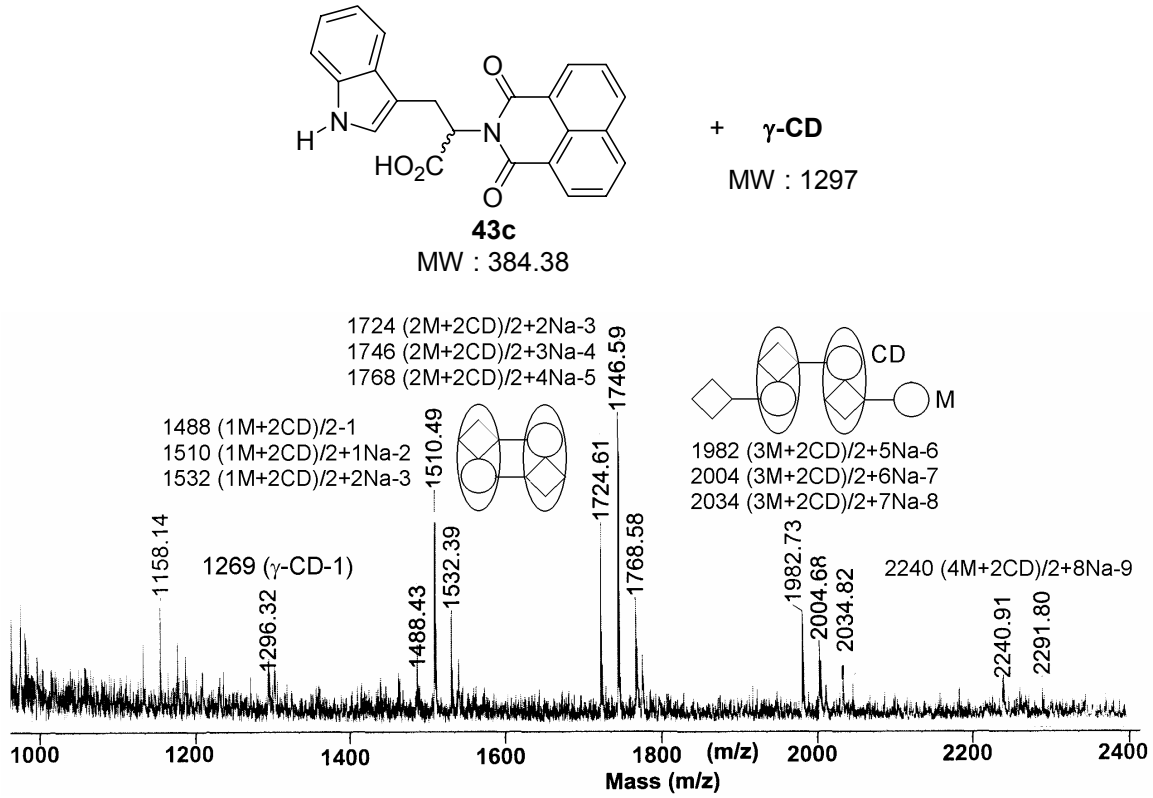


Figure 2.10 Positive MALDI-MS spectra of aqueous γ-CD and **43c**

To better understand the enantiomeric recognition capability of α, β and γ-cyclodextrins with guest compounds **43c** (Figure 2.5), association constants (K_a, M^{-1}) of **43c** with all three

cyclodextrins were measured by ^{1}H NMR (500 MHz) at different temperatures. The solution of **43c (**0.001 M) in aqueous $NaHCO_3$ (0.003 M) was titrated with a host cyclodextrin solution. Each time the change in chemical shift was measured by NMR spectroscopy. Non-linear regression analysis (Figure 2.11) using Origin 6.1 (Aston Scientific Ltd.) generated the association constants K_a according to equation (1).[264]

$$\Delta = \frac{\Delta_{max} K_a [H]}{1 + K_a [H]} \quad \text{---------- Equation (1)}$$

Where Δ = the peak shift in ppm, Δ_{max} = the maximum peak shift in ppm, and [H] = the concentration of host. At least two experiments were performed for each system.

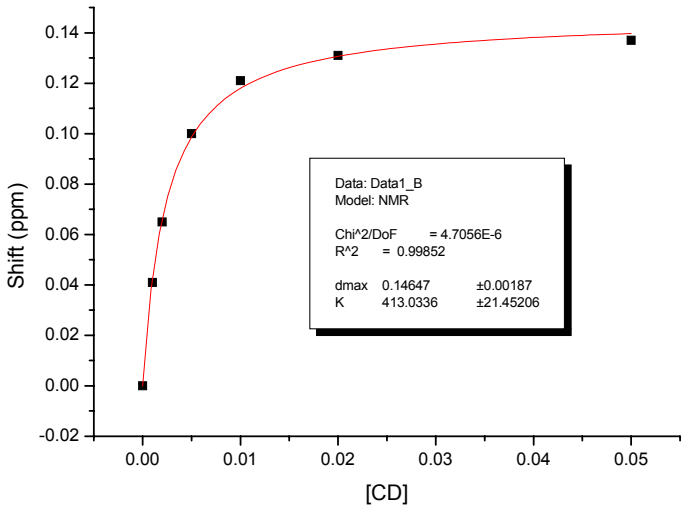


Figure 2.11 Binding isotherm for the complexation of **43c*S*** and γ-CD at 25 °C

In Table 2.1, association constants K_a, standard free energy ΔG^o, standard entropy term $T\Delta S°$ and standard enthalpy $\Delta H°$ of guest **43c** with β and γ cyclodextrin are listed. The chemical

shift change of the NMR signal of guest **43c** in the presence of α–cyclodextrin was too small to measure (as shown in Figure 2.5), and the association constant was considered too small to measure practically. Standard free energy was calculated using equation (2).

$$\Delta G° = -RT \ln K_a \quad \text{---------- Equation (2)}$$

Standard enthalpy $\Delta H°$ was determined according to the Van't Hoff equation (3).

$$d \ln K / d (1/T) = - \Delta H° / R \quad \text{----------Equation (3)}$$

Where R = 8.3144 J $K^{-1}mol^{-1}$ and $\Delta H°$ in J mol^{-1}.

$T\Delta S°$ was calculated using equation (4).

$$\Delta G° = \Delta H° - T\Delta S° \quad \text{---------- --------Equation (4)}$$

Table 2.1 ^{1}H NMR derived thermodynamic parameters for the binding of guest molecule **43c** to host β and γ–CD. All *K* values are in mol^{-1} unit and ΔG^o, $T\Delta S^o$, ΔH^o values are in kJ/mol unit.

Temp.	*S*-enantiomer		*R*-enantiomer	
	β-CD	**γ-CD**	**β-CD**	**γ-CD**
298 K	K= 63 ± 20 ΔG^o= -10.3 ± 0.7 $T\Delta S^o$= -4.84 ± 2.9	K = 413 ± 21 ΔG^o= -14.93 ± 0.13 $T\Delta S^o$= -9.70 ± 0.12	K = 47 ± 7 ΔG^o= -9.6 ± 0.3 $T\Delta S^o$= -28.9 ± 1.5	K = 321 ± 20 ΔG^o= -14.31 ± 0.15 $T\Delta S^o$= -18.7 ± 3.5
313 K	K = 43 ± 10 ΔG^o= -9.2 ± 0.6 $T\Delta S^o$= -5.7 ± 3.1	K = 262 ± 12 ΔG^o= -13.81 ± 0.11 $T\Delta S^o$= -10.83 ± 0.11	K = 20 ± 4 ΔG^o= -7.4 ± 0.4 $T\Delta S^o$= -31.1 ± 1.6	K = 211 ± 6 ΔG^o= -13.26 ± 0.07 $T\Delta S^o$= -19.7 ± 3.4
343K	K = 27 ± 3 ΔG^o= -8.2 ± 0.3 $T\Delta S^o$= -6.8 ± 3.4 ΔH^o= -15 ± 3.7	K = 113 ± 5 ΔG^o= -11.73 ± 0.11 $T\Delta S^o$= -12.9 ± 0.1 ΔH^o= -24.63 ± 0.01	K = 6 ± 1 ΔG^o= -4.5 ± 0.5 $T\Delta S^o$= -33.9 ± 1.7 ΔH^o= -38.4 ± 1.2	K = 59 ± 13 ΔG^o= -10.12 ± 0.49 $T\Delta S^o$= -22.9 ± 3.8 ΔH^o= -33 ± 3.4

As shown in Table 2.1, the association constant of guest **43c** is more than seven times higher for γ–CD compared to the association constant for β–CD. This is exactly what was

predicted from Figure 2.5. The cyclodextrin cavity must be large enough to accommodate two aromatic rings. The association constant for *S*-enantiomer is higher than that of *R*-enantiomer and the association constant (K_a) decreases as temperature increases.

In the guest compound **43b** (scheme 2.1), the electron rich indole moiety was replaced by a phenyl moiety, resulting weaker π-π stacking interaction between aromatic moieties. Therefore, as shown in Figure 2.12, α-CD gives no enantiomeric recognition, while β- and γ-CD gives a little enantiomeric recognition.

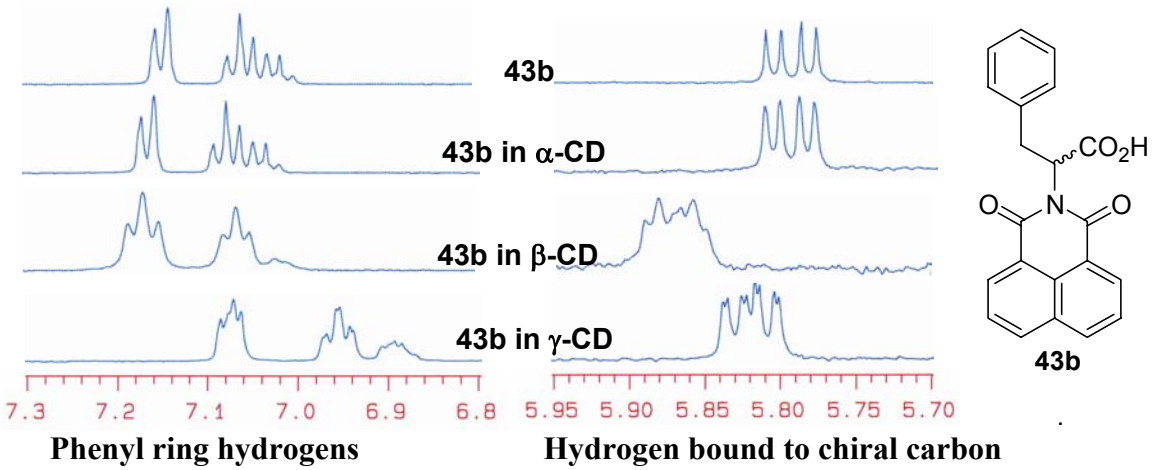


Figure 2.12 A portion of NMR spectra of racemic **43b** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) with α, β, and γ-CD (0.01 M), respectively.

Guest compound **43a** has only one aromatic ring therefore only γ-CD can give little enantiomeric recognition (figure 2.13) while α- and β-CD can not show any enantiomeric recognition because neither of these two CD can accommodate the aromatic ring inside their cavity.

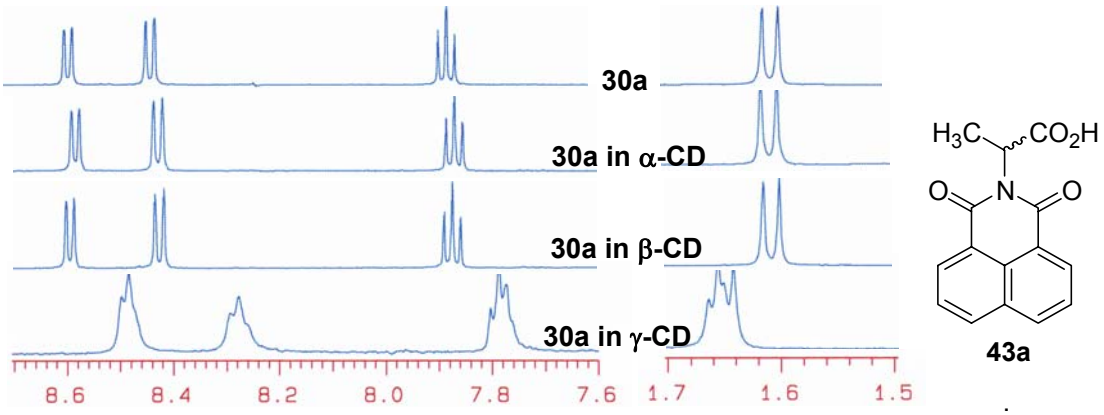
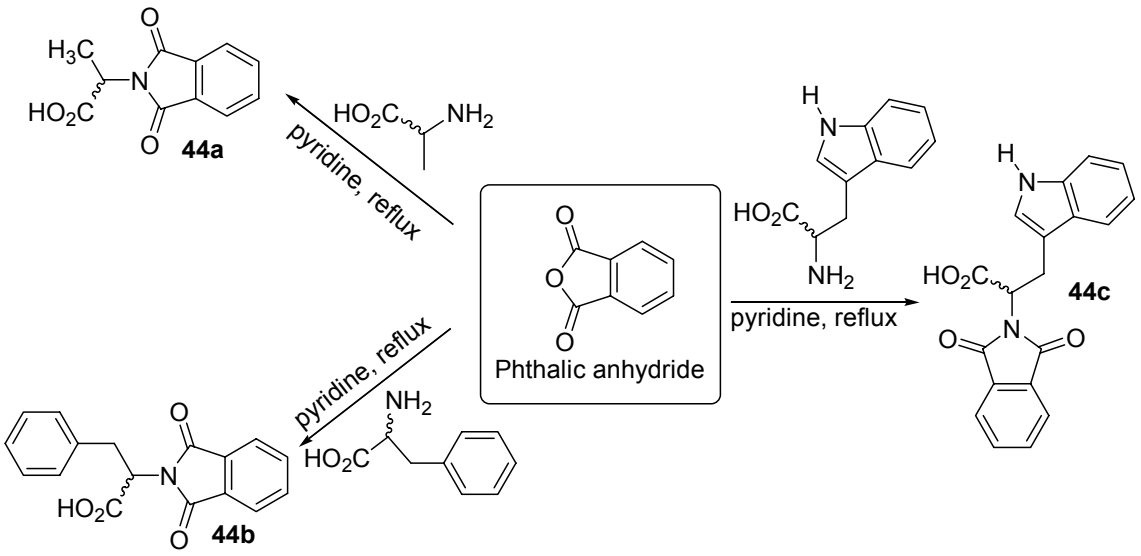


Figure 2.13 A portion of NMR spectra of racemic **43a** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) with α, β, and γ-CD (0.01 M) respectively.

2.1.3 Isoindole-1,3-Dione Derived Amino Acids

The π-π stacking interactions between two aromatic moieties strongly depend on the magnitude of electron acceptor and electron rich aromatic moiety. The stronger the π-π stacking interaction between two aromatic moieties, the better enantiomeric recognition in the racemate NMR of the guest compound in the presence of the appropriate host cyclodextrin. Moreover, the size of naphthalene ring was responsible for the smaller association constants of guest compound **43c** with α- and β-cyclodextrins. By reduction of acceptor ring size from naphthalene to benzene, a series of guest compounds **44a**, **44b** and **44c** have been synthesized and the changes in enantiomeric recognition and association constants are studied.

The synthetic conditions for the preparation of these amino acid derivatives are presented in Scheme 2.2.[265] Reactions were performed with phthalic anhydride in pyridine with the amino acid alanine, phenylalanine and tryptophan (Scheme 2.2). The isolated yields range between 79-95%.



Scheme 2.2 Preparation of guest compounds **44a**, **44b** and **44c**

To observe the formation of self-assembly molecular associates, as demonstrated in Figure 2.4 for compound 4**3c**, ^{1}H NMR spectra of compound **44c** were recorded using different enantiomeric compositions of *R*: *S* (1:4 and 4:1), as well as using different concentrations, ranging from 10^{-4} to 10^{-1}. In none of the recorded NMR spectra could we detect any resolution of enantiomers. One reasonable explanation could be that in both compounds **43c** and **44c**, the only difference is in the size of the electronic acceptor ring. In compound **44c**, the naphthalene ring is replaced with a benzene ring, which makes it slightly less electron poor, so the LUMO energy of this compound would be lower than that of **43c**. In the case of **44c,** the formed weak self-assembly molecular associates cannot provide a sufficient different environment for both enantiomers to be seen by NMR spectroscopy.

Negative electrospray ionization mass spectra (ESIMS) of compound **44c*S*** in alkaline aqueous solution was performed in order to detect the formation of a molecular associate. Figure 2.14 indicates the formation of the molecular dimer with MW 667.1. Failure to observe the NMR enantiomer nonequivalence might be due to a weak enantiomer association constant.

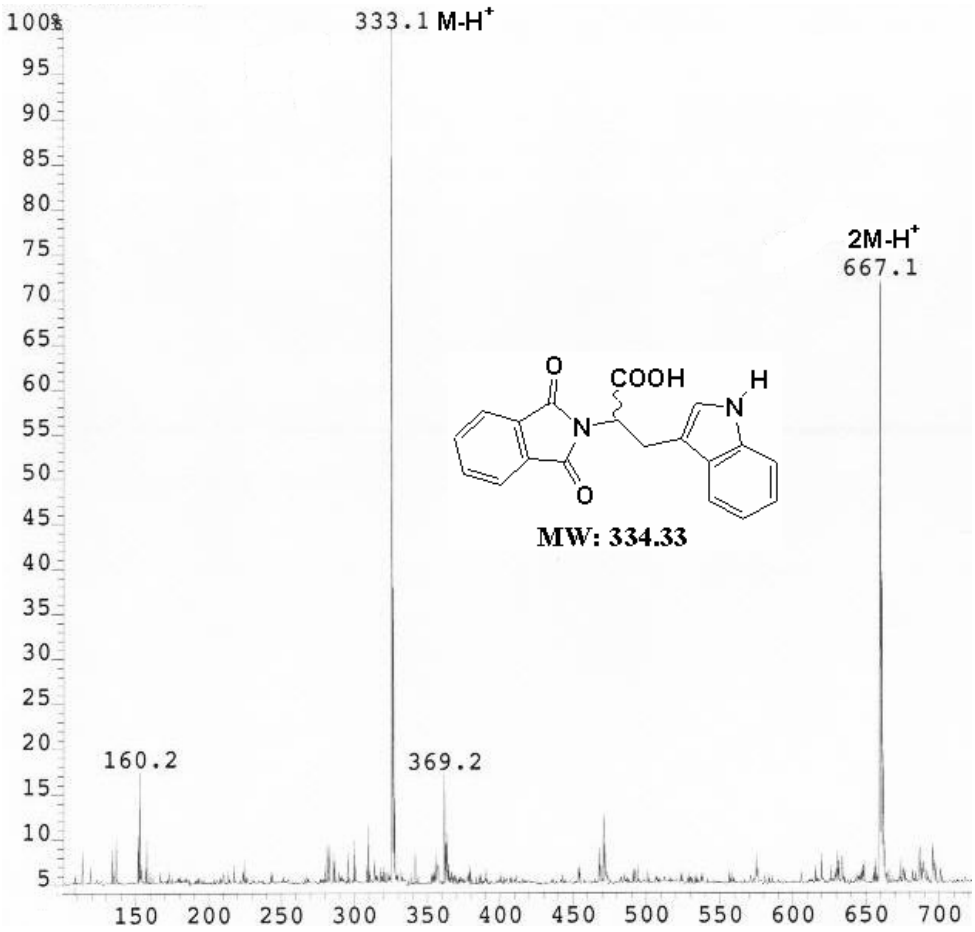


Figure 2.14 Negative ESIMS of sodium salt of **44c** in aqueous solution

If the association constant for the formation of the self-assembly molecular associate is very small due to weak nonbonding π-π stacking interactions of the aromatic moieties, the presence of cyclodextrin should stabilize these associates by the binding of the guest molecule inside the CD cavity. In such a case, the NMR spectroscopic recognition could be enforced. This is demonstrated in Figure 2.15 for cyclodextrin assisted enantiomeric recognition of racemic **44c**. The chemical shift change in the racemate NMR in the presence of α-CD indicates a complex formation between **44c** and outside of the α-CD cavity because the α-CD cavity is not sufficiently large enough to form neither the 1:1 nor the other complexes shown in Figure 2.6. The best results were obtained in γ-cyclodextrin in comparison with β-cyclodextrin. The β-CD

cavity can accommodate either of two aromatic rings of **44c** and can form a 1:1 complex but not any higher order complexes; therefore, only weak enantiomeric discrimination was observed.

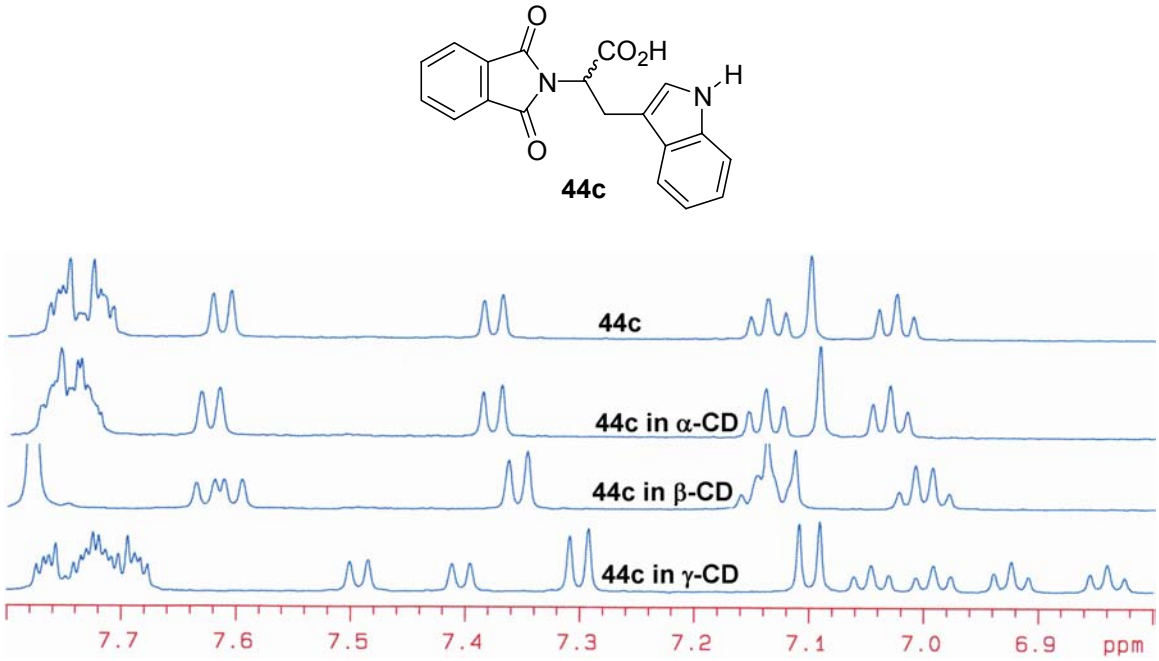


Figure 2.15 A portion of NMR spectra of **44c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M), respectively

From the comparison of the racemate NMR with individual enantiomer NMRs in γ-CD, the enantiomeric composition and signal assignment can be identified, but more importantly, from the proton signal shift it is also possible to deduce which enantiomer will bind more strongly with cyclodextrin (Figure 2.16). For example, if the racemate NMR is compared with both enantiomer NMRs in the presence of γ-cyclodextrin, there is less influence of γ-CD on chemical shift of **44c*S***. On the other hand, there is a substantial up field chemical shift of indole hydrogens (6.8-7.5 ppm) and a downfield shift (7.7 ppm) for phthalimido hydrogens of **44c*R*** in the presence of γ-CD. Based on this observation, it might be suggested that γ-CD forms preferentially a stronger complex with **44c*R*** compared to **44c*S***.

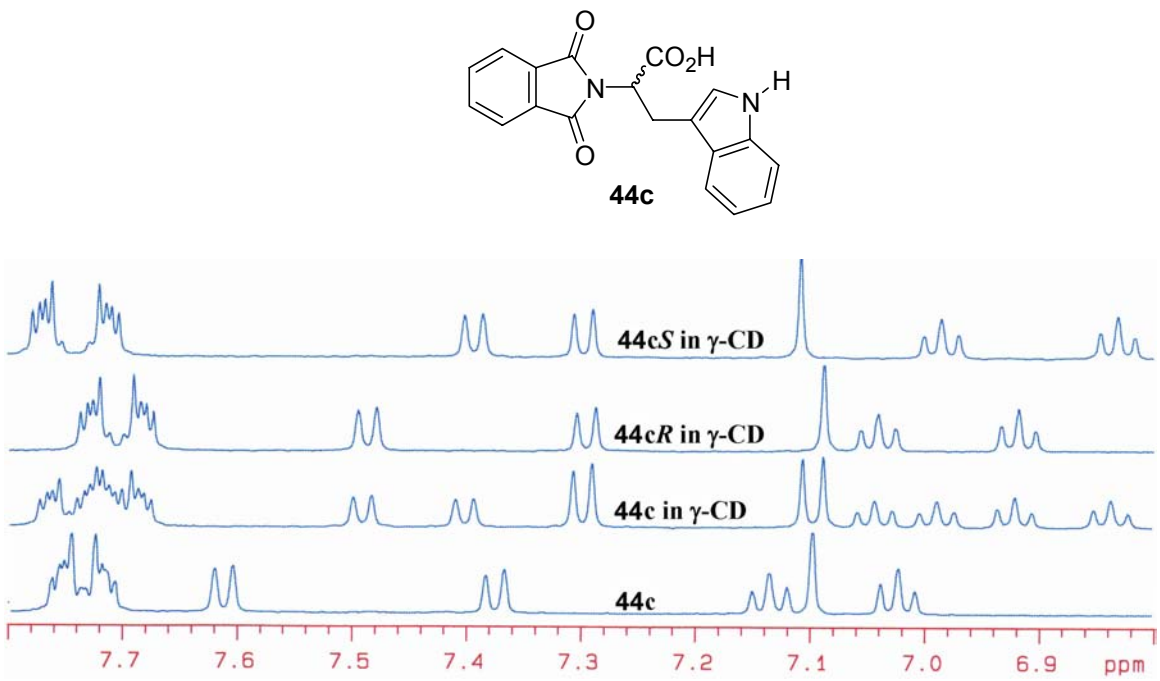


Figure 2.16 Comparison of γ-CD induced NMR non-equivalency of **44c**

Considering that the γ-CD cavity is sufficient enough to accommodate both phthalimido and indole moieties, it should form a ternary and higher order complexes with **44c**. Since γ-CD would form a ternary complex with a **44c** dimer, it should not be possible to detect a "free" **44c** dimer signal at 667.1 m/z (Figure 2.13) in the solution, but rather it should be complexed with one γ-CD (981.9 m/z), two γ-CDs (1630 m/z), and three γ-CDs (2278.6 m/z), respectively. This is perfectly demonstrated in ESIMS of **44cS** (Figure 2.17) with γ-CD.

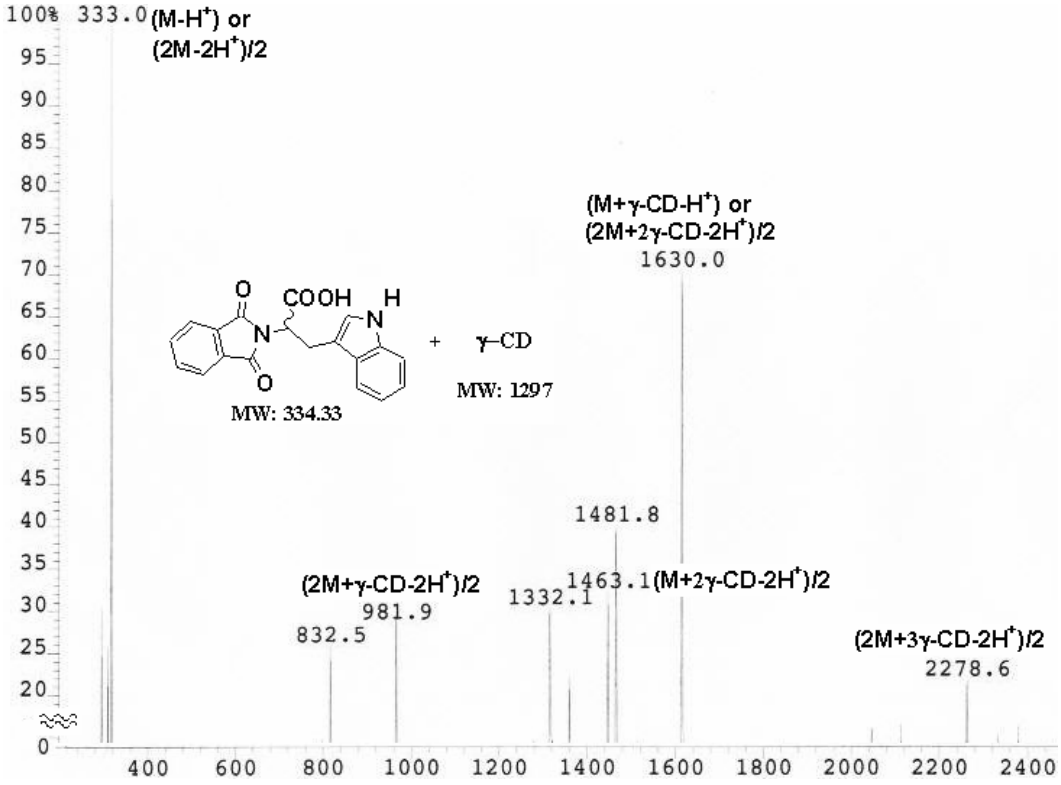


Figure 2.17 ESIMS of **44cS** (0.001 M) in DMSO (1 drop) and γ–CD (0.01 M)

To show the π-π interaction of the electron poor phthalimido moiety hydrogen with the electron rich indole moiety hydrogen, which leads to the formation of self-assembly molecular aggregates (Figure 2.1), NOESY (Nuclear Overhauser Effect Spectroscopy) NMR spectrum (Figure 2.18) of **44cS** (0.01 M) in $NaHCO_3$ (0.03 M) was performed on 500 MHz NMR using D_2O as solvent.

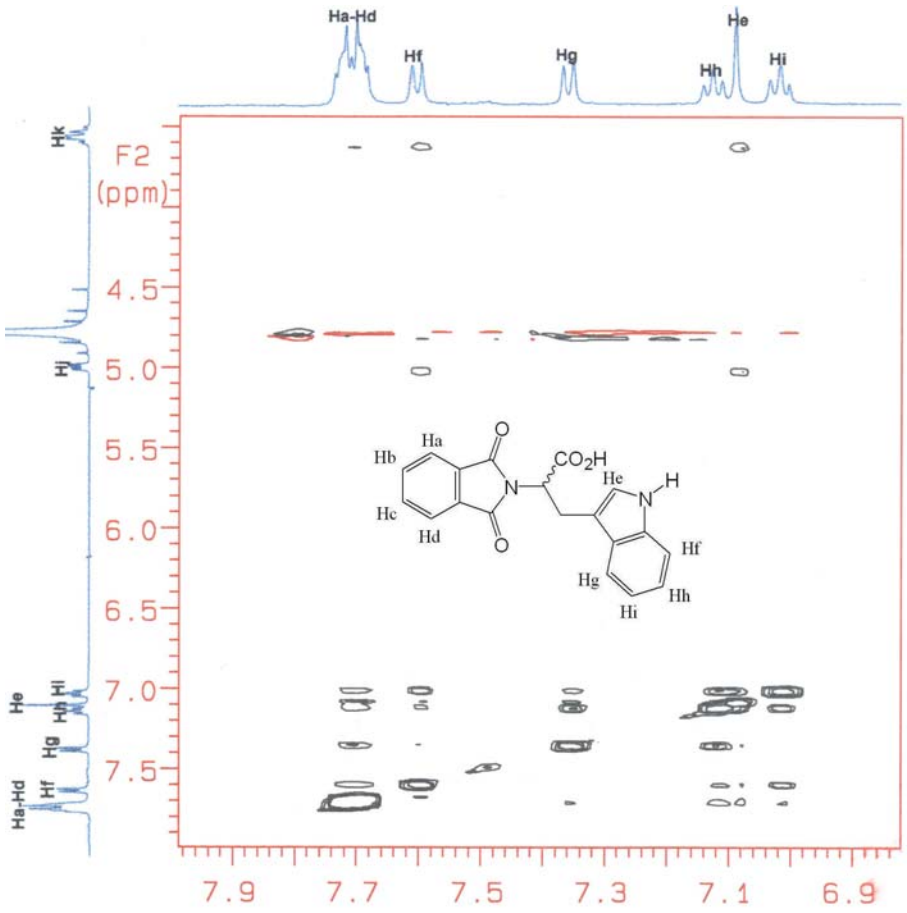


Figure 2.18 NOESY NMR spectrum of mixture of **44c*S*** (0.01 M) in aqueous $NaHCO_3$ (0.03 M)

The π–π stacking interaction between two guest molecules of **44c*S*** is shown in Figure 2.18. NOE evidence of π–π stacking interaction between hydrogens of the phthalimido moiety (Ha-Hd) and the electron rich indole moiety (He-Hi) are very clear.

Formed self-assembly molecular aggregates due to π-π stacking interactions between electron rich and electron poor aromatic moieties are very weak. Therefore these aggregates stabilize by binding into the cyclodextrin cavity (Figure 2.1). To show the interaction between the guest compound **44c** hydrogen and host γ-cyclodextrin hydrogen, a NOESY NMR

spectrum (Figure 2.19) of mixture of **44c*S*** (0.01 M) and γ-CD (0.01 M) and was performed on a 500 MHz NMR using D_2O as solvent.

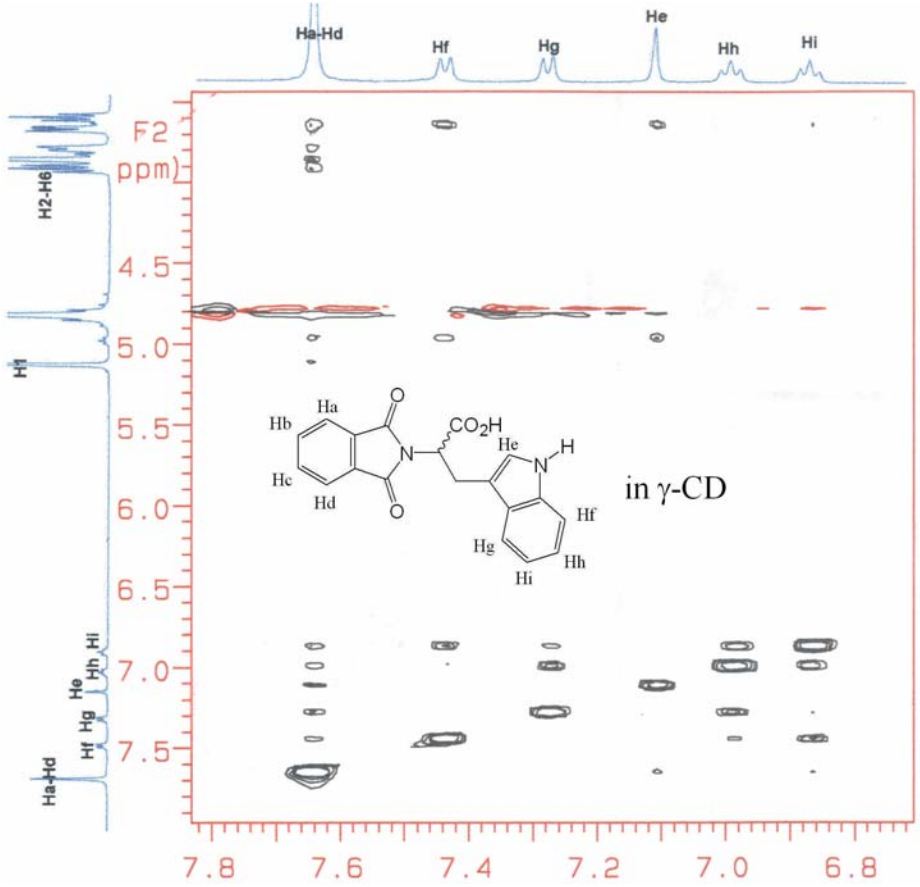


Figure 2.19 NOESY NMR spectrum of mixture of **44c*S*** (0.01 M) in aqueous $NaHCO_3$ (0.03 M) and γ-CD (0.01 M)

The interaction between the host γ-cyclodextrin hydrogen and the guest **44c*S*** hydrogen (Figure 2.19) can be seen in this NOESY spectrum. For example, all γ-cyclodextrin hydrogens (H_2-H_6) show strong space coupling interactions with guest **44c*S*** hydrogens (Ha-Hd, Hf, He).

To better understand the binding of guest compound **44c** with host cyclodextrins, association constants (K_a, M^{-1}) were measured by ^1H NMR (500 MHz). The solution of **44cS** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) was titrated with host CD solution at different temperatures and after each titration the change in chemical shift was measured.

Using equation (1), the binding isotherms for the guest **44cS** with α–, β– and γ–cyclodextrins were generated to calculate the association constant K_a from which the standard free energy was calculated, using the equation (2).

Table 2.2 Association constant K_a and standard free energy $\Delta G°$ for the binding of guest molecule **44c*S*** to host α, β and γ–CD at 25 °C

Host Compd.	Association Const. K_a mol^{-1}	Standard Free Energy $\Delta G°$ kJ mol^{-1}
α-CD	67 ± 13	-10.4 ± 0.5
β-CD	105 ± 14	-11.5 ± 0.3
γ-CD	226 ± 13	-13.4 ± 0.2

As shown in Table 2.2, the association constant of the guest **44c*S*** is highest for γ–CD. This is exactly what we predicted from the spectrum indicated in Figure 2.15, and are consistent with what we understand from our model Figure 2.1. The cyclodextrin cavity must be large enough to accommodate two aromatic rings. It was also found that the association constant for the *R*-enantiomer is higher than that of the *S*-enantiomer (Table 2.3). It is also interesting to note that the association constant of **44c*S*** with α- and β- cyclodextrin is much higher compared to guest compound **43c*S***. As explained earlier, this is due to better fitting of the guest compound

inside the cyclodextrin cavity. In guest compound **44c*S***, the naphthalene ring is replaced by a smaller phthalimido ring (scheme 2.2).

Table 2.3 Association constant and standard free energy for the binding of guest molecule **44cS** and **44c*R*** to host γ–CD at 25 °C

Enantiomer	Association Const. K_a mol^{-1}	Standard Free Energy $\Delta G°$ kJ mol^{-1}
S	226 ± 13	−13.4 ± 0.2
R	1050 ± 63	−17.2 ± 0.2

In Table 2.4, the association constant K_a, standard free energy $\Delta G°$, standard entropy term $T\Delta S°$ and standard enthalpy $\Delta H°$ of the guest **44c*S*** with γ-cyclodextrin at different temperatures are listed. As shown in the table, the association constant K_a decreases as the temperature increases.

Table 2.4 ^{1}H NMR derived thermodynamic parameters for the binding of guest molecule **44cS** to host γ–CD

Temperature	Association Const. K_a Mol^{-1}	$\Delta G°$ kJ Mol^{-1}	$\Delta H°$ kJ Mol^{-1}	$T\Delta S°$ kJ Mol^{-1}
298 K	226 ± 13	-13.4 ± 0.2	-24.4 ± 2	-10.9 ± 1.8
313 K	187 ± 8	-12.9 ± 0.1		-11.4 ± 1.9
333 K	74 ± 6	-10.7 ± 0.2		-13.7 ± 1.8

To show that for the successful cyclodextrin assistance in the formation of π–π stacking molecular complexes, the guest compound must contain both electron-poor and electron-rich

aromatic moieties; guest compound **44b** and **44a** (Scheme 2.2) were synthesized by replacing the electron rich indole moiety with phenyl or hydrogen, respectively. By doing so, the π-π interaction should be diminished (**44b**) or removed (**44a**). As expected, both guest compound's NMR spectra with all three cyclodextrins show no noticeable enantiomeric recognition. However, the phthalimido and phenyl ring hydrogen signals in compound **44b** and the methyl hydrogen signal in compound **44a** split into two sets of signals due to perfect fitting of the phthalimido ring into β-CD (Figure 2.20, 2.21). This finding is not surprising considering that the cyclodextrin cavity must be large enough to accommodate the ring of the guest compound. The α-CD cavity is too small while γ-CD cavity is too big to perfectly accommodate the guest compounds.

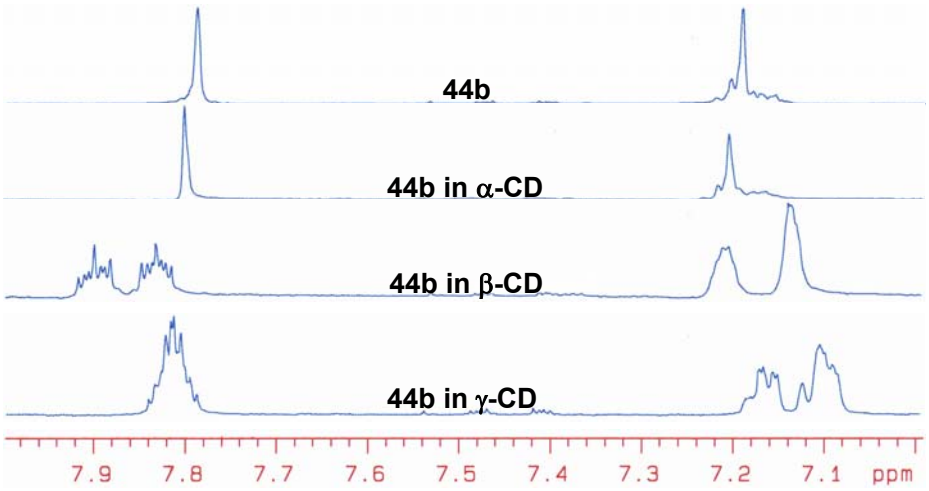


Figure 2.20 A portion of NMR spectra of **44b** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M), respectively.

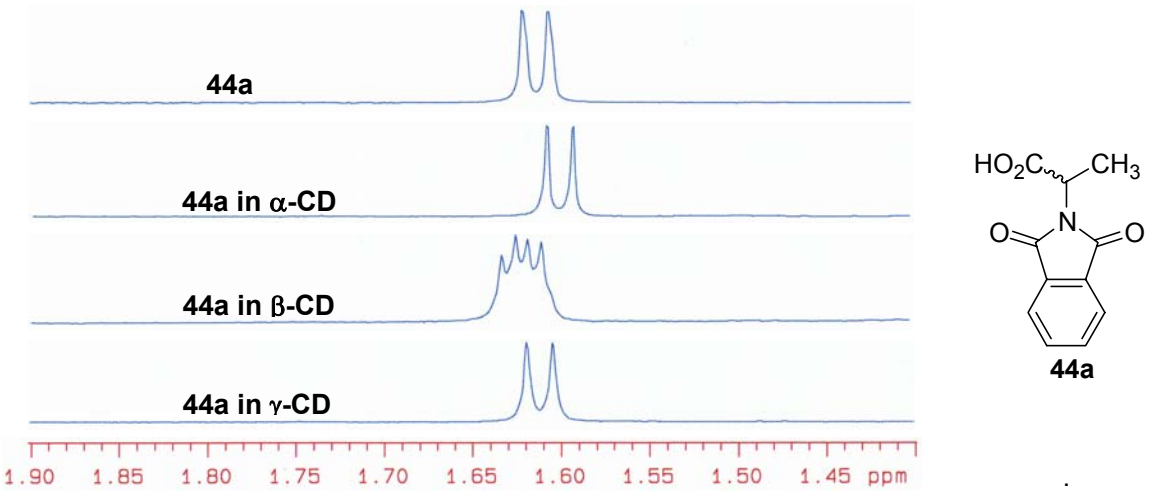


Figure 2.21 A portion of NMR spectra of **44a** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M), respectively.

Association constants (K_a) for guest compound **44a*S*** with all three cyclodextrins are listed in Table 2.5. As explained earlier, guest compound **44a*S*** shows better enantiomeric recognition with β-CD (Figure 2.21), and the association constant with β -CD is higher than that with α- and γ-CD.

Table 2.5 Association constant and standard free energy ΔG° for the binding of guest molecule **44a*S*** to host α, β and γ–CD

Host Compd.	Association Const. K_a mol^{-1}	Standard Free Energy ΔG^o kJ mol^{-1}
α-CD	64 ± 10	-10.3 ± 0.4
β-CD	227 ± 17	-13.5 ± 0.2
γ-CD	82 ± 6	-10.9 ± 0.2

The association constant (K_a) is different for both enantiomers of the racemate. It was also found that the association constant for **44a*R*** is higher than that of **44a*S*** with γ-CD (Table 2.6).

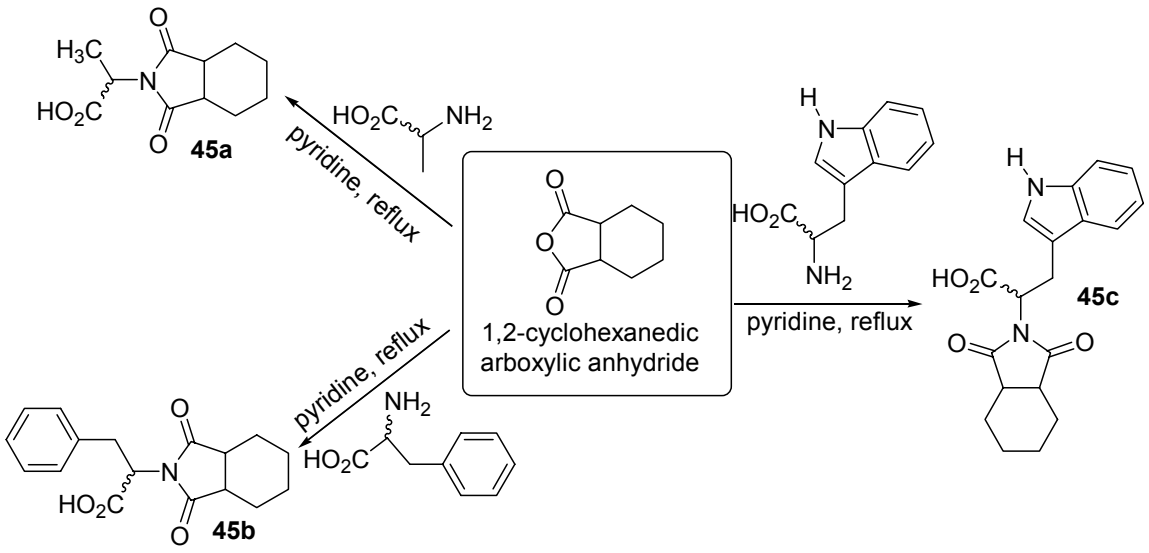
Table 2.6 Association constant and standard free energy ΔG° for the binding of guest molecule **44a*S*** and **44a*R*** to host γ–CD at 25 °C

Enantiomer	Association Const. K_a mol^{-1}	Standard Free Energy $\Delta G°$ kJ mol^{-1}
S	82 ± 6	-10.9 ± 0.2
R	141 ± 30	-12.3 ± 0.6

2.1.4 Hexahydro-isoindole-1,3-dione derived amino acids

To show that the electron acceptor aromatic moiety is also very important for π–π stacking molecular complex formation between host cyclodextrin and guest compounds, we synthesized guest compounds **45a**, **45b** and **45c** (Scheme 2.3), and studied their binding capability with different cyclodextrins. None of these series of guest compounds have electron acceptor aromatic moieties.

The synthetic conditions for the preparation of these amino acid derivatives are presented in Scheme 2.3. Reactions were performed with 1,2-cyclohexanedicarboxylic anhydride in pyridine with the amino acids alanine, phenylalanine and tryptophan (Scheme 2.3). The isolated yields range between 90-93 %.



Scheme 2.3 Preparation of guest compounds **45a**, **45b** and **45c.**

^{1}H NMR spectroscopic study of the guest compounds **45a**, **45b** and **45c** with all three α, β, and γ CD does not show significant enantiomeric recognition. This was expected considering that for strong π–π stacking interactions the guest compound should have both an electron acceptor and an electron donor aromatic moiety.

The ^{1}H NMR spectra of guest compound **45c** shows a slight shift of proton signals in the presence of α, β, and γ–CD. In the presence of β-CD, there is slight enantiomeric recognition (Figure 2.22). The two triplets of the indole ring split into two sets of triplets that are slightly overlapped. We believe that this observed enantiomeric spectroscopic non-equivalence might come from only the 1:1 cyclodextrin complex with indole moiety in β-CD.

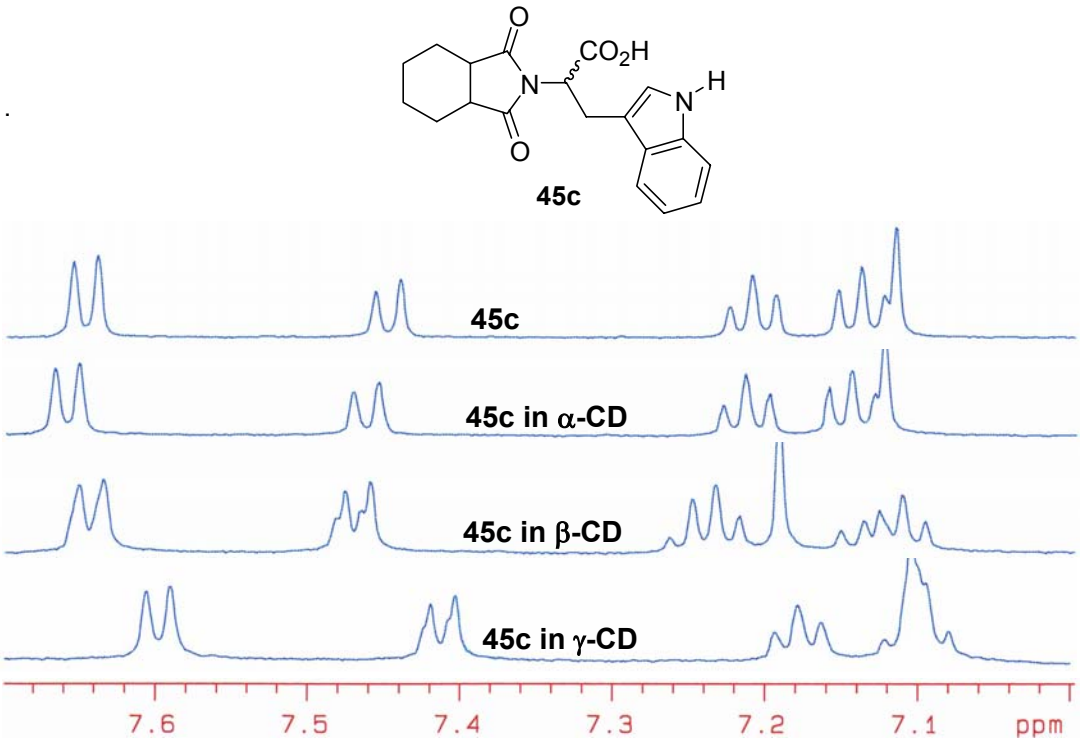


Figure 2.22 A portion of NMR spectra of **45c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M), respectively.

The ^1H NMR spectra of guest compound **45b** shows a slight shift of proton signals in the presence of α–, β– and γ–CD, but no significant enantiomeric recognition (Figure 2.22).

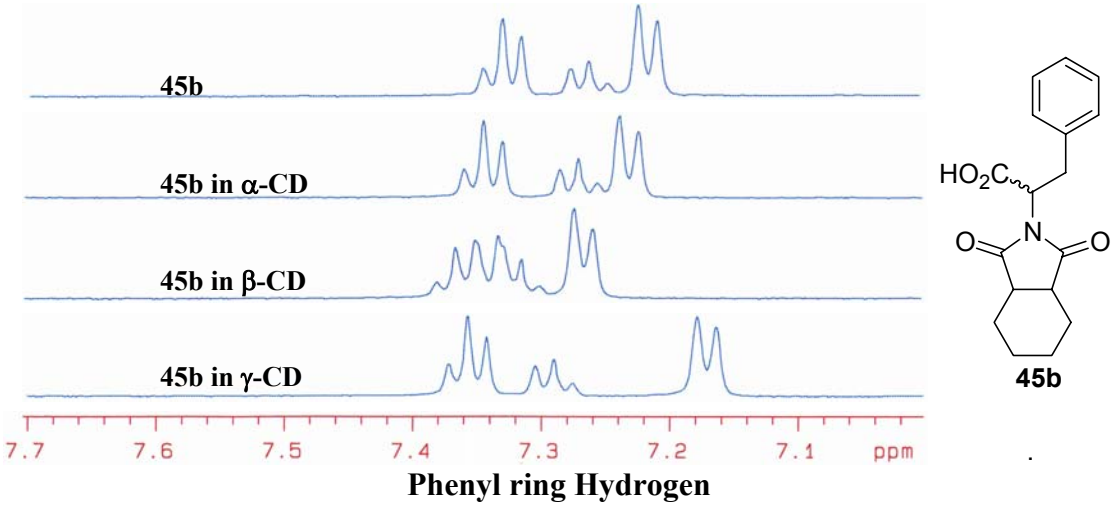


Figure 2.23 A portion of NMR spectra of **45b** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M)

The ^{1}H NMR spectra of racemate **45a** in the presence of α–, β– and γ–CD are shown in Figure 2.24. Compound **45a** has no aromatic moiety. The racemate NMR of **45a** in the presence of β-CD shows diminutive enantiomeric recognition. Two quartets, each from different enantiomers are slightly shifted, giving an impression of a pentet. The methyl hydrogen gives two sets of doublets. Signals from both enantiomers are shifted more with β-CD compared to γ-CD. Thus, β-CD gives slightly better enantiomeric recognition compared to γ-CD.

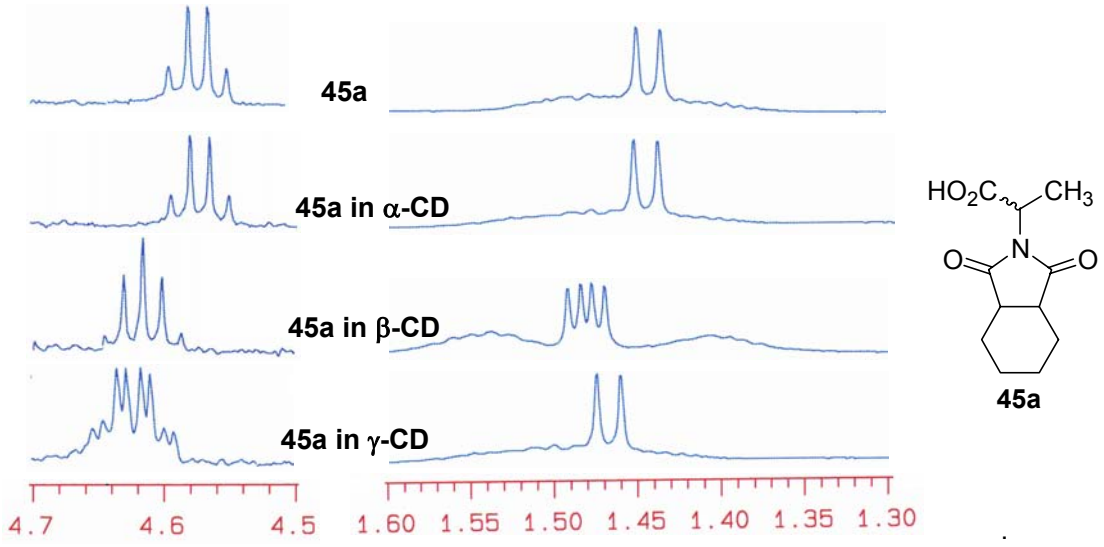
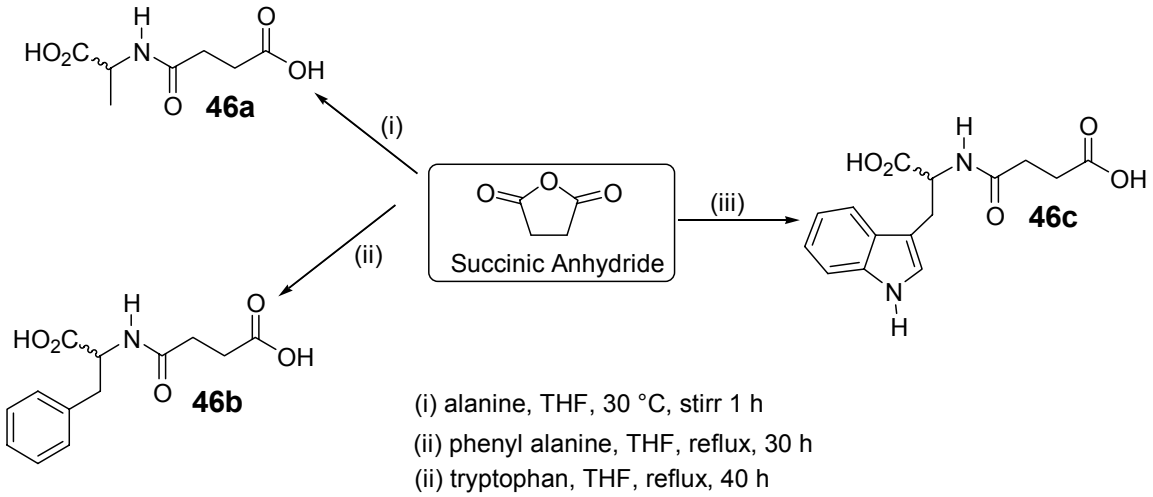


Figure 2.24 A portion of NMR spectra of **45a** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ–CD (0.01 M)

2.1.5 Succinamic Acid Derived Amino Acids

To show that the presence of the electron-poor aromatic moiety is crucial for the formation of the molecular assemblies discussed in Figure 2.1, mono amino acid amides with succinic acid were prepared (Scheme 2.4). The reaction progress was optimized and monitored by NMR

spectroscopic studies. After all reaction conditions were optimized, the isolated yields of the target compounds were between 83-96 %.



Scheme 2.4 Preparation of guest compounds **46a**, **46b**, and **46c**

These compounds, **46a**, **46b**, and **46c,** are very soluble in water. Spectroscopic studies of racemic and non-racemic solutions of these guest compounds at higher concentrations and different enantiomeric ratios were studied in DMSO and water. We did not observe NMR non-equivalency of enantiomers for any of these compounds. Even the racemate NMR of **46a**, **46b**, and **46c** in the presence of α-, β- and γ-cyclodextrin did not show enantiomeric recognition either. However, we were able to detect the formation of CD complexes with guest compounds **46b** and **46c** through ESIMS studies. From this finding it is obvious that these cyclodextrin complexes cannot produce a different NMR environment to observe enantiomeric recognition through NMR spectroscopy. As shown in Figures 2.25, 2.26 and 2.27, none of these guest

compounds shows enantiomeric recognition in the presence of α–, β– and γ–CD. Only a slight change in the NMR signal can be seen, which indicates the formation of inclusion complexes.

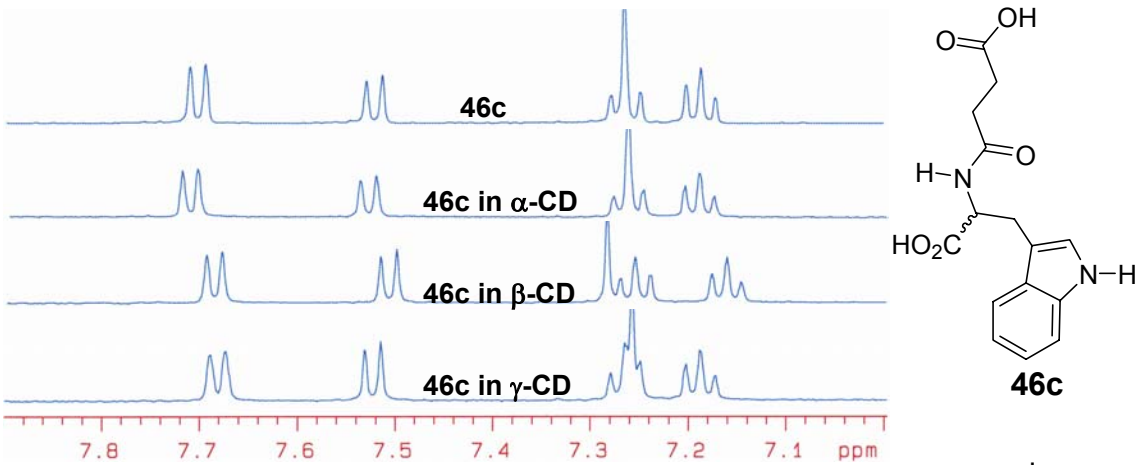


Figure 2.25 A portion of NMR spectra of **46c** (0.001 M) in α, β, γ–CD (0.01 M)

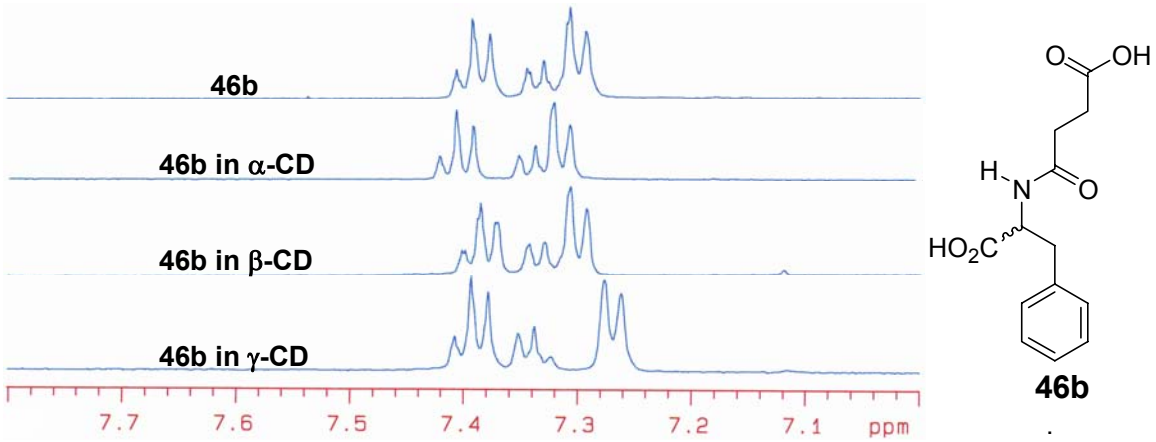


Figure 2.26 A portion of NMR spectra of **46b** (0.001 M) in α, β, γ–CD (0.01 M)

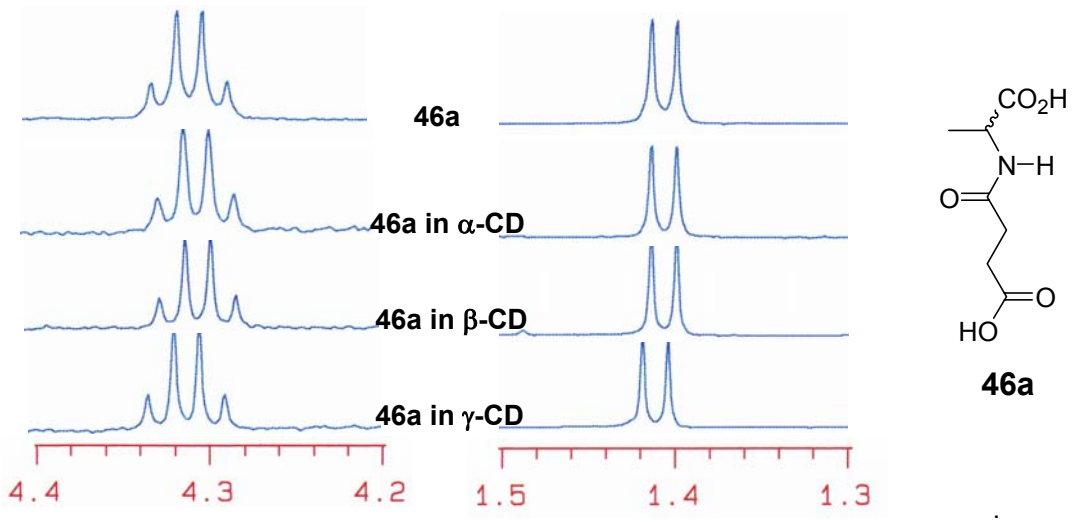


Figure 2.27 A portion of NMR spectra of **46a** (0.001 M) α, β, γ–CD (0.01 M)

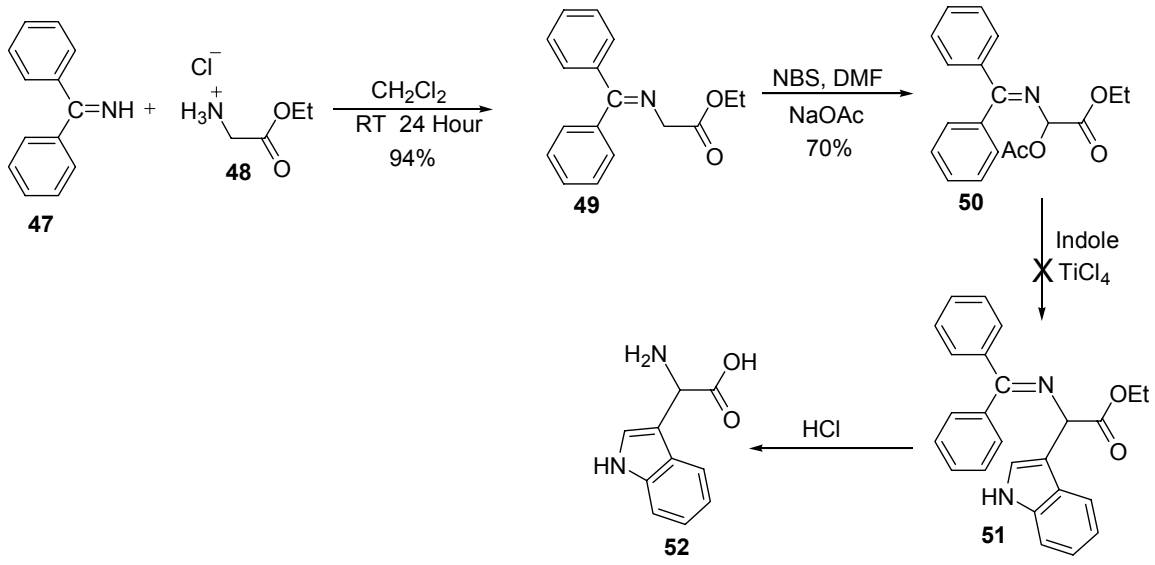
Association constants (K_a) of guest compound **46c*S*** with all three cyclodextrins are listed in Table 2.6. Compound **46c*S*** has a higher association constant with γ-CD compared to α– and β-CD. This is probably due to better fitting of the indole moiety inside γ-CD cavity.

Table 2.7 Association constant and standard free energy ΔG° for the binding of guest molecule **46c*S*** to host α, β and γ–CD

Host Compd.	Association Const. K_a mol^{-1}	Standard Free Energy ΔG^o kJ mol^{-1}
α-CD	56 ± 10	-9.9 ± 0.7
β-CD	81 ± 9	-10.9 ± 0.3
γ-CD	188 ± 17	-12.9 ± 0.2

2.1.6 Amino-(1H-indol-3-yl)-Acetic Acid Derived Guest Compounds

Guest compounds **43c**, **44c**, **45c**, and **46c** have been synthesized using different electron acceptor moieties and tryptophan as the electron donor aromatic moiety. In tryptophan, there is a $-CH_2$ group that connects the chiral carbon with the indole ring, which makes the indole ring very flexible, resulting in poor interaction with the host cyclodextrin during complex formation. Inoue and coworkers, during their study of complexation ability of different guest compounds with cyclodextrins, proposed that the change in the length of tether which connects two aromatic moieties of guest compounds can dramatically change the ability of the aromatic rings to adjust their position within CD cavity and thus enantiomeric recognition property.[266] To check this, we have planned to synthesize derivatives of the guest compounds **43c**, **44c**, **45c**, and **46c** by replacing tryptophan with amino-(1H-indol-3-yl)-acetic acid **(52)** (Scheme 2.5). The new guest compounds with amino acid **52** as the electron rich moiety will be more rigid and should form more stable diastereomeric inclusion complexes with cyclodextrins. The synthetic strategy for the synthesis of amino acid **52** is outlined in Scheme 2.5.



Scheme 2.5 Synthetic strategy for synthesis of **52**

Our initial synthetic preparations include the following results: Transimination of the reactive benzophenone equivalent, benzophenone imine **47** with the amino ester salt, glycine ethyl ester hydrochloride **48** in dichloromethane at room temperature for 24 hour gave **49** in 94% yield.[267] The stable electrophilic glycine synthon **50** was prepared directly from the Schiff base **49** by bromination in the presence of sodium acetate in 70% yield.[268] Attempts to convert the electrophilic glycine cation equivalent **50** into **51** using $TiCl_4$ as a Lewis acid and indole as a nucleophile were not successful.

2.2 Amino Acid Imide Guest Compounds with Two Chiral Centers

2.2.1 Cyclodextrin Assisted Formation of Homochiral Polymer-Like Associate in Two Chirality System

Cyclodextrins can also form diasteromeric inclusion complexes with racemates that have two chiral centers, one relatively large aromatic acceptor, and two smaller electron-rich aromatic moieties. A graphical representation of proposed guest compound is shown in Figure 2.28. Considering structural properties, molecule M should also form molecular self-assembly as described earlier in figure 2.1.[259]

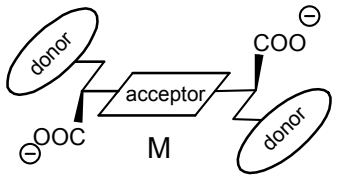


Figure 2.28 Chiral molecule with two donors and one acceptor

Since compound **M** has both electron-deficient and electron-rich aromatic moieties, it should form molecular associates through dimerization, trimerization, etc. by nonbonding aromatic interactions. The stability of molecular associates can be enhanced by binding into the cyclodextrin cavity and forming inclusion complexes (Figure 2.29).

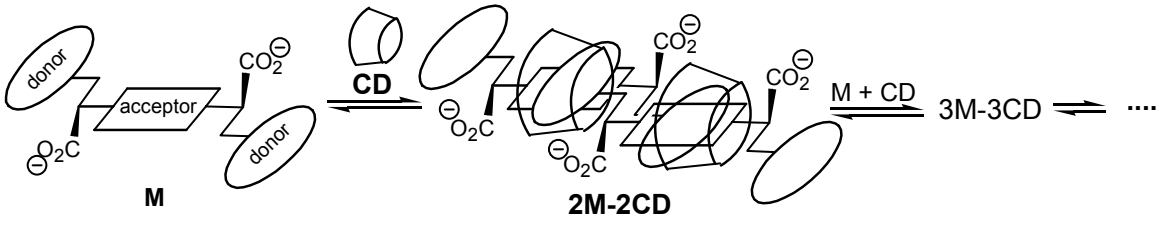
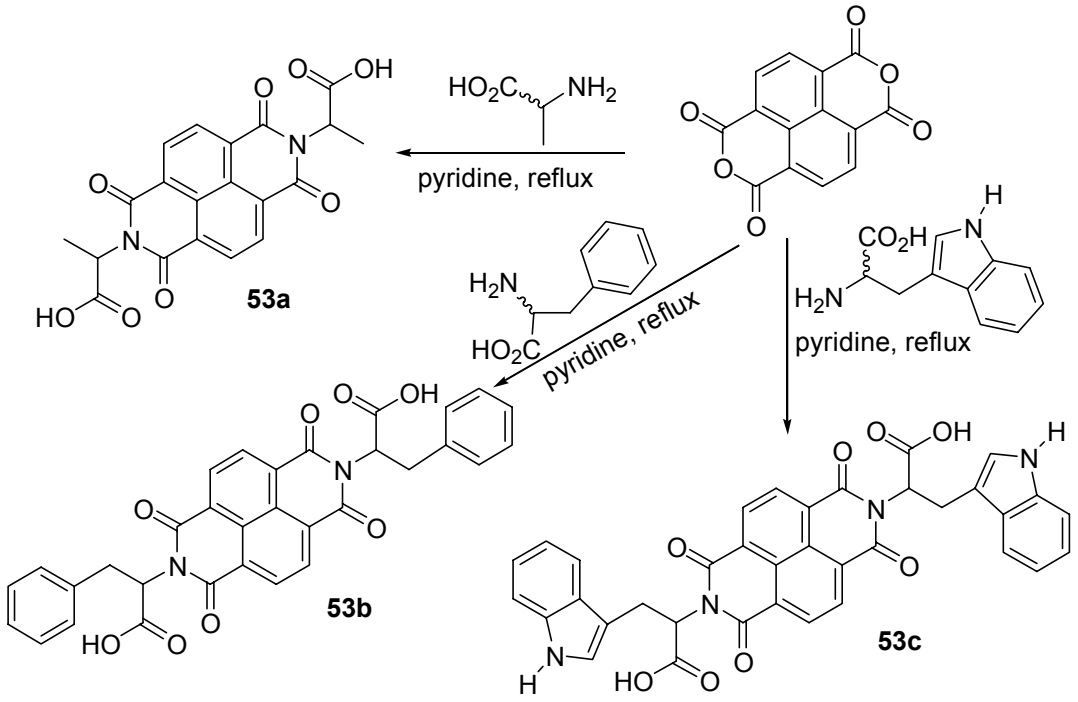


Figure 2.29 Possible molecular associates in aqueous cyclodextrin solutions

2.2.2 Benzo[*lmn*][3,8]Phenanthroline Derived Amino Acids

Three amino acids were chosen from which guest compounds **53a**, **53b** and **53c** were prepared for the study of the formation of molecular aggregates (Scheme 2.26).[269] The central aromatic portion of the guest compounds **53a**, **53b** and **53c** is electron-deficient containing a naphthalene ring with four carbonyl groups. The two indole moieties of tryptophan in **53c** and phenyl moiety of phenylalanine in **53b** make these parts of these compounds electron rich. Aromatic π-π interactions in compounds **53c** and **53b** make it possible to form self-assembly molecular associates. The formation of these associates is not possible for compound **53a**, which does not contain an additional aromatic moiety, but was synthesized to demonstrate the necessity of second aromatic moiety (Scheme 2.6). Isolated yields are between 90-97%.



Scheme 2.6 Synthetic transformations of three amino acids into **53a**, **53b**, and **53c**

Electro-spray mass spectroscopic studies of three cyclodextrins, α–, β– and γ–cyclodextrins with guest compounds **53a**, **53b** and **53c** were carried out to study the formation

of molecular associates. In aqueous solution, the guest molecules **53a**, **53b** and **53c** can form molecular aggregation by dimer, trimer, etc. For example, negative ESIMS of **53a** in methanol-water solution shows a molecular peak at 819.3 corresponding to dimer (**2M)** of **53a** (Figure 2.30). This finding indicates the formation of molecular self- assembly of **53a**, **53b**, and **53c** in solution.

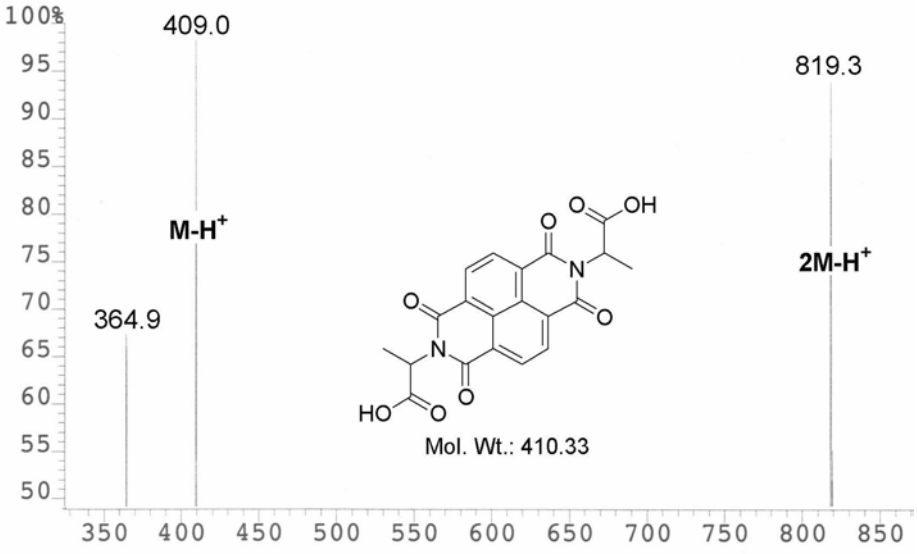


Figure 2.30 Negative ESIMS of **53a*S*** in methanol-water

In the ESIMS spectra of all three guest compounds **53a**, **53b** and **53c**, molecular aggregates in aqueous media were observed with and without cyclodextrins. This was demonstrated with the negative ESIMS of **53a** in aqueous α-cyclodextrin (Figure 2.31). The signal intensity of the molecular dimer (**2M-H**$^+$=818.8 m/z) of **53a** increased with reference to the molecular signal (**M-H**$^+$=409 m/z) (Figure 2.29). Formation of the molecular trimer (**3M-H**$^+$=1229 m/z) was also observed. Complex formation with α-cyclodextrin (1:1) was also observed (1381.7 m/z), but higher order complex formation was not observed. Since compound

53a does not have an electronic rich moiety present, it cannot form self-assembly molecular aggregates through aromatic stacking interactions, only through hydrogen bonding interactions present in two carboxylic acid groups.

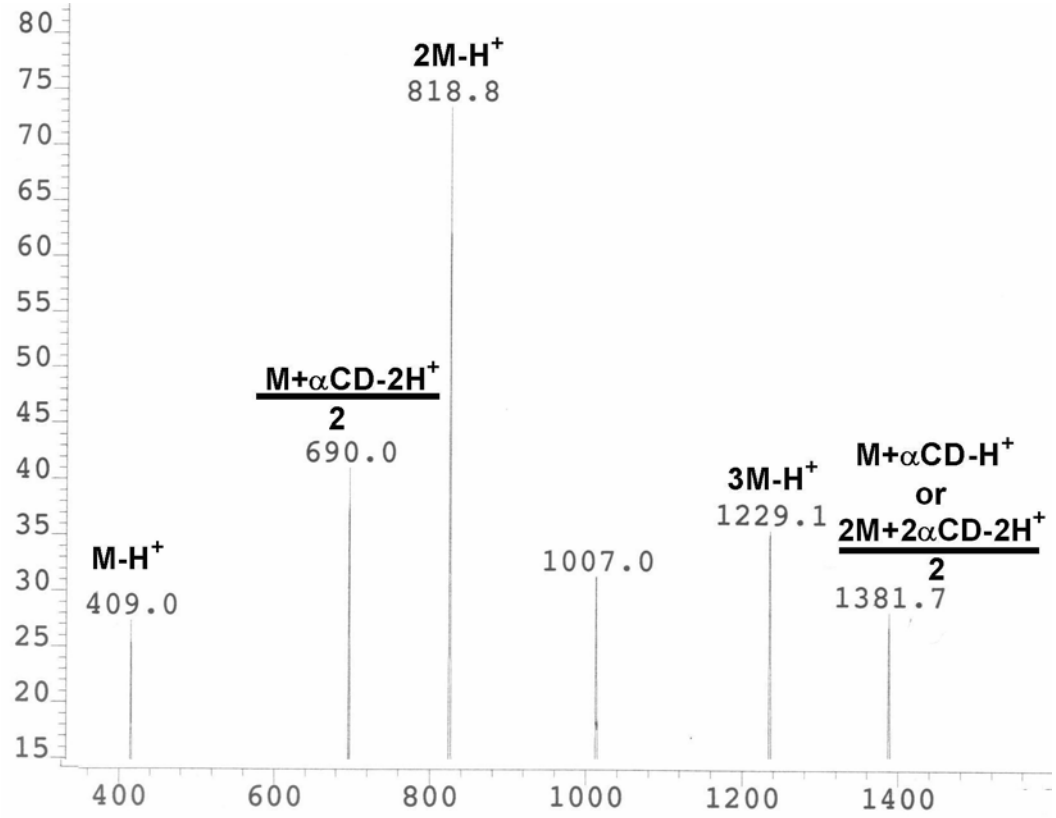


Figure 2.31 Negative ESIMS of **53aS** in aqueous α-cyclodextrin solution

Since molecule **53c** contains both electron rich and electron poor aromatic moieties, it is capable of forming molecular associates through aromatic stacking interactions, and γ-cyclodextrin (largest cavity size) might stabilize these molecular associates by forming inclusion complexes with the aggregates. Negative ESIMS spectra indicates the formation of self-assembly molecular dimers (2M, 1279.4 m/z) as well as formation of molecular complexes with cyclodextrin that are of higher order than 1:1, such as a 2:1 (2M+1γCD) and 1:2 (1M+2γCD)

(Figure 2.32). This suggests that due to the formation of high order diastereomeric molecular complexes, spectroscopic enantiomeric discrimination of **53c** should occur.

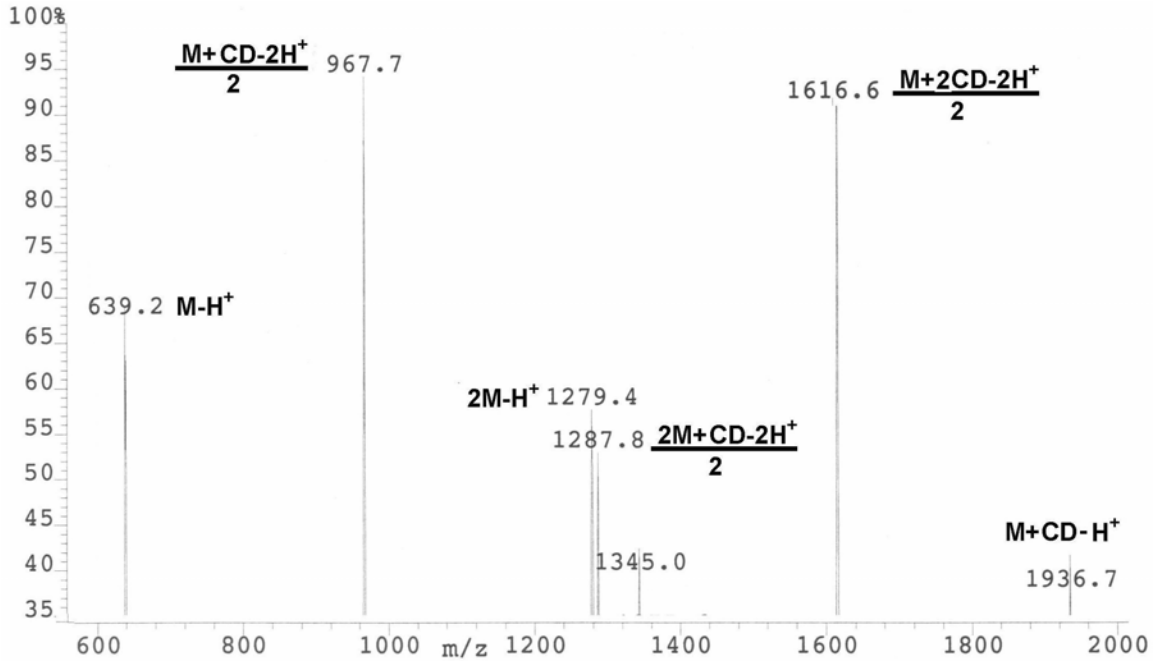


Figure 2.32 Negative ESIMS of **53cS** (0.001 M) in DMSO (1drop) and γ-cyclodextrin (γ–CD, 0.001 M).

The ESIMS is very good in demonstrating the formation of molecular complexes through molecular peaks, but to study the enantiomeric recognition or discrimination capability of cyclodextrins with guest compounds **53a**, **53b** and **53c,** the NMR spectroscopic studies were performed. ^{1}H spectra of racemic **53a**, **53b** and **53c** were recorded in aqueous cyclodextrin solutions.

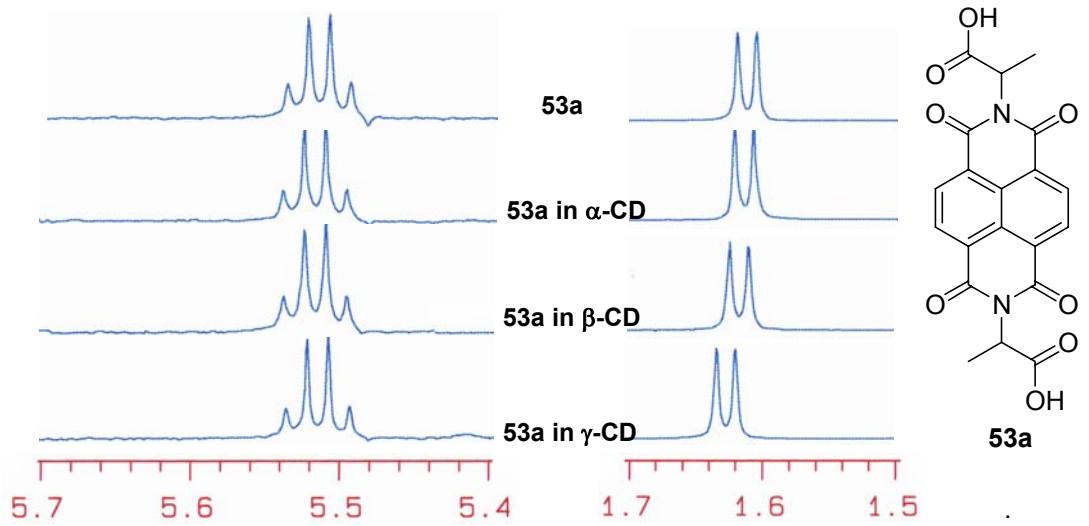


Figure 2.33 A portion of NMR spectra of **53a** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ-CD (0.01 M).

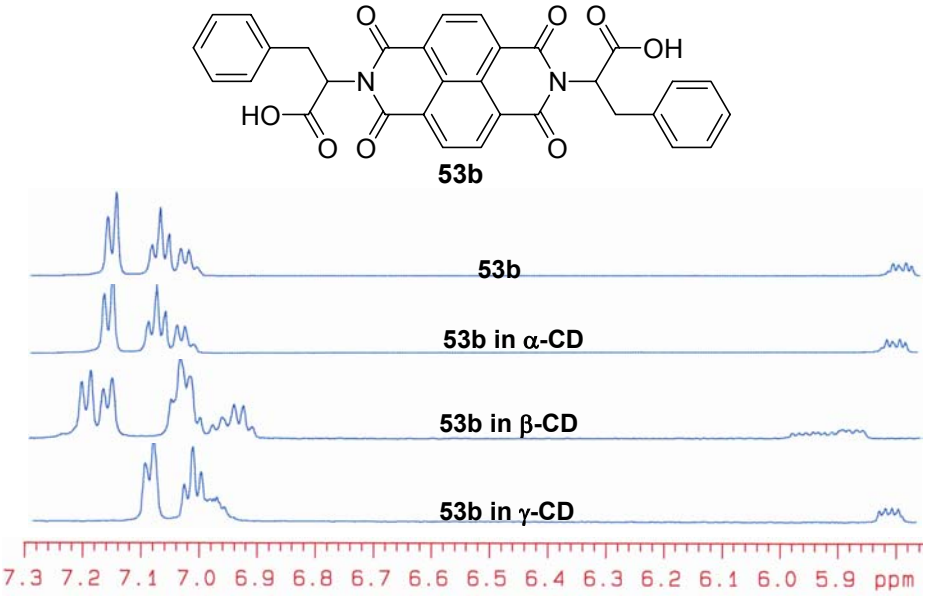


Figure 2.34 A portion of NMR spectra of **53b** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ-CD (0.01 M)

NMR discrimination of alkaline aqueous solutions of racemic **53a (**Figure 2.33) was not observed in the presence of α-, β- and γ-cyclodextrin. There is little enantiomeric recognition of

compound **53b (**Figure 2.34) seen with β-CD. This is surprising, considering that the ESIMS spectra of **53a** and α-cyclodextrin (Figure 2.31) clearly show the formation of molecular complexes. The reasonable explanation could be that these molecular complexes are not inclusion complexes but rather formed by hydrogen bonding interactions on the outside of the cyclodextrin cavity. What we see in the NMR spectrum is the average association of a molecule that is in fast equilibrium with many different aggregates. For better enantiomeric recognition it is important that guest molecules **53a**, **53b** and **53c** form strong diastereomeric complexes with cyclodextrin as the molecular dimer. For instance, the formation of a ternary complex between **53a** in α-cyclodextrin is not evident in the ESIMS spectra (Figure 2.31). Contrary to this, there is a strong signal for the ternary β-cyclodextrin complex with the **53cS** dimer (Figure 2.35).

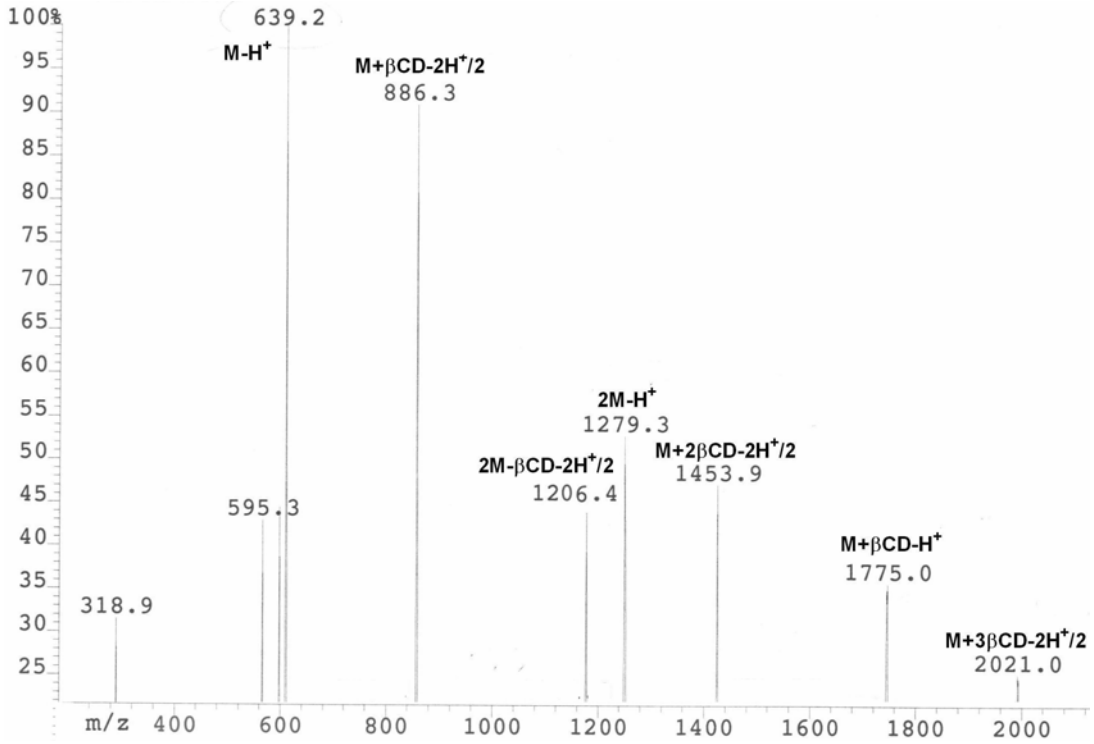


Figure 2.35 Negative ESIMS spectra of **53cS** in aqueous β-cyclodextrin

This is demonstrated in the ^{1}HNMR spectroscopic study of the enantiomeric discrimination of **53c** in aqueous cyclodextrins (Figure 2.36). The racemic mixture of **53c** in

water shows only one set of signals for both enantiomers. Enantiomeric recognition is not observed in the presence of α-CD considering that the guest molecule **53c** is too big to enter the α-cyclodextrin cavity and a diastereomeric inclusion complex cannot be formed (Figure 2.36). The cavity of β-cyclodextrin can accommodate the molecular size of **53c**; therefore, diastereomeric inclusion complexes are formed and NMR recognition is observed (Figure 2.36). Considering the large cavity size of γ-cyclodextrin, it can form a ternary inclusion complex (2M+ γ-CD, 1616.6 m/z in Figure 2.32). Formation of a strong ternary complex resulted in its lower water solubility and difficulty in NMR characterization (Figure 2.36).

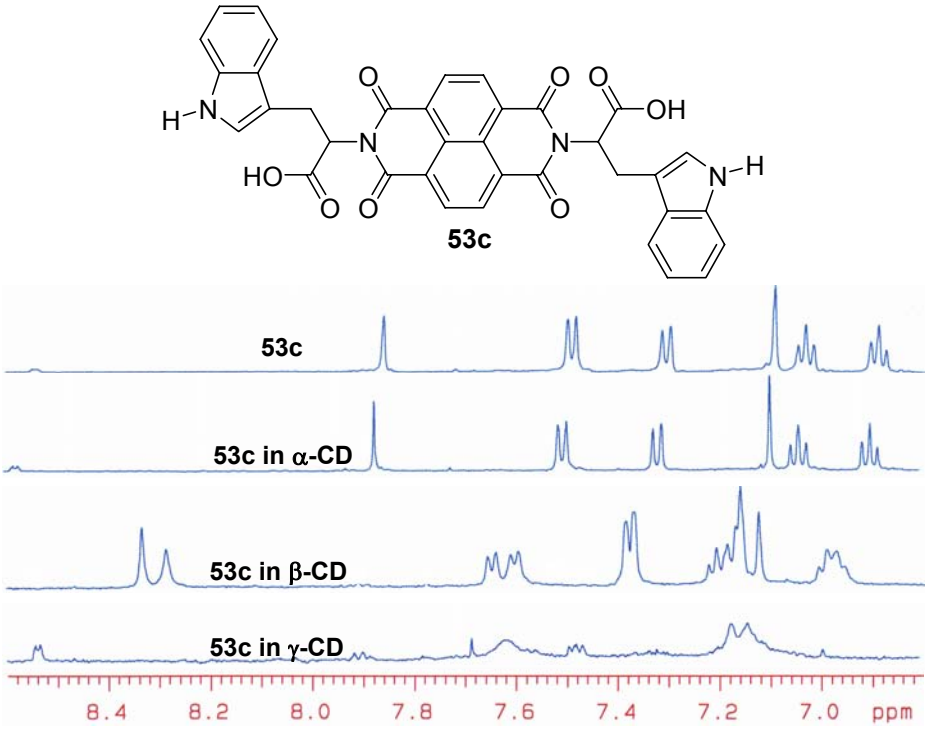
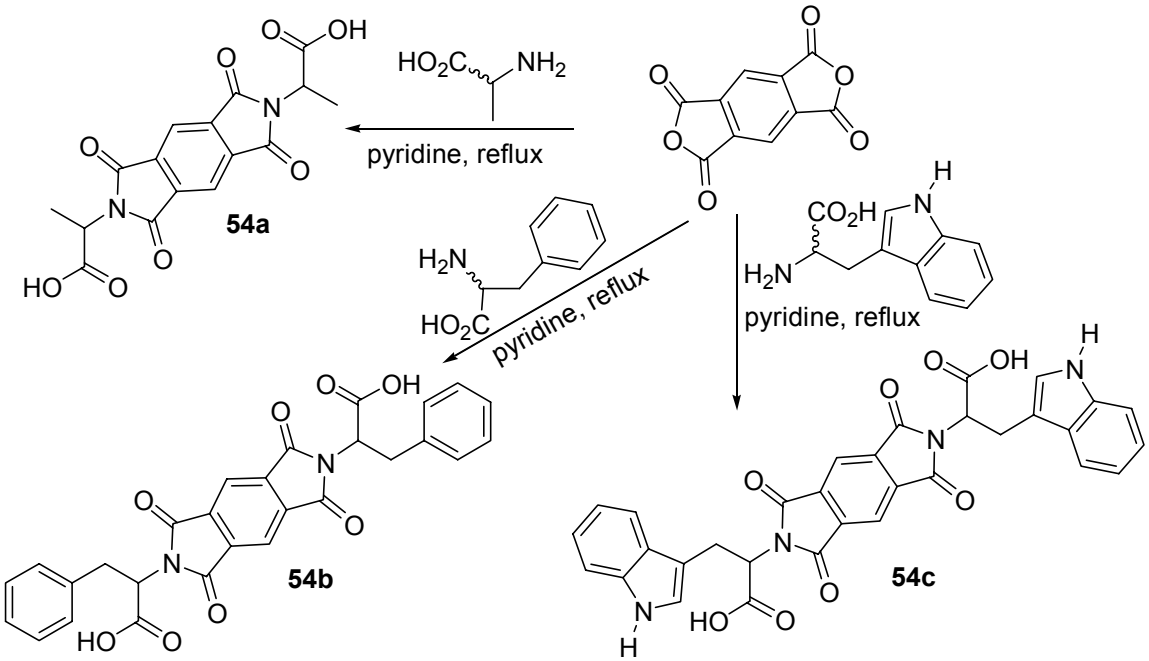


Figure 2.36 A portion of NMR spectra of **53c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ-CD (0.01 M), respectively

2.2.3 Pyrrolo[*3,4-f*]Isoindole Derived Amino Acids

To study the formation of molecular aggregates (Figure 2.29); another series of guest molecules were synthesized by replacing the naphthalene ring of the central portion with a smaller benzene ring. By doing so, the newly synthesized guest compounds **54a**, **54b** and **54c** now have smaller electron acceptor aromatic moieties in comparison to **53a**, **53b** and **53c**.

The synthetic conditions for the preparation of these amino acid derivatives are presented in Scheme 2.7. Reactions were performed with 1,2,4,5-benzenetetracarboxylic dianhydride in pyridine with the amino acids alanine, phenylalanine and tryptophan. The isolated yields of the target compounds range between 81-90 %.



Scheme 2.7 Preparation of guest compounds **54a**, **54b** and **54c**

NMR spectroscopic studies of the cyclodextrin aqueous solution of racemic **54a**, **54b** and **54c** were performed with the intent to study the enantiomeric recognition ability of these guest

compounds. Guest compounds **54a** (Figure 2.37) and **54b** (Figure 2.38) in the presence of β-CD show little enantiomeric recognition by NMR spectroscopy.

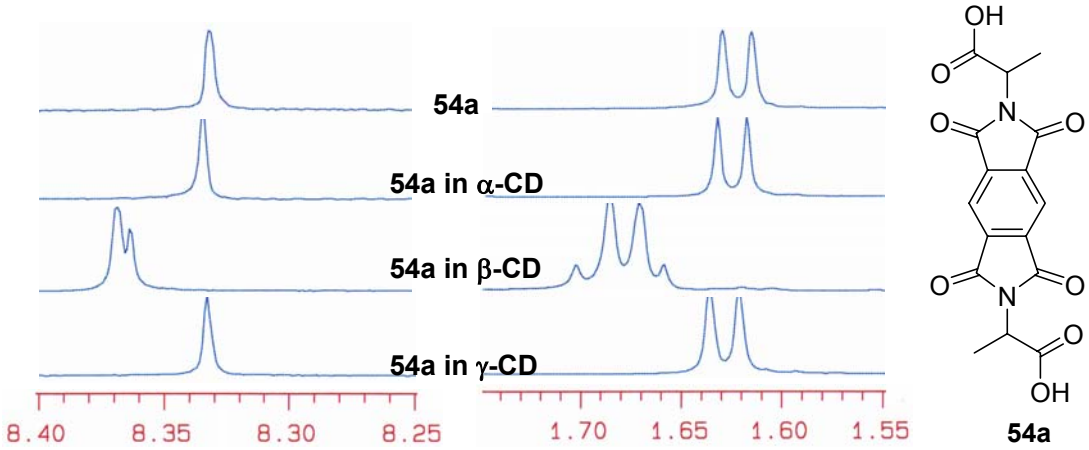


Figure 2.37 A portion of NMR spectra of **54a** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ-CD (0.01 M), respectively

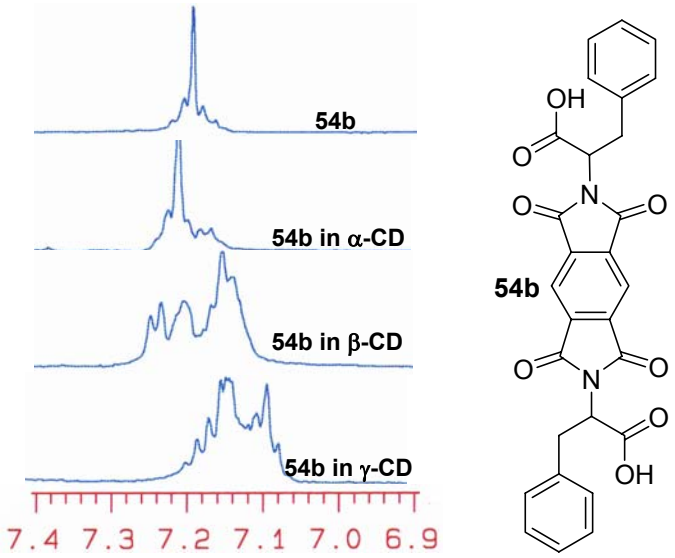


Figure 2.38 A portion of NMR spectra of **54b** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α, β, γ-CD (0.01 M), respectively

The NMR spectra of **54c** in α–, β– and γ–CD are presented in Figure 2.39. The cyclodextrin cavity must be large enough to accommodate two aromatic rings. In α-cyclodextrin,

no NMR spectroscopic recognition was observed due to the fact that neither the indole nor the 1,2,4,5-benzenetetracarboxylic dianhydride aromatic moieties can bind into the α-cyclodextrin cavity. The β-cyclodextrin cavity is large enough to accommodate part of the molecule, therefore, slight enantiomeric discrimination is observed in the racemate NMR of **54c** in β-CD. Guest molecule **54c** forms molecular aggregates through aromatic stacking interactions and this aggregate binds into the cyclodextrin cavity, forming strong diastereomeric complexes. The lower solubility of these complexes in water makes it difficult to characterize the NMR spectra (Figure 2.39).

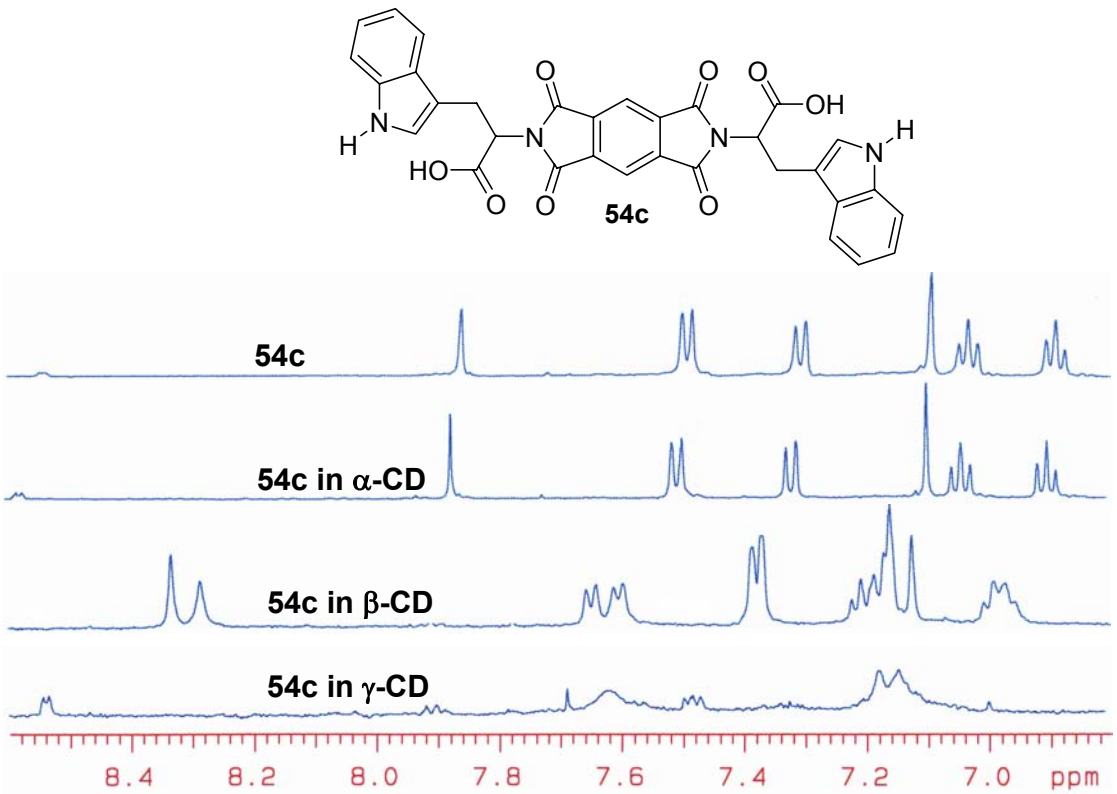


Figure 2.39 A portion of NMR spectra of **54c** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and α,β,γ–CD (0.01 M), respectively

Due to lower solubility of the γ-CD assisted inclusion complexes of **54c** in water, we found it difficult to observe enantiomeric resolution in NMR spectroscopy, but negative ESIMS

of **54cS** (Figure 2.40) with γ-CD shows the complex formation of 1:1, 2:1 and 3:1 γ-CD complexes with **54cS** (signals at 942.74, 1590.88 and 2239.59, respectively). All other molecular complexes are present as well.

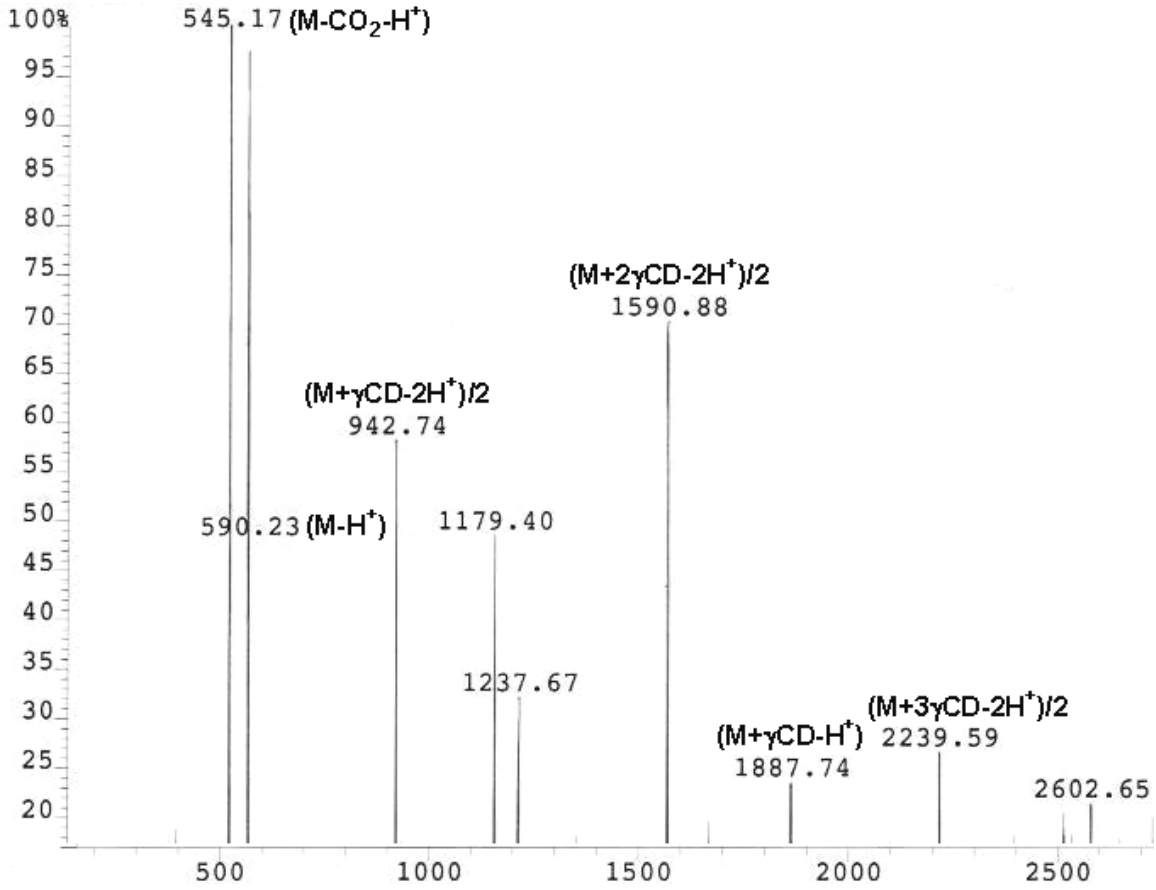
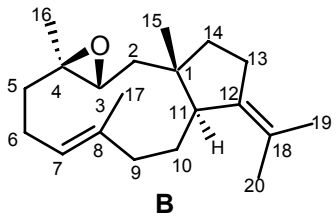


Figure 2.40 Negative ESIMS of **54cS** (0.001 M) in DMSO (1drop) and γ-cyclodextrin (γ–CD, 10^{-2} M)

III. Toward Synthesis of Dolabellane Diterpenoid B

3.1 Retro-Synthetic Scheme of Dolabellane Diterpenoid B



B

Among the four dolabellane diterpenoids isolated from the Okinawan soft coral of the genus *Clavularia* by Iguchi and co-workers (Figure 1.10),[225] the dolabellane diterpenoid **B** is unique in that it has an absence of a ketone moiety on carbon 13, which makes it very difficult to synthesize this diterpenoid. Conversion of dolabellane diterpenoid **C** into **B** by reduction of the ketone moiety on C-13 using general reduction methods such as Clemmensen reduction, Wolff-Kishner reduction or other methods cannot be applied because the epoxide moiety cannot survive the harsh conditions associated with these types of reductions. Since there is no synthetic study of compound **B**, we have decided to carry out the total synthesis of compound **B** and produce enough material to further explore the biological properties (antibacterial, antimicrobial, and antitumor activities) of this diterpeniod.[233, 196-198]

The retro-synthetic scheme of dolabellane diterpenoid **B** is outlined in Figure 3.1. Convergent synthetic approach is applied by synthesizing Fragment 1 and Fragment 2 individually, and then conducts the final ring closing step in a reaction between these two fragments.

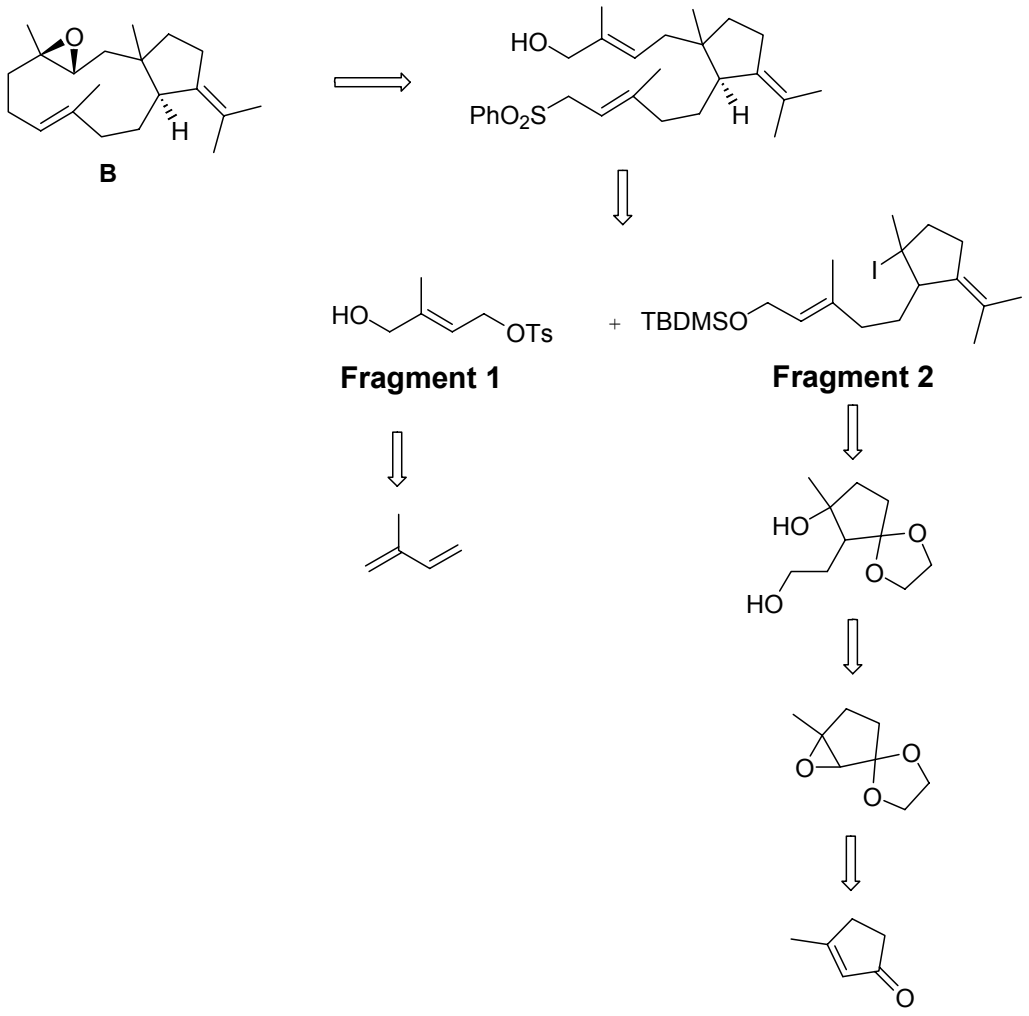
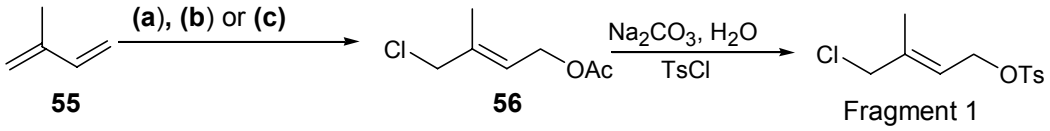


Figure 3.1 Retro-synthetic schemes for diterpenoid **B**

3.2 Proposed Synthetic Strategy for Target Molecule B

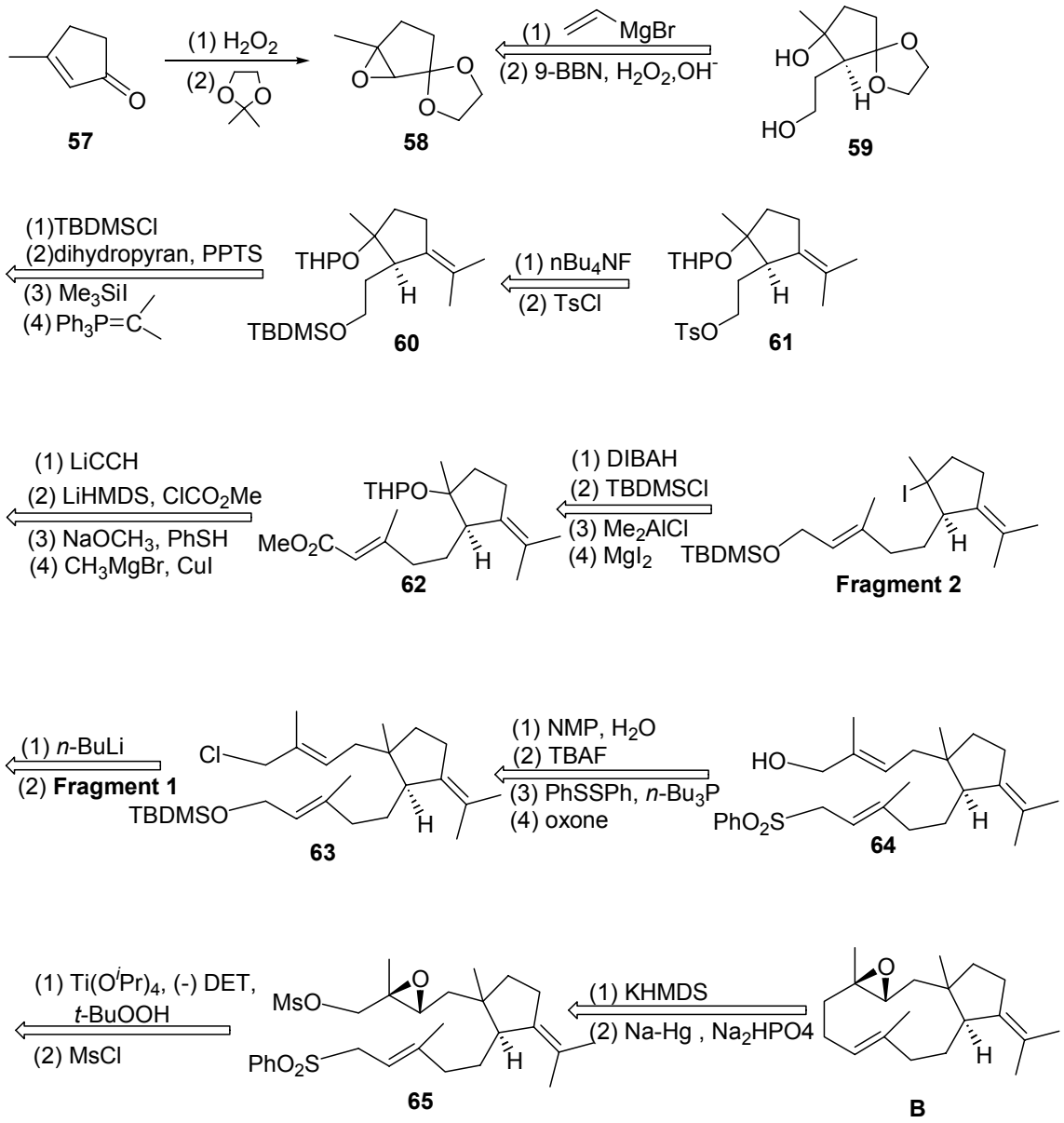
Our synthetic approach to Fragment 1 was selected due to the fact that this compound could be prepared from 2-methyl-1,3-butadine (**55**) by following previously reported synthetic procedures. The first step involves acetoxychlorination[270-272] of the diene **55** using procedure **a**, **b** or **c** (Scheme 3.1). The acetate intermediate **56** could be hydrolyzed and tosylated to afford Fragment 1 (Scheme 3.1).



(a) Benzoquinone, AcOH, LiCl, LiOAc, $Pd(OAc)_2$
(b) t-Butylhypochlorite, AcOH
(c) NCS, AcOH

Scheme 3.1 Literature preparation scheme for Fragment 1.

Considering that the literature procedure for the preparation of Fragment 1 is available, major attention was focused on developing a reliable synthetic procedure for the preparation of Fragment 2 and then its coupling to Fragment 1 to produce diterpenoid **B**. Based on our literature search, the proposed reaction sequence and the reaction conditions are presented in Scheme 3.2.

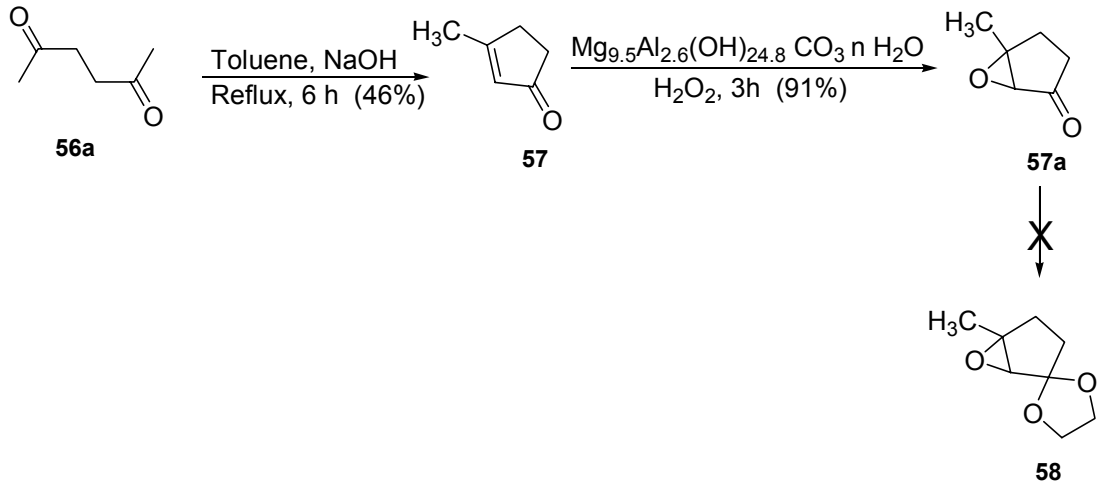


Scheme 3.2 Proposed synthetic scheme and reaction conditions for the preparation of Fragment 2 and its coupling to Fragment 1.

3.3 Accomplished synthesis toward Target Molecule B

The first step in the realization of synthetic Scheme 3.2 is the preparation of epoxyketal **58** (Scheme 3.2). Commercially available expensive starting material 3-methyl-2-cyclopentenone **57** can be prepared very easily from the comparably inexpensive starting material acetonyl

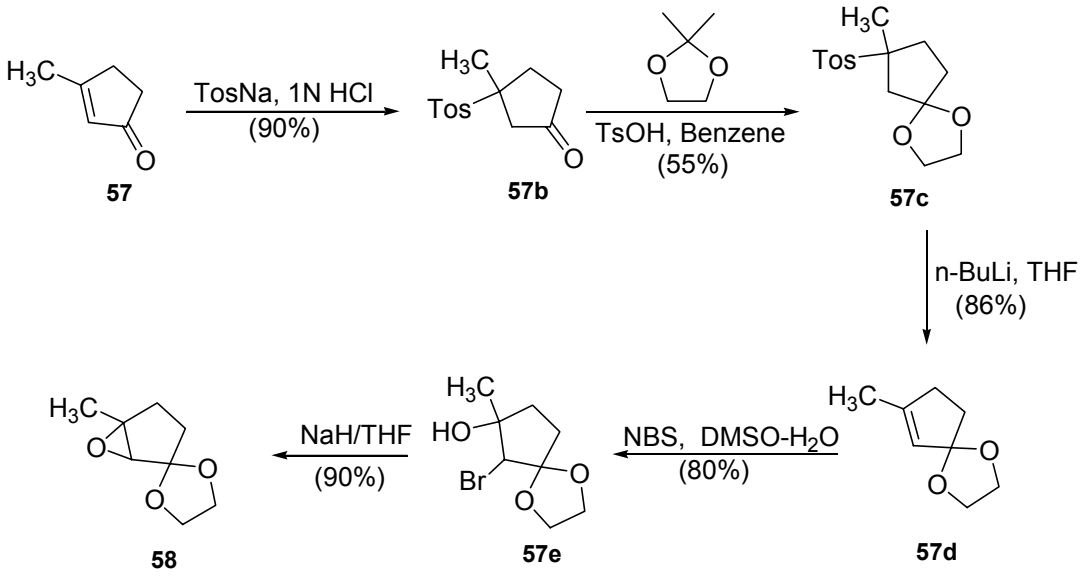
acetone **56a** via a base-induced intramolecular aldol reaction (Scheme 3.3).[274] Refluxing **56a** with NaOH in toluene followed by distillation under reduced pressure gave pure product **57**. The double bond of **57** was epoxidized[273] in high isolated yield using H_2O_2 in the presence of a freshly prepared alloy catalyst HT(MO)[273] ($Mg_{9.5}Al_{2.6}(OH)_{24.8}CO_3.nH_2O$) (Scheme 3.3).



Scheme 3.3 Epoxidation-ketalization approach

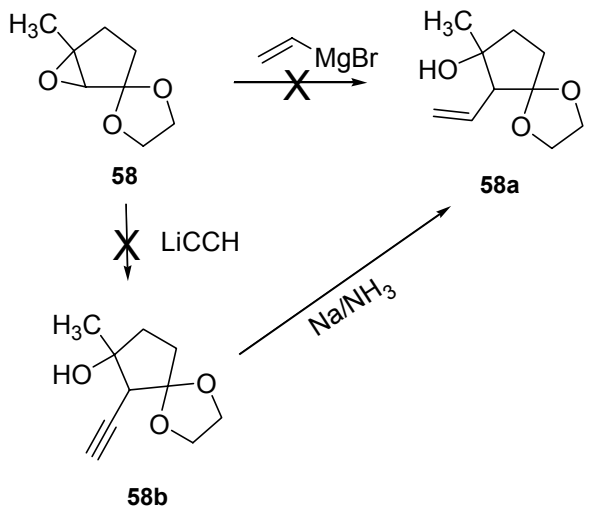
Unfortunately, we were not successful in protecting the keto group of **57a** without epoxide ring opening. Therefore, we designed a new synthetic route for the conversion of **57** into **58** (Scheme 3.4). A literature search showed that direct ketalization of α–β unsaturated ketones is very difficult. So epoxyketal **58** was synthesized using an indirect route.[275] To make ketalization more efficient, the double bond was protected by the addition of *p*-tolunesulfinic acid which gave Michael product **57b.** Now ketone **57b** could easily be protected by refluxing with 2,2-dimethyl-1,3-dioxolane[276] (1,2-ethanediol equivalent) and a trace amount of *p*-toluenesulfonic acid in benzene. The double bond was regenerated by elimination of the *p*-tolunesulfinic acid group of **57c** using a strong base, n-butylithium. The α–β unsaturated ketal

57d was oxidized[277,278] into an epoxide in two steps: (i) the addition of HOBr to the double bond using NBS as a source of positive bromine and DMSO as solvent gave **45e** (ii) the bromohydrin adduct **57e** was converted to epoxide **58** by ring closing using NaH in anhydrous THF (Scheme 3.4).



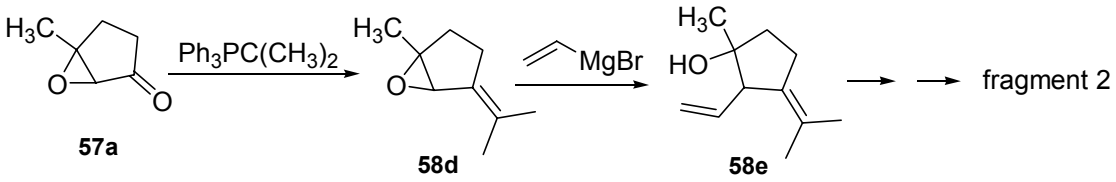
Scheme 3.4 Multi-step preparation of epoxyketal **58**

The next step of our proposed synthetic route (Scheme 3.2) is the transformation of epoxyketal **58** into dialcohol **59** by selective epoxide ring opening with vinylmagnesium bromide. Unfortunately, neither vinylmagnisium bromide nor lithium acetylide produced satisfactory products of epoxide ring opening (Scheme 3.5). Through a study of the product distribution of these two reactions, it was obvious that the presence of the ketal group hampered the nucleophilic addition to the epoxide ring. Again a new synthetic route was developed to bypass this synthetic intermediate.



Scheme 3.5 Nucleophilic epoxide ring opening of **58**

In our new approach, instead of ketalizing, we planned to olefinate the keto group of **57a** using a Wittig reagent (Scheme 3.6). Nucleophilic epoxide ring opening can now be accomplished with vinylmagnesium bromide to generate the desired alcohol **58e,** which would later be converted into fragment 2 after accomplishing a few more steps as described in scheme 3.2.



Scheme 3.6 Proposed synthetic approach for the preparation of **58e**

IV. Conclusions

We have shown spectroscopic evidence for the formation of cyclodextrin assisted molecular aggregates with chiral amino acid imides. If the amino acid guest compounds contain both electronic rich and electronic poor aromatic moieties, strong π–π stacking interactions between these aromatic moieties are responsible for the formation of molecular associates that bind into the cyclodextrin cavity. This results in spectroscopic enantiomeric discrimination. The cyclodextrin cavity must be large enough to accommodate the two aromatic rings. Cyclodextrins with smaller cavities show little or no enantiomeric recognition. In the absence of any of these aromatic moieties (electronic rich or electronic poor), the polymer-like molecular aggregate cannot be formed or is only weakly formed. In amino acids containing no aromatic moiety or only an electron deficient aromatic moiety, the aggregate formation occurs through carboxylic hydrogen-bonding interactions, which is not sufficient to produce a different NMR environment needed to cause the NMR spectroscopic recognition.

The unique structure of dolabellane diterpenoid **B** makes its synthesis difficult. Some of the methods used for the construction of general *trans*-bicyclo[9.3.0]tetradecane ring structure cannot be used in the synthesis of dolabellane diterpenoid **B**. During the synthesis of dolabellane diterpenoid **B**, the first epoxydation-ketalization approach was unsuccessful because of the sensitive epoxide ring functionality. In the second indirect approach, the presence of the ketal moiety hampered the nucleophilic epoxide ring opening of the epoxyketal intermediate. A new synthetic approach has been designed to overcome these problems toward the synthesis of dolabellane diterpenoid **B**.

V. Experimental Section

5.1 General

Melting points were taken on an Electrothermal IA 9000 Digital Melting Point Apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were run on Varian Unity 300, 400 and Varian INOVA 500 MHz spectrophotometer with $CDCl_3$, D_2O or DMSO-d_6 as a solvent. The mass spectra were recorded on a Micromass Quattro 2 Triple Quadropole Mass Spectrometer; Optical rotations of guest compounds were detected at 25°C with the light of sodium D-line (589 nm) using Autopo III automatic polarimeter Rudolph Research, Flanders, New Jersey. Elemental Analysis was performed by Atlantic Microlab, Inc., Norcross, GA. NOESY spectra were recorded on 500 MHz spectrophotometer using D_2O as a solvent and mixing time was kept 0.3 sec.

5.2 Synthesized Compounds

Preparation of (*S*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)propionic acid (43a*S*). Dimethyl sulfoxide (30 mL) solution of benzo[de]isochromene-1,3-dione (1.98 g; 0.01 mol) and alanine (0.89 g; 0.01 mol) was refluxed for 1 h. Reaction mixture was cooled to room temperature and slowly added into stirring water (200 mL). Formed precipitate was separated by filtration, washed with water (3x30 mL) and dried at 110 °C for a few hours to afford 2.15 g (80%) pure product. mp 257-261 ºC. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.51 (dd, 4H, J_1 10.5 Hz ; J_2 7.5Hz), 7.89 (t, 2H, J=7.5Hz), 5.54 (q, 1H, J 7 Hz), and 1.53 (d, 3H, J 6.5 Hz).

^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.4, 162.9 (carbonyls), 134.7, 131.3, 131.1, 127.4, 121.7 (aromatic carbons), 48.5(chiral carbon CH), 14.5 (methylene carbon). MS-ES$^+$ (CH_3OH) m/z 292.2 (65%, M+Na$^+$), 324.1 (85%, M+CH_3OH+Na$^+$), 561.3 (100%, 2M+Na$^+$), and 829.8 (50%, 3M+Na$^+$). Anal. Calcd for $C_{15}H_{11}NO_4$ (269.07): C, 66.91; H, 4.12; N, 5.20 Found: C, 66.85; H, 4.21; N, 5.11.

Preparation of (*R*)-2-(1,3-dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)propionic acid (43a*R*). The stereoisomer *R* was prepared in 87% yield by following procedure for preparation of *S* isomer. mp 258-261 ºC. ^{1}H NMR (DMSO-d_6, 300 MHz): δ ppm: 8.50 (dd, 4H), 7.89 (t, 2H, *J*=7.5Hz), 5.57 (q, 1H, *J* 6.9 Hz), and 1.52 (d, 3H, *J* 6.6 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.5, 162.9 (carbonyls), 134.7, 131.3, 131.1, 127.3, 121.7 (aromatic carbons), 48.5(chiral carbon CH), 14.5 (methylene carbon).

Preparation of (*S*)-2-(1,3-Dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)-3-phenyl-propionic acid (43b*S*). Pyridine solution (150 mL) of benzo[de]isochromene-1,3-dione (0.99 g; 5 mmol) and L-phenylalanine (0.825 g; 5 mmol) was refluxed for 6 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50 concd. HCl). Formed solid precipitate was separated by filtration, washed with water (3x20 mL) and dried at 110 °C for a few hours to afford 1.6 g (93%) pure product. mp 250-252 ºC ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.41 (d, 2H naphthalene ring , *J*=7 Hz), 8.37 (d, 2H naphthalene ring , *J* 7 Hz), 7.78 (t, 2H naphthalene ring, *J* 7.5 Hz,), 7.14 (d, 2H, *J* 7.5 Hz), 7.07 (2H, t, *J* 7.5Hz) 7.00 (t, 1H, *J* 7.5 Hz) 5.92 (dd, 1H, J_1 10 Hz ; J_2 5.5 Hz) 3.58 (dd, 1H, J_1 14 Hz ; J_2 5.5 Hz), and 3.39(dd, 1H, J_1 14.5 Hz ; J_2 10 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 170.9, 163.0 (carbonyls), 137.9, 134.7, 131.2, 129.0, 128.1, 127.5, 127.3, 127.2, 126.3, 121.2 (10 aromatic carbons), 53.9 (chiral carbon CH), and 34.3 (methylene

carbon). MS-ES$^-$ m/z 344 (100% M-H$^+$). Anal. Calcd for $C_{21}H_{15}NO_4$ (345.10): C, 70.03; H, 4.38; N, 4.06. Found: C, 69.91; H, 4.42; N, 3.95.

Preparation of (*R*)-2-(1,3-Dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)-3-phenyl-propionic acid (43b*R*). Stereoisomer *R* was prepared in 91% yield and has same spectroscopic characteristics as stereoisomer *S*. mp 250-252 ºC ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.39 (d, 2H naphthalene ring , *J*=7 Hz), 8.29 (d, 2H naphthalene ring , *J* 7 Hz), 7.73 (t, 2H naphthalene ring, *J* 8 Hz,), 7.16 (d, 2H, *J* 7.5 Hz), 7.07 (2H, t, *J* 7Hz) 7.00 (t, 1H, *J* 7 Hz) 5.97 (dd, 1H, J_1 10 Hz ; J_2 5.5 Hz) 3.61 (dd, 1H, J_1 12.5 Hz ; J_2 5.5 Hz), and 3.39(dd, 1H, J_1 13.5 Hz ; J_2 10.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 170.8, 163.0 (carbonyls), 137.9, 134.7, 131.2, 129.0, 128.1, 127.5, 127.3, 127.2, 126.3, 121.2 (10 aromatic carbons), 53.9 (chiral carbon CH), and 34.3 (methylene carbon).

Preparation of (*S*)-2-(1,3-Dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)-3-(1*H*-indol-3-yl)-propionic acid (43c*S*). Dimethyl sulfoxide (30 mL) solution of benzo[de]isochromene-1,3-dione (0.99 g; 5 mmol) and L-tryptophan (1.04 g; 5 mmol) was heated at 150 ºC for 30 min. Red colored reaction mixture was slowly added into stirring water (200 mL). Formed yellow precipitate was separated by filtration, washed with water (3x20 mL) and dried at 110 °C to give 1.6 g (83%) of pure product. mp 235-239 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 12.80 (br s, 1H), 10.63 (1H, s), 8.44 (dd, 4H, J_1 4.4 Hz, J_2 7.2 Hz), 7.85 (t, 2H, J 7.6 Hz), 7.46 (d, 1H, J 8.0 Hz), 7.17 (d, 1H, J 7.6 Hz), 6.99 (s, 1H,), 6.92 (t,1H, J 7.6 Hz), 6.79 (t,1H, *J* 7.2 Hz), 5.88 (dd, 1H, J_1 9.6 Hz, J_2 5.6 Hz), 3.65 (dd, 1H, J_1 14.8 Hz, J_2 5.6 Hz), 5.54 (dd, 1H, J_1 14.8 Hz, J_2 9.6 Hz),^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.0, 163.1, 135.9, 134.7, 131.2, 127.4, 127.2, 123.5, 121.4, 120.8, 118.2, 118.0, 111.3, 110.3, 53.7, and 24.0 ppm. ESI$^+$ (CH_3CO_2H) m/z 385

(100, $M+H^+$) and 769 (45%, $2M+H^+$). Anal. Calcd for $C_{23}H_{16}N_2O_4$ (384.38): C, 71.87; H, 4.20; N, 7.29 Found: C, 71.55; H, 4.31; N, 7.18.

Preparation of (*R*)-2-(1,3-Dioxo-1*H*,3*H*-benzo[*de*]isoquinolin-2-yl)-3-(1*H*-indol-3-yl)-propionic acid (43c*R*). The *R* stereoisomer has same spectroscopic characteristics as *S* isomer and is prepared in 87% isolated yield by following the *S* preparation procedure. mp 235-238 ºC. ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 12.80 (br s, 1H), 10.64 (1H, s), 8.46 (dd, 4H), 7.86 (t, 2H, J 8 Hz), 7.48 (d, 1H, J 7.6 Hz), 7.18 (d, 1H, J 8 Hz), 7.0 (s, 1H,), 6.93 (t, 1H, J 8 Hz), 6.8 (t, 1H, *J* 7.2 Hz), 5.89 (dd, 1H, J_1 9.6 Hz, J_2 5.2 Hz), 3.65 (dd, 1H, J_1 15.2 Hz, J_2 5.2 Hz), 5.56 (dd, 1H, J_1 14.8 Hz, J_2 9.6 Hz),^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 171.2, 163.3, 136.1, 134.9, 131.5, 127.6, 127.4, 123.7, 121.6, 121.0, 118.5, 118.2, 111.5, 110.4, 53.9, and 24.2 ppm.

Preparation of (*S*)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-propionic acid (44a*S*). Pyridine solution (600mL) of phthalic anhydride (2.0 g; 0.013 mol) and L- alanine (1 g; 0.011 mol) was refluxed for 10 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to mixture of ethyl acetate (250 ml) and aq. hydrochloric acid (250 mL water and 50 mL concd. HCl). Formed solid precipitate was separated by filtration, washed with water (3x20 mL) and recrystalized with water to afford 2.23 g (91%) pure white crystals. Mp 148.5-150 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 7.85-7.90 (m, 4H) 4.87 (q, 1H, *J* 7 Hz), 1.55 (3H, d, J_2 7.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 171.1, 167.2 (carbonyls), 134.8, 131.3, 123.3 (3 aromatic carbons), 46.9(chiral carbon CH), and 14.8 ppm (methylene carbon). MS-ES^- (CH_3OH) m/z 218.2 (100% $M-H^+$).

Preparation of (*R*)- 2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-propionic acid (44a*R*). The *R* stereoisomer was prepared in 88% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral

characteristics of the *S* stereoisomer. Mp 148.5-150 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 7.85-7.90 (m, 4H) 4.86 (q, 1H, *J* 7 Hz), 1.54 (3H, d, J_2 7.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 171.1, 167.2 (carbonyls), 134.8, 131.3, 123.3 (3 aromatic carbons), 47.0 (chiral carbon CH), and 14.8 ppm (methylene carbon).

Preparation of (*S*)-2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-phenyl-propionic acid (44b*S*). Pyridine solution (600 mL) of phthalic anhydride (2.0 g; 0.013 mol) and L-phenyl alanine (1.65 g; 0.01 mol) was refluxed for 12 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aq. hydrochloric acid (150 mL water and 50 mL concd. HCl). Formed solid white precipitate was separated by filtration, washed with water (3x20 mL) and dried in oven at 60 °C for a few hours to afford 2.8 g (95%) pure product. Mp 186-188 °C. ^{1}H NMR (500 MHz; DMSO-d_6) 7.82 (s, 4H) 7.15 (m, 5H), 5.13 (dd, 1H, J_1 11.5 Hz; J_2 4.5 Hz), 3.49 (dd, 1H, *J1* 15.5 Hz; J_2 4.5 Hz), 3.35 (dd, 1H, J_1 14 Hz; J_2 11.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 170.1, 167.1 (carbonyls), 137.3, 134.9, 130.7, 128.7, 128.3, 126.6, 123.4 (7 aromatic carbons), 52.9 (chiral carbon CH), and 33.9 ppm (methylene carbon). MS-ES$^-$ (CH_3OH) m/z 250.3 (15%, M-CO_2-H$^+$), 294.2 (100%, M-H$^+$), 589.4 (25%, 2M-H$^+$).

Preparation of (*R*)- 2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-phenyl-propionic acid (44b*R*). The *R* stereoisomer was prepared in 93% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer. Mp 186-188 °C. ^{1}H NMR (500 MHz; DMSO-d_6) 7.80 (s, 4H) 7.14 (m, 5H), 5.14 (dd, 1H, J_1 12 Hz; J_2 5 Hz), 3.50 (dd, 1H, *J1* 14 Hz; J_2 5 Hz), 3.37 (dd, 1H, J_1 14.5 Hz; J_2 11.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 170.3, 167.3 (carbonyls), 137.4, 135.0, 130.8, 128.8, 128.4, 126.7, 123.5 (7 aromatic carbons), 53.1 (chiral carbon CH), and 34.1 ppm (methylene carbon).

Preparation of (*S*)- 2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-(1H-indol-3-yl)-propionic acid (44c*S*). Pyridine solution (600 mL) of phthalic anhydride (1.48 g; 0.01 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed for 12 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50 concd. HCl). Formed solid precipitate was separated by filtration, washed with water (3x20 mL) and dried in oven at 60 °C for a few hours to afford 2.8 g (84%) pure product. mp 180-182 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.74 (s, 1H, pyrrol ring NH) 7.79 (s, 4H, benzene ring), 7.48 (d, 1H, *J* 7.5 Hz), 7.26 (d, 1H, *J* 7.5 Hz) 7.03 (s, 1H, pyrrol ring), 7.00 (1H, t, *J* 8 Hz) 6.90 (t, 1H, *J* 7.5 Hz) 5.12 (dd, 1H, J_1 9.5 Hz ; J_2 6.5 Hz) 3.58 (ddd, 2H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 170.5, 167.3 (carbonyls), 136.1, 134.9, 130.9, 127.0, 123.5, 123.4, 121.1, 118.5, 118.0, 111.6, 109.8 (11 aromatic carbons), 52.7 (chiral carbon CH), and 24.2 ppm (methylene carbon). MS-ES$^-$ (CH_3OH) m/z 333.1 (100%, M-H$^+$), 667.1 (70%, 2M-H$^+$). Anal. Calcd for $C_{19}H_{14}N_2O_4$ (334.33) : C, 68.26; H, 4.22; N, 8.38. Found: C, 68.31; H, 4.22; N, 8.35.

Preparation of (*R*)- 2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-3-(1H-indol-3-yl)-propionic acid (44c*R*). The *R* stereoisomer was prepared in 79% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer. Mp 180-182 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.72 (1H, s, pyrrol ring NH) 7.80 (4H, s, benzene ring), 7.47 (d, 1H, *J* 7.5 Hz), 7.24 (d, 1H, *J* 8 Hz) 7.01 (s, 1H, pyrrol ring), 7.98 (t, 1H, *J* 7.5 Hz) 6.88 (t, 1H, *J* 7.5 Hz) 5.09 (dd, 1H, J_1 10 Hz ; J_2 6 Hz) 3.55 (ddd 2H,). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 170.5, 167.4 (carbonyls), 136.1, 134.9, 130.9, 127.0, 123.5, 123.4, 121.1, 118.0, 111.6, 109.8 (11 aromatic carbons), 52.8 (chiral carbon CH), and 24.2 (methylene carbon).

Preparation of (*RS*)- 2-(1,3-Dioxo-octahydro-isoindol-2-yl)-propionic acid (45a*RS*). Pyridine solution (500 mL) of 1,2-cyclohexanedicarboxylic anhydride (3.2 g; 0.021 mol) and DL alanine (1.78 g; 0.02 mol) was refluxed for 10 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to mixture of ethyl acetate (250 mL) and aq. hydrochloric acid (250 mL water and 50mL concd. HCl). The ethyl acetate layer was separated and washed with water (3x20 mL) and then removed under reduced pressure. The product was recrystalized from water and dried in air for a few hours to afford 4.05 g (90 %) pure product. Mp 148-149 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 4.62 (q, 1H, J 7.5 Hz), 2.94 (m, 2H), 1.73-1.61(m, 4H), 1.38 (d, 3H, J 7.5 Hz), 1.29-1.26 (m, 4H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 178.9, 178.7, 170.9 (carbonyls), 46.9 (chiral carbon), 39.0, 23.1, 21.3, 21.3, 21.1, 14.1 (aliphatic carbon). MS-ES$^-$ (CH_3OH) m/z 224.1 (100% M-H$^+$).

Preparation of (*RS*)- 2-(1,3-Dioxo-octahydro-isoindol-2-yl)-3-phenyl-propionic acid (45b*RS*). Pyridine solution (500 mL) of 1,2-cyclohexanedicarboxylic anhydride (3.85 g; 0.025 mol) and DL-phenyl alanine (3.3 g; 0.02 mol) was refluxed for 10 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to aqueous hydrochloric acid (300 mL water and 50 concd. HCl). Formed oily residue was stirred for 1 hour and obtained precipitate was separated by filtration, washed with water (3x20 mL) and dried in air for a few hours. Recrystalization from water gives 5.5 g (92 %) pure product. Mp 145-147 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 7.23 (t, 2H, J 7.5 Hz), 7.17(t, 1H, J 7 Hz), 7.13(d, 2H, J 6.5 Hz), 4.93 (dd, 1H, J_1 12 Hz ; J_2 5 Hz), 3.36 (dd, 1H, J_1 14 Hz ; J_2 5 Hz), 3.26 (dd, 1H, J_1 13.5 Hz ; J_2 12 Hz).2.75 (m, 2H), 1.56-0.95 (m, 8H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 178.8, 178.6, 169.9 (carbonyls), 137.1, 128.9, 128.2, 126.6 (4 aromatic carbons), 52.9 (chiral carbon), 52.2,

38.6, 38.5, 33.3, 23.4, 22.4, 21.2, 21.1 ppm (aliphatic carbon). MS-ES$^-$ (CH_3OH) m/z 300.1 (100%, M-H$^+$) 601.2 (20%, 2M-H$^+$).

Preparation of (*S*)- 2-(1,3-Dioxo-octahydro-isoindol-2-yl)-3-(1H-indol-3-yl)-propionic acid (45c*S*). Pyridine solution (600 mL) of 1,2-cyclohexanedicarboxylic anhydride (1.7 g; 0.011 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed for 12 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50mL concd. HCl). Formed solid precipitate was separated by filtration, washed with water (3x20 mL) and dried in oven at 60°C for a few hours to afford 2.1 g (93%) pure product. Mp 200-201 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.83 (s, 1H, pyrrol ring NH) 7.44 (d, 1H, *J* 8 Hz), 7.30 (d, 1H, *J* 8 Hz) 7.04(s, 1H, pyrrol ring) 7.03 (t, 1H) 6.95 (t, 1H, *J* 7 Hz) 4.91 (dd, 2H, J_1 11 Hz ; J_2 5 Hz) 3.45 (ddd, 2H), 2.72 (q, 1H, J_1 7 Hz), 2.67 (q, 1H, J_1 7 Hz), 1.50-0.95 (m, 8H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 178.9, 178.6, 170.3 (carbonyls), 136.0, 127.1, 123.6, 120.9, 118.4, 118.1, 111.4, 109.5, (8 aromatic carbons), 52.3, 38.8, 38.6, 23.4, 23.3, 22.3, 21.2, 21.0 (8 aliphatic carbon). MS-ES$^-$ (CH_3OH) m/z 339.1 (100% M-H$^+$) 679.2 (100% 2M-H$^+$). Anal. Calcd for $C_{19}H_{20}N_2O_4$ (340.37) : C, 67.05; H, 5.92; N, 8.23. Found: C, 66.91; H, 5.92; N, 8.23.

Preparation of (*R*)- 2-(1,3-Dioxo-octahydro-isoindol-2-yl)-3-(1H-indol-3-yl)-propionic acid (45c*R*). The *R* stereoisomer was prepared in 92% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer. Mp 200-201 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.82 (s, 1H, pyrrol ring NH) 7.44 (d, 1H, *J* 8 Hz), 7.30 (d, 1H, *J* 7.5 Hz), 7.04 (s, 1H, pyrrol ring), 7.03 (t, 1H), 6.95 (t, 1H, *J* 7 Hz), 4.91 (dd, 2H, J_1 11 Hz ; J_2 5 Hz), 3.48 (dd, 2H, J_1 15 Hz ; J_2 11 Hz), 3.40 (dd, 2H, J_1 15.5 Hz ; J_2 5 Hz), 2.73 (q, 1H, J_1 7 Hz), 2.67 (q, 1H,

J_1 7.5 Hz), 1.50-0.95 (m, 8H). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 178.9, 178.5, 170.2 (carbonyls), 136.0, 127.1, 123.6, 120.9, 118.4, 118.1, 111.3, 109.5, (8 aromatic carbons), 52.2, 38.7, 38.6, 23.3, 23.4, 22.2, 21.2, 21.0 (8 aliphatic carbon).

Preparation of (*S*)-*N*-(1-carboxyethyl)succinamic acid (46a*S*). Water solution (5 mL) of *S*-Alanine (1.5 g; 0.017 mol) was slowly added into stirring tetrahydrofuran solution (200 mL) of succinic anhydride (1g; 0.01 mol). Resulting suspension was stirred at 30ºC for 1 h. Solid was separated by filtration and filtrate was evaporated under reduced pressure. Liquid residue was mixed with ethyl acetate and the resulting mixture was dried over anhydrous sodium sulfate. Drying reagent was removed by filtration and filtrate was evaporated to solid residue and solid residue was slurried in petroleum ether. Product was isolated by filtration of white suspension in 79% yield. Mp 152-155 ºC. ^{1}H NMR (D_2O, 500 MHz) δ ppm: 4.35 (q, 1H, chiral CH; *J* 7 Hz), 2.69 (s, 4H, two CH_2 groups of the succinic acid moiety) and 1.42 (d, 3H, *J* 7.5 Hz). ^{13}C NMR (D_2O, drop of DMSO-d_6 added for reference, 500 MHz) δ ppm: 177.5, 177.4, 175.2 (carbonyl carbon), 49.4, 30.5, 29.8, 29.5 (four aliphatic carbon). ES-MS$^-$ *m/z* 188.1 (85%, M-H$^+$), 377.3 (100%, 2M-H$^+$), and 566.0 (30%, 3M-H$^+$). Anal. Calcd for $C_7H_{11}NO_5$ (189.06): C, 44.45; H, 5.86; N, 7.40 Found: C, 44.37; H, 5.98; N, 7.33.

Preparation of (*R*)-*N*-(1-carboxyethyl)succinamic acid (46a*R*).

The *R* isomer was prepared by following the same synthetic procedure with 83% isolated yield. The spectroscopic characteristics are identical to the *S* isomer. . mp 1510-153 ºC. ^{1}H NMR (D_2O, 500 MHz) δ ppm: 4.34 (q, 1H, chiral CH; *J* 7 Hz), 2.68(s, 4H, two CH_2 groups of the succinic acid moiety) and 1.41 (d, 3H, *J* 7 Hz). ^{13}C NMR (D_2O, drop of DMSO-d_6 added for reference, 500 MHz) δ ppm: 177.7, 177.4, 175.1 (carbonyl carbon), 49.3, 30.4, 29.7, 29.4(four aliphatic carbon).

Preparation of (*S*)-*N*-(1-carboxy-2-phenylethyl)succinamic acid (46b*S*). Tetrahydrofuran (500 mL) suspension of phenylalanine (1.65 g; 0.01 mol) and succinic anhydride (1.0 g; 0.01 mol) was refluxed until the suspension becomes clear solution (approximately 30 h). Solvent was evaporated under reduced pressure. Solid residue was sclurried in petroleum ether (100 mL), separated by filtration, and washed with petroleum ether (3x20 mL) and dried at 60 ºC for a few hours. The isolated yield is 2.5 g (95%) of pure product. mp. 127-129 ºC. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.15 (d, 1H, amide NH, J 8 Hz) 7.25 (m, 5H benzene ring), 4.50 (ddd, 1H chiral), 3.05 (dd, 1H, J_1 13.5 Hz; J_2 5 Hz), 2.86 (dd, 1H, J_1 13.5 Hz; J_2 9.5 Hz), and 2.35 (s, 4H aliphatic two CH_2 group). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm: 173.8, 173.1, 171.1 (carbonyls), 129.2, 128.2, 126.4 (aromatic carbons), 53.6, 37.0, 29.9, and 29.1 (four aliphatic carbon). MS-ES$^+$ m/z 266.1 (22%, M+H$^+$) and 288.1 (100%, M+Na$^+$). Anal. Calcd for $C_{13}H_{15}NO_5$ (265.10): C, 58.86; H, 5.70; N, 5.28 Found: C, 58.92; H, 5.77; N, 5.15.

Preparation of (*R*)-*N*-(1-carboxy-2-phenylethyl)succinamic acid (46b*R*). The *R* stereoisomer was prepared in 93% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer. Mp. 127-129 ºC. ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 8.14 (d, 1H, amide NH, J =8Hz) 7.21 (m, 5H benzene ring), 4.69 (ddd, 1H chiral), 3.14 (dd, 1H, J_1 13 Hz; J_2 5 Hz), 2.96 (dd, 1H, J_1 13 Hz; J_2 8 Hz), and 2.48 (s, 4H aliphatic two CH_2 group). ^{13}C-NMR (DMSO-d_6, 500 MHz) δ ppm: 174.5, 173.3, 172.7 (carbonyls), 130.5, 129.4, 127.7 (aromatic carbons), 54.6, 38.4, 31.2, and 29.9 (four aliphatic carbon).

Preparation of (*S*)-*N*-[1-carboxy-2-(1*H*-indol-3-yl)ethyl]succinamic acid (46c*S*). Tetrahydrofuran (1 L) suspension of L-tryptophan (2 g; 0.01 mol) and succinic anhydride (1 g;

0.01 mol) was refluxed until it became solution (approximately 40 h). Solvent was evaporated and solid residue was mixed with petroleum ether (200 mL). Solid material was separated by filtration, washed with petroleum ether (3x 20 mL), and dried at 60 °C for several hours to afford 2.8 g (92%) of pure product. Mp (hydroscopic). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 10.82 (s, 1H the pyrrole ring NH) 8.15 (d, 1H amide hydrogen, J 7.5 Hz), 7.52 (d, 1H benzene ring H, J 7.5 Hz), 7.32 (d, 1H, J 8 Hz), 7.14 (s, 1H the pyrrole ring CH) 7.05 (t, 1H, J 7 Hz) 6.97 (t, 1H, J 7 Hz) 4.46 (ddd, 1H) 3.14 (dd, 1H, J_1 14 Hz; J_2 5.5 Hz) 3.00 (dd, 1H, J_1 14 Hz ; J_2 8 Hz), 2.35 ppm (s, 4H aliphatic two CH_2 group hydrogen). ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 173.8, 173.5, 171.1 (carbonyls), 136.1, 127.3, 123.6, 121.0, 118.4, 118.2, 111.4, 109.9 (8 aromatic carbons), 53.1, 29.9, 29.1, and 27.2 ppm (four aliphatic carbon). MS-ES$^-$ m/z 303.1 (35%, M-H$^+$), 431.3 (15%, 3M-CO_2-2H$^+$), and 607.1(100%, 2M-H$^+$). Anal. Calcd for $C_{15}H_{16}N_2O_5$ (304.30): C, 59.21; H, 5.30; N, 9.21 Found: C, 59.15; H, 5.38; N, 9.16.

Preparation of (*R*)-*N*-[1-carboxy-2-(1*H*-indol-3-yl)ethyl]succinamic acid (46c*R*). The *R* stereoisomer was prepared in 96% yield by following same procedure outlined for the *S* stereoisomer. All spectra of *R* stereoisomer are identical to the S stereoisomer spectra. Mp. (hygroscopic). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm: 10.83 (s, 1H the pyrrole ring NH) 8.16 (d, 1H amide hydrogen, J 7.5 Hz), 7.54 (d, 1H benzene ring H, J 8 Hz), 7.34 (d, 1H, J 8 Hz), 7.15 (s, 1H the pyrrole ring CH) 7.07 (t, 1H, J 7.5 Hz) 6.99 (t, 1H, J 7 Hz) 4.47 (ddd, 1H) 3.17 (dd, 1H, J_1 14.5 Hz; J_2 5.5 Hz) 3.02 (dd, 1H, J_1 14 Hz ; J_2 8 Hz), 2.37 ppm (s, 4H aliphatic two CH_2 group hydrogen). ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 173.9, 173.5, 171.1 (carbonyls), 136.1, 127.3, 123.7, 121.0, 118.4, 118.3, 111.4, 109.9 (8 aromatic carbons), 53.1, 29.9, 29.1, and 27.3 ppm (four aliphatic carbon).

Preparation of (Benzhydrylidene-amino)-acetic acid ethyl ester (49): A solution of benzophenone imine **47** (1 g, 0.0052 mol) and glycine ethyl ester hydrochloride **48** (0.77 g, 0.0052 mol) in 20 mL methylene chloride was stirred in 100 mL round bottom flask covered with CaCl2 tube at room temperature for 24 h. The reaction mixture was filtered to remove NH4Cl and the filtrate was evaporated to dryness on a rotary evaporator. The residue was stirred in 20 mL of ether, filtered, washed with 20 mL of water, and dried with anhydrous MgSO4. Filtered and removal of solvent gave **49** which can be recrystallized using ether/hexane. Weight of white crystals 1.31 g (94%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm: 7.0-7.6 (m, 10H), 4.13 (s, 2H), 4.14 (q, 2H, *J* 7.5 Hz), 1.2 (t, 3H, *J* 7.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz) δ ppm:172.1, 170.9, 130.7, 129.0 128.9, 128.8, 128.3, 127.9, 61.1, 55.9, 14.4.

Preparation of acetoxy-(benzhydrylidene-amino)-acetic acid ethyl ester (50): A solution of N-bromosuccinamide (1.31 g; 0.005 mol) dissolved in 10 mL dry DMF was added dropwise at room temperature with stirring over three hours to a mixture of shiff base **49** (1.16 g, 0.0065 mol), anhydrous sodium acetate (1.23 g; 0.015 mol) in 10 mL DMF. The mixture was stirred at room temperature overnight with exclusion of moisture (CaCl2 tube), poured into 100 mL of water, extracted with ether (3x 15 mL), the ether extracts were washed with water, dried anhydrous MgSO4, filtered through a 3 cm pad of silica gel and the solvent was removed under reduced pressure. Resulting yellow liquid weight 1.14 g (70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ ppm: 7.2-7.7 (m, 10H), 6.18 (s, 1H), 4.22 (q, 2H, *J* 7 Hz), 2.18 (s, 3H), 1.28 (t, 3H, *J* 7 Hz).

Preparation of (*S*)- 2-[7-(1-Carboxy-ethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1H-benzo[lmn][3,8]phenanthrolin-2-yl]-propionic acid (53a*S*). A pyridine solution (600 mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and alanine (0.89 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~10 mL and the

hot reaction mixture was added to aq. hydrochloric acid (300 mL water and 100 mL concd. HCl). The formed solid precipitate was separated by filtration, washed with water (3x20 mL) and oven dried at 100 ºC for a few hours to afford 1.86 g (91%) pure product. Mp. 343-345 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.69 (s, 4H naphthalene ring) 5.59 (q, 2H, *J* 6.5 Hz) 1.57 (d, 3H, *J* 7 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 171.2 (two equivalent carbonyls of carboxylic acid) 162.1(four equivalent carbonyls), 131.1, 126.2 (aromatic carbons), 49.2 (chiral carbon), 14.5(methylene carbon). MS-ES$^-$ (CH_3OH) *m/z* 364.9 (65%, M-CO_2-H$^+$), 410 (100%, M-H$^+$), 818 (95%, 2M-2H$^+$) . Anal. Calcd for $C_{20}H_{14}N_2O_8$ (534.47) : C, 58.54; H, 3.44; N, 6.83. Found: C, 57.50; H, 3.49; N, 6.70.

Preparation of (*R*)- 2-[7-(1-Carboxy-ethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1H-benzo[lmn][3,8]phenanthrolin-2-yl]-propionic acid (53a*R*). The stereoisomer *R* was prepared in 90% yield by following the procedure for preparation of the *S* isomer. The NMR and ESIMS spectra of *R* and *S* stereoisomers are identical. Mp 343-345 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.66 (s, 4H, naphthaline ring) 5.57 (q, 2H, *J* 7 Hz) 1.55 (d, 3H, *J* 6.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ 171.3 (two equivalent carbonyl of carboxylic acid) 162.1(four equivalent carbonyls), 131.1, 126.3 (aromatic carbons), 49.2 (chiral carbon), 14.5(methylene carbon).

Preparation of (*RS*)- 2-[7-(1-Carboxy-2-phenyl-ethyl)-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1H-benzo[lmn][3,8]phenanthrolin-2-yl]-3-phenyl-propionic acid (53b*RS*). A pyridine solution (500 mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and DL phenyl alanine (1.65 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~ 10 mL and the hot reaction mixture was added to aq. hydrochloric acid (300 mL water and 100 concd. HCl). The formed yellow solid precipitate was separated by filtration, washed with water (3x20 mL) and oven dried for a few hours to afford 2.81 g (96 %)

pure product. M.p. 352-354 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.64 (s, 4H naphthalene ring), 7.16 (d, 4H phenyl ring, *J* 7 Hz) 7.11(t, 4H, phenyl ring *J* 7.5 Hz) 7.04 (t, 2H phenyl ring, *J* 7 Hz) 5.86 (dd, 2H, J_1 5.5 Hz ; J_2 9.5Hz) 3.59 (dd, 2H, J_1 14 Hz ; J_2 5.5 Hz) 3.32 (dd, 2H, J_1 14 Hz ; J_2 9.5 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm: 170.3 (two equivalent carbonyls of carboxylic acid) 162.0(four equivalent carbonyls), 137.8, 131.3, 128.9, 128.2, 126.4, 126.0, 125.7(aromatic carbons), 54.6(chiral carbon), 34.3(methylene carbon). MS-ES$^-$ (CH_3OH) *m/z* 517.2 (85%, M-CO_2-H^+), 562 (100%, M-H^+). Anal. Calcd for $C_{32}H_{22}N_2O_8$ (562.53) : C, 68.32; H, 3.94; N, 4.98. Found : C, 67.70; H, 3.89; N, 4.94.

Preparation of (*S*)- 2-{7-[1-Carboxy-2-(1H-indol-3-yl)-ethyl]-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1H-benzo[*lmn*][3,8]phenanthrolin-2-yl}-3-(1H-indol-3-yl)-propionic acid (53c*S*). A pyridine solution (600mL) of 1,4,5,8-naphthalenetetracarboxylic dianhydride (1.34 g; 0.005 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed for 12 h. The volume of the reaction mixture was reduced to ~ 10 mL and the hot reaction mixture was added to aq. hydrochloric acid (300 mL water and 100 concd. HCl). The formed black solid precipitate was separated by filtration, washed with water (3x20 mL) and oven dried for a few hours to afford 3.1 g (97%) pure product. M.p. 286-288 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.65 (s, 2H pyrrol ring NH) 8.60 (s, 4H naphthalene ring), 7.47 (d, 2H, *J* 8 Hz), 7.19 (d, 2H, *J* 8.5 Hz) 7.05 (2H, s, pyrrol ring) 6.93 (t, 2H, *J* 7.5 Hz) 6.80 (t, 2H, *J* 7.5 Hz) 5.86 (dd, 2H, J_1 9.5 Hz ; J_2 5.5 Hz) 3.69 (dd, 2H, J_1 14.5 Hz ; J_2 5.5 Hz) 3.49 (dd, 2H, J_1 15 Hz ; J_2 9 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm: 170.7 (two equivalent carbonyl of carboxylic acid) 162.1(four equivalent carbonyls), 135.9, 131.2, 127.1, 125.9, 125.7, 123.8, 120.9, 118.3, 118.0, 111.4 and 110.2 (11 aromatic carbons), 54.3(chiral carbon), 24.2(methylene carbon). MS-ES$^-$ (CH_3OH) *m/z* 595.8

(65%, M-CO_2-H^+), 640.7 (100%, M-H^+),. Anal. Calcd for $C_{36}H_{24}N_4O_8$ (640.6): C, 65.70; H, 3.78; N, 8.75. Found: C, 65.06; H, 4.08; N, 8.38.

Preparation of (*R*)- 2-{7-[1-Carboxy-2-(1H-indol-3-yl)-ethyl]-1,3,6,8-tetraoxo-3,6,7,8-tetrahydro-1H-benzo[*lmn*][3,8]phenanthrolin-2-yl}-3-(1H-indol-3-yl)-propionic acid (53c*R*). The stereoisomer *R* was prepared in 94% yield by following the procedure for the preparation of the *S* isomer. The NMR and MS-ES spectra of *R* and *S* stereoisomers are identical. M.p. 286-288 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.64 (s, 2H pyrrol ring NH) 8.59 (s, 4H naphthalene ring), 7.46 (d, 2H, *J* 8 Hz), 7.19 (d, 2H, *J* 8.5 Hz) 7.04 (2H, s, pyrrol ring) 6.92 (t, 2H, *J* 7 Hz) 6.79 (t, 2H, *J* 7 Hz) 5.85 (dd, 2H, J_1 9 Hz ; J_2 5.5 Hz) 3.68 (dd, 2H, J_1 14.5 Hz ; J_2 5.5 Hz) 3.49 (dd, 2H, J_1 15 Hz ; J_2 9 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm: 170.7 (two equivalent carbonyl of carboxylic acid) 162.1(four equivalent carbonyls), 135.9, 131.2, 127.1, 125.9, 125.7, 123.8, 120.9, 118.3, 118.0, 111.4 and 110.2 (11 aromatic carbons), 54.3(chiral carbon), 24.1(methylene carbon).

Preparation of (*RS*)- 2-[6-(1-Carboxy-ethyl)-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrrolo[3,4-f]isoindol-2-yl]-propionic acid (54a*RS*). Pyridine solution (600 mL) of 1,2,4,5-benzenetetracarboxylic dianhydride (1.42 g; 0.0065 mol) and DL- alanine (1 g; 0.011 mol) was refluxed for 11 h. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50mL concd. HCl). Formed solid precipitate was separated by filtration, washed with water (3x20 mL) and dried in oven for a few hours to afford 1.6 g (81%) pure product. M.p. 297-298 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.28 (s, 2H) 4.94 (q, 2H), 1.57 (d, 6H; *J* 7 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 170.7, 165.5 (carbonyls), 136.8, 118.0 (2 aromatic carbons), 47.6

(chiral carbon CH), and 14.7 ppm (methyl carbon). MS-ES$^-$ (CH_3OH) m/z 315.1 (65%, M-CO_2-H^+), 359 (100%, M-H^+), 718.8 (100% 2M-H^+).

Preparation of (*RS*)- 2-{6-[1-Carboxy-2-(1H-indol-3-yl)-ethyl]-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrrolo[3,4-f]isoindol-2-yl}-3-(1H-indol-3-yl)-propionic acid (54b*RS*). Pyridine solution (600 mL) of 1,2,4,5-benzenetetracarboxylic dianhydride (1.09 g; 0.005 mol) and DL-phenyl alanine (1.65 g; 0.01 mol) was refluxed approximately for 12 h.Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50 mL concd. HCl). Formed solid white precipitate was separated by filtration, washed with water (3x20 mL) and dried in oven for a few hours to afford 2.3 g (90%) pure product. Mp 296-297.5 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 8.21 (s, 2H) 7.10-7.15 (m, 10 H), 5.16 (dd, 2H, J_1 11 Hz ; J_2 5 Hz), 3.50(dd, 2H, J_1 14 Hz ; J_2 5 Hz), 3.30 (dd, 2H, J_1 14 Hz ; J_2 11.5 Hz). ^{13}C NMR (DMSO-d_6, 500 MHz): δ ppm: 169.6, 165.2 (carbonyls), 137.1, 136.2, 128.7, 128.4, 126.7 (6 aromatic carbons), 53.6 (chiral carbon CH), and 33.9 ppm (methylene carbon). MS-ES$^-$ (CH_3OH) m/z 467.3 (100%, M-CO_2-H^+), 511.3 (50%, M-H^+).

Preparation of (*S*)- 2-{6-[1-Carboxy-2-(1H-indol-3-yl)-ethyl]-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrrolo[3,4-f]isoindol-2-yl}-3-(1H-indol-3-yl)-propionic acid (54c*S*). Pyridine solution (600 mL) of 1,2,4,5-benzenetetracarboxylic dianhydride (1.09 g; 0.005 mol) and L-tryptophan (2.04 g; 0.01 mol) was refluxed overnight. Volume of the reaction mixture was reduced to ~ 5 mL and hot reaction mixture was added to ice cooled aqueous hydrochloric acid (150 mL water and 50 mL concd. HCl). Formed solid yellow precipitate was separated by filtration, washed with water (3x20 mL) and dried at 60 °C for few hours to afford 2.5 g (85%) pure product. Melting point range 192-194 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.71 (s,

2H, pyrrol ring NH) 8.13 (s, 2H, benzene ring), 7.47 (d, 2H, *J* 8 Hz,), 7.24 (d, 2H, *J* 8 Hz) 7.03(s, 2H, pyrrol ring) 6.99 (t, 2H, *J* 7 Hz) 6.88 (t, 2H, *J* 7.5 Hz) 5.15 (dd, 2H, J_1 11.5 Hz ; J_2 5Hz) 3.58 (dd, 2H, J_1 14.5 Hz ; J_2 5 Hz) 3.52 (dd, 2H, J_1 14.5 Hz ; J_2 11 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm: 169.9 (two carbonyl of carboxylic acid) 165.4(four equivalent carbonyls), 136.3, 136.1, 126.9, 123.7, 121.1, 118.5, 118.3, 117.9, 111.5 and 109.5 (10 aromatic carbons), 52.3(chiral carbon CH), 24.2(methylene carbon). MS-ES$^-$ (CH_3OH) m/z 545.3 (35%, M-CO_2-H^+), 589.4 (100%, M-H^+),. Anal. Calcd for $C_{32}H_{22}N_4O_8$ (590.54): C, 65.08; H, 3.75; N, 9.49. Found: C, 63.58; H, 3.91; N, 9.26.

Preparation of (*R*)- 2-{6-[1-Carboxy-2-(1H-indol-3-yl)-ethyl]-1,3,5,7-tetraoxo-3,5,6,7-tetrahydro-1H-pyrrolo[3,4-f]isoindol-2-yl}-3-(1H-indol-3-yl)-propionic acid (54c*R*). The *R* stereoisomer was prepared in 89% isolated yield by following the procedure described for the *S* stereoisomer. The spectral characteristics for this compound are identical to the spectral characteristics of the *S* stereoisomer. ^{1}H NMR (DMSO-d_6, 500 MHz): δ ppm: 10.73 (s, 2H, pyrrol ring NH) 8.15 (s, 2H, benzene ring), 7.47 (d, 2H, *J* 8 Hz,), 7.24 (d, 2H, *J* 8.5 Hz) 7.03(s, 2H, pyrrol ring) 6.99 (t, 2H, *J* 7.5 Hz) 6.88 (t, 2H, *J* 7.5 Hz) 5.13 (dd, 2H, J_1 10.5 Hz ; J_2 5 Hz) 3.57 (dd, 2H, J_1 15 Hz ; J_2 5 Hz) 3.51 (dd, 2H, J_1 14.5 Hz ; J_2 11 Hz). ^{13}C NMR (DMSO-d_6, 400 MHz): δ ppm: 169.8 (two carbonyl of carboxylic acid) 165.3 (four equivalent carbonyls), 136.3, 136.0, 126.8, 123.6, 121.0, 118.4, 118.3, 117.8, 111.4 and 109.4 (10 aromatic carbons), 53.2 (chiral carbon CH), 24.1 (methylene carbon).

Preparation of 3-methyl-2-cyclopenten-1-one (57). Acetonyl acetone (0.4 mol, 45.6 g) and toluene (52 mL) were placed in a 250 mL 3 necked round bottom flask equipped with a thermometer and a magnetic stirring bar. A mixture of NaOH (4.0 g, 0.1 mol) and water (40 mL) was added at 22 ºC into the above flask. This two-layer mixture was then stirred and heated at

80-85 ºC for a period of six hours. The color of the two-phased mixture was dark brown and some tar formation was observed. The toluene layer was separated and washed once with 20 mL of saturated NaCl solution. The toluene was stripped of at 30-40º C (20 mm Hg) at reduced pressure and the **57** was collected by distillation at 45-70 ºC (5-15 mm Hg) to afford 17.7 g (46%) pure pale yellow product; bp 72-74 ºC/15 mm Hg. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.83 (s, 1H, =CH), 2.3-2.5 (m, 4H, $2CH_2$), 2.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 210.3 (carbonyl), 179.1, 130.6, 35.7, 33.0, 19.4.

Preparation of 2,3-epoxy-3-methylcyclopentanone (57a). Into a reaction vessel with a reflux condenser were successively placed the catalyst HT(MO) (0.15 g), 3-methyl-2-cyclopenten-1-one 3 (1.93 g, 20 mmol) and 30% aqueous hydrogen peroxide (3.4 mL, 30 mmol). After the resultant heterogeneous mixture was stirred at 40 ºC for 3 h, the hydrotalcite was separated by filtration. The filtrate was treated with aqueous Na_2SO_3 followed by extraction with ethyl acetate. Removal of the solvent under reduced pressure afforded 2.04 g (91%) of pure **57a**; bp 65-67 ºC/ 15 torr. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.16 (s, 1H), 1.9-2.4 (m, 4H, $2CH_2$), 1.6 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 210.2 (carbonyl), 65.4, 60.7, 32.4, 27.2, 17.4.

Preparation of 3-methyl-3-tosylcyclopentanone (57b). To a stirred solution of 3-methyl-2-cyclopentenone **57** (12 g, 0.125 mol) and sodium 4-methylbenzenesulfinate (27.8 g, 0.156 mol) in water (100 mL), 1N HCl (200 mL) is added slowly in a thin stream. Stirring is continued for 18 h and the solid product then isolated by suction, washed with water and air dried; yield: 28.4 g (90%); mp 86-88 ºC. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.78 (d, 2H, AA' Tos-H, *J* 8.1 Hz), 7.40 (d, 2H, BB' Tos-H, *J* 8.4 Hz), 1.8-3.1 (m, 6H, $3CH_2$), 2.48 (s, 3H, Tos-CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 213.6 (carbonyl), 145.5, 132.1, 130.2, 129.9, 65.7, 46.4, 36.7, 29.9, 22.2, 21.8.

Preparation of 3-methyl-3-tosylcyclopentanone 1,2-ethanediyl acetal (57c). A stirred mixture of 3-methyl-3-tosylcyclopentanone **57b** (10 g, 0.04 mol), 2,2-dimethyl-1,3-dioxolane (13 mL, 0.12 mol), a trace amount of p-toluenesulfonic acid, and benzene (80 mL) was heated at 65-70 ºC and the liberated acetone distillate slowly through a small Claisen-Vigreux column at atmospheric pressure for a period of 2 hour. The progress of reaction was followed by NMR. After 2 hour saturated sodium hydrogen carbonate solution (50 mL) is added and heating is continued for 15 min. the mixture is then cooled and layers are separated. The organic layer is washed with water (3x50 mL) and dried with potassium carbonate. The solvent is removed under reduced pressure and crystallize from ice-cold diisopropyl ether to afford **57c** in6.5 g (55%) yield; mp 86-88 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73 (d, 2H, AA' Tos-H, *J* 7.6 Hz), 7.30 (d, 2H, BB' Tos-H, *J* 7.6 Hz), 3.8-3.9 (m, 4H, O-CH2-O-CH2), 1.4-2.7 (m, 6H, $3CH_2$), 2.41 (s, 3H, Tos-CH_3), 1.39 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 144.8, 133.0, 130.2, 129.7, 116.5, 66.7, 64.7, 64.4, 43.4, 35.6, 31.7, 23.4, 21.7.

Preparation of 3-methyl-2-cyclopentenone 1,2-ethanediyl acetal (57d). To a stirred solution of 3-methyl-3-tosylcyclopentanone 1,2-ethanediyl acetal **57c** (3 g, 0.01 mol) in dry THF (10 mL) added very slowly 1.6 M solution of *n*-Butyl lithium (13.7 mL, 0.022 mol) in hexane at 0 ºC. After 30 min, ether (10 mL) and water (2 mL) are added. The organic layer is separated, washed with water (3x5 mL), dried with anhydrous sodium sulfate, and evaporated to afford product **57d** in 1.2 g(86%) yield; bp 47-48 ºC/4.5 torr. ^{1}H NMR (CCl_4, 400 MHz): δ 5.26 (s, 1H, =CH), 3.77 (s, 4H, O-CH_2-O-CH_2), 2.0-2.3 (m, 4H, $2CH_2$), 1.75 (s, 3H, CH_3). ^{13}C NMR (CCl_4, 400 MHz): δ 145.8, 125.8, 120.4, 64.2, 35.6, 33.6, 17.0.

Preparation of 2-Bromo-3-hydroxy-3-methyl-cyclopentanone 1,2-ethanediyl acetal (57e). Under an atmosphere of nitrogen, the olefin **57d** (3 g, 0.022 mol) in DMSO (60 mL) was

treated with water (0.8 mL) and cooled to 10 ºC. With stirring, NBS (7.6 g, 0.043 mol) was added as one portion. After a short induction period of 2-3 min a yellow color developed and the solution became quite warm. The color of the solution deepened as the reaction progressed. Stirring for an additional 10-15 min was followed by quenching the reaction mixture in dilute $NaHCO_3$ solution with concomitant discharge of color and extraction of the product into ether to afford **57e** in 4.2 g (80%) yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.27 (s, 1H), 3.8-4.1 (m, 4H, O-CH_2-O-CH_2), 1.9-2.2 (m, 4H, 2CH_2), 1.45 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 113.5, 77.7, 66.6, 65.6, 64.8, 35.2, 33.4, 25.1.

Preparation of 2,3-epoxy-3-methylcyclopentanone 1,2-ethanediyl acetal (58). To a stirred solution of the bromohydrin adduct **57e** (4 g, 0.017 mol) in dry THF (10mL) added NaH under an atmosphere of nitrogen. The resulting mixture was stirred for 1 h followed by filtration and evaporation of solvent under reduced pressure afford α−β epoxy ketal **58** in 2.4 g (90%) yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.9-4.1 (m, 4H, O-CH_2-O-CH_2), 3.06 (s, 1H), 1.6-2.0 (m, 4H, 2CH_2), 1.45 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 400 MHz): δ 115.1, 65.3, 64.9, 61.9, 31.2, 29.5, 17.9.

5.3 Optical Rotations of Guest Compounds 43 and 46

Table 5.1 Optical rotations of guest compounds **43a**, **43b** and **43c**

Guest Compd.	Optical Rotation	
	L-enantiomer	D-enantiomer
43a	-19.5 (c 0.1, MeOH)	+18.0 (c 0.1, MeOH)
43b	-31.6 (c 1, MeOH)	+28.4 (c 1, MeOH)
43c	-17.0 (c 0.1, H_2O)	+16.2 (c 0.1, H_2O)

Table 5.2 Optical rotations of guest compounds **46a**, **46b** and **46c**

Guest Compd.	Optical Rotation	
	L-enantiomer	D-enantiomer
46a	+22.0 (c 1, MeOH)	-21.6 (c 1, MeOH)
46b	-14.9 (c 1, MeOH)	+13.4 (c 1, MeOH)
46c	+37.1 (c 1, MeOH)	-32.8 (c 1, MeOH)

5.4 ESIMS of Guest Compounds with CDs

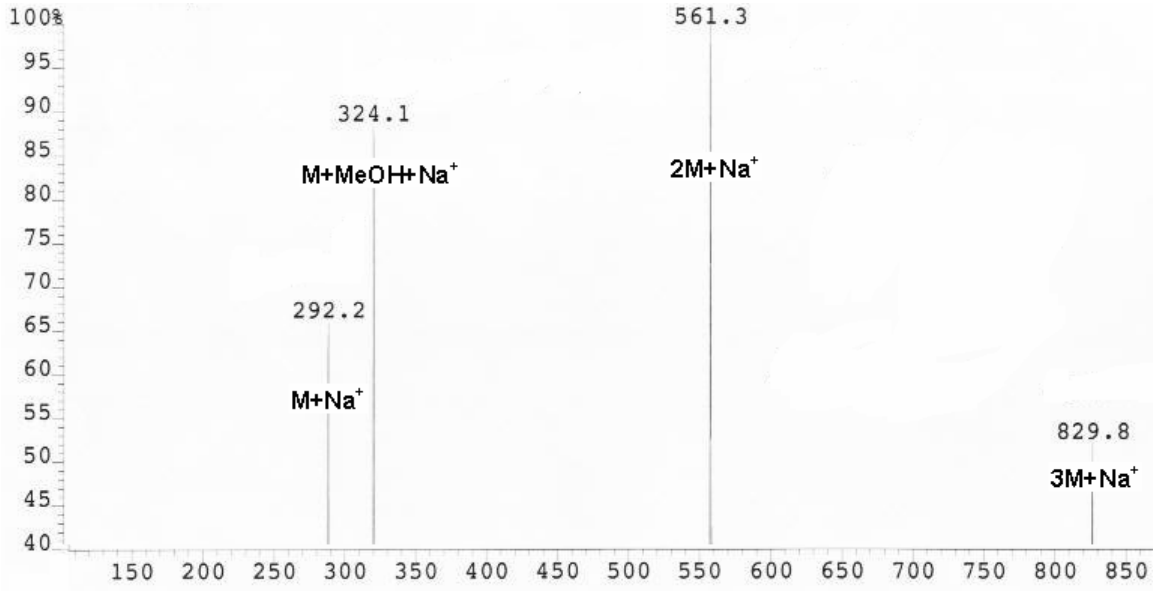


Figure 5.1 Positive ESIMS of **43a*S*** in methanol-water

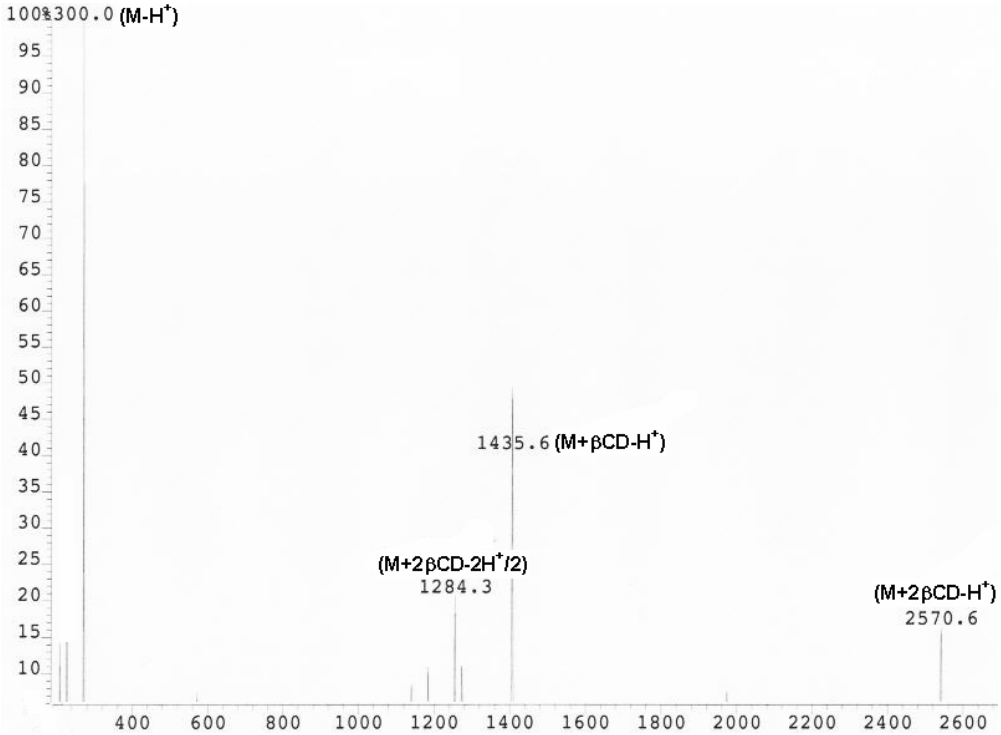


Figure 5.2 Negative ESIMS of **45b*RS*** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and β- CD (0.01 M)

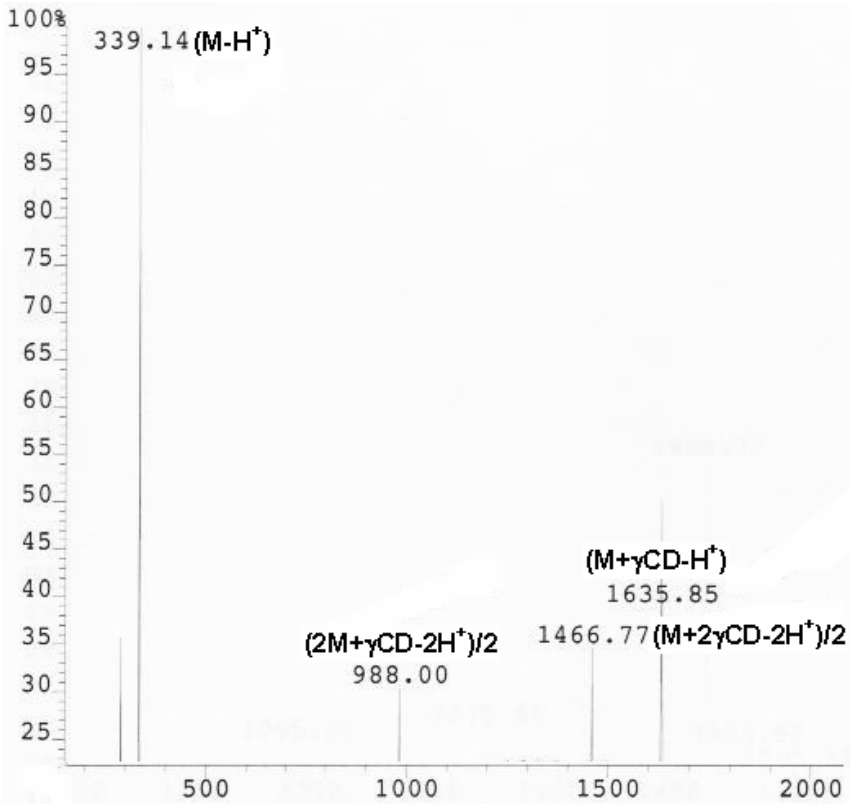


Figure 5.3 Negative ESIMS of **45c*S*** (0.001 M) in aqueous $NaHCO_3$ (0.003 M) and γ-cyclodextrin (γ–CD, 0.01 M)

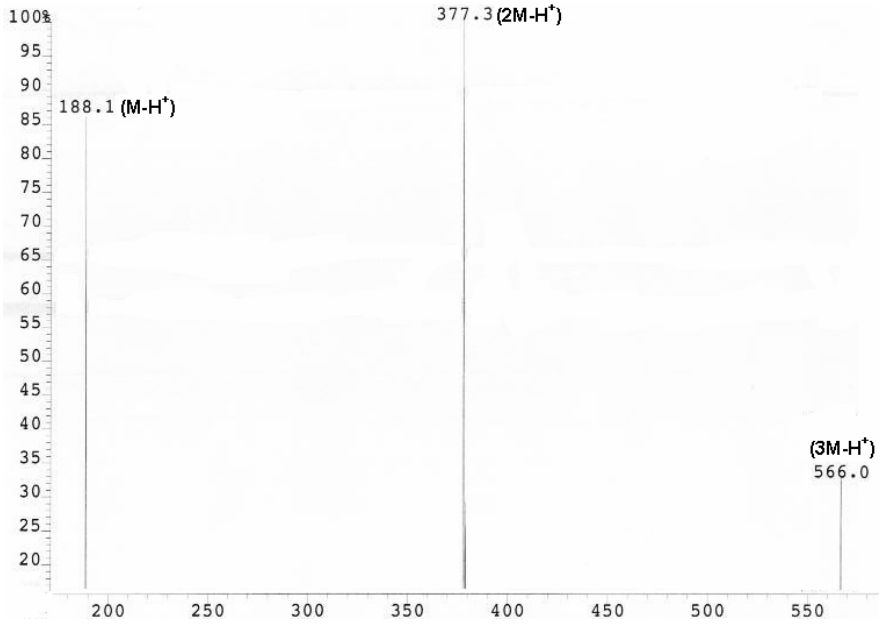


Figure 5.4 Negative ESIMS of **46a*S*** in methanol-water

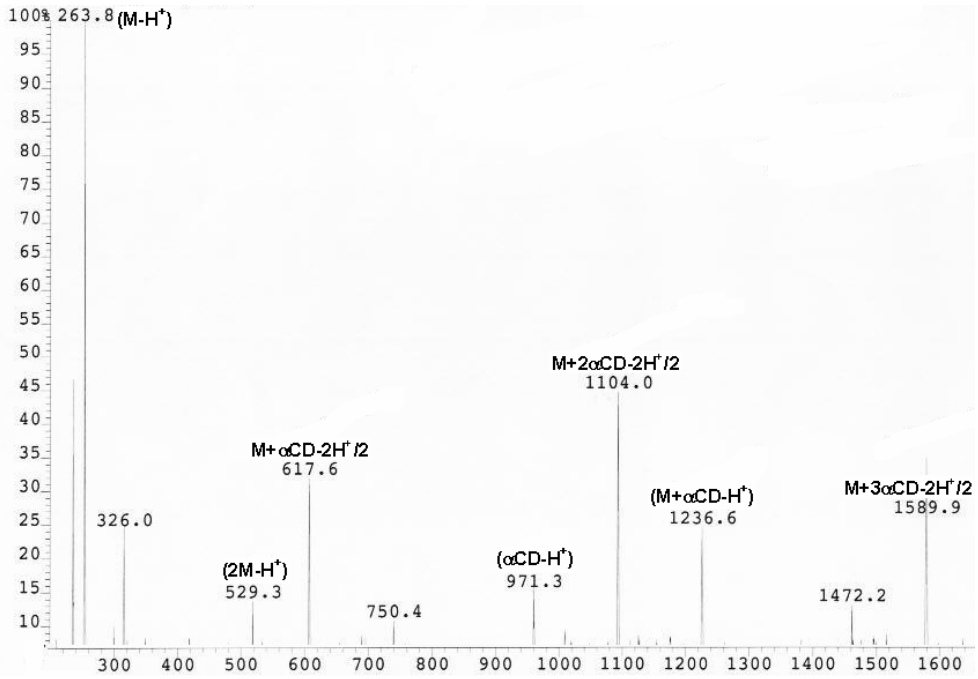


Figure 5.5 Negative ESIMS of **46b*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and α-cyclodextrin (γ–CD, 10^{-2} M)

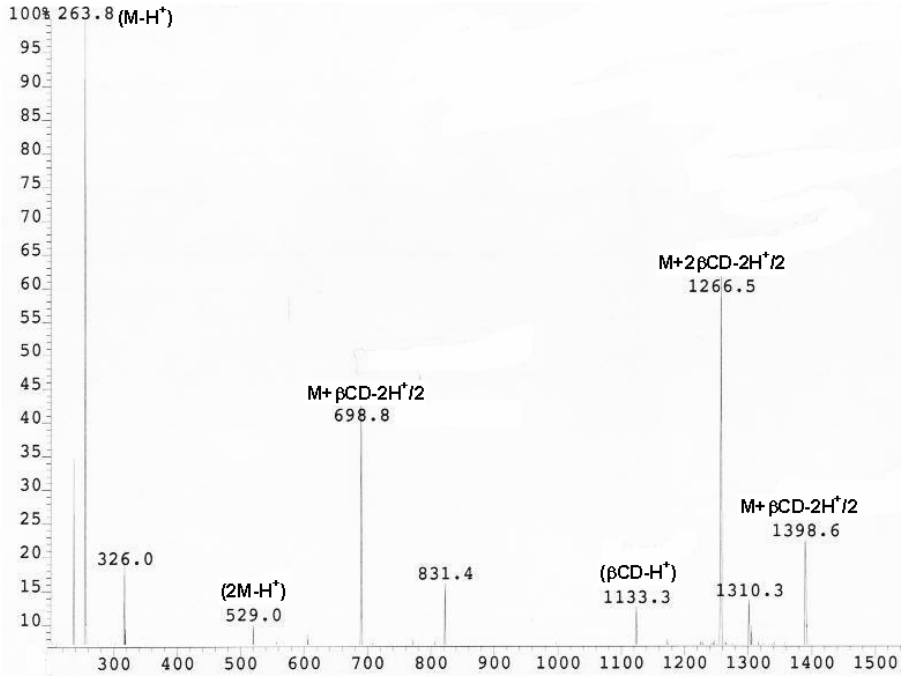


Figure 5.6 Negative ESIMS of **46b*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and β-cyclodextrin (γ–CD, 10^{-2} M)

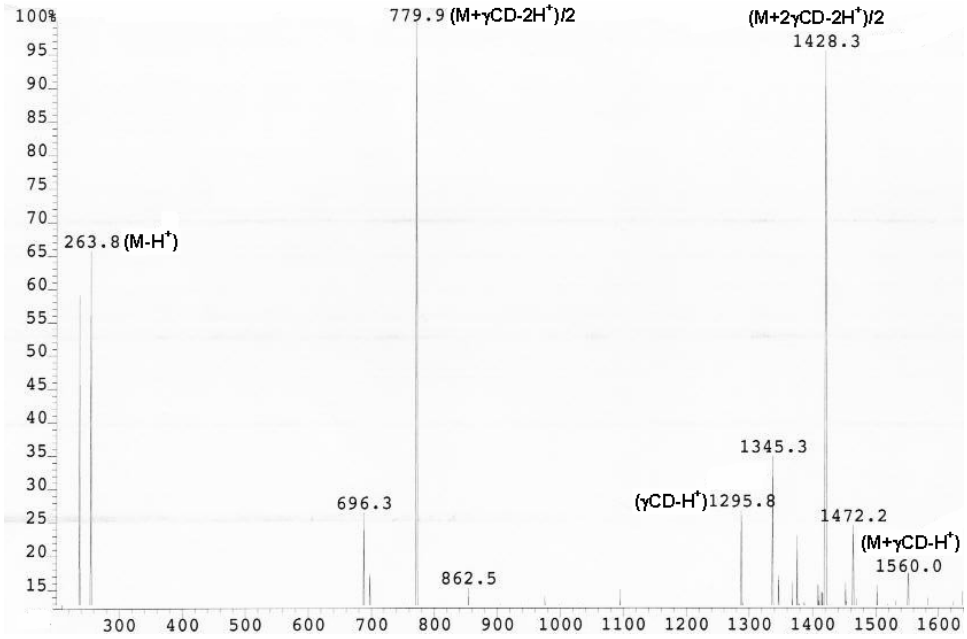


Figure 5.7 Negative ESIMS of **46b*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

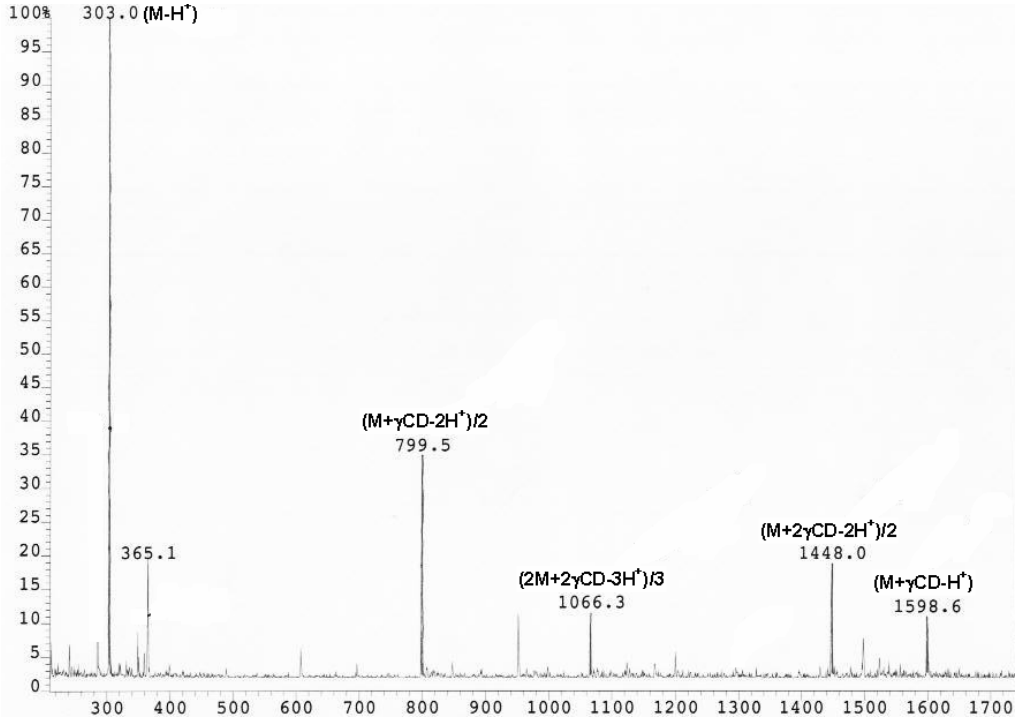


Figure 5.8 Negative ESIMS of **46c*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

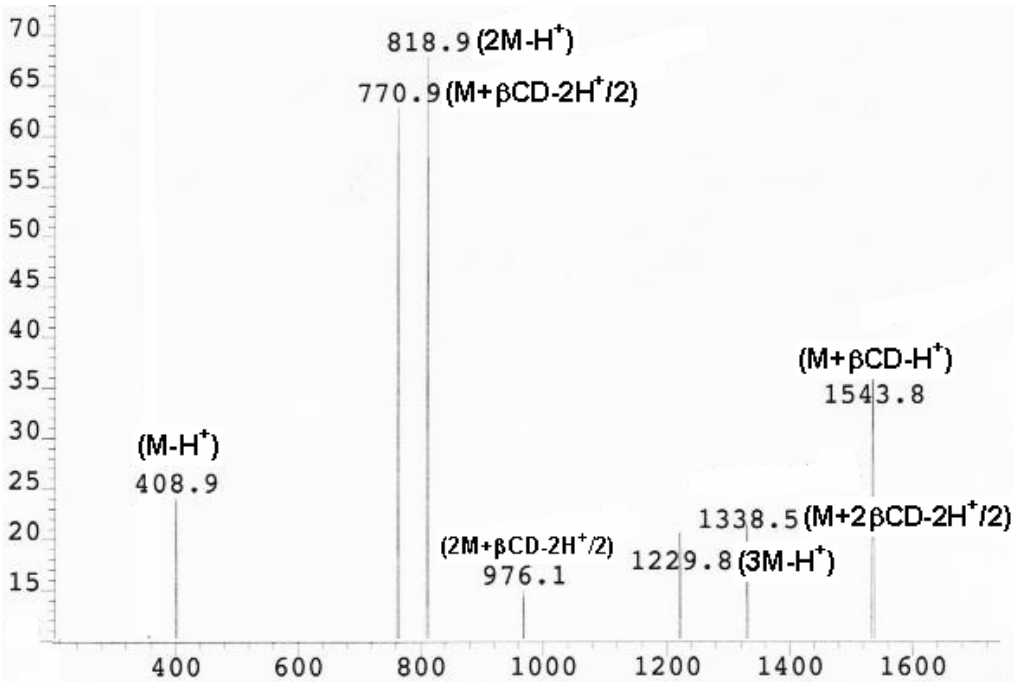


Figure 5.9 Negative ESIMS of **53a*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and β-cyclodextrin (γ–CD, 10^{-2} M)

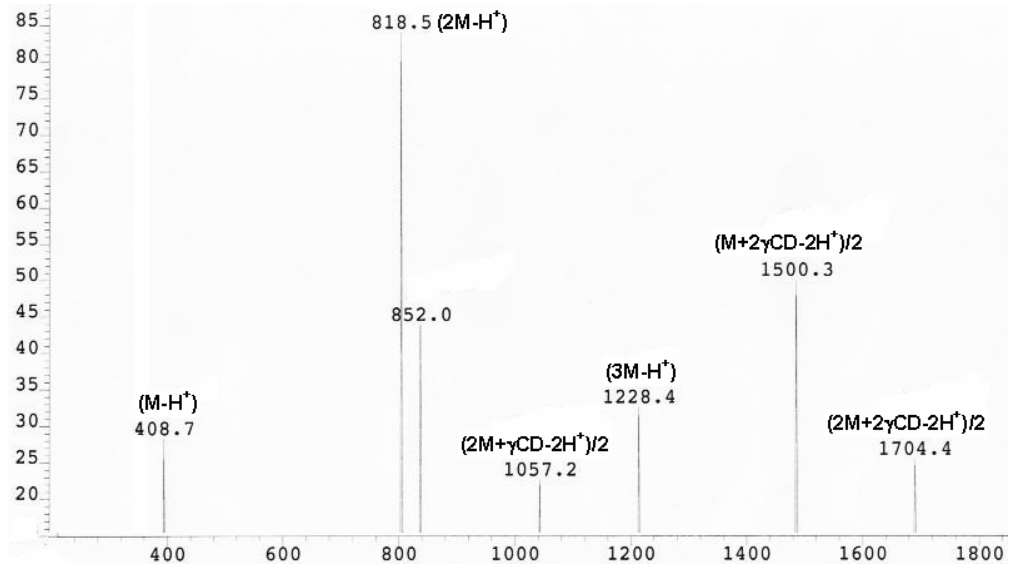


Figure 5.10 Negative ESIMS of **53a*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

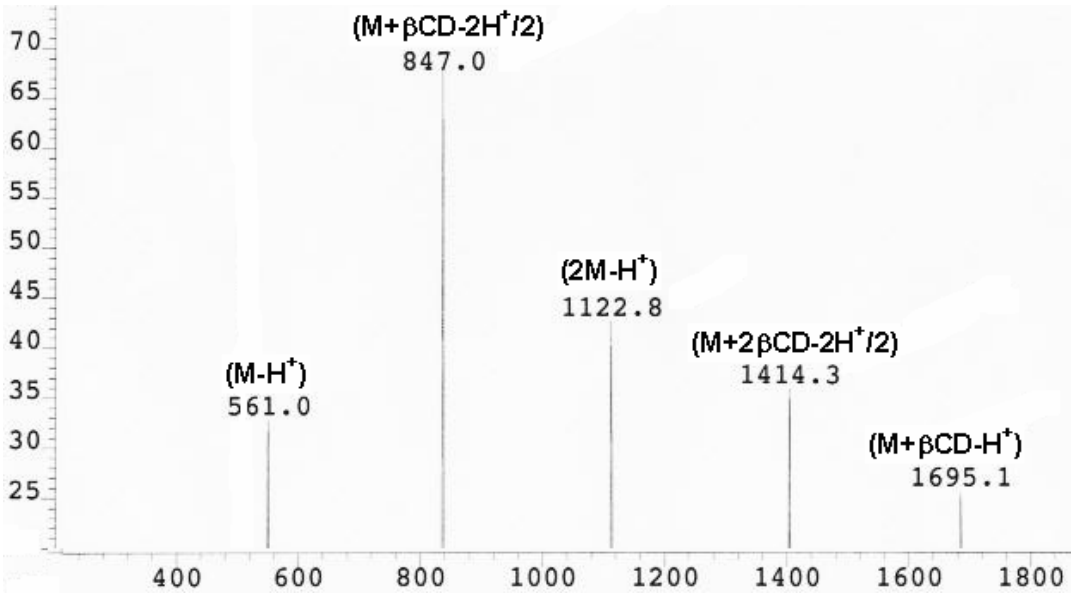


Figure 5.11 Negative ESIMS of **53b*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and β-cyclodextrin (γ–CD, 10^{-2} M)

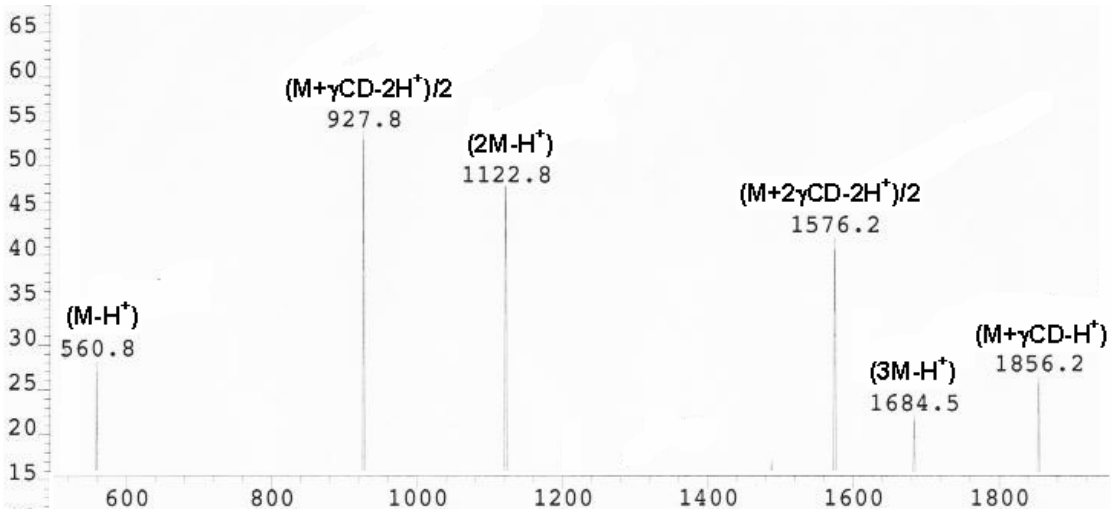


Figure 5.12 Negative ESIMS of **53b*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

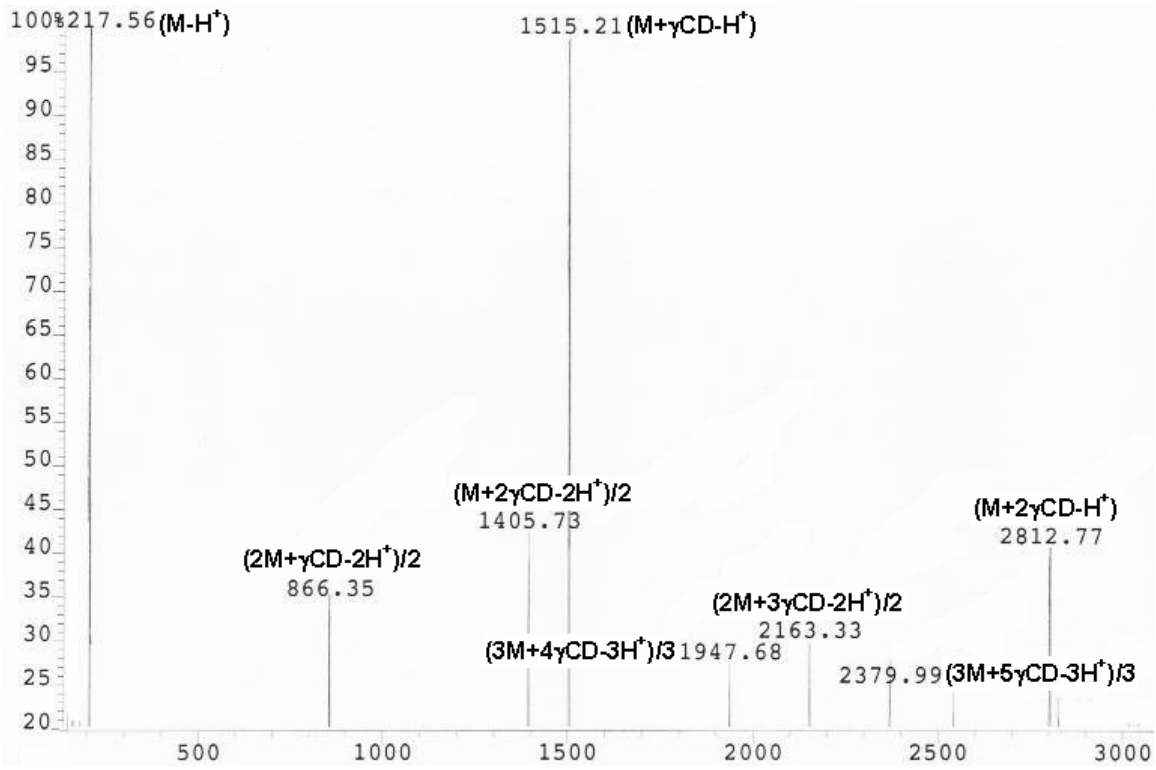


Figure 5.13 Negative ESIMS of **54a*S*** (0.001 M) in aqueous $NaHCO_3$ ($3x10^{-3}$ M) and γ-cyclodextrin (γ–CD, 10^{-2} M)

5.5 Binding Isotherms of Guest Compounds with CDs

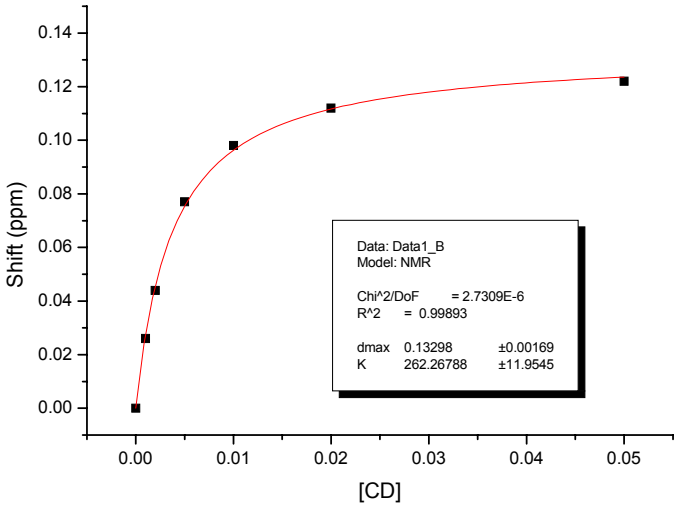


Figure 5.14 Binding isotherm for the complexation of **43c*S*** and γ-CD at 40 °C

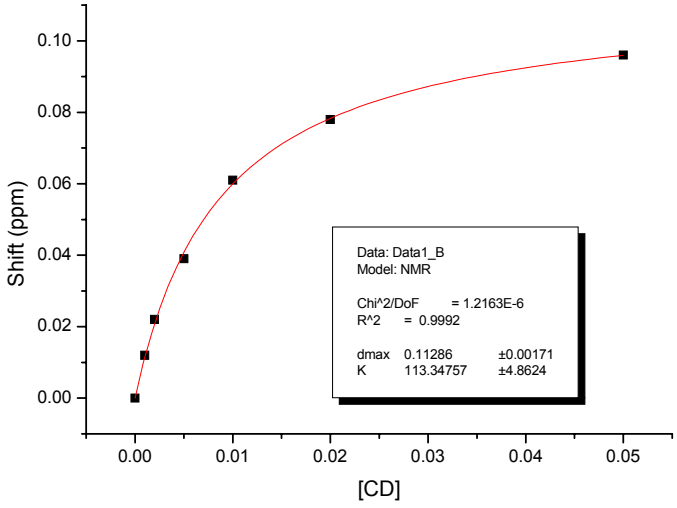


Figure 5.15 Binding isotherm for the complexation of **43c*S*** and γ-CD at 70 °C

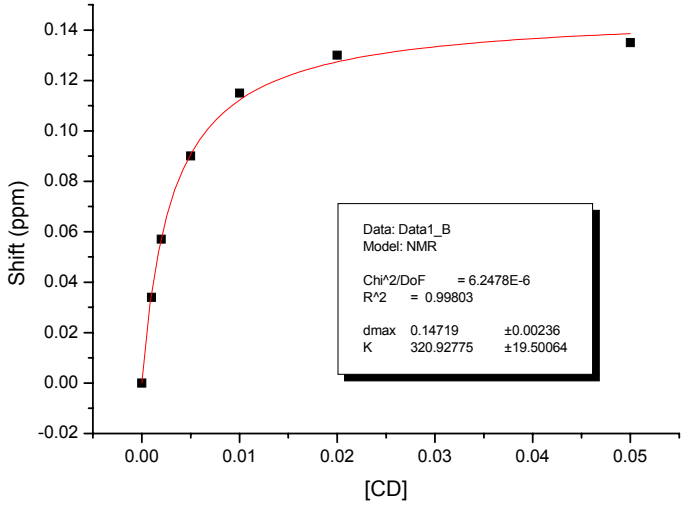


Figure 5.16 Binding isotherm for the complexation of **43c*R*** and γ-CD at 25 °C

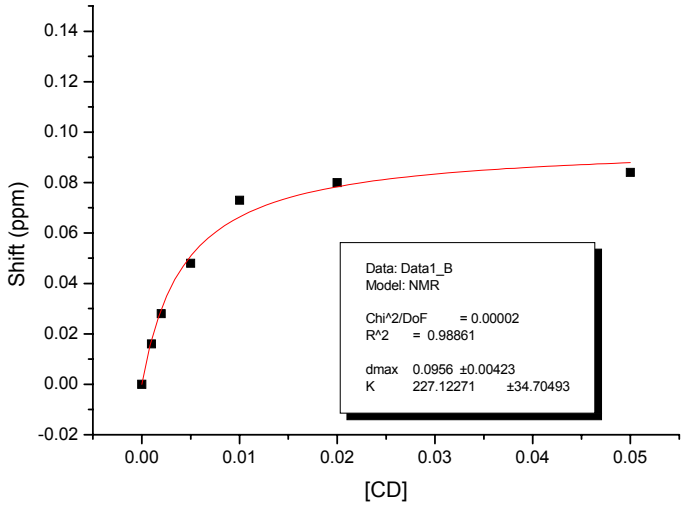


Figure 5.17 Binding isotherm for the complexation of **44a*S*** and β-CD at 25 °C

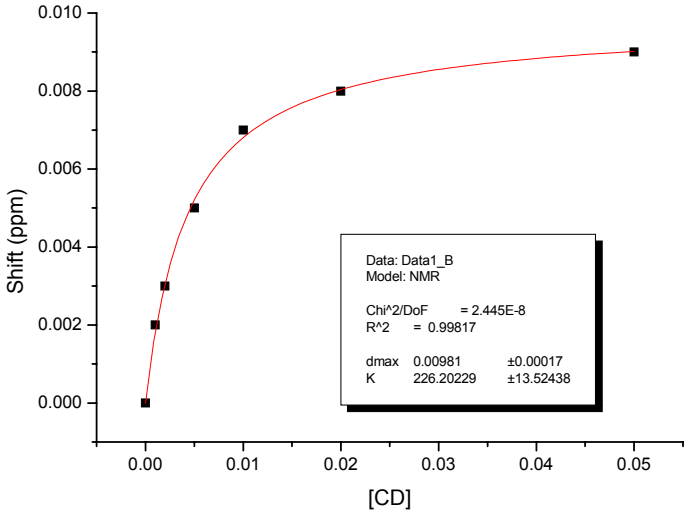


Figure 5.18 Binding isotherm for the complexation of **44cS** and γ-CD at 25 °C

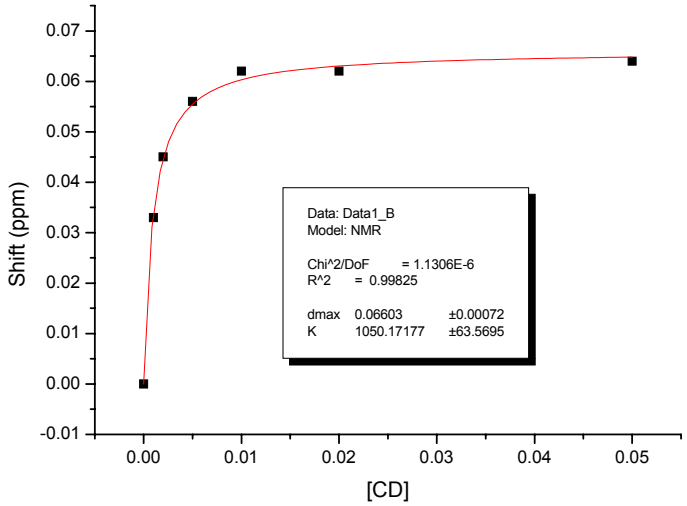


Figure 5.19 Binding isotherm for the complexation of **44c*R*** and γ-CD at 25 °C

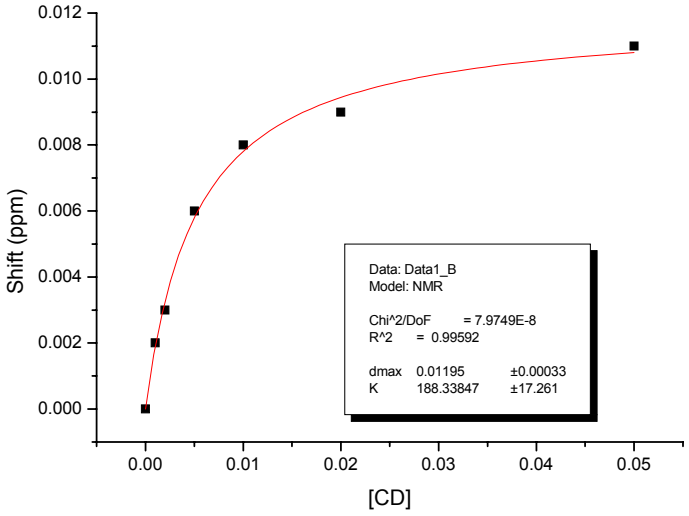


Figure 5.20 Binding isotherm for the complexation of **46c*S*** and γ-CD at 25 °C

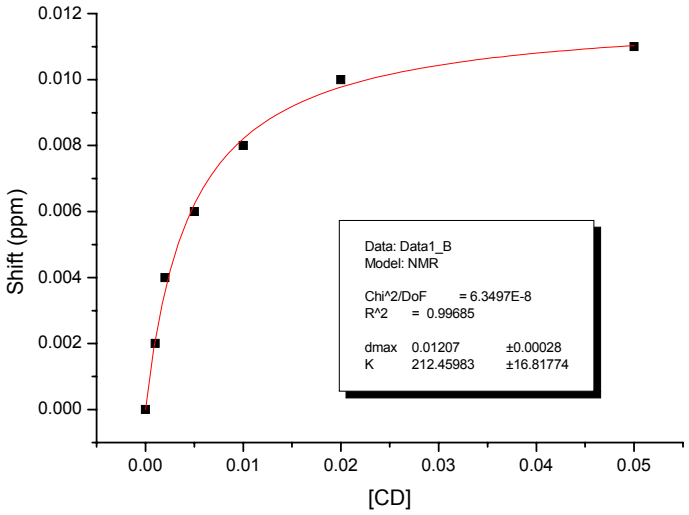


Figure 5.21 Binding isotherm for the complexation of **46c*R*** and γ-CD at 25 °C

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13 April 2005

Our ref: HG/ct/Apr 05.J054

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TETRAHEDRON, *Vol 61, 2005, pp 919-926, Branko et al, "Cyclodextrin Assisted ..."*

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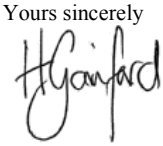

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CARBOHYDRATE RESEARCH, Vol 340, No 7, 2005, pp 1413-1418, Branko et al: "Cyclomaltooligosachharide …"

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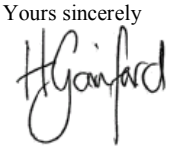

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The author was born in Ahmedabad, India. in 1994, he began his undergraduate study in chemistry at Gujarat University, where he received a B.S. degree in 1997. He continued his graduate study in physical chemistry at University School of Science and earned an M.S. degree in 1999. After graduation, he worked at Mirix Laboratory Ltd. In 2001, he came to US to pursue his Ph.D. degree in organic chemistry at the University of New Orleans, under the supervision of Professor Branko S. Jursic.