CHAPTER 5

Enantioselective fluorescent sensors for α-hydroxymono and dicarboxylic acids and their salts
Enantioselective fluorescent sensors for $\alpha$ - hydroxy mono and dicarboxylic acids and their salts

Outline

This chapter deals with the enantioselective recognition of $\alpha$-hydroxymono and dicarboxylic acids and their salts using various synthetic receptors. The geometrical feature and the chirality have been taken into account to design the receptors for enantioselective recognition. To describe all these aspects, this chapter has been divided in two sections.

Section A: Review on enantioselective fluorescent sensors for $\alpha$-hydroxymono and dicarboxylic acids and their salts.

Section B: Present work on enantioselective fluorescent sensors for $\alpha$-hydroxy mono and dicarboxylates.
Chapter 5

Section A

Review on enantioselective fluorescent sensors for \( \alpha \)-hydroxymono and dicarboxylic acids and their salts

Outline

Chiral recognition, a particular case of molecular recognition, is central to the most biological processes such as drug-biomolecule interaction. Therefore, the chiral recognition has been prime focus in recent years. This chapter describes the introductory part in enantioselective fluorescent recognition of \( \alpha \)-hydroxymono and dicarboxylic acids and their salts.
Chapter 5A

5A.1. Review on chiral fluorescent sensors for enantioselective α-hydroxymono and dicarboxylic acids and their salts

Design and synthesis of a simple and easy-to-make chiral receptor that shows selective sensing of enantiomers of a particular chiral target with respect to its mirror image is an important aspect in the area of chiral recognition. Chiral recognition, a particular case of molecular recognition, is central to most biological processes such as drug-biomolecule interaction. Therefore, the chiral recognition has been prime focus in recent years. Analytical techniques such as NMR, UV-vis, circular dichroism and fluorescence spectroscopy are commonly used to study the chiral recognition. Among these, fluorescence technique is widely used now-a-days as fluorescence-based enantioselective sensors can potentially provide high sensitivity and real-time measurement. Of the different chiral targets, carboxylate-based substrates are considered to be important as enzymes, antibodies, amino acids and different metabolic intermediates contain carboxylate functionalities. Even various natural products that show different biochemical behaviours contain a range of carboxylate functionalities. In this review, we have focused on enantioselective fluorescent sensors that are capable of showing chiral recognition of chiral hydroxycarboxylic acids. Hydroxycarboxylic acids are found to be the structural units of many natural products and drug molecules. They can also serve as multifunctional precursors to a great variety of organic compounds. For enantioselective discrimination there are several chiral chemosensors. But enantioselective fluorescent sensors for recognition of hydroxycarboxylic acids and their salts are less explored.

Mendoza and coworkers reported chiral receptors 5A.1. It showed chiral complexation of enantiomers of the sodium salt of N-acetyltryptophan. Moran et al. introduced compounds 5A.2 and 5A.3 for recognition of hydroxycarboxylates. They used bis-chromenylurea and a spirobiuorene linker for the synthesis of macrocyclic receptor 5A.3. Bis-chromenyl urea-based open structure 5A.2 bound carboxylates without showing any chiral discrimination. In comparison, 5A.3, allowed chiral discrimination of enantiomerically pure acids, such as naproxen, leucine-CBZ, lactic and mandelic acids.
Lin Pu contributed significantly in chiral recognition by introducing a series of BINOL-based compounds. His group introduced binaphthyl-based chiral molecule 5A.4 for enantioselective recognition of α-hydroxycarboxylic acids. By theoretical energy minimization they have reported that receptor 5A.4 strongly binds (S)-mandelic acid through three definite hydrogen bonds. The fluorescence intensity of 5A.4 was enhanced during the complexation of both (R) and (S)-mandelic acids but to the different extents. The fluorescence intensity of 5A.4 was enhanced 2.87 times than that of the original value by (S)-mandelic acid where the enhancement in presence of (R)-mandelic acid was only 1.75 times. This large difference in the enhancement of intensity makes the receptor suitable for enantioselective recognition of α-hydroxycarboxylic acids.

Lin Pu et al. further developed bisbinaphthyl-based macrocycles 5A.5 and 5A.6 for enantioselective recognition of α-hydroxycarboxylic acids. During the complexation of 5A.5 with chiral acids, enhancement of fluorescence intensity took place due to the protonation of nitrogen atoms which inhibited the photoinduced-electron-transfer. S-mandelic acid enhanced the emission intensity of receptor 5A.5 by large extent, in comparison to (R)-mandelic acid. On the other hand, R-mandelic acid significantly enhanced the fluorescence intensity of 5A.6.

290
To improve fluorescence signals and better enantioselective recognition, Lin Pu et al. synthesized chiral molecule 5A.7 and its dendritic analogue 5A.8 and also studied the interaction with both (R)- and (S)-mandelic acids.

They introduced dendritic branches to the chiral receptor unit due to which the fluorescence signals of the receptors were significantly amplified because of the light-harvesting effect of the dendritic structure. This greatly increased the sensitivity of the sensors in the fluorescent recognition. Study of the three generation sensors demonstrated that the generation zero sensor was the best choice for the recognition of mandelic acid because of its greatly increased fluorescence signal over the core and its high enantioselectivity.

Lin Pu and coworkers also synthesized a series of bisbinaphthyl- based optically active acyclic and macrocyclic receptors for recognition of α-hydroxycarboxylic acids. They demonstrated that receptors (S)- 5A.5 and (R) - 5A.6 behaved differently in fluorescence with (R)- and (S)- mandelic acids. Introduction of conjugated substituents to the 6, 6'-positions of the binaphthyl units in the macrocycles 5A.5 and 5A.6 led to greatly amplified fluorescence signals in 5A.9 and 5A.10. Using the 6, 6'-substituted
bisbinaphthyl macrocycles in place of the unsubstituted macrocycles allowed a 2 orders of magnitude reduction in the sensor concentration for the fluorescence measurements. These macrocycles exhibited highly enantioselective fluorescent enhancements in the presence of chiral α-hydroxy carboxylic acids and N-protected α-amino acids. The macrocycle showed much greater enantioselectivity in the substrate recognition than their acyclic analogue.

Mandelic acid also enhanced the fluorescence of another acyclic compound (S)-5A.11 in methylene chloride, but almost no enantioselectivity was observed. 6, 6-styryl-substituted macrocycle (S)-5A.12 showed similar behavior as (S)-5A.10. In presence of (S)-mandelic acids, intensity of (S)-5A.12 at shorter wavelength is enhanced and intensity at longer wavelength, is quenched to the greater extent than (R)-mandelic acid, resulting enantioselective recognition with an ef of 2.

Song et al. successfully synthesized chiral nitrogen-containing calix[4]arene-based chiral receptor 5A.13 for chiral discrimination of enantiomers of mandelic acid and 2,3-dibenzoyltartaric acid. Chiral receptor 5A.13 attains cone conformation and during the addition of racemic mandelic acid to the solution of 5A.13, methane proton signal in H
Chapter 5A

NMR underwent upfield shift and split into two signals. In $^1$H NMR, (S) isomer of mandelic acids caused more downfield shift of complex signals. Again upon addition of racemic solution of 2, 3-dibenzoyltartaric acid, not only the methine proton signals of 2,3-dibenzoyltartaric acid moved to the upfield direction with splitting but also the aromatic proton signals of it underwent upfield shift. The signals of the complex of $D$-2, 3-dibenzoyltartaric acid with 5A.13 were in lower field than those of $L$-2, 3-dibenzoyltartaric acid which supported the ability of 5A.13 towards enantioselective recognition.

Yang and coworkers reported C$_2$-symmetric receptor structure 5A.14 which showed enantioselective recognition between tetrabutylammonium salts of (R)- and (S)-mandelic acids. In $^1$H NMR, (R)- and (S)-mandelates faced different chiral environment which was responsible for nonequivalent chemical shifts of $\alpha$-protons in the molecules of (R)- and (S)-mandelates. The difference in chemical shift of corresponding protons of two enantiomeric mandelates in the presence of receptor 5A.14 was the indicative of chiral discrimination.

Wolf and Mei have synthesized C$_2$-symmetric 1, 8-diacridylnaphtalene N, N'-dioxide compound 5A.15 for enantioselective recognition of different analytes. It was more selective towards (R)-mandelic acid compared to (S)-mandelic acid.
Lin Pu et al. developed a series of cyclohexane-1,2-diamine-based bisbinaphthyl macrocycles \((S)/{(R)}\) \(\textbf{5A.21}\) and their acyclic analogues for recognition of \(\alpha\)-hydroxycarboxylic acids.\(^{19}\) Receptor \(\textbf{5A.21}\) although showed small interaction in ground state, remarkable change in fluorescence intensity was observed during the titration of \((S)\)- \(\textbf{5A.21}\) with \((R)\)- and \((S)\)- mandelic acids. While the fluorescence intensity of \((S)\)- \(\textbf{5A.21}\) was enhanced over 20-fold at the monomer emission by \((S)\)-mandelic acid in benzene/0.05% DME, \((R)\)- mandelic acid produced insignificant effect. In order to establish the fact behind large difference in the fluorescence responses of \((S)\)- \(\textbf{5A.21}\) toward \((R)\)- and \((S)\)- mandelic acids, which was due to an inherent chiral recognition, they further studied the interaction of \((R)\)- \(\textbf{5A.21}\), with \((R)\)- and \((S)\)- mandelic acids. The \((R)\)- \(\textbf{5A.21}\) was also able to discrimination \((R)\)- and \((S)\)- mandelic acids.
Chapter 5A

They also studied acyclic receptor 5A.22 with chiral (R)- and (S)-mandelic acids. Receptor 5A.22 showed very small enantioselectivity towards (R)- and (S)-mandelic acids. To improve the enantioselectivity they devised compounds 5A.23 and macrocyclic (S)-(R) - 5A.24. In the presence of (R)-mandelic acid rather than its (S)-isomer, intensity of (R) - 5A.23 was enhanced at its excimer emission. Similar to (S)- 5A.21, (S)- 5A.23 also showed mirror image enantioselection. (S)- 5A.24, synthesized by the same group, showed similar enantioselectivity. They treated (S) - 5A.24 with mandelic acid and found the greater fluorescence enhancement at the monomer emission with (S) - mandelic acid over its mirror image isomer.

Bing et al. prepared chiral fluorescent receptors 5A.25 and 5A.26 for enantioselective recognition of D and L- dibenzoyl tartrate anions.20 The receptor 5A.25 which is more rigid and preorganised than 5A.26. As a result to it, 5A.25 inferred greater enantioselective recognition ability. To establish the discrimination, they performed 1H NMR titration experiment. During the interaction of racemic dibenzoyl tartrate with 5A.25, the methane protons split into two peaks, indicating different interaction of D and L- dibenzoyl tartrate with 5A.25. The larger downfield shift of -CH proton of L- dibenzoyl tartrate suggested the better interaction between 5A.25 and L- dibenzoyl tartrate. Receptor 5A.26 showed similar binding feature giving selectivity for L- dibenzoyl tartrate.

He and coworkers synthesized (S)- 1, 1'-bi- 2- napthol based chemosensors 5A.27-5A.29 for enantioselective recognition of dibenzoyl tartrate anions.21 Compounds
Chapter 5A

5A.27-5A.29 did not show selectivity towards D- and L- malates. But all of them fluorametrically preferred D- enantiomer of dibenzoyl tartrate anion. While the fluorescence intensity of 5A.27 was almost quenched on addition of 99 equivalent amounts of D- dibenzoyl tartrate, the same change in intensity was observed upon addition of 935 equivalent of L- dibenzoyl tartrate. Receptors 5A.28 and 5A.29 showed similar enantioselectivity like 5A.27. All the receptors bound D- dibenzoyl tartrate more strongly than L- dibenzoyl tartrate.

Juaristi et al. synthesized thiourea- based chiral receptors 5A.30 and 5A.31 for enantioselective recognition of α- hydroxy as well as α- amino carboxylic acids. They studied the carboxylate binding properties of both the receptors by NMR analysis in solution and established their enantiomeric discriminating ability.

He et al developed (S)-BINOL and thiourea unit- based receptors 5A.32 and 5A.33 for chiral recognition. Receptor 5A.32 has small enantioselective recognition power.
Chapter 5A

towards $D$- and $L$- mandelates. In presence of $D$- and $L$- mandelates, fluorescence intensity of 5A.33 was quenched to the different extents.

\[ \text{5A.30} \quad \text{5A.31} \]

The different quenching efficiencies indicated that receptor 5A.33 has excellent enantioselective recognition ability for the enantiomers of mandelate. They further reported that receptor 5A.32 have similar change in UV-vis spectra during titration with both $D$- and $L$- mandelates. In case of both the isomers, during the titration the colour of the receptor solution of 5A.32 changed from pale yellow to dark yellow. In case of receptor 5A.33 similar features were observed in UV-vis spectra upon addition of $D$- and $L$- mandelate; but notable color changes were observed when either $D$- or $L$- mandelate anion was added into solution of 5A.33 in DMSO. Upon a gradual addition of $D$- mandelate, into the receptor 5A.33 in DMSO, the color changed from light yellow to light orange, while the color of the solution of 5A.33 in the presence of the same amount of $L$-mandelate changed from light yellow to red.

Griesbeck et al. developed phthalimide containing thiourea-based chemosensor 5A.34 for recognition of anions as well as chiral carboxylates.\textsuperscript{24} The sensor 5A.34 was able to recognize $F^-$, $Cl^-$, $AcO^-$ and $H_2PO_4^-$ anions. In addition to this, the chemosensor 5A.34 was able in chiral discrimination between enantiomers of lactate. Although in presence of
Chapter 5A

$D$- and $L$- lactates, the absorption spectra of 5A.34 did not show significant changes, but the association constants for $D$- and $L$-lactate revealed an enantioselectivity coefficient of 1.93, indicating moderate chiral discrimination ability of receptor 5A.34 towards enantiomers of lactate.

Luis et al. synthesized a series of bis (amino amides) derivatives 5A.35 and 5A.36, for determination of enantiomeric excess of $\alpha$-hydroxy and arylpropionic acids.25 Open-chain peptidomimetics 5A.35 and 5A.36 were derived from $L$-valine and $L$-phenylalanine.

Lin Pu et al. synthesized benzylaminomethyl group containing BINOL- based receptor 5A.37 for enantiomeric recognition.26 Upon addition of (R)- mandelic acid to the solution of (S)- 5A.37, fluorescence intensity was enhanced to the considerable extent in comparison to the case with (S)- mandelic acid. The same group also prepared (R)- 5A.37 and investigated the chiral discrimination ability towards enantiomers of mandelic acid. In this case, receptor (R)- 5A.37 showed large fluorescence enhancement in the presence of (S)- mandelic acid than (R)-mandelic acid and exhibited mirror image selectivity with respect to (S)- 5A.37. They further investigated the structural influence of 5A.37 towards enantioselective recognition by synthesizing compounds 5A.38-5A.41. While (R)- mandelic acid enhanced the fluorescence intensity of 5A.38 by 4.8 fold, enhancement of 3 fold was recorded by (R)- mandelic acid. Due to the presence of steric groups on the nitrogen atoms of 5A.38, both the fluorescence increase and decrease in enantioselectivity are observed compared to the receptor 5A.37. The chiral discrimination ability of the diasteriomeric compound 5A.39 was much lower than that of 5A.38. The 3-alkylaminomethyl substituent in 5A.40 and 5A.41 modified the binding selectivity and sensitivity. For them, the enantioselectivity ($\text{ef}$) was found to be less than 1.4.

298
Chapter 5A

Introduction of more number of hydrogen bonding group in the substituents modified the recognition behavior of the binol-based chiral molecules. In this regard, Lin Pu et al. addressed receptors (R)/ (S)-5A.42 which exhibited enantioselective precipitation and solid-state fluorescence enhancement in the recognition of α-hydroxycarboxylic acids.27 Upon addition of (S)-mandelic acid into the solution of (S)-5A.42 in benzene containing 0.4 vol% dimethoxyethane (DME), the clear solution immediately became a white suspension. Under similar condition, upon addition of (R)-mandelic acid the solution remained clear. The same experiment was done with receptor (R)-5A.42 and the reverse behavior was observed.

To prove the structural features of receptors (R)/ (S)-5A.42 towards enantioselective precipitation, they further developed compounds (S)-5A.43 and (S)-5A.44. Upon addition of (R)- or (S)-mandelic acids in benzene containing 0.4 vol% DME to the (S)-5A.43 and (S)-5A.44 no precipitate was formed. Change of substituents in the amino
alcohol side chain gave interesting observations in chiral recognition. Introduction of less steric to more steric group altered the enantioselectivity.\textsuperscript{28}

Lin Pu et al. reported pseudoenantiomeric pairs (S)-5A.45 and (R)-5A.46 of opposite chiral configuration at both the axially chiral biaryl centers and the amino alcohol units for enantioselective recognition of \( \alpha \)-hydroxycarboxylic acids.\textsuperscript{29} Upon addition of (R)-mandelic acid to the solution of (S)-5A.45, fluorescence intensity was enhanced significantly but (S)-mandelic acid merely perturbed the fluorescence intensity in this region. For (R)-5A.46 opposite selectivity was observed. Fluorescence intensity was enhanced by (S)-mandelic acid rather than by (R)-mandelic acid.

Pandey and coworker recently showed that L-phenylalanine and L-valine- based chiral receptors 5A.47 and 5A.48 are useful for visual chiral recognition of mandelic acid and \( \alpha \)-amino acid derivatives by enantioselective gel formation and precipitation.\textsuperscript{30} Compound 5A.47 formed gel selectively with R-enantiomer of mandelic acid salt in 10% DMSO/CHCl\(_3\) but with S- enantiomer of mandelic acid no gel formation was observed.

Song and coworkers reported salen-based chiral fluorescent polymers 5A.49 and 5A.50 for enantioselective recognition of \( \alpha \)-hydroxycarboxylic acids.\textsuperscript{31} The interaction property of both the receptors was carried out in a mixed solvents of toluene and DME upon gradual addition of (L)- or (D) - mandelic acid.
Change in emission character of the polymers in the presence and absence of chiral mandelic acids was considered to establish their enantioselective recognition ability. Li et al. synthesized BINOL-based dual chirogenic centers containing bisboronic acid $5A.51$ as a chiral sensor for enantioselective recognition of tartaric acid and $D$-sorbitol. In acidic pH, the fluorescence intensity of $S, S$-$5A.51$ was quenched in the presence of $D$-tartaric acid, but the emission was enhanced in the presence of $L$-tartaric acid. The diastereomer $S, R$-$5A.51$ exhibited reverse enantioselectivity towards tartaric acid.

Anthracene- labeled boronic acid-based chiral fluorescent sensors $5A.52$ and $5A.53$ were reported by James et al. They investigated the chiral discrimination ability of $R$ and $S$-$5A.52$ with enantiomer of mandelic acid in acetonitrile. In the presence of $L$-mandelic acid fluorescence intensity of $S$-$5A.52$ was enhanced to the greater extent. With $R$-$5A.52$ mirror image selectivity was noted. In their experiment they also
Chapter 5A

demonstrated that receptor 5A.53 did not have such discrimination ability towards mandelic acid.

Yang et al. reported trans-cyclohexane-1, 2- diamine and BINOL- based chemosensors 5A.54 and 5A.55 for enantioselective recognition of enantiomer of mandelic acid. In presence of (R)- mandelic acid, fluorescence intensity of receptor 5A.54b was enhanced to the greater extent in comparison to (S)- mandelic acid and gave \( I_R/I_S = 1.45 \). On the other hand 5A.54a exhibited reverse fluorescence response towards mandelic acid \( (I_S/I_R = 1.55) \). They also noted the interaction property of 5A.55a and 5A.55b. In case of both these sensors fluorescence intensity was greatly enhanced by (R)- mandelic acid than (S)- mandelic acid. From these observations, they revealed that when a sensor contains multiple chiral units, each chiral unit might play a different role in the recognition process. They also synthesized 5A.54c which exhibited mirror image selectivity towards enantiomers of mandelic acid.

![Chemical structures](image)

5A.54a (a= R, = R)
5A.54b (a= S, = R)
5A.54c (a= S, = S)
5A.55a (a= R, = R)
5A.55b (a= S, = R)
5A.55c (a= S, = S)

Carbazole- based chiral fluorescent bisboronic acid sensor 5A.56 devised by Zhao et al. was used for enantioselective recognition of mandelic acid. In the design they introduced thiophene unit to extend the n-conjugation of the carbazole and to enhance the electron-donating ability of the fluorophore. The fluorescence intensity of \((S, S)\) 5A.56 was greatly enhanced in the presence of \(L\)- tartaric acid only. With \((R, R)\)- 5A.56, the reverse fluorescence response was documented.

James and coworkers reported 3, 6-disubstituted carbazole labeled chiral boronic acid sensor 5A.57 for enantioselective recognition of tartaric acid. 
(S, S)- 5A.57 showed higher fluorescence enhancement in the presence of L- tartaric acid rather than D- tartaric acid in the pH range of 2.0-6.0. In contrast, (R, R)- 5A.57 exhibited mirror image enantioselective recognition towards enantiomer of tartaric acid. Wang and coworkers synthesized BINOL- based chiral sensors 5A.58 and 5A.59 for enantioselective recognition of mandelate in aq. solution.37

Chen et al. introduced chiral fluorescence receptors 5A.60 and 5A.61 and investigated their recognition ability in aq. solution (Tris–HCl buffer pH 7.4, MeOH/H2O = 1:1).38 They first studied the interaction of both the receptors with metal salts and established them as good binder of Cu2+ ion. In presence of Cu2+ ions, fluorescence intensity of receptors was decreased to the significant extent. They further used this receptor- Cu2+ complex as an ensemble for enantioselective recognition of mandelate. Upon gradual addition of L- mandelate to the 5A.60- Cu2+ complex, fluorescence intensity was retrieved by 27.5% where as the enhancement by D- mandelate was only 5.3%. They also carried out
Chapter 5A

similar experiment with 5A.61- Cu²⁺ complex and established its poor response to enantioselective recognition.
Banerjee and coworkers synthesized chiral Schiff-base 5A.62 for enantioselective recognition.³⁹ In the presence of (S)- mandelic acid, emission of (R, R)- 5A.62 was enhanced more significantly than in the presence of (R)- mandelic acid. They also studied the insertion of (S, S)- 5A.62 with enantiomer of mandelic acid. In case of receptor (S, S)- 5A.62 fluorescence intensity was greatly affected by (R)- mandelic acid than (S)- mandelic acid.

Xu and coworkers developed amino acid bearing fluorescent sensors 5A.63 and 5A.64 for chiral discrimination of D/ L- bis (tetrabutylammonium) dibenzoyl tartrate in DMSO.⁴⁰ Upon addition of tartrate salt to 5A.64, the emission intensity was gradually increased due to the inhibition of PET process. In presence of D- dibenzoyl tartrate, fluorescence intensity enhancement was larger compared to the case with L- dibenzyl tartrate. Similar enantioselectivity was observed with 5A.63. Lin Pu et al. synthesized bisbinaphthyl chiral macrocycle 5A.65 like 5A.23.⁴¹ Macrocycle 5A.65 showed two characteristic bands in emission. It is worth mentioning that compound 5A.65 did not show enantioselectivity in the concentration range 1.0 X 10⁻⁵ M in presence of enantiomer of mandelic acid. But at the concentration 1.0 X 10⁻⁴ M, the receptor showed the enantioselectivity in its excimer emission in the presence of mandelic acid. (S)- mandelic acid enhanced the excimer emission of (S)- 5A.65 to the greater extent than (R)- mandelic acid.
In an effort to synthesize effective receptor for hydroxycarboxylate such as tartrate, Schmidtchen et al. synthesized guanidinium group containing macrocyclic receptor 5A.66. This was used for enantioselection of tartrate and aspartate.\textsuperscript{42} For enantiodifferentiation of tartrate and aspartate using compound 5A.66, they carried out isothermal calorimetry titration experiment by the formation of diastereomeric complexes between chiral host 5A.66 and enantiomeric carboxylates. The association entropy component TAS was considered as an indicator in the enantioselection of tartrate and aspartate.

Bencini et al. recently published (S)- BINOL linker based chiral ditopic polyammonium compound 5A.67 for selective binding of (S, S)- tartaric acid over its (R, R)/meso forms in water.\textsuperscript{43} As the fluorescence property of compound 5A.67 varied with pH, they investigated the binding property of the compound at different pH. At pH 7 the receptor showed highest binding constant with (S, S)- tartrate over other guest analytes. Similar result was found in acidic pH during the formation of higher percentage of 5A.67. (S, S)- tartaric acid adducts.

In addition to the above examples, there are other chiral receptor modules which are effective in enantioselection of tartaric acid.\textsuperscript{44}
Chapter 5A

5A.2 References


Chapter 5A

Chapter 5A

CHAPTER 5

Section B

Fluorescent sensors for enantioselective sensing of chiral carboxylates

Outline

This section addresses the design and synthesis of easy-to-make simple chiral chemosensors based upon amide-urea functionalities along with benzimidazole and pyridinium motifs. The enantioselective binding abilities of the designed receptors were investigated towards enantiomers of tetrabutylammonium salts of tartaric and mandelic acids. The enantioselectivity in the binding was ascertained by observing the changes in fluorescence, UV and \(^1\)H NMR.
Chapter 5B

5B.1. Objective
Thorough review in section A of this chapter reveals that the chiral receptors are numerous in number and many of them are used for the enantioselective sensing of α-hydroxymono and dicarboxylic acids rather than their carboxylate salts. Therefore, in spite of significant progress, there is a need to develop structurally simpler chiral receptor molecules which will be efficient and competent in enantioselective sensing of α-hydroxymono and dicarboxylate anions. To fulfill this, we intended to design and synthesize benzimidazole-based chiral sensors using α-amino acid as chiral source.

5B.2. Present work
To fulfill our objective, we designed and synthesized benzimidazole-based chiral receptors 5B.1-5B.4 which are neutral in nature. They were explored in enantioselective recognition of enantiomer of α-hydroxymono and dicarboxylates. In addition to this, pyridinium based charged chiral fluorescent molecule 5B.5 was designed and synthesizes to explore its potential in chiral recognition.
Chapter 5B

5B.2.1. Synthesis of 5B.1

The receptor 5B.1 was accomplished according to the Scheme 5B.1. For 5B.1, the N-Boc-valine was coupled with o-phenylenediamine to generate the compound 5B.6, which was converted to benzimidazole derivative 5B.7 under acidic condition. Deprotection of the amine functionality in 5B.7, followed by coupling with 5-octyloxyisophthaloyl diacid chloride, gave the desired compound 5B.1 in appreciable yield.

Scheme 5B.1. (i) o-phenylenediamine, DCC, DMAP, stirred in CH₂Cl₂ for 19 h, yield: 63%; (ii) AcOH, heat, 2 h, yield: 80%; (iii) a. 50% TFA in CH₂Cl₂, stirred for 3 h, yield: 88%; b. 5-octyloxyisophthaloyl diacid chloride, dry CH₂Cl₂, Et₃N, stirred for 4 h, yield: 88%.

5B.2.2. Binding studies on receptors 5B.1

To evaluate the hydrogen bonding properties of the receptor 5B.1 towards enantiomers of tetrabutylammonium salt of tartaric acid and mandelic acid in solution, UV-vis and fluorescence experiments were performed in DMSO containing 15% DME (1, 2 dimethoxy ethane). In DMSO containing 15% DME, on excitation of 5B.1 at 280 nm, a non structured strong emission at 333 nm was observed. However,
Chapter 5B

Figure 5B.1. Fluorescence titration spectra of 5B.1 (c = 4.0 x 10^{-5} M) in DMSO containing 15% DME upon addition of tetrabutylammonium salt of (a) L-tartaric, (b) D-tartaric, (c) R-mandelic, S-mandelic acids (c = 8.0 x 10^{-4} M) (λ<sub>ex</sub> = 280 nm).

Upon addition of the tetrabutylammonium salts of D-/L-tartaric and R-/S-mandelic acids to the solution of 5B.1, monomer emission at 333 nm underwent quenching to the different extents. Upon gradual addition of L-tartrate (c = 8.0 x 10^{-4} M) to the receptor solution the fluorescence intensity of receptor was significantly quenched but the extent of quenching in presence of D-tartrate was much smaller (Figure 5B.1). Other carboxylates such as D- and L-isomers of mandelate brought about minor change in emission spectra. Different extent of fluorescence quenching indicated the ability of receptor 5B.1 in chiral discrimination.

Figure 5B.2. Change in fluorescence ratio of 5B.1 (c = 4.0 x 10^{-5} M) at 333 nm upon addition of 15 equiv. amounts of anions.
Figure 5B.2 shows plot of change in emission of \textbf{5B.1} at the monomer emission (333 nm) in the presence of 15 equiv. amounts of the different anions. It is apparent from Figure 5B.2 that the receptor is much selective to \textit{L-} tartarate. Other studied \textit{\alpha-} hydroxy acid salts weakly perturbed the emission of \textbf{5B.1} at this wavelength.

During the titration the emission centered at 333 nm is decreased; a new band at 444 nm begins to appear on progression of the titration of \textbf{5B.1} with \textit{L-} tartrate only and exhibits a ratiometric change. The ratiometric calibration curve is displayed in Figure 5B.3a.

![Figure 5B.3](image)

**Figure 5B.3.** (a) Ratiometric calibration curve for \textbf{5B.1} (c = 4.0 x 10^{-5} M) with \textit{L-} tartarate, (b) Ratiometric fluorescent response of \textbf{5B.1} (c = 4.0 x 10^{-5} M) with the addition of 15 equiv. amounts of each chiral anion (c = 8.0 x 10^{-4} M) in DMSO containing 15% DME.

This experimental finding (i.e., ratiometric fluorescence change) distinguishes \textit{L-} tartrate ion from the other chiral anions examined. The ratiometric fluorescent sensors have an important feature that they can use to evaluate the analyte concentration and provide built-in correction for environmental effects.

To our knowledge, such chiral amide based receptor for ratiometric enantioselection is a new addition to the existing reports in the literature as discussed in section A of this chapter.\textsuperscript{1} Further, the plot of ratio of fluorescence response at the wavelengths 333 nm and 444 nm is cited in Figure 5B.3b, which indicates that the response factor is noteworthy for \textit{L-} tartrate.

To our opinion, the ratiometric response of \textbf{5B.1} towards \textit{L-} tartarate ion is attributed to the strong interaction in the chiral cavity leading the closer approach of benzimidazole units (Figure 5B.4). MM3 optimized geometry shows that the amide -NHs and the
benzimidazole ring nitrogens are well arranged to form H-bonds. The steric feature of the isopropyl groups around the binding cavity presumably governs the fitting of one isomer over the other in the complexation.

![Figure 5B.4](image_url)

(a) MM3 optimized geometry of 5B.1; (b) Probable structure of the complex.

In the interaction process, the stoichiometry of the L-tartrate complex was evaluated to be 1:1, as evident from the Job's method of continuous variations (Figure 5B.5).

![Figure 5B.5](image_url)

Figure 5B.5. Fluorescence Job plot for 5B.1 ([H] = [G] = 5.67 x 10^{-5} M) with L-tartrate at 338 nm.

From the titration data, the binding constant (K_a) for the formation of complex of 5B.1 with L-tartrate complex was estimated by non linear curve fitting procedure and it was found to be 8.0 x 10^{2} M^{-1}. We were unable to fit the emission titration data for 5B.1 with D-tartrate to have reliable binding constant value. This underlines the fact...
that the strong interaction of L-tartrate over the rest of the chiral anions is presumably due to its steric fit into the cavity.

\[
\text{Figure 5B.6. Fluorescence quenching of } \textbf{5B.1} \text{ in DMSO containing 15\% DME versus concentration of chiral guests } (\lambda_{\text{ex}} = 280 \text{ nm}).
\]

The plot in Figure 5B.6 demonstrates the quenching aspect. The fluorescence difference ratio \( ef = (I_0 - I_1)/(I_0 - I_2) \), is determined to be 35.5 (Figure 5B.6). This \( ef \) value reflects that chiral receptor \textbf{5B.1} is capable of recognizing L-tartrate over its D-isomer in DME-DMSO co solvent. We believe that enantioselectivity in 15\% DME in DMSO is attributed to the role of DME. DME being less polar reduces the polarity of the solvent mixture and increases the host-guest interaction.

The interaction of \textbf{5B.1} with the same chiral anions in the ground state was also understood from UV-vis titration experiments. The UV-vis study of \textbf{5B.1} in the presence of all chiral guests showed minor change in absorbance. Figure 5B.7 shows the change in absorbance of \textbf{5B.1} upon gradual addition of guest anions.
Chapter 5B

Figure 5B.7. Change in absorbance of 5B.1 (c = 4.0 x 10^{-5} M) upon gradual addition of (a) L-tartaric, (b) D-tartaric, (c) R-mandelic and (d) S-mandelic acids (c = 8.0 x 10^{-4} M) in DMSO containing 15% DME.

In the titration profile, decrease in absorbance at 282 nm upon successive addition of guest anions to receptor solutions, indicates the weak interaction of all the chiral salts in the ground state. This fact reveals that compound 5B.1 is not efficient in discriminating the enantiomers of either tartrate in the ground state. The stoichiometry of the complex in the ground state was found to be similar to that of the excited state (Figure 5B.8). Furthermore, the interaction of 5B.1 with D/L-tartrate was also realized by $^1$H NMR spectroscopy in d_6-DMSO (Figure 5B.9). On gradual addition of L-tartrate to 5B.1 in d_6-DMSO, the amide proton H_a and benzimidazole ring H_b moved to the downfield directions with splitting. While the signal for H_a appeared at 8.87 ppm moved to 9.67 ppm ($\Delta\delta = 0.80$ ppm), H_b proton showed a downfield shift of 1.11 ppm (Figure 5B.9) at [G]/[H] = 1. The larger downfield shifts of amide protons and the benzimidazole -NH protons interpreted their involvement in
binding. However, under identical condition, at [G]/[H] = 1 with D-tartrate, both H\textsubscript{a} and H\textsubscript{b} protons also underwent downfield chemical shifts but it was smaller than the previous case (\(\Delta \delta = 0.76\) ppm for H\textsubscript{a} and \(\Delta \delta = 0.84\) for H\textsubscript{b}).

![Figure 5B.9](image)

**Figure. 5B.9.** \(^1\)H NMR titration of 5B.1 (c = 4.59 \times 10^{-3} \text{ M}) with (a) D-tartrate and (b) L-tartrate.

Thus these experimental findings, demonstrate that L-valine bearing benzimidazole-based chiral receptor is able in enantioselective recognition of tetrabutyl ammonium salt of L-tartrate over its D-isomer. Up to now, many investigations were conducted to make ratiometric fluorescent probes for various metal ions such as Zn\(^{2+}\), Hg\(^{2+}\), Pb\(^{2+}\), and Cu\(^{2+}\). In contrast, ratiometric fluorescent sensors for anions mainly focused on small-sized anions such as F\(^-\) and CN\(^-\), are known, but hardly were developed for enantiomers of \(\alpha\)-hydroxycarboxylate. Thus in the present case, compound 5B.1 is an example of ratiometric fluorescent sensor for L-tartrate with high selectivity.

As it is well known that among the different metal binding motifs, benzimidazole is an important one, as the ring nitrogen can beneficially be used in metal coordination, we further became interested in exploring 5B.1 towards metal ion sensing and then the metal complex in chiral discrimination of chiral carboxylates. In order to do that, we had to synthesize model compound 5B.8. The synthesis of the model compound 5B.8 was achieved according to the Scheme 5B.2, which follows similar reaction sequences as applied for the synthesis of 5B.1.
Chapter 5B

Scheme 5B.2. (i) o-phenylenediamine, DCC, DMAP, stirred in CH$_2$Cl$_2$ for 19 h, yield: 63%; (ii) AcOH, heat, 2 h, yield: 80%; (iii) a. 50% TFA in CH$_2$Cl$_2$, stirred for 3 h, yield: 88%; b. 5-octyloxyisophthaloyl diacid chloride, dry CH$_2$Cl$_2$, Et$_3$N, stirred for 4 h, yield: 88%;

Figure 5B.10. (a) Change in fluorescence ratio of 5B.1 (c = 2.98 x 10$^{-5}$ M) upon addition of 15 equivalent of cations at 336 nm; (b) Change in emission of 5B.1 (c = 2.98 x 10$^{-5}$ M) upon gradual addition of Hg$^{2+}$ ion at 336 nm.

The ability of 5B.1 towards sensing of different metal ions (taken as their perchlorate salts) was investigated in CH$_3$CN containing 0.2% DMSO using fluorescence and UV–vis spectroscopic tools. Additionally, $^1$H NMR was studied in CDCl$_3$ containing 5% d$_6$-DMSO to understand the nature of interaction in the binding site.

Figure 5B.10a shows the change in emission of 5B.1 in presence of 15 equiv. amounts of the different metal ions in CH$_3$CN containing 0.2 % DMSO on excitation at 290 nm. It is evident from Figure 5B.10a that the receptor 5B.1 shows measurable interaction with Hg$^{2+}$ by exhibiting a different mode of emission from the other metal ions except Zn$^{2+}$. Figure 5B.10b represents the change in emission of 5B.1 (c = 2.98 x 10$^{-5}$ M) upon addition of Hg$^{2+}$ ions. Upon gradual addition of Hg(ClO$_4$)$_2$ solution to the solution of 5B.1 in CH$_3$CN containing 0.2 % DMSO, the emission at 336 nm increased to a significant
extent without having any other change in the emission spectra. During progression of the titration with mercuric ion, receptor 5B.1 showed a red shift of 5 nm of the emission peak at 336 nm and the light blue color of the receptor solution turns to deep blue as noted upon illumination with UV light (inset of Figure 5B.10b).

This red-shift in the emission spectra is presumably the result of stabilization of the excited state of the fluorophore relative to its ground state on cation binding. It is evident from Figure 5B.10 that among the metal ions studied, Cd$^{2+}$, Co$^{2+}$, Fe$^{2+}$, Mn$^{2+}$, Cu$^{2+}$, Mg$^{2+}$, Ni$^{2+}$ and Pb$^{2+}$, cations show quenching of fluorescence, whereas addition of Hg$^{2+}$ and Zn$^{2+}$ under similar conditions enhances the emission of 5B.1. In this aspect, the fluorescence enhancement factor (Z) for Hg$^{2+}$ and Zn$^{2+}$ ions was determined to be 7.40 and 2.04, respectively which is represented in Figure 5B.11. However, the significant change in emission of 5B.1 in the presence of Hg$^{2+}$ is attributed to the better coordination of Hg$^{2+}$ in the open cleft involving the amides and benzimidazole rings. We believe that the intensity enhancement during complexation may be a consequence of a restriction in the conformational flexibility that would otherwise lead to non radiative decay through rotatory motion of the amide group. Quantum yield measurement using tryptophan (Q = 0.13 in water) as the standard one, also indicated an increase in quantum yield of the receptor 5B.1 (Q = 0.28) while it is involved in complexation with Hg$^{2+}$ (Q = 0.33 in 1:1 complex) in CH$_3$CN. To our opinion, the steric feature of isopropyl groups in 5B.1 plays a crucial role in the orientation of the benzimidazoles due to which the cavity selectively prefers Hg$^{2+}$ ion.

![Figure 5B.11: Fluorescence enhancement factor (Z) of 5B.1 (c = 2.98 x 10$^{-5}$ M) upon addition of 15 equiv. amounts of Zn$^{2+}$ and Hg$^{2+}$ ions.](image)
Chapter 5B

Considering the model receptor 5B.8 where the isopropyl groups are absent proved this truth. Interestingly, the fluorometric titration of the same metal ions with 5B.8 under similar condition did not show any characteristic selectivity in the recognition process. Figure 5B.12 shows the change in emission characteristics of 5B.8 (c = 3.77 x 10^{-5} M) upon the addition of 15 equiv. amounts of the metal ions. Moreover, in comparison to 5B.1, the emission of 5B.8 was quenched in the presence of all metal ions studied.

Figure 5B.12. Change in fluorescence ratio of 5B.8 (c = 3.77 x 10^{-5} M) upon addition of 15 equiv. of cations at 341 nm.

The stoichiometry of the Hg^{2+} complex with 5B.1 was evaluated to be 1:1 by Job plot\(^2\) (Figure 5B.13).

Figure 5B.13. Fluorescence Job plot for 5B.1 with Hg^{2+} in CH₃CN containing 0.2\% DMSO ([H] = [G] = 2.98 x 10^{-5} M).

Figure 5B.14. Fluorescence titration curves ([Guest]/[Host] vs change in emission) of 5B.1 (c = 2.98 x 10^{-5} M) measured at 336 nm in CH₃CN containing 0.2\% DMSO.
Chapter 5B

Using non-linear curve fitting method\textsuperscript{2b} the association constant for 5B.1 with Hg\textsuperscript{2+} was determined to be $(3.50 \pm 0.90) \times 10^3$ M\textsuperscript{-1}. We were unable to determine the association constants for the other metal ions due to weak interaction. The close spacing (Figure 5B.14) of the fluorescence titration curves for 5B.1 with all the metal ions except Hg\textsuperscript{2+} corroborated the weak interaction in the recognition process. Figure 5B.15 shows change of emission of 5B.1 with selected metal salts.

![Figure 5B.15](image)

**Figure 5B.15.** Change in emission of receptor 5B.1 (c = 2.98 x 10\textsuperscript{-5} M) upon addition of (a) Zn\textsuperscript{2+}, (b) Cu\textsuperscript{2+}, (c) Fe\textsuperscript{2+}, in CH\textsubscript{3}CN containing 0.2% DMSO (in all cases [cation] = 5.96x10\textsuperscript{-4} M) upon excitation at 290 nm.

However, to comprehend the selectivity in the sensing of Hg\textsuperscript{2+} by 5B.1, we recorded the emission spectra of 5B.1 upon adding 5 equiv. amounts of Hg\textsuperscript{2+} ions in the presence of 5 equiv. amounts of other metal ions, examined in the present study.

![Figure 5B.16](image)

**Figure 5B.16.** Change in emission of (a) 5B.1 (c = 3.07 x 10\textsuperscript{-5} M), (b) upon addition of 5 equiv. amount of Hg\textsuperscript{2+} in 5B.1, (c) upon addition of 5 equiv. amount of other studied metal ions to 5B.1 containing no Hg\textsuperscript{2+} ion, (d) upon addition of 5 equiv. amount of Hg\textsuperscript{2+} to 5B.1 containing other metal ions.
Chapter 5B

Figure 5B.16 displays the relative view on the change in emission of **5B.1** in the presence of Hg\(^{2+}\) when the other metal ions are absent and present in the receptor solution. Large increase in emission upon addition of Hg\(^{2+}\) to the solution of **5B.1** is noted when other metal ions are absent, but in the presence of other metal ions the enhancement of emission is marginally reduced.

Thus Figure 5B.16 represents the small interference of other metal ions during complexation of Hg\(^{2+}\). The interaction of **5B.1** with the same metal ions in the ground state was also realized by UV-vis titration experiments. The UV-vis study of **5B.1** in the presence of all metal ions except Hg\(^{2+}\) and Cu\(^{2+}\) showed minor change in absorbance (Figure 5B.17). Figure 5B.17a shows the change in absorbance of **5B.1** upon gradual addition of Hg\(^{2+}\). In the titration profile decrease in absorbance at 277 nm with small blue shift (\(\Delta\lambda = 3\) nm) also indicates the strong interaction of Hg\(^{2+}\) in the ground state.

Figure 5B.18 UV-vis Job plots for receptor **5B.1** with Hg\(^{2+}\) measured at 280 nm ([H] = [G] = 2.79 \times 10^{-5} \text{ M}).
Chapter 5B

The stoichiometry\(^{2a}\) of the complex in the ground state was found to be similar to that of the excited state which represented in Figure 5B.18.

![Figure 5B.19. Absorption titration spectra for 5B.8 (c = 3.77x 10^{-5} M) with (a) Cu\(^{2+}\), (b) Hg\(^{2+}\), (c) Fe\(^{2+}\) in CH\(_3\)CN containing 0.04% DMSO (in all cases [cation] = 7.54 x 10^{-4} M).](image)

The appreciable increase in absorbance of 5B.1 at 306 nm in the presence of Cu\(^{2+}\) is presumably due to strong metal benzimidazole interaction in the ground state. This was also true for the receptor 5B.8, shown in Figure 5B.19.

This is in accordance with the previous observation made by other group.\(^{8}\) However, based on the experimental findings the binding structure for 5B.1 with Hg\(^{2+}\) ion was proposed in Figure 5B.20.

![Figure 5B.20. (a) MM3 optimized geometry of 5B.1, (b) Suggested binding structure of 5B.1 with Hg\(^{2+}\).](image)

322
Chapter 5B

The isophthaloyl diamide is known to exhibit different conformations. Among them the conformation in which the two amide NHs are pointed into the cavity was optimized at MM3 level to realize the disposition of the benzimidazole ring nitrogens. From the MM3 optimized geometry of 5B.1 in Figure 5B.20b it is evident that the rotation of the benzimidazole amine segments along the amide bonds is quite restricted due to the steric feature of the isopropyl groups. Thus the coordination of Hg$^{2+}$ ion can be rationalized through the suggested mode in Figure 5B.20b. In this aspect, the receptor 5B.1 can attain a two-to-two or a polymeric 1:1 binding mode involving the anti orientation of the amide arms. However, these can not be ignored in solution and they may remain in equilibrium with the one-to-one binding structure involving the syn orientation of the amide arms as shown in Figure 5B.20b. We believe that the rapid rotation of the amide bonds in 5B.8 due to absence of the isopropyl groups may bring several rotameric structures in solution. As a result of which the model structure 5B.10 is unable to bring any selectivity in the binding process.

To be acquainted with the binding structure of 5B.1 with Hg$^{2+}$ we tried to record the $^1$H NMR of 5B.1 in the absence and presence of equiv. amount of Hg$^{2+}$ in CDCl$_3$ containing 5% d$_6$-DMSO.

![Figure 5B.21. Partial $^1$H NMR (400 MHz, d$_6$-DMSO) of (a) 5B.1 ($c = 5.24 \times 10^{-3}$ M) and (b) with equiv. amount of Hg$^{2+}$ions.](image)

However, addition of Hg$^{2+}$ to the solution of 5B.1 ($c = 5.24 \times 10^{-3}$ M) caused significant broadening of the signals in the aromatic region. The benzimidazole ring protons moved to the downfield direction by 0.10 to 0.30 ppm and thereby suggested the participation
Chapter 5B

of the benzimidazole ring in coordination of Hg\(^{2+}\). Even the amide signal underwent downfield shifting to the extent of 0.23 ppm with broadening (Figure 5B.21). FTIR analysis was additionally done to confirm the nature of interaction of the amide linkage with the Hg\(^{2+}\). The amide stretching at 1627 cm\(^{-1}\) appeared as a sharp singlet in 5B.1 became broad maintaining its position upon complexation of Hg\(^{2+}\). This observation nullified the possibility of the deprotonation of the amide protons during complexation.

After establishing the selectivity of 5B.1 towards Hg\(^{2+}\), we next explore the metal complex ensemble for identifying the interaction with different amino and hydroxy acids. For this purpose we added various amino acid solution (dissolved in water) to the complex of 5B.1 with Hg\(^{2+}\) in semi aqueous system (DMSO: H\(_2\)O = 4:1). Enhanced fluorescence of 5B.1 (c = 3.07 \times 10^{-5} M) induced by 10 equiv of Hg\(^{2+}\) (c = 6.15 \times 10^{-4} M) ions returned to the emission intensity of free receptor 5B.1 at 336 nm upon addition of 15 equiv of L-cysteine (Figure 5B.22c).

Farther addition resulted in slight increase in fluorescence intensity. During this titration, a weak non-structured band at 415 nm appeared along with the decrease in intensity at \(~ 345\) nm. However, under similar condition, addition of other amino acids such as L-alanine, L-glycine, L-valine, L-proline did not disturb the emission of the Hg\(^{2+}\) complex of 5B.1 suggesting their inability to break the complex which shown in Figure 5B.23.

Like cysteine, homocysteine induced similar decomplexation by showing gradual decrease in emission at \(~ 345\) nm (Figure 5B.24).
Chapter 5B

Figure 5B.23. Change in emission upon addition of (a) L-glycine, (b) L-proline, (c) L-valine to the solution of 5B.1.Hg$^{2+}$ (c = 3.07 x 10$^{-5}$ M) in aq. DMSO upon excitation at 290 nm (concentration of guests = 8.15 x 10$^{-4}$ M).

A small peptide glutathione also showed the same behavior (Figure 5B.24). In comparison, addition of dedecanethiol to the solution of 5B.1 containing Hg$^{2+}$ ion brought about marginal change in emission indicating its inefficiency to cause decomplexation effectively. Thus, this simple experimental observation indicates that the thiol group containing amino acids such as cysteine, homocysteine and small peptide glutathione (reduced form) can be selectively identified from other amino acids with no thiol group in the structure.

Figure 5B.24. Change in emission upon addition of (a) Homocysteine, (b) Glutathione to the solution of 5B.1.Hg$^{2+}$ (c = 3.07 x 10$^{-5}$ M) in aq. DMSO upon excitation at 290 nm (concentration of guests = 8.15 x 10$^{-4}$ M).

The emission titration data for the ensemble of 5B.1.Hg$^{2+}$ with cysteine, homocysteine and glutathione were fitted in 1:1 non linear binding isotherm$^{2b}$ and the binding constant values were found to be in the order of $\sim 10^3$ M$^{-1}$ [$K_a$ for L-cysteine = (2.69 ± 0.069) x 10$^3$ M, $K_a$ for DL-homocysteine = (5.89 ± 1.2) x 10$^3$ M, $K_a$ for glutathione = (4.71 ± 0.8) x 10$^3$ M].
Chapter 5B

x $10^3$ M]. In the series, homocysteine shows two fold greater affinities than cysteine and glutathione remains in between them. The Hg$^{2+}$ complex of 5B.1 was also explored to study the interaction with D/L-mandelic acids and no interesting results were found. Thus simple host 5B.1, derived from L-valine showed efficient cation-binding behaviour. The steric feature of the isopropyl group influences 5B.1 to discriminate Hg$^{2+}$ ion from the rest of the metal ions very effectively, showing considerable enhancement in emission instead of quenching. The absence of such steric effect at the vicinity of the binding center in 5B.8 confirmed this truth. This observation is appealing in the detection of Hg$^{2+}$ using a simple host in addition to the existing receptor modules of simple to complex architectures present in the literature.10,11 Moreover the mercury complex of 5B.1 senses thiol group containing amino acids such as L-cysteine, DL-homocysteine and also a small peptide glutathione from the other L-amino acids with no thiol group in the structure as well as mandelic acid isomers. This occurs due to the binding of thiol of cysteine, homocysteine and reduced form of glutathione to the Hg$^{2+}$ bound probe. Binding constant values revealed the greater affinity of the mercury ensemble for homocysteine in the series.

However, promising results on the chiral discrimination of tartrate by 5B.1 inspired to undertake the designs 5B.2 and 5B.3.

5B.2.3. Studies on chiral receptors 5B.2 and 5B.3

5B.2.3.1. Synthesis

The chiral receptors 5B.2 and 5B.3 were synthesized according to the Scheme 5B.3. The L - valine derived benzimidazole amine 5B.10 was synthesized from L-Boc-valine after performing a series of reactions as highlighted in the Scheme 5B.3. Boc- protected
Chapter 5B

Scheme 5B.3. i. o-phenylenediamine, DCC, DMAP, stirred in CH$_2$Cl$_2$ for 19 h; ii. AcOH, heat, 2 h; iii. NaH, THF, n-octyl bromide, heat 4 h; iv. 50% TFA in CH$_2$Cl$_2$, stirred for 3 h; v. 1, 3-diisocyanatobenzene, N, N-diisopropyl ethylamine stirred in CH$_2$Cl$_2$ for 9 h.

$L$-valine-based benzimidazole 5B.7 was treated with octyl bromide in the presence of NaH in dry THF to afford alkylated benzimidazole 5B.9 which was 95% enantiomerically pure (see the chiral HPLC result in the appendix). The enantiomeric purity was judged with the $D$-isomer of 5B.9. The $D$-isomer of 5B.9 was obtained from $D$-valine following the same reaction protocols as given in Scheme 5B.3. Coupling of the amine 5B.10 with 1, 3-diisocyanatobenzene in CH$_2$Cl$_2$ afforded the desired compound 5B.2 in 71% yield.

On the other hand, deprotection of amine in 5B.7 in the presence of TFA in CH$_2$Cl$_2$ gave amine 5B.11 which on reaction with 1, 3-diisocyanatobenzene afforded compound 5B.3 in 66% yield. All the compounds were characterized by $^1$H NMR, $^{13}$C, FT-IR, and mass analyses.

5B.2.3.2. Binding studies on receptors 5B.2 and 5B.3

The interaction of chiral receptor 5B.2, toward enantiomers of tetrabutylammonium salts of tartaric and mandelic acids were investigated in DMSO. On excitation at 280 nm, a nonstructured strong emission at 315 nm and a weak emission at 405 nm were observed. Weak emission at 405 nm is attributed to the solvent coordination effect at the binding center of 5B.2 for which probable formation of excimer/exciplex is expected.
Chapter 5B

This was confirmed by recording emission of 5B.2 in polar solvent CH3OH (Figure 5B.25). Limited number of solvents were taken in the study due to the solubility issue of 5B.2. As can be seen from figure 5B.25, the emission at the longer wavelength is absent in CH3OH.

![Figure 5B.25](image)

**Figure 5B.25.** Change in emission of receptor 5B.2 (c = 5.2 x 10^-5 M) in different solvents [λ_\text{exc} = 280 nm].

However, upon addition of the tetrabutylammonium salts of D-/L-tartaric and R-/S-mandelic acids to the solution of 5B.2 in DMSO, monomer emission at 315 nm underwent quenching to the different extents. In relation to this, the calculated fluorescence ratio at 315 nm for all anions except L-tartrate was found to be almost same in magnitude. Figure 5B.26 shows the change in emission of 5B.2 in the presence of 12 equiv. amounts of tetrabutylammonium salts of D/L-tartaric and R/S-mandelic acids in DMSO. As can be seen from Figure 5B.26, while the receptor 5B.2 shows sharp fluorometric discrimination between D- and L-tartrates, enantiomers of mandelate are scarcely discriminated.

![Figure 5B.26](image)

**Figure 5B.26.** Change in fluorescence ratio of 5B.2 (c = 5.67 x 10^-5 M) at 315 nm upon addition of 12 equiv. amounts of anions (λ_\text{exc} = 280 nm).

Figure 5B.27a indicates the effect of L-tartrate on fluorescence intensity of receptor 5B.2. Upon gradual addition of L-tartrate (c = 1.13 x 10^-3 M) to the receptor solution in DMSO
Chapter 5B

the emission intensity at 315 nm gradually was decreased. Inset of Figure 5B.27a explains this aspect. In comparison, upon addition of D- tartrate (c = 1.13 x 10⁻³ M) the emission intensity at this wavelength was decreased to the smaller extent (Figure 5B.27b and inset of Figure 5B.27b). Under similar condition enantiomers of mandelate quenched the emission to the smaller extent (Figure 5B.28). Stern-Volmer plot in Figure 5B.29 corroborates the quenching phenomenon. As the concentration of guests increases, only L-tartrate among the others quenches the emission more effectively. The non linear nature of the curves in Figure 5B.29 demonstrates the mixing of both static and dynamic quenchings.

Figure 5B.27. Fluorescence titration spectra of 5B.2 (c = 5.67 x 10⁻⁵ M) in DMSO upon addition of tetrabutylammonium salts of (a) L- tartaric (Inset: change in emission at 315 nm with [G]/[H]) and (b) D- tartaric acids (Inset: change in emission at 315 nm with [G]/[H]) (concentration of guests was 1.13 x 10⁻³ M) (λexc = 280 nm).

Figure 5B.28. Change in emission of receptor 5B.2 (c = 5.67 x 10⁻⁵ M) in DMSO with tetrabutylammonium salts of (a) (R) - mandelic and (b) (S) - mandelic acids (in all cases [anion] = 1.13 x 10⁻³ M; λexc = 280 nm).
Chapter 5B

Figure 5B.29. Fluorescence quenching of 5B.2 with concentration of chiral guests in DMSO ($\lambda_{\text{exc}} = 280$ nm).

The selective recognition effect on the guest of the $D$- and $L$- isomers of tartrate can be understood from the enantiomeric fluorescence difference ratio, $ef = (I_L - I_0)/(I_D - I_0)$. $I_0$ represents the fluorescence emission intensity in the absence of the chiral substrate. $I_L$ and $I_D$ are the fluorescence intensities in the presence of $L$- and $D$-tartrates, respectively. The value of $ef$ is 2.46 for the chemosensor 5B.2, which indicates that this chemosensor can exhibit measurable enantioselective response toward $L$- tartrate. The reason may be attributed to the steric fit of the $L$- isomer into the chiral cavity of 5B.2 that augments strong complexation. In the interaction process, the stoichiometry of the complexes of 5B.2 with both $D$- and $L$- tartrates was determined to be 1:1 as confirmed by Job’s plot.\textsuperscript{2a} Figures 5B.30a and 5B.30b, for example, show the Job plot for 5B.2 with $L$- and $D$- tartrates.
Chapter 5B

Figure 5B.30. Fluorescence Job plots for 5B.2 with (a) L- tartrate, (b) D- tartrate in DMSO ([H] = [G] = 5.67 x 10^-5 M) and (c) binding constant curve for 5B.2.

The binding constant value was determined from non linear fit of the emission titration data and it was found to be (1.3 ± 0.13) x 10^4 M^-1 (Figure 5B.30c). The binding constant value for D-tartrate was determined to be (4.6 ± 0.75) x 10^3 M^-1 which is ~ 3 times less compared to L-tartrate. Thus, like the difference in $e_f$, the considerable difference in binding constant values also precisely describes the enantioselectivity.

The enantioselectivity of 5B.2 towards a particular stereoisomer was further understood from the change in emission of 5B.2 in the presence of its mirror image isomer. Figure 5B.31 demonstrates these features.

Figure 5B.31. Fluorescent response of receptor 5B.2 (c = 5.62 x 10^-5 M) to (a) L- tartrate (c = 1.13 x 10^-3 M) in presence of D- tartrate (c = 1.13 x 10^-3 M) and (b) D- tartrate (c = 1.13 x 10^-3 M) in presence of L- tartrate (c = 1.13 x 10^-3 M) in DMSO.
Chapter 5B

While $D$-tartrate induced change in emission of 5B.2 was further perturbed to the considerable extent upon addition of $L$-tartrate (Figure 5B.31a), the reverse one was noticed to be insignificant (Figure 5B.31b).

**Figure 5B.32.** Change in absorbance of 5B.2 ($c = 5.67 \times 10^{-5}$ M) upon gradual addition of (a) $L$-tartrate, (b) $D$-tartrate in DMSO.

UV-vis titrations of 5B.2 with all the guests studied in DMSO exhibited minor change in absorbance (Figure 5B.32). In all cases, the absorption of 5B.2 was decreased to the small extent when guest solutions were progressively added to the receptor solution. This indicated that the receptor 5B.2 is not efficient in discriminating the enantiomers of either tartrate in the ground state. Furthermore, the interaction of 5B.2 with $D/L$-tartrate was also realized by NMR spectroscopy in d$_6$-DMSO (Figure 5B.33).

**Figure 5B.33.** $^1$H NMR titration of 5B.2 with ($L$) - tartrate ($c = 4.59 \times 10^{-3}$ M).

**Figure 5B.34.** $^1$H NMR titration of 5B.2 ($c = 4.59 \times 10^{-3}$ M) with ($D$) - tartrate.
On gradual addition of L-tartrate to **5B.2** in $d_6$-DMSO, the urea proton $H_a$ and $H_b$ moved to the downfield direction with splitting. While the signal for $H_a$ appeared at 8.48 ppm moved to 8.63 ppm ($\Delta \delta = 0.15$ ppm), $H_b$ proton showed a downfield shift of 0.13 ppm (Figure 5B.33) at $[G]/[H] = 1$. During interaction, the bifurcation of the signals for urea protons reveals an asymmetric nature of the complex formed in solution. However, under identical condition, at $[G]/[H] = 1$ with D-tartrate, both $H_a$ and $H_b$ protons underwent small downfield chemical shifts ($\Delta \delta = 0.10$ ppm for $H_a$ and $\Delta \delta = 0.03$ for $H_b$) (Figure 5B.34). Importantly, further addition of L-tartrate to **5B.2** in $d_6$-DMSO caused greater shift of the urea protons ($\Delta \delta = 0.37$ for $H_a$).

In comparison, it was not so meaningful for D-tartrate. Moreover, when equiv. amount of L-tartrate was added to the solution of **5B.2**, CH proton of L-tartrate underwent a small upfield shift of 0.02 ppm. To determine the binding constant values for **5B.2** with both the isomers of tartrate, we tried to fit the downfield chemical shift values of urea protons both in linear and non linear fit equations.

But we failed to encompass any reliable binding constant value. Careful observation of NMR titration spectra reveals that during interaction, the benzimidazole ring protons underwent negligible downfield shift and it was thus anticipated that the benzimidazole rings were non participants in the interaction. Only the urea protons are involved in the bonding. In this context, we also presume that not only the urea protons alone are liable for discrimination of D-/L-tartrate but also the steric features of the isopropyl and octyl chains around the binding zone are responsible too. To prove this we considered the new structure **5B.3** where the octyl chains are absent. $^1$H NMR of **5B.3** itself and in the presence of equivalent amount of both D- and L-tartrates was recorded in $d_6$-DMSO with difficulty as compound **5B.3** in the NMR concentration range ($\sim 10^{-3}$ M) was moderately soluble in DMSO. Indeed, in the presence of L-tartrate the benzimidazole ring NHs appeared at 12.36 ppm moved to 13.4 ppm. The urea protons $H_a$ and $H_b$ showed downfield chemical shifts of 0.66 ppm and 0.03 ppm, respectively. In the presence of D-tartrate, benzimidazole ring NHs, urea protons $H_a$ and $H_b$ exhibited relatively small downfield chemical shifts of 0.20 ppm, 0.19 ppm and 0.02 ppm, respectively. This change in chemical shift values indicated that both the urea and benzimidazole rings were involved in complexation.

With this background in NMR, we further studied its emission behavior toward the same anions to enquire its chiral recognition ability. Due to the solubility issue, only the emission properties of **5B.3** were investigated in DMSO in the same concentration as taken for
Chapter 5B

5B.2. Interestingly, compound 5B.3 under identical condition, exhibited a small preference for D-tartrate (Figure 5B.35).

![Figure 5B.35](image1)

**Figure 5B.35.** Change in fluorescence ratio of 5B.3 ($c = 5.67 \times 10^{-5}$ M) at 318 nm upon addition of 26 equiv. amounts of anions ($\lambda_{\text{exc}} = 280$ nm).

The enantiomeric fluorescence difference ratio ($ef$) in DMSO for 5B.3 was determined to be 1.44, which is much less compared to the case of 5B.2 in DMSO. Thus, despite of the involvement of the urea and benzimidazole moieties in complexation,

![Figure 5B.36](image2)

**Figure 5B.36.** Fluorescence quenching of 5B.2 with concentration of chiral guests in DMSO ($\lambda_{\text{exc}} = 280$ nm).

the enantioselective recognition ability of 5B.3 is observed to be poor (Figure 5B.36). This underlines the truth that the steric crowding around the binding zone in 5B.2 is the key feature for its enantioselective sensing of tartrate.

To realize the steric feature of the receptors 5B.2 and 5B.3, MMX calculations\(^\text{12}\) on L-
tartrate complexes were performed. As can be seen from Figure 5B.37A, the alignment of the octyl chains permits the urea groups only to complex tartrate keeping the benzimidazoles away. One of the octyl chains flies over the benzene ring on account of CH- π interaction (shortest distance between the methylene proton and benzene ring = 2.79 Å). In comparison, 5B.3 binds tartrate involving both the urea and benzimidazole moieties (Figure 5B.36B). No steric crowding is observed in the complex.

Thus, L-valine derived benzimidazole, linked to the m-xylene spacer through urea linkages, is able to discriminate D-/L- tartrate fluorometrically in polar solvent DMSO if necessary steric feature is imposed in the design. Thus in the present report, while receptor 5B.2 is capable of sensing L-tartrate with ef 2.46, less steric receptor 5B.3 having no octyl chains shows a marginal preference for D-tartrate with ef 1.44. The disposition of isopropyl groups and also the octyl chains around the binding center in 5B.2 modulates the cavity dimension in such a way that L-tartrate binds more comfortably than D-tartrate. Thus the receptor steric effect, structural rigidity and hydrogen bond altogether enforce the simple design 5B.2 to distinguish L-tartrate from D-tartrate fluorometrically.
Chapter 5B

5B.2.4. Studies on chiral receptors 5B.4

5B.2.4.1. Syntheses

The receptor 5B.4 was accomplished according to the Scheme 5B.4. In the reaction scheme, Boc-protected benzimidazole 5B.6 was reacted with 1, 3-

Scheme 5B.4. (i) NaH, THF, 1, 3-bis(bromomethyl)benzene, heat 6 h, yield: 80 %; (ii) 50% TFA in CH₂Cl₂, stirred for 5 h, yield: 88%; (iii) triphosgene, pyrene amine, N, N – diisopropyl ethylamine stirring in CH₂Cl₂ for 12 h yield: 66%.

bis(bromomethyl)benzene to afford the compound 5B.12. Deprotection of the Boc groups in 5B.12 resulted in amine 5B.13. Next, the amine was coupled with pyrene isocyanate obtained from the reaction of pyrene with triphosgene in CH₂Cl₂ to give the desired compound 5B.4. All the compounds were fully characterized spectroscopically.

5B.2.4.2. Binding studies

The complexation properties of 5B.4 towards enantiomers of D/ L tartrate and R/ S- mandelate were studied by fluorescence and UV-vis methods in 3% DMSO in DME. We first probed binding ability of 5B.4 towards the tetrabutylammonium salt of (D)/ (L) tartrate and (R)/ (S)- mandelic acids based on fluorescence change. In the absence of the carboxylates, the fluorophore pyrene unit in 5B.4 exhibited strong structured monomer emission at 416 nm. A weak peak at ≈ 525 nm was assumed to be due to the excimer between the pendant pyrene units. However, addition of the tetrabutylammonium salt of (D)/ (L) – tartrate (c = 7.58 x 10⁻⁴ M) as well as (R)/ (S)-mandelate (c = 7.58 x 10⁻⁴ M) to the solution of 5B.4 hardly perturbed the fluorescence intensity of the monomer band. Figure 5B.38 represents the change in emission of 5B.4 upon addition of 20 equiv. amounts of a particular anion.
Chapter 5B

Figure 5B.38. Change in fluorescence ratio of 5B.4 (c = 3.8 x 10^{-5} M) upon addition of 20 equiv. amounts of (D)/(L) tartrate and (R)/(S)- mandelate (c = 7.58 x 10^{-4} M) in 3% DMSO in DME at 416 nm.

Figure 5B.39 indicates the change of emission of 5B.4 upon successive addition of the enantiomers of (R)/(S)- mandelate and (D)/(L)- tartrate to the receptor solution in DMSO- DME, on excitation at 370 nm. Small change in fluorescence reflected weak interaction and hence poor enantioselectivity. This is presumed to be due to structural feature of 5B.4 for which poor hydrogen bonding interaction occurs.

Figure 5B.39. Fluorescence titration spectra of 5B.4 (c = 3.8 x 10^{-5} M) in DME containing 3% DMSO upon addition of tetrabutylammonium salts of (a) L- tartaric, (b) D-tartaric, (c) R- mandelic and (d) S- mandelic acids (c = 7.58 x 10^{-4} M) (λ_{ex} = 370 nm).
Chapter 5B

To see the interaction in the ground state we also carried out the UV-vis titration experiments in the same solvent. In the titration, change in absorption for pyrene unit was same in magnitudes for all the chiral carboxylates and thereby indicated poor selectivity in the ground state also.

Figure 5B.40. Change in absorbance of 5B.4 (c = 5.67 x 10^{-5} M) upon gradual addition of tetrabutylammonium salts of (a) (L) tartaric, (b) (D) tartaric, (c) (R) mandelic, (S) mandelic acids (c = 7.58 x 10^{-4} M) DME containing 3% DMSO.

Figure 5B.40, for example, shows the titration spectra of 5B.4 with tetrabutylammonium salts of L/D - tartaric and R/ S- mandelic acids.

The binding constant values for 5B.4 with L- tartarate was observed to be (3.27 ± 0.67) x 10^{2} M^{-1} and with D- tartarate was (3.08 ± 0.657) x 10^{2} M^{-1} obtained by UV-vis method. Similarly, analysis of the absorption data provided the association constants (K_a) (2.99 ± 0.45) x 10^{2} M^{-1} for R- mandelate and (2.89 ± 0.77) x 10^{2} M^{-1} for S- mandelate. We were unable to determine the association constants for the isomers of tartebrate and mandelate by the fluorescence method due to weak interaction.

Thus, the experimental observations reveal that the pyrene based receptor 5B.4 is not a suitable candidate in enantioselective recognition of α- hydroxymono and dicarboxylates.
Chapter 5B

Further, we carried out similar spectroscopic investigation, taking higher concentration of chiral anions ($\approx 10^{-3}$ M) in the same solvent.

![Figure 5B.41](image)

**Figure 5B.41.** Change in fluorescence ratio of 5B.4 ($c = 5.99 \times 10^{-5}$ M) upon addition of 20 equiv. amounts of (D)/(L) tartrate and (R)/(S) - mandelate ($c = 1.13 \times 10^{-3}$ M) in 3% DMSO in DME at 416 nm.

Figure 5B.41 shows the change in emission of 5B.4 in the presence of 20 equiv. amounts of the different chiral anions in DME containing 3% DMSO on excitation at 370 nm. It is evident from Figure 5B.41 that in the concentration range the receptor is again unable for enantiomeric discrimination of $\alpha$- hydroxymono and dicarboxylates.

Instead of chiral benzimidazole we planned to use the chiral pyridinium motif to devise chiral fluorescent molecule for the better chiral discrimination of the chiral carboxylates. For this, we designed and synthesized the symmetrical anthracene- labeled chiral charged receptor 5B.5.

5B.2.5. Studies on receptor 5B.5

5B.2.5.1 Synthesis

Chiral fluororeceptor 5B.5 was synthesized by quarternization of the pyridine ring nitrogen in 5B.15 using 9, 10-bis(chloromethyl)anthracene followed by Cl$^-$ exchange with PF$_6^-$ ions. The intermediate compound 5B.15 in the scheme was obtained from N-protected L-valine ester by following usual protection/deprotection and coupling reactions as mentioned in the Scheme 5B.5.
Chapter 5B

Scheme 5B.5. (i) LiOH, MeOH - H2O, stirring, 4h; (ii) 3 - aminopyridine, DCC- DMAP, dry CH2Cl2, stirring, 19h; (iii) a) 9,10-bis(chloromethyl)anthracene, CH3CN, reflux, 72h; b) NH4PF6, MeOH - H2O.

5B.2.5.2. Binding studies of 5B.5

All the receptors 5B.1- 5B.4 discussed above are neutral in charge but here we introduce positively charged sensor 5B.5 containing two symmetrical chiral chain connected through spacer, anthracene. Purpose of designing such cationic receptor is to acquire the better enantioselection of isomers of α-hydroxymono and dicarboxylate anions involving hydrogen bonding and charge-charge interaction.

Similar to the receptors 5B.1- 5B.4, the solution phase binding interaction of the tetrabutylammonium salts of D-/L-tartaric and R-/S- mandelic acids with 5B.5 was investigated in DMSO by UV-vis and fluorescence techniques.

The chemosensor 5B.5 showed an intense emission at 432 nm when excited at 380 nm in DMSO. However, upon progressive addition of the tetrabutylammonium salts of D-/L-tartaric and R-/S- mandelic acids to the solution of 5B.5 (c = 1.12 x 10^-4 M) in DMSO, the intensity of monomer emission at 432 nm underwent change to the different extents. During fluorometric titration only for L- isomer of tartrate, emission of 5B.5 at 432 nm was increased significantly. The increase in emission of 5B.5 at 432 nm in the presence of tetrabutylammonium salt of L-tartaric acids is moderate and distinguishable from its D- isomer. In relation to this, the fluorescence ratio at 432 nm for all anions except L- tartrate was found to be almost same in magnitude. In this regard, Figure 5B.42 shows the change in emission of 5B.5 in the presence of 20 equiv. amounts of tetrabutylammonium salts of D / L- tartaric and R / S- mandelic acids in DMSO. As can be seen from Figure 5B.42, although the enantiomers of mandelate are scarcely discriminated, receptor 5B.5 shows sharp fluorometric discrimination between D- and L- tartrates.
Fig 5B.42. Change in fluorescence ratio of 5B.5 (c = 1.12 x 10^{-4} M) at 332 nm upon addition of 20 equiv. amounts of anions (λ_{exc} = 380 nm).

Figures 5B.43 and Figure 5B.44 show the change in emission of 5B.5 upon increasing the quantity of tetrabutylammonium salts of D / L- tartaric and R / S - mandelic acids in DMSO, respectively.

Fig 5B.43. Fluorescence titration spectra of 5B.5 (c = 1.12 x 10^{-4} M) in DMSO upon addition of tetrabutylammonium salts of (a) L- tartaric (Inset: change in emission at 432 nm with [G]/ [H]) and (b) D- tartaric acids (concentration of guests was 2.2 x 10^{-3} M) (λ_{exc} = 380 nm).

From Figure 5B.43a it is clear that upon gradual addition of L- tartrate (c = 2.2 x 10^{-3} M) to the receptor solution in DMSO, the emission intensity at 432 nm is considerably enhanced.

In contrast, upon addition of D- tartrate (c = 2.2 x 10^{-3} M) the emission intensity at the same region is decreased to the smaller extent (Figure 5B.43b).
Chapter 5B

Fig 5B.44. Fluorescence titration spectra of **5B.5** (c = 1.12 x 10^-4 M) in DMSO upon addition of tetrabutylammonium salts of (a) R- mandelic and (b) S- mandelic acids (concentration of guests was 2.2 x 10^-3 M) (λexc = 380 nm).

The selective recognition effect on the guest of the D- /L- isomers of tartrate was understood from the enantiomeric fluorescence difference ratio, \( ef = (I_L - I_0)/(I_D - I_0) \). \( I_0 \) represents the fluorescence emission intensity in the absence of the chiral substrate. \( I_L \) and \( I_D \) are the fluorescence intensities in the presence of \( L- \) and \( D- \) tartrates, respectively. The value of ‘ef’ is 29.38 for the chemosensor **5B.5**.

![Diagram of probable conformations of 5B.5 and their preferential interactions](image)

*Figure 5B.45. Probable conformations of 5B.5 and their preferential interactions*
Chapter 5B

This signifies that chemosensor **5B.5** exhibits enantioselective response toward *L*-tartrate. The steric fit of the *L*-isomer into the *syn* conformation of **5B.5** presumably indicates strong hydrogen bonding interaction. The equilibrium *anti* conformation **5B.5X**\(^1\) will go to the *syn* conformation **5B.5Y** in the presence of tartrate (dicarboxylates) due to formation of greater number of H-bonds (Figure 5B.45).

To be confirmed with the mode of interaction in Figure 5B.45, \(^1\)H NMR studies of **5B.5** in the presence of equiv. amount of *L*- and *D*-tartrates were performed in \(d_6\)-DMSO (Figure 5B.46). The amide proton \(H_a\) and carbamate proton \(H_f\) in **5B.5** underwent downfield chemical shift during complexation. The pyridinium ring protons \(H_c\) compared to \(H_a\) moved more to the downfield direction and thereby indicated their involvement in the complexation. Careful scrutiny reveals that the chemical shift of the indicated protons is slightly more in the presence of *L*-tartrate than the case with *D*-tartrate.

![Figure 5B.46.](image)

**Figure. 5B.46.** \(^1\)H NMR titration of (i) **5B.5** \((c = 2.9 \times 10^{-3} \text{ M})\) with (ii) *D*-tartrate and (iii) *L*-tartrate.

In the interaction process, the stoichiometry of the complexes of **5B.5** with both *D*- and *L*-tartrates was determined to be 1:1 as confirmed by Job’s plot.\(^2\) Figure 5B.47, for example, shows the Job plot for **5B.5** with *L*-tartrates.
Non linear fit of the emission titration data gave the binding constant \( (k_a)^{2b} \) value of \((6.50 \pm 0.75) \times 10^3 \text{ M}^{-1}\) for \(L\)-tartrate (Figure 5B.48). We were unable to determine \(K_a\) values for \(D\)-tartrate due to weak interaction.

The binding constant \((k_a)\) value\(^{2b}\) was also determined for the complex of \(5B.5\) with \(R-/S-\) mandelate. Binding constant values for the isomers of mandelates were found to be \((3.55 \pm 0.77) \times 10^3 \text{ M}^{-1}\) and \((2.72 \pm 0.41) \times 10^3 \text{ M}^{-1}\) for \(R-\) mandelate and \(S-\) mandelate (Figure 5B.49) respectively. As before with sensor \(5B.2\) the enantioselectivity of \(5B.5\) towards a particular stereoisomer of tartrate was further realized from the change in emission of \(5B.5\) in the presence of its mirror image isomer.
Chapter 5B

Figure 5B.49. Binding constant curves for 5B.5 with (a) R- mandelate; (b) S- mandelate.

Figure 5B.50 clearly demonstrates these aspects. As can be seen from Figure 5B.50, D- tartrate induced change in emission of 5B.5 was further perturbed to the considerable extent upon addition of L-tartrate (Figure 5B.50a), the reverse one was noticed to be insignificant (Figure 5B.50b).

Figure 5B.50. Fluorescent response of receptor 5B.5 (c = 1.14 x 10^{-4} M) to (a) D- tartrate (c = 2.2 x 10^{-3} M) in presence of L- tartrate (c = 2.2 x 10^{-3} M) and (b) L- tartrate (c = 2.2 x 10^{-3} M) in presence of D- tartrate (c = 2.2 x 10^{-3} M) in DMSO.

The UV-vis study of 5B.5 in the presence of tetrabutylammonium salts of D / L- tartaric and R / S - mandelic acids in DMSO showed marginal change in absorbance (Figure 5B.51) for anthracene.
Chapter 5B

Figure 5B.51. Change in absorbance of 5B.5 (c = 1.12 x 10^{-4} M) upon gradual addition of (a) L-tartrate, (b) D-tartrate, (c) R-mandelate, (d) S-mandelate in DMSO.

In comparison, the change in absorbance in the region $\approx 300$ nm (attributed to the pyridinium binding site) was mentionably, especially for tartrates. The stoichiometries of the tartrate complexes with 5B.5 were also 1:1 as determined from Job plot\textsuperscript{13} by Uv-method (Figure 5B.52).

Figure 5B.52. UV-vis Job plot for 5B.5 with (a) L-tartrate, (b) D-tartrate in DMSO ([H] = [G] = 5.00 x 10^{-4} M)
The binding constant values for the isomers of tartrate with 5B.5 were determined to be \((9.56 \pm 2.3) \times 10^3\) M\(^{-1}\) and \((8.55 \pm 1.9) \times 10^3\) M\(^{-1}\) for D- and L- tartrate respectively. Similarly, analysis of the absorption data provided the association constants \((K_a)\) \((1.47 \pm 0.19) \times 10^3\) M\(^{-1}\) for R- mandelate and \((1.08 \pm 0.24) \times 10^3\) M\(^{-1}\) for S- mandelate.

Thus, at a glance, chemosensor 5B.5 is successfully capable of discriminating L- tartrate from D- tartrate fluorimetrically with an ‘ef’ value of 29.38. The mandelates being smaller in size than tartrate are unable to bridge the two pyridinium motifs in 5B.5 and thereby induce small change in emission without showing any measurable distinctive feature.

5.B.3. Summary and outlook

We have introduced a series of L-valine derived neutral benzimidazole and pyrene-based, chiral receptors comprising of urea and amide motifs. Among them chemosensors 5B.1, 5B.2 and 5B.3 are promising for enantioselective discrimination of tartrate. The disposition of isopropyl groups around the binding center of receptors modulates the cavity dimension in such a way that one enantiomer binds more comfortably over its mirror image isomer. On the other hand, if necessary steric feature is imposed around the benzimidazole unit in 5B.3, the discrimination between D-/L-tartrates becomes possible fluorometrically. These findings, thus, underline the fact that the presence of steric effect, structural rigidity and hydrogen bond altogether are the responsible factors for the enantiomeric recognition of hydroxy dicarboxylates. Metal binding studies and subsequent use of metal complex of 5B.1 were explored in the discrimination of chiral amino acids and hydroxy acids. But no promising results were observed for application. In addition, pyrene coupled benzimidazole-based new system 5B.4 did not act as effective sensor in chiral recognition. We hope that further tuning of the structures may bring fruitful results of enantioselection from a variety of chiral hydroxyl group containing mono and dicarboxylates. On the other hand, the use of charge centre near the hydrogen binding site is an elegant choice of making good sensor for anions. In relation to this, the chemosensor 5B.5, an easy- to- make simple structure, has been established as an effective candidate for chiral discrimination of L-tartrate from its mirror image isomer. The enantioselection ability of 5B.5 has been
Chapter 5B

found to be noteworthy than 5B.2 with greater 'ef' value. The hydrogen bonding and charge-charge interactions during complexation play the critical role in the event.

5B.4. Experimental

General

Reagents and solvents were purified using standard techniques. Solvents were dried over the appropriate drying agent following standard procedure. Solvents for spectroscopic measurements were of spectroscopic or HPLC grade. Infrared spectra were recorded on Perkin Elmer L120-00A spectrophotometer. $^1$H NMR spectra were recorded at 400 MHz using Bruker instrument. Mass spectra were recorded on API 2000 LCMS/MS instrument. Fluorescence measurements were done using Perkin Elmer LS 55 and UV-vis absorption spectra were recorded at room temperature using Perkin Elmer Lambda 25.

tert-Butyl (1H-benzo[d]imidazol-2-yl)methylcarbamate (5B.7):

To a stirred solution of N-Boc-protected L-valine acid (0.323 g, 1.85 mmol), in dry CH$_2$Cl$_2$ (30 mL), DCC (0.383 g, 1.85 mmol) and a catalytic amount of DMAP were added at 0 °C. The solution was stirred for 30 min. and then a solution of o-phenylenediamine (1.0 g, 9.25 mmol) in dry CH$_2$Cl$_2$ was added to it under nitrogen atmosphere. After stirring for 19 h, the reaction mixture was filtered off to remove insoluble DCU. The filtrate was removed in vacuo and the crude product was purified by silica gel column chromatography using 1% methanol in chloroform to afford the compound 5B.6 (0.321 g, yield: 65.4%), FT-IR $\tilde{\nu}$ cm$^{-1}$ (KBr): 3420, 3339 , 3257 , 3044, 2980 , 1703 , 1678 , 1538.

Compound 5B.6 (0.2 g, 0.754 mmol) was next heated in acetic acid (0.3 ml) at 60°C for 2 h. Then the reaction was quenched by addition of aq solution of NaHCO$_3$ (15 mL). The reaction mixture was next extracted with CHCl$_3$-MeOH mixture solvent (CHCl$_3$: MeOH = 3:1 v/v; 15 mL). Organic layer was washed with brine, dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using 40% petroleum ether in ethyl acetate to afford the compound 5B.7 (0.165 g, yield: 88.5%), mp 174°C. $^1$H NMR (400 MHz, CDCl$_3$ containing one drop of $d_6$-DMSO): δ 10.70 (s, 1H), 7.71 (d, 1H, $J= 8$ Hz), 7.35 (d, 1H, $J = 8$ Hz), 7.21- 7.19 (m, 2H), 5.67 (d , 1H, $J= 8$ Hz), 4.61 (t , 1H, $J = 8$ Hz), 2.47 – 2.45 (m , 1H), 1.43 (s, 9H), 1.06 (d, 3H, $J = 4$ Hz), 0.93 (d, 3H, $J= 4$ Hz ); $^{13}$C NMR (100 MHz, CDCl$_3$ containing
one drop of $d_6$-DMSO): $\delta$ 155.6, 154.9, 121.7 (four carbons unresolved), 79.2, 55.1, 33.2, 28.2, 19.2, 18.3; FT-IR: $\nu$ cm$^{-1}$ (KBr): 3333, 2974, 2930, 1675, 1628, 1536.

tert-butyl (1H-benzo[d]imidazol-2-yl)methylcarbamate (2B.10):

Compound 2B.10 and amine 2B.11 was obtained according to the procedure depicted in the experimental section of chapter 2B.

tert-butyl (1-octyl-1H-benzo[d]imidazol-2yl)methylcarbamate (5B.9):

To a stirred solution of 5B.7 (0.2 g, 0.81 mmol), in dry THF (20 mL), sodium hydride (0.02 g, 0.89 mmol) was added. The solution was refluxed for 30 min. and a solution of 1-bromooctane (0.156 g, 0.81 mmol) in dry THF was added to it under nitrogen atmosphere. Reaction mixture was refluxed for further 4 h. After completion of reaction, monitored by TLC, THF was evaporated and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent gave crude mass which was purified by column chromatography to afford pure compound 5B.9 (0.23 g, yield: 79%) as light yellow solid, mp 148 °C. $^1$H NMR (400 MHz, CDCl$_3$ containing two drops of $d_6$-DMSO): $\delta$ 7.42 (d, 1H, $J$= 8 Hz), 7.35 (d, 1H, $J$= 8 Hz), 7.27- 7.25 (m, 2H), 5.32 (d, 1H, $J$ = 8 Hz), 4.76 (t, 1H, $J$ = 8 Hz), 4.30 - 4.12 (m, 2H), 2.37 – 2.32 (m, 1H), 1.84 – 1.77 (m, 2H), 1.43 (s, 9H), 1.28 – 1.25 (m, 10H), 1.08 (d, 3H, $J$= 8 Hz), 0.90 (d, 3H, $J$= 8 Hz), 0.86 (t, 3H, $J$ = 4 Hz); FT-IR: $\nu$ cm$^{-1}$ (KBr): 3328, 3235, 3040, 2941, 2885, 1683, 1615, 1515.

$N^1,N^3$-bis([1H-benzo[d]imidazol-2-yl]-2-methylpropyl)-5-(octyloxy)isophthalamide (5B.1):

Compound 5B.7 (0.2 g, 0.691 mmol) was dissolved in 50% TFA in CH$_2$Cl$_2$ and the solution was stirred for 3 h. After that, the reaction was quenched by the addition saturated aq solution of NaHCO$_3$ (15 mL). The aqueous solution was next extracted with CHCl$_3$-MeOH mixture solvent (CHCl$_3$-MeOH = 3:1 v/v; 15 mL) and the organic layer washed with brine, dried over Na$_2$SO$_4$ to afford the desired amine (0.115 g, yield 88%) which was almost pure to use in the next step.

To a stirred solution 5-(octyloxy) isophthaloyl dichloride (0.2 g, 0.603 mmol) in dry CH$_2$Cl$_2$ (20 mL), amine (0.228 g, 1.21 mmol) dissolved in dry CH$_2$Cl$_2$ (10 mL) was added dropwise followed by the addition of Et$_3$N (0.603 mmol, 0.87mL). Stirring was continued
Chapter 5B

for 4 h. After completion of reaction, monitored by TLC, CH$_2$Cl$_2$ was evaporated and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Purification of the crude mass by silica gel column chromatography using 40% petroleum ether in ethyl acetate to afford the compound 5B.1 (0.24 g, 88%). Grey solid, Mp 263 °C, $[\alpha]_D^{25} = +16.3$ (c = 0.344 gm/100 mL, MeOH); $^1$H NMR (400 MHz, CDCl$_3$ containing three drops of d$_6$-DMSO): $\delta$ 12.20 (brs, 2H), 8.30 (d, 2H, $J = 8$Hz), 8.13 (s, 1H), 7.61 – 7.58 (brs, 6H), 7.04 – 6.91 (m, 4H), 5.34 (t, 2H, $J = 8$ Hz), 4.01 (t, 2H, 4 Hz), 3.53 – 3.50 (m, 2H), 2.41 (m, 2H), 1.86 – 1.60 (m, 2H), 1.40 – 1.30 (m, 8H), 1.07 (d, 6H, $J = 8$ Hz), 0.95 (d, 6H, $J = 8$ Hz), 0.88 (t, 3H, $J = 6.40$ Hz); $^{13}$C (100 MH, d$_6$-DMSO) $\delta$ 165.7, 158.3, 154.6, 142.9, 135.5, 133.7, 121.7, 121.0, 119.2, 118.4, 116.1, 111.2, 67.9, 54.2, 33.3, 31.5, 31.1, 28.7, 28.6, 25.5, 22.0, 19.5, 19.2, 13.9; FT-IR: $\nu$ cm$^{-1}$ (KBr): 3227, 2960, 2927, 1644, 1538; Mass (HRMS, TOF, MS ES$^+$): calcd, 636.3788 (M$^+$); found, 637.3953 (M + H)$^+$. 

N,N'-Bis-(1H-benzoimidazol-2-ylmethyl)-5-octyloxy-isophthalamide (5B.8): 

To a stirred solution 5-(octyloxy) isophthaloyl dichloride (0.259 g, 0.781 mmol) in dry CH$_2$Cl$_2$ (20 mL), amine 2B.11 (0.230 g, 1.56 mmol) dissolved in dry CH$_2$Cl$_2$ (10 mL) was added dropwise followed by the addition of Et$_3$N (1.56 mmol, 0.225 mL). Stirring was continued for 4 h. After completion of reaction, monitored by TLC, CH$_2$Cl$_2$ was evaporated and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Purification of the crude mass by silica gel column chromatography using 40% petroleum ether in ethyl acetate to afford the compound 5B.8 (0.38 g, 88%), mp 238°C; $^1$H NMR (400 MHz, CDCl$_3$ containing three drops of d$_6$-DMSO): $\delta$ 12.30 (br s, 2H), 8.64 (br m, 2H), 8.06 (s, 1H), 7.63 (s, 2H), 7.54 (m, 4H), 7.71–7.18 (m, 4H), 4.85 (br d, 4H, $J = 4$ Hz), 4.00 (t, 2H, $J = 4$ Hz), 1.99–1.97 (m, 2H), 1.44–1.42 (m, 2H), 1.31–1.21 (m, 8H), 0.87 (m, 3H); $^{13}$C (100 MH, d$_6$-DMSO): d 165.8, 158.0, 152.1, 142, 135.4, 128.0, 121.4 (two carbons unresolved), 119.0, 116.1, 114.0, 111.0, 67.9, 44.0, 37.8, 31.1, 28.6 (two carbons unresolved), 25.4, 22.0, 13.9; FT-IR: $\nu$ cm$^{-1}$ (KBr): 3230, 2924, 2854, 1644, 1590, 1553; Mass (HRMS, TOF, MS ES$^+$): calcd, 552.285 (M$^+$); found, 553.3084 (M + H)$^+$. 

350
Chapter 5B

1,1’-(1,3-phenylene)bis(3-((S)-2-methyl-1-(1-octyl-1H-benzo[d]imidazol-2-yl)propyl)urea) (5B.2):

Compound 5B.9 (0.15g, 0.42 mmol) was dissolved in 50% TFA in CH$_2$Cl$_2$ and the solution was stirred for 3 h. After completion of reaction, monitored by TLC, CH$_2$Cl$_2$ and excess TFA was completely evaporated of and residue was dried in vacuuo to afford the amine 5B.10 which remained in protonated form. This amine was used directly in the next step without characterization. To a stirred solution of 1, 3-diisocyanatobenzene (0.25 g, 0.16 mmol) in CH$_2$Cl$_2$ (10 mL), amine 5B.10 (0.085 g, 0.327 mmol) dissolved in CH$_2$Cl$_2$ (10 mL) was added dropwise followed by addition of N, N-diisopropyl ethylamine (0.125 mL, 0.735 mmol). Stirring was continued for 9 h. After completion of reaction, monitored by TLC, CH$_2$Cl$_2$ was evaporated and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Purification of the crude mass by silica gel column chromatography using 3% CH$_3$OH in CHCl$_3$ as eluent yielded the product 5B.2 (0.08 g, yield: 67%), mp 194°C, $[\alpha]_{D}^{25} = +12.58 ([C] = 0.254$ gm/100 mL in DMSO).

$^1$H NMR (400 MHz, $d_6$-DMSO): δ 8.48 (s, 2H), 7.59 (d, 2H, $J= 8$ Hz), 7.53 (d, 2H, $J = 8$ Hz), 7.42 (s, 1H), 7.21 - 7.17 (m, 4H), 7.08 (br t, 1H), 6.99 (d, 1H, $J = 8$ Hz), 6.93 (d, 1H, $J = 8$ Hz), 6.63 (d, 2H, $J = 8$ Hz), 6.35 (t, 2H, $J = 8$ Hz), 4.90 (t, 2H, $J = 8$ Hz), 4.35 - 4.21 (m, 4H), 2.28 - 2.27 (m, 2H), 1.71 (m, 4H), 3.23 - 1.12 (m, 20H), 1.02 (d, 6H, $J = 8$ Hz), 0.87 (d, 6H, $J = 8$ Hz), 0.74 (br t, 6H); $^{13}$C NMR (100 MHz, $d_6$-DMSO): δ 155.4, 155.0, 142.5, 140.9, 135.0, 122.4, 121.9, 119.1, 110.9, 50.8, 43.4, 33.0, 31.5, 30.1, 29.1, 28.9, 26.6, 22.4, 20.1, 18.8, 14.3; FT-IR: ν cm$^{-1}$ (KBr): 3308, 2926, 2853, 1636, 1555; Mass (ESI): 764.2 (M + 1)$^+$; Anal.Calcd for C$_{46}$H$_{66}$N$_8$O$_2$: C, 72.40; H, 8.72; N, 14.68. Found: C, 72.42; H, 8.71; N, 14.67.

1,1’-(1,3-phenylene)bis(3-((S)-1H-benzo[d]imidazol-2-yl)-2-methylpropyl)urea) (5B.3):

Compound 5B.7 (0.15g, 0.42 mmol) was dissolved in 50% TFA in CH$_2$Cl$_2$ and the solution was stirred for 3 h. After completion of reaction, CH$_2$Cl$_2$ and excess TFA was completely evaporated of and residue was dried in vacuuo to afford the protonated amine 5B.11. This amine was used directly in the next step without characterization. To a stirred solution of 1,3-diisocyanatobenzene (0.042 g, 0.264 mmol) in CH$_2$Cl$_2$ (10 mL), amine 5B.11 (0.1 g, 0.528 mmol) dissolved in CH$_2$Cl$_2$ (10 mL) was added dropwise
followed by addition of $N, N$-diisopropyl ethylamine (0.204 mL, 1.18 mmol). Stirring was continued for 9 h. After completion of reaction, CH$_2$Cl$_2$ was evaporated of and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Purification of the crude mass by silica gel column chromatography using 4% CH$_3$OH in CHCl$_3$ as eluent gave the product **5B.3** (0.094 g, yield: 66%), mp (decompose) 270 °C. $[^{[a]}]_D^{25}$ = -38.12 ([C] = 0.144 gm/ 100 mL in DMSO). $^1$H NMR (400 MHz, d$_6$-DMSO): $\delta$ 12.37 (s, 2H), 8.70 (s, 2H), 7.57 (d, 2H, $J$ = 8 Hz), 7.46 (d, 2H, $J$ = 8 Hz), 7.14 (br m, 4H), 7.04 (br m, 2H), 6.96 (d, 2H, $J$ = 8 Hz), 6.69 (d, 2H, $J$ = 8 Hz), 4.87 (br t, 2H), 2.24 (m, 2H), 0.88 (br s, 12H); $^{13}$C NMR (100 MHz, d$_6$-DMSO): $\delta$ 155.8, 155.3, 143.7, 141.1, 134.3, 129.5, 122.2, 121.5, 120.4, 118.8, 111.6, 107.0, 53.6, 33.0, 19.6, 18.3; FT-IR: v cm$^{-1}$ (KBr): 3310, 2958, 2925, 1644, 1560; Mass (ESI): 537.6 (M + 1)+; Anal.Calcd for C$_{30}$H$_{34}$N$_8$O$_2$: C, 66.89; H, 6.36; N, 20.80. Found: C, 66.92; H, 6.34; N, 20.83.

tert-butyll,1',l,l'-((1,3-phenylenebis(methylene))bis(1H-benzo[d]imidazole-2,1-diyl))bis(2-methylpropane-1,1-diyl)dicarbamate (5B.12):

To a stirred solution of **5B.7** (0.3 g, 1.04 mmol), in dry THF (20 mL), sodium hydride (0.036 g, 1.52 mmol) was added. The solution was refluxed for 30 min. and a solution of 1,3- bis(bromomethyl)benzene (0.2 g, 0.757 mmol) in dry THF was added to it under nitrogen atmosphere. Reaction mixture was refluxed for further 4 h. After completion of reaction, monitored by TLC, THF was evaporated and water was added to the residue. The aqueous layer was extracted with CHCl$_3$ (25 mL x 3) and dried over anhydrous Na$_2$SO$_4$. Evaporation of solvent gave crude mass which was purified by column chromatography to afford pure compound **5B.12** (0.41 g, yield: 80%) as light yellow solid, mp 148 °C. $^1$H NMR (400 MHz, CDCl$_3$ containing one drops of d$_6$-DMSO): $\delta$ 7.73 (d, 2H, $J$ = 8 Hz), 7.25- 7.23 (m, 4H), 7.20- 7.11 (m, 4H), 7.04 (d, 2H, $J$ = 8 Hz), 5.40 (dd, 4H, $J$ = 16 Hz), 5.31 (d, 2H, $J$ = 8 Hz), 4.70 (t, 2H, $J$ = 8 Hz), 2.24- 2.23 (m, 2H), 1.37 (s, 18H), 0.96 (d, 6H, $J$ = 4 Hz), 0.68 (d, 6H, $J$ = 4 Hz); FT-IR: v cm$^{-1}$ (KBr): 3328, 3235, 3040, 2941, 2885, 1683, 1615, 1515; Mass (ESI): 681.4 (M + 1)+.
Chapter 5B

1,1’-(1S,1’R)-1,1’-(1,3-phenylenebis(methylene))bis(1H-benzo[d]imidazole-2,1-diyl))bis(2-methylpropane-1,1-diyl)bis(3-(pyren-4-yl)urea) (5B.4):

Compound 5B.12 (0.12 g, 0.123 mmol) was dissolved in 50% TFA in CH2Cl2 and the solution was stirred for 5 h. After that, the reaction was quenched by the addition saturated aq solution of NaHCO3 (15 mL). The aqueous solution was next extracted with CHCl3-MeOH mixture solvent (CHCl3-MeOH = 3:1 v/v; 15 mL) and the organic layer washed with brine, dried over Na2SO4 to afford the desired amine (0.076 g, yield 88%) which was almost pure to use in the next step. To a stirred solution of 1, 3-diisocyanatobenzene (0.025 g, 0.158 mmol) in CH2Cl2 (10 mL), amine 5B.13 (0.076 g, 0.158 mmol) dissolved in CH2Cl2 (10 mL) was added dropwise followed by addition of N,N-diisopropyl ethylamine (0.06 mL, 0.347 mmol). Stirring was continued for 12 h. After completion of reaction, CH2Cl2 was evaporated of and water was added to the residue. The aqueous layer was extracted with CHCl3 (25 mL x 3) and dried over anhydrous Na2SO4. Purification of the crude mass by silica gel column chromatography using 4% CH3OH in CHCl3 as eluent gave the product 5B.4 (0.1 g, yield: 66%), mp(decompose) 270 °C. [α]D25 = +12.58, DMSO, [C] = 0.254 gm/ 100 mL.

1H NMR (400 MHz, CDCl3 containing two drops of d6-DMSO): δ 8.60 (s, 2H), 8.08 (d, 2H, J = 8Hz), 7.98 (d, 4H, J = 8Hz), 7.95-7.76 (m, 6H), 7.65 (d, 2H, J = 8Hz), 7.37 (d, 2H, J = 8Hz), 7.27-6.98 (m, 14H), 5.40(q, 4H, J = 16 Hz), 5.07 (t, 2H, J = 8Hz), 2.53-2.48 (m, 2H), 1.07 (d, 6H, J = 6Hz), 0.66 (d, 6H, J = 4Hz); 13C NMR (100 MHz, d6-DMSO): 160.1, 159.4, 151.3, 148.8, 142.7, 142.0, 137.0, 136.8, 134.2, 132.0, 131.3, 129.8, 129.4, 129.2, 128.7, 128.2, 128.0, 127.8, 127.4, 127.0, 126.6, 126.3, 126.0, 125.2, 124.9, 116.0, 114.1, 113.5, 56.26, 38.2, 37.8, 24.6, 23.3; m/z (ES+): 967.6 (M+H)+; Anal.Calcd for C64H54N8O2: C, 79.48; H, 5.63; N, 11.69. Found: C, 79.52; H, 5.68; N, 11.73

tert-butyl 3-methyl-1-oxo-1-(pyridin-3-ylamino)butan-2-ylcarbamate (5B.15):

To a stirred solution of N-Boc-protected L-valine acid (0.5 g, 2.3 mmol), in dry CH2Cl2 (30 mL), DCC (0.475 g, 2.3 mmol) and a catalytic amount of DMAP were added at 0 °C. The solution was stirred for 30 min. and a solution of pyridin-3-amine (0.216 g, 2.3 mmol) in dry CH2Cl2 was added to it under nitrogen atmosphere. After stirring for 19 h, the reaction mixture was filtered off to remove insoluble DCU. The filtrate was removed in vacuo and the crude product was purified by silica gel column chromatography using 20% petroleum ether in ethyl acetate to afford the compound 5B.15 (0.48 g, yield: 353
Chapter 5B

70%), $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.58 (d, 2H, $J = 4$ Hz), 8.32 (d, 1H, $J = 4$ Hz), 8.08 (d, 1H, $J = 4$ Hz), 7.23- 7.20 (m, 1H), 5.19 (d, 1H, $J = 4$ Hz), 4.06 (t, 1H, $J = 8$ Hz), 2.25- 2.23 (m, 1H), 1.46 (s, 9H), 1.04- 1.0 (m, 6H) FT-IR $\nu$ cm$^{-1}$ (KBr): 3420, 3339, 3257, 3044, 2980, 1703, 1678, 1538; Mass (ESI): 293.2 (M + 1)$^+$.  

$1,1'$-(anthracene-9,10-diylbis(methylene))bis(3-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)pyridinium) hexafluorophosphate(V) 5B.5:

Compound 4C.15 (0.14g, 0.473 mmol) was dissolved in dry CH$_3$CN (15 mL) with warming. 9, 10-bis(chloromethyl)anthracene (0.065 g, 0.238 mmol.) was added and the reaction mixture heated under reflux for 3 days under a nitrogen atmosphere. During the reaction, chloride salt was precipitated. The salt was filtered and washed several times with CH$_3$CN. The chloride salt was dissolved in aq. MeOH with warming and chloride ion was exchanged by the addition of NH$_4$PF$_6$. The yellow precipitate of 5B.5 was collected by filtration. Repeated recrystallisation of the salt 5B.5 from a CHCl$_3$/CH$_3$OH mixture solvent gave the pure product (0.15 g, yield 77%); $\alpha$ 182 °C; $[\alpha]_D^{25} = + 150$ (c = 0.344 gm/100 mL, MeOH). $^1$H NMR (400 MHz, CDCl$_3$ containing one drop of $d_6$-DMSO): $\delta$ 10.79 (s, 2H), 9.21 (s, 2H), 8.79 (d, 2H, $J = 8$ Hz), 8.38- 8.28 (m, 6H), 7.88 (t, 2H, $J = 8$ Hz), 7.74 (d, 4H, $J = 8$ Hz), 6.89 (s, 4H), 5.52 (d, 2H, $J = 8$ Hz), 4.07 (br t, 2H), 2.08- 2.01 (m, 2H), 1.41 (s, 18H), 0.96- 0.90 (m, 12H); $^{13}$C NMR (100 MHz, CDCl$_3$ containing one drop of $d_6$-DMSO): $\delta$ 172.6, 155.8, 140.1, 138.0, 134.8, 133.9, 131.3, 129.0, 128.7, 124.5, 123.6, 80.0, 60.5, 56.8, 31.0, 28.2, 19.3, 17.6; FTIR (u in cm$^{-1}$, KBr): 3379, 3108, 2974, 1706, 1633, 1595, 1506, 1441.9; Mass (HRMS, TOF, MS ES$^+$): calcd, 935.4054 [(M-PF$_6$)$^+\text{;}$; 790.4407 [(M-2PF$_6$)]$^+\text{;}$; found, 935.4111 [(M-PF$_6$)$^+\text{;}$; 790.4411 [(M-2PF$_6$)]$^+$.  

General procedure of fluorescence titration

Stock solutions of the hosts and guests were prepared in DMSO and DMSO- DME and 2 mL of the individual host solution was taken in the cuvette. The solution was irradiated at the excitation wavelength maintaining the excitation and emission slits. Upon addition of guest, the change in fluorescence emission of the host was noticed. The corresponding emission values during titration were noted and used for the determination of binding constant values.
Chapter 5B

General procedure of UV-vis titration

The receptors were dissolved in dry UV grade DMSO and DMSO-DME and 2 ml of the individual host solution was taken in the cuvette. Then, guests, dissolved in similar solvent combination were individually added in different amounts to the receptor solution. The corresponding absorbance values during titration were noted and used for the determination of binding constant values.

Binding constant determination

Binding constant values for receptors with the anionic guests were determined by nonlinear curve fitting procedure. The non linear fit was done using the equation 1.

\[ I = I_0 + \frac{(I_{\text{lim}} - I_0)/2C_H}{C_H + C_G + 1/K_a - \left[(C_H + C_G + 1/K_a)^2 - 4C_HC_G\right]^{1/2}} \]

Where \( I \) represents the intensity; \( I_0 \) represents the intensity of pure host; \( C_H \) and \( C_G \) are corresponding concentrations of host and anionic guest; \( K_a \) is the binding constant. The binding constant \( (K_a) \) and correlation coefficients \( (R) \) were obtained from a non-linear least-square analysis of \( I \) versus \( C_H \) and \( C_G \).

Method for Job plot

The stoichiometry was determined by the continuous variation method (Job Plot). In this method, solutions of host and guests of equal concentrations were prepared in dry DMSO and DMSO-DME. Then host and guest solutions were mixed in different proportions maintaining a total volume of 3 mL of the mixture. The related compositions for host:guest (v/v) were 3:0, 2.8:0.2, 2.5:0.5, 2:1, 1.8:1.2, 1.5:1.5, 1:2, 0.8:2.2, 0.5:2.5, 0.2:2.8. All the prepared solutions were kept for 1 h with occasional shaking at room temperature. Then emission and absorbance of the solutions of different compositions were recorded. The concentration of the complex i.e., \([HG]\) was calculated using the equation \([HG] = \Delta I/I_0 \times [H] \) or \([HG] = \Delta A/A_0 \times [H] \) where \( \Delta I/I_0 \) and \( \Delta A/A_0 \) indicate the relative emission and absorbance intensities, respectively. \([H]\) corresponds the concentration of pure host. Mole fraction of the host \( (X_H) \) was plotted against concentration of the complex \([HG] \). In the plot, the mole fraction of the host at which the concentration of the host-guest complex concentration \([HG]\) is maximum, gives the stoichiometry of the complex.
Chapter 5B

5B.5. References


Chapter 5B


12. MMX calculation has been performed using PC model v 9.0, Serena Software.

General Remark on the Thesis

The development of convenient, directly responsive and cost effective techniques for the detections of cationic and anionic substrates of environmental and biological significances is extremely important in the area of supramolecular chemistry. During the research, a number of structural features for the synthesis of target orientated receptor modules have been rationalized and in the present state of art, it has been possible to rationally design hydrogen bonding receptors for selective binding of target cationic and anionic substrates. In order to recognize the biologically relevant species, a series of fluorophore labeled receptors have been designed and synthesized. Fluorescence – based chemosensors are of significant attention both in terms of their cost effectiveness and high sensitivity and availability of wide number of opportunities for modulation of physical properties of a fluorophore. The host-guest interaction of the newly designed receptors of this thesis has been studied by exploiting fluorescence, UV-vis and NMR techniques. In few cases, single crystal X-ray structures have been analyzed to support the experimental findings. Also theoretical studies wherever necessary have been performed to correlate the experimental observations. In major cases, the binding interactions of the receptors have been performed in organic solvent. The similar study in water was impossible in the present case due to limited solubility of the designed receptors in water. In some cases, as a reasonable compromise, a 4:1 aqueous organic solution was used in the subsequent study. To study the host-guest interaction in pure water modulation of receptor structures is necessary. This is in progress in the laboratory. Throughout the thesis, binding constants related to the stability of the receptor-substrate complexes were determined by using the fluorescence, absorbance data.