CHAPTER 2

Quinoline-based molecular receptors
for carboxylic acids
Chapter 2

Outline

This chapter describes the present work, related to the design and synthesis of quinoline and naphthalene-based receptors of various topologies for sensing of hydroxy and non-hydroxy carboxylic acids. The quinoline-based receptor shows monomer emission quenching followed by intramolecular excimer emission upon hydrogen bond mediated complexation with hydroxy carboxylic acids guests. The excimer emission has been used to confirm the selective recognition of various hydroxy carboxylic acids as guest molecules. In contrast to quinoline-based receptor, naphthalene-based receptor did not show any excimer emission upon complexation of hydroxy acids. This establishes the fact that quinoline ring nitrogen plays an important role in the formation of excimer. The related review on the recognition of hydroxy di- and tricarboxylic acids are also cited.
Present work

This Chapter mainly highlights the following important items in detail.

(a) Design and synthesis of quinoline-based receptor for fluorometric discrimination of tartaric acids from its non-hydroxy analogue.

(b) Design and synthesis of poly ether linked quinoline-based receptor for fluorometric discrimination of carboxylic acids.

(c) Design and synthesis of quinoline-based tripod-shaped receptor for citric acid.

2.1. Importance of carboxylic acids and carboxylates as guest species

Carboxylic acids and carboxylates are important as guest species in host-guest chemistry because of their biological relevance. A large number of drugs like antibiotics, analgesics, anti-inflammatory agents and other biologically active molecules e.g., folic acid, bile acid, prostaglandin, bilirubin etc., contain carboxylic acid functionality\(^1\). Enzymes, antibodies, amino acids and metabolic intermediates as well as other natural products contain a range of carboxylate functionalities that account for the characteristic biochemical behavior. Di- and tri-carboxylates are essential components of numerous metabolic processes, including for instance, the citric acid glyoxalate cycle\(^2\). They are also involved in the formation of high energy phosphate bonds in our body. The binding of carboxyl and carboxylate groups is involved in many biological recognition processes such as peptide recognition by vancomycin in which amide-carboxylate binding is crucial\(^3\) and biotin dependent carboxylate catalyzed reactions\(^4\) (which proceed through a carboxylated enzyme complex intermediate in which the covalently bound biotinyl prosthetic group acts as a mobile carbonyl carrier between the remote catalytic sites). The carboxylic acid in carboxypeptidase-A enzyme is involved in complexation as carboxylate.\(^5\) Enantioselective recognition of carboxylates plays an important role in asymmetric synthesis and drug discovery.\(^6\) Zalups et al. reported that small dicarboxylic acids such as succinic, glutaric and adipic acids inhibit renal uptake of administered mercury in rats.\(^7\) Similarly, \(\alpha\)-hydroxy acids, or alpha hydroxy acids (AHAs), are a class of chemical compounds that consist of a carboxylic acid substituted with a hydroxy group on the adjacent carbon. They may be either naturally occurring or synthetic. AHAs are
well-known for their use in the cosmetics industry. They are often found in products claiming to reduce wrinkles or the signs of aging, and improve the overall look and feel of the skin. They are also used as chemical peels available in a dermatologist's office, beauty and health spas and home kits, which usually contain a lower concentration. Although their effectiveness is documented numerous cosmetic products have appeared on the market with unfounded claims of performance. Many well-known \( \alpha \)-hydroxy acids are useful building blocks in organic synthesis: the most common and simple are glycolic acid, lactic acid, citric acid, mandelic acid.

![Krebs Cycle](image)

**Figure 2.1.** Different components of Krebs cycle.

Citric acid is an example of hydroxy tricarboxylic acid that plays an important role in Krebs cycle (Figure 2.1) to provide the greater part of energy used by aerobic cells in human beings. Maleic acid is a well-known inhibitor of this cycle and its implication in different kidney diseases has been widely described.

### 2.2. Brief review on the different approaches to the recognition of hydroxy di- and tricarboxylic acids and their salts

Selective recognition of hydroxy di- and tricarboxylic acids by synthetic receptors is an interesting area in molecular recognition research. In relation to this, considerable efforts
have been directed from various laboratories to the synthesis of molecular receptors of various architectures for the recognition of these substrates. The different approaches are highlighted below.

Moran et al. introduced a chromoneamino-benzoxazole binding site-based molecular receptor 7, which was resolved in the presence of optically pure dibenzoyl tartaric acid through chiral recognition process.\(^{12}\)

Recognition of D-tartaric acid by boronic acid receptor is very interesting. Zhao et al. reported the selective binding of D- and L-tartaric acids with chiral fluorescent sensors \(R,R-8\) and \(S,S-8\). The addition of D-tartaric acid to \(R,R-8\) caused a larger increase in fluorescence. While addition of L-tartaric acid to \(R,R-8\) only produced small changes in fluorescence.\(^{13}\)

Frontera et al. reported the receptor 9, which bound methylmethanetriacid (MMTA) through the \textit{endo}-binding mode due to favorable C-H/\pi interaction.\(^{14}\) Recent years have witnessed a rapid growth of interest in multi-component crystals (cocrystals)\(^{15}\) as functional solid-state materials. Frscic \textit{et al.} studied the cocrystallization of caffeine, theophylline with tartaric (D- and DL-tartaric acid) and malic (D- and DL-malic acid) acids. In this regard, Figure 2.2 displays the crystal structure of the cocrystal of theophylline with \textit{rac}-malic acid. In the study they followed a trend reminiscent of Wallach's rule, the racemic cocrystal appears more stable than its chiral counterpart.\(^{17}\)
Kim et al. reported the non covalent synthesis of self-assembled helical capsule-like structure (via cocrystallization technique), composed of two tris(imidazoline) bases 10 and three tartaric acids through charged hydrogen bonds in aqueous solution (Figure 2.3).\textsuperscript{18}

Based on the idea of Hamilton, Goswami et al. synthesized the symmetrical and unsymmetrical bisamide receptors, which bound insoluble tartaric acid in CHCl\textsubscript{3}.\textsuperscript{19a} Calixarene-based receptors 11 and 12 exhibited the recognition properties of hydroxy acids.\textsuperscript{19b}
Calixarenes 13a and 13b bearing optically pure α, β- amino alcohol groups at their lower rim was found to exhibit exceptional and efficient chiral recognition abilities in the discrimination of rac-mandelic acid, 2,3-dibenzoyltartaric acid and 2-hydroxy-3-methylbutyric acid.\textsuperscript{20}

Recently, boronic acid-based C\textsubscript{3} symmetric fluorescent chemosensors 14 have been prepared by Zheng et al.\textsuperscript{21} These compounds showed remarkable ability to recognize α-hydroxy carboxylic acids (such as tartaric acid) and sugar acids over most saccharides. The fluorescence intensity of the receptors decreased upon adding the α-hydroxy acids in a pH 8.71 buffer of methanol–water, which can be explained with the internal charge transfer (ICT) mechanism.

Kuroda et al. reported a unique trench type-binding site on a porphyrin 15a-15f, which specifically recognized tartaric acid derivatives with four-point hydrogen bonding.\textsuperscript{22}
From our laboratory naphthyridine-based receptor 16 has been designed and synthesized for fluorometric recognition of tartaric, citric, succinic and malic acids, etc.

Recently, the chirality and the geometry of Troger’s base skeleton have been exploited to discriminate the enantiomers of different carboxylic acids. Tartaric acid is one of them. Periasamy et al. reported this by using the Troger’s base derivatives 17, 18 and 19.
Efforts have also been given to the recognition of salts of hydroxy di- and tricarboxylic acids (e.g., tartarate, malate, citrate etc.) by various synthetic receptors. In this connection, the different approaches are addressed below.

Many of the receptors that bind citrate instead of citric acid, are based on positively charged, hydrogen bond groups or unsaturated metal centers coordinated to 1,3,5-trialkylbenzene scaffolds, which adopt a “fly-trap” conformation. Schmuck and co-workers synthesized guanidino carbonyl pyrrole-based receptor 20 that also bound citrate in aqueous buffer in the presence of a 1000-fold excess of chloride anions with an association constant of $K \approx 10^5$ M$^{-1}$. Even the receptor 20 distinguished citrate by naked eye from other substrates such as tartarate, malate etc., in aqueous solutions using an indicator displacement assay with carboxyfluorescein. Another approach that employs the indicator displacement method was elegantly demonstrated by Anslyn et al. In their
approach the ‘sensing ensemble’ for the detection of citrate included the receptor 21 and the indicator 5-carboxyfluorescein 22. In methanol–water (3:1 v/v), the binding constant between 21 and citrate was $2.9 \times 10^5$ M$^{-1}$ (determined by UV-vis method).

An indicator displacement sensing ensemble 23a-23c, composed of bis-Cinchona alkaloid diimide triad (A) and bromophenol blue (I), was developed by Kacprzak et al. for easy and efficient assay of \( \alpha \)-hydroxycarboxylic acids with a high affinity for tartarates, for which the detection limit of 0.015 mg/ml was achieved.\(^{28}\)

This system also discriminated enantiomeric \( \alpha \)-hydroxycarboxylic acids and could be used for quick quantitative determination of natural tartaric acid in wine.

A bibrachial lariat aza-crown 24 containing appended naphthalene fluorophore was designed and synthesized by Clares et al. The receptor showed specific interaction of citrate by exhibiting an increase in emission. The smaller Krebs cycle components like...
Chapter 2

In fact, whereas fumarate is generated in Krebs cycle, maleate is a well-known inhibitor of this cycle and its implication in different kidney diseases is known. The glucose receptor developed by Shinkai was synthesized by known methods and with modifications involving the final synthetic step, installation of the phenylboronic acid moieties.

binding of the bis(R-hydroxycarboxylate) and tartarate, was assessed and compared to the corresponding bis(diol), erythritol, as well as the corresponding mono(R-hydroxycarboxylate), malate. These results suggested that bisboronate/bis(R-hydroxycarboxylate) interactions were stronger than the corresponding
bisboronate/bis(diol) interactions. Furthermore, the receptor was an order of magnitude more selective for tartarate than malate.\textsuperscript{31} Xu \textit{et al.} developed fluorescent receptors 26a and 26b, which exhibited different chiral recognition abilities toward the enantiomers of D- and L-bis(tetrabutylammonium)dibenzoyl tartarate.\textsuperscript{32}

![Diagram of 26a, n=2 and 26b, n=3]

Based on the cooperative action of boronic acid and guanidinium groups, a first fluorescent sensor 27 for D-glucarate was established by Yang \textit{et al.}\textsuperscript{33}

![Diagram of Sensor 27]

Similarly, a chiral macrocyclic host, devised by Schmidtchen \textit{et al.}, was found to exhibit good enantio differentiation of various closely related hydroxyl and amino dicarboxylic acids. In the macrocycle, they used guanidinium ion as well as urea as the hydrogen bonding sites for complexation of dicarboxylates (Figure 2.4)\textsuperscript{34}
The survey of various reports and reviews on the recognition of hydroxy di- and tricarboxylic acids and their salts reveals that although significant progress has been made in designing new receptors, fluorescent receptors for sensing them are less explored. The use of pyridine amide especially, which is a well known motif for carboxylic acid binding, has not been used in designing fluorescent receptor. At the same time, receptors that are capable of showing fluorometric discrimination among hydroxy di- and tricarboxylic acids of different kinds are less in number. In recent days, fluorescence detection has been widely used as a versatile tool in analytical chemistry, biochemistry, cell biology, etc. From consideration of all the above points, we decided to use pyridine amide and quinoline moieties to synthesize new fluorescent receptors of different architectures for the recognition of tartaric, citric and malic acids and also non hydroxy analogue succinic acid.
Chapter 2

Present work

2.3. Design and synthesis of Quinoline-based receptors for fluorometric discrimination of tartaric acid from its non hydroxy analogue

Due to many applications in analytical chemistry and biomedical research, the development of receptors which have the ability to selectively bind and sense neutral molecules, anions and cations through an optical response has attracted much attention in recent years. In this context, one of the recent approaches to the design of fluorescent signaling systems relies on guest-induced folding of flexible receptors, which brings the fluorophores close enough as to function as excimer. This excimer emission formation is sometimes used to 'read out' the molecular recognition process more conveniently. Given the importance of dicarboxylic acids due to their biological relevance, the need for fluorescent receptors as sensors for carboxylic acids in different contexts of molecular recognition research has recently been of paramount interest. In this respect, tartaric acid, a common natural product in wines and other grape derived beverages, has received attention due to its structural features possessing several hydrogen bond donors and acceptors. Many hydrogen bonding receptors for the binding of tartaric acid and its derivatives have been reported. This has been discussed in details in Section 2.2.

In this chapter, we report the design, synthesis and photophysical behavior of quinoline-based receptor 28 for the selective recognition of hydroxy carboxylic acids. The quinoline motif was part of our design strategy both as hydrogen bond acceptor as well as fluorophore to communicate the recognition process. In order to establish the role of quinoline ring nitrogen in the recognition event, we synthesized the naphthalene-based receptor 29. In this context, the hetero bis amide receptor 30, where both naphthalene and quinoline motifs are present, was also synthesized. All the three receptors were explored in molecular recognition studies on hydroxy dicarboxylic acids and their non hydroxy analogues.
2.3.1. Synthesis of the receptors 28, 29 and 30

The receptor 28 was synthesized according to Scheme 2.1 and was isolated in 55% yield. The lumophore, 8-hydroxyquinoline, was first coupled with 2-N-pivaloylamino-6-bromomethylpyridine (obtained from 2-N-pivaloylamino-6-methylpyridine by reaction with NBS in dry CC\textsubscript{4}) to give compound 31.

Amide hydrolysis of 31 then afforded compound 32 in 80% yield. On coupling of 32 with 5-octyloxy-1,3-benzenedicarbonyl chloride (prepared by ethenification of diethyl 5-hydroxyisophthalate with octyl bromide in dry acetone using K\textsubscript{2}CO\textsubscript{3} and hydrolysis of the esters followed by reaction with oxaloyl chloride) yielded the desired receptor 28.

Similarly, the receptor 29 was synthesized according to Scheme 2.2. Initially, 1-naphthol was reacted with 2-N-pivaloylamino-6-bromomethylpyridine to give compound 33.
Chapter 2

Amide hydrolysis of 33 furnished compound 34 in 82% yield. On coupling of compound 34 with isophthaloyl dichloride yielded the desired receptor 29 in 62% yield.

![Synthesis of receptor 29](image)

Scheme 2.2. Synthesis of receptor 29: Reagents and conditions. (i) 2-N-pivaloylamino-6-bromomethylpyridine, K$_2$CO$_3$ in dry CH$_3$CN (n) 4N KOH in aqueous-ethanol, reflux, 12 h, (iii) Isophthaloyl dichloride, dry CH$_2$Cl$_2$, Et$_3$N.

On the other hand, reaction of the two amines 32 and 34 with isophthaloyl dichloride in dry THF under high dilution condition gave the desired hetero bis amide receptor 30 in 32% yield (Scheme 2.3) All the compounds were characterized by $^1$H NMR, $^{13}$C, FT-IR, mass and elemental analyses.

![Synthesis of receptor 30](image)

Scheme 2.3. Synthesis of receptor 30: Reagents and conditions. (i) High dilution condition, dry THF, Et$_3$N.

2.3.2. Molecular modeling

Isophthaloyl diamide can assume both syn and anti conformations (Figure 2.5), which may remain in equilibrium in solution. Usually in the syn form A, both amide hydrogens are involved in repulsive interactions with aromatic ortho proton. Prior to study the interaction of the receptors in solution we initially optimized the structures in gas phase.
Chapter 2

**Figure 2.5.** Different conformations of isophthaloyl diamide.

Energy minimization<sup>40</sup> of 28 in the *syn* conformation shows the nearly parallel arrangement of the pendant quinolines with a separation of distance of 4.01 Å and the open binding cleft assumes a non-planar shape (Figure 2.6). The hydrogen bonding groups in the cavity are well arranged for complexation of hydroxy dicarboxylic acids. Similarly the receptors 29 and 30 were energy optimized and Figure 2.6 shows the optimized geometries. In receptor 29 the pendant naphthalenes are 3.50 Å apart. Similarly, in 30 quinoline and naphthalene rings are separated by 3.46 Å.

**Figure 2.6.** Energy minimized structures of (a) 28 [E = 19.92 kcal/mol], (b) 29 [E = -11.62 kcal/mol] and (c) 30 [E = -5.19 kcal/mol].
2.3.3. Complexation studies on receptors 28, 29 and 30

\[^1\text{H NMR study}\]

The hydrogen bonding complexation of the receptors were mutually established by observing the change in \[^1\text{H NMR}\] of the receptors in the presence of equivalent amount of the diacids in CDCl\(_3\). In all the cases the amide protons of the receptors underwent downfield chemical shift upon complexation of the diacids. For example, \[^1\text{H NMR}\] of the receptor 28 in CDCl\(_3\) (5.83 \times 10^{-3} \text{ M}) revealed the position of the amide protons at 8.96 ppm. The addition of powdered D-(-)-tartaric acid to this solution showed clear dissolution after sonication. This was evident from the downfield shift of the amide protons (0.94 ppm; 8.96 ppm to 9.90 ppm) of 28 as well as from the appearance of a new peak at 4.97 ppm due to methine protons in the 1:1 complex (Figure 2.7). The integration ratio of tartaric acid methine protons to the receptor amide protons in the NMR spectrum of the complex (Figure 2.7; top) clearly revealed the formation of a 1:1 complex. On
dilution of the 1:1 complex, there was practically no shift of the receptor amide protons.
This suggested strong complexation of tartaric acid into the open cleft of receptor 28 in
the mode shown in complex A (Figure 2.7).
Similarly, 'H NMR of the receptor 29 (c = 2.72 x 10^-3 M) with pendant naphthalenes was
recorded in the presence and absence of D-(-)-tartaric acid following the same
experimental technique as followed in receptor 28. The amide protons of 29, appeared at
8.74 ppm, moved downfield (8.74 ppm to 9.62 ppm; \( \Delta \delta = 0.88 \)) upon complexation with
D-(-)-tartaric acid although less in magnitude compared to the case of 28 with D-(-)-
tartaric acid. The methine protons of tartaric acid appeared at 4.75 ppm. The integration
ratio of tartaric acid methine protons to the receptor amide protons in NMR spectrum of
the complex (Figure 2.8; top) clearly indicated the formation of a 1:1 complex. Receptor
30 also bound D-(-)-tartaric acid in CDCl₃ and formed 1:1 complex as indicated in Figure
2.9 Determination of the association constants by NMR method was, however, 
impossible because of the negligible change in position of the amide protons of the
receptors after forming 1:1 complex.

![Figure 2.8. Partial 'H NMR (400 MHz, CDCl₃) spectra of receptor 29 (bottom) and
the 1:1 complex with tartaric acid (top)](image-url)
Figure 2.9. Partial $^1$H NMR (400 MHz, CDCl<sub>3</sub>) spectra of receptor 30 (bottom) and the 1:1 complex with tartaric acid (top).

Thus the change in $^1$H NMR of the receptors in the presence of diacids demonstrate that the pyridine amides serve as binding site under the mastery of isophthaloyl spacer.

**UV-vis and fluorescence studies**

Prior to the interaction studies on receptors 28, 29 and 30 with the hydroxy dicarboxylic acids and their non hydroxy analogues, the absorbance and fluorescence behaviors of the receptors were studied in solvents of different polarities. Figures 2.10 and 2.11 show the changes in absorbance and fluorescence of 28, respectively, in different solvents.

**Figure 2.10.** UV-vis spectra of 28 ($c = 2.63 \times 10^{-5} \text{ M}$) in different solvents.

**Figure 2.11.** Fluorescence spectra of 28 ($c = 2.63 \times 10^{-5} \text{ M}$) in different solvents.
Chapter 2

It is evident from Figure 2.10 that the receptor 28 exhibits solvatochromic effect. In the excited state, the intensity of the emission peak for quinoline in 28 is also changed with polarity of the solvent (Figure 2.11). Similarly, the solvent effect on receptors 29 and 30 was investigated. Figures 2.12 and 2.13 represent the changes in absorbance and fluorescence of 29, respectively in different solvents as were used for 28. In a similar way, Figures 2.14 and 2.15 highlight the change in absorbance and emission of 30, respectively.
In case of 29, the absorption peak at 290 nm did not exhibit any characteristic positional movement. On the contrary, the emission peak for naphthalene in 29 moved in either direction with change in intensity as the polarity of solvent was changed. In less polar solvent CHCl₃, an emission peak for 29 at longer wavelength (492 nm) was observed. This was presumably attributed to the formation of an excimer between two closely spaced naphthalene moieties in 29. This is also evident from molecular modeling (Figure 2.6). This peak was not found in other solvents taken in the study.

With the change in solvent polarity of the intensity of the absorption band at 291 nm in 30 was changed. The same was true in the excited state also. No additional peak at higher wavelength for excimer or exciplex formation was observed in CHCl₃ (Figures 2.14 and 2.15).

However, we choose CHCl₃ as the desired solvent medium (non interference in hydrogen bonding between receptors and substrates) to study the non-covalent interactions of 28, 29 and 30 with dicarboxylic acids. The absorption spectra of 28 and its 1:1 complexes with D-(-)-tartaric, rac-malic, and succinic acids in CHCl₃ were recorded to investigate the interactions in the ground state Chloroform solutions of the 1:1 complexes were diluted gradually with CHCl₃, and the change in intensity as a function of the concentration was linear in each case. Figure 2.16 shows the effect of dilution on the UV spectra of 1:1 complex of D-(-)-tartaric acid with 28. Figure 2.17 represents the change in absorbance with complex concentration. This change in the UV-vis spectra was used conveniently to study the binding since the lower concentration used led to a more accurate determination of the values of the association constants for the acids (Table 2.1). Figure 2.18, for example, represents the binding constant curve for D-(-)-tartaric acid with 28. The hydroxy analogues of succinic acid show higher binding constants due to the greater number of hydrogen bonds. Interestingly, the binding values were reduced ten-fold as the number of -OH groups decreases.

The fluorescence spectra of 28 were simultaneously recorded in CHCl₃ both in the presence and absence of dicarboxylic acid guests. Figure 2.19 shows the fluorescence spectra of 28 and its 1:1 complexes with D-(-)-tartaric, rac-malic and succinic acids in CHCl₃. On complexation with these acids, significant fluorescence quenching took place. However, the degree of quenching is dependent on the nature of the acid. The magnitude
Chapter 2

Figure 2.16. UV spectra of complex 28 (c = 3.15 x 10^{-3} M) with D-(−)-tartaric acid and its change of absorbance on dilution with CHCl₃.

Figure 2.17. Plot of absorbance vs concentration of the complexes of the acids with 28.

Table 2.1: Association constants determined by UV method in CHCl₃.

<table>
<thead>
<tr>
<th>Guest</th>
<th>Kₐ in M⁻¹ for 28</th>
<th>Kₐ in M⁻¹ for 29</th>
<th>Kₐ in M⁻¹ for 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-(−)-Tartaric acid</td>
<td>9.81 x 10⁵</td>
<td>1.90 x 10⁴</td>
<td>2.96 x 10⁴</td>
</tr>
<tr>
<td>rac-Malic acid</td>
<td>4.96 x 10⁴</td>
<td>1.78 x 10⁴</td>
<td>2.39 x 10⁴</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>9.38 x 10³</td>
<td>1.56 x 10³</td>
<td>2.89 x 10³</td>
</tr>
</tbody>
</table>

a. Errors in Kₐ were ≤ 10%.

The quenching efficiency (φq)⁴₂ follows the order of D-(−)-tartaric acid (0.92) > rac-malic acid (0.87) > succinic acid (0.53), reflecting the stabilities of the complexes. In the case of D-(−)-tartaric acid, an additional peak at 453 nm along with monomer emission at 377 nm was noticed due to excimer formation.

The excimer emission resulted from the intramolecular excimer, rather than intermolecularly, as indicated by the dilution experiments at different concentrations in which the intensities of the ratio of excimer to monomer emission changed gradually (Figure 2.20). It is of note that this excimer formation was found to be dependant on the
solvent polarity. Complexation of D(-)-tartaric acid into the open cleft of 28 in polar solvents such as DMSO, CH$_3$OH, THF did not produce any peak at higher wavelength for excimer. In CH$_3$CN, the characteristic emission at 480 nm for excimer was observed and it was found to be more intense than in CHCl$_3$ (Figure 2.21). The associated change in absorbance of the 1:1 complex of 28 with D(-)-tartaric acid in different solvents are shown in Figure 2.22. However, the formation of this excimer in the presence of D(-)-tartaric acid could be attributed to the tartaric acid templated hydrogen bond induced
organization of the quinoline moieties. Such excimer formation was not observed in the case of succinic acid due to the lack of -OH groups, which are necessary to bring the pendant quinoline groups of the binding arms via hydrogen bond formation. This was confirmed using rac-malic acid where the excimer emission was observed (Figure 2.19), due to the possibility of hydrogen bonding structure 35 which may remain in equilibrium with 36 and 37 (Figure 2.23). It is, therefore, worth noting that the conformation of 28 was changed substantially only on binding with hydroxy dicarboxylic acids rather than with a dicarboxylic acid of the same chain length.

The role of quinoline nitrogens of 28 in the binding interaction with the hydroxy dicarboxylic acids as shown in Figure 2.23 was substantiated by considering the naphthalene appended receptor 29 and also the hetero bis amide receptor 30. The binding interaction was monitored under identical conditions as was maintained for 28. However, to compare the non-covalent interaction of 29 with the receptor 28, we considered CHCl₃ as the desired medium. Chloroform solutions of 1:1 complexes of receptor 29 with the diacids such as D-(-)-tartaric, rac-malic and succinic acids were diluted gradually with CHCl₃, and the change in intensity as a function of the complex concentration was plotted. Figure 2.24, for example, represents the absorption spectra of 1.1 complex of D-(-)-tartaric acid with 29 upon dilution with CHCl₃, and Figure 2.25 shows the change in absorbance as a function of complex concentration of 29 with the diacids. The linear nature of the curve in Figure 2.25 indicates that 1:1 stoichiometry of the complexes is maintained throughout the course of titration.
The emission spectra of 29 were recorded in the presence and absence of diacids in CHCl₃. In this context, Figure 2.26 shows the emission spectra of 29 and its 1:1 complexes with the three diacids in CHCl₃. Upon complexation, intensity of excimer at 492 nm was reduced to the different extents accompanying an increase in the monomer emission of naphthalene. The excimer intensity was significantly reduced upon complexation with D-(-)-tartaric acid, which suggested strong complexation into the cleft. Upon complexation, the closeness of the naphthalene moieties are disrupted or modulated for which a weak excimer was noticed (Figure 2.26). The related absorption spectra of 29 in the presence of the equivalent amount of the diacids are shown in Figure 2.27.

A similar UV-vis and emission studies on the hetero bis amide receptor 30 were conducted in CHCl₃. Upon complexation, the emission of 30 at 385 nm was reduced to the different extents. The quenching of emission was significant in the presence of D-(-)-tartaric acid. Figure 2.28 indicates the change in emission of 30 in the presence of the diacids as Figure 2.29 displays the change in absorbance in CHCl₃. It also of note that the absorbance at 290 nm was substantially decreased upon complexation of D-(-)-tartaric acid. Like receptors 28 and 29 the chloroform solution of the receptor substrate complexes for 30 were diluted with CHCl₃. For example, Figure 2.30 represents the absorption spectra of 1:1 complex of D-(-)-tartaric acid with 30 upon dilution with CHCl₃, and Figure 2.31 shows the change in absorbance as a function of complex
concentration of 30 with the dicarboxylic acids. The linear nature of the curve in Figure 2.31 indicates the maintenance of 1:1 stoichiometry of the complexes throughout the course of titration.

The binding constant values for 29 and 30 were determined by Benesi–Hildebrand plots and are collected in Table 2.1. As can be seen from Table 2.1, the binding constant values for 29 are less compared to receptor 28. Succinic and rac-malic acids interacted into the binding site of 29 with less number of hydrogen bond formation.

It is worth noting that the binding constant values for 30 are slightly greater than receptor 29. This is due to the presence of one quinoline motif at the lower rim of 30, which contributes to the binding event. These experimental results establish that quinoline motif...
Chapter 2

in 28 has a key role in hydrogen bonding for which the binding constant values are found to be higher.

Conclusion

We have demonstrated that hydrogen bond-mediated complexation of D-(-)-tartaric with 28 results in monomer emission quenching followed by intramolecular excimer emission. This excimer emission is moderate and convenient for practical use to distinguish D-(-)-tartaric from its nonhydroxy analogue succinic acid. The hydrogen bonding behavior of quinoline in 28 for the formation of excimer has been established by considering the receptors 29 and 30. Further work on the modulation of structure 28 is underway in the laboratory for chiral discrimination of hydroxy acids.

2.4. Design and synthesis of polyether linked quinoline-based receptor for fluorometric discrimination of carboxylic acids

With a view of modifying the lower rims of the receptors 28 and 29 by introducing flexible links with hydrogen bond acceptor sites, we designed and synthesized receptors 38 and 39. In the designs, the binding sites are pyridine amides, which are linked to the
quinoline and naphthalene moieties via polyether chains. It is worth noting that receptor 38 is able to bind citric, gluconic and tartaric acids strongly in less polar solvent CHCl₃. The guests can be clearly distinguished by observing the strong excimer emission formed by the pendant quinoline probes upon complexation. To establish the role of quinoline ring nitrogen in complexation, an alternative naphthalene-based receptor 39, where quinoline has been replaced by naphthalene keeping all the other hydrogen bonding groups fixed was taken into account. The key in all the designs is the appropriate flexible ether linkage to hold the fluorophore probes viz. quinoline and naphthalene in such a manner as to create open clefts of different topologies.

2.4.1. Synthesis of the receptors 38 and 39

The receptors 38 and 39 were accomplished according to Schemes 2.4 and 2.5. The alcohols 40 and 43, obtained from 8-hydroxyquinoline and 1-naphthol, were coupled with 2-N-pivaloylamino-6-bromomethylpyridine (obtained from 2-N-pivaloylamino-6-methylpyridine by reaction with NBS in dry CCl₄) to give compounds 41 and 44, respectively. Amide hydrolysis of 41 and 44 afforded the corresponding amines 42 and 45 in good yields. Coupling of these amines with isophthaloyl dichloride yielded the desired receptors 38 and 39. All the compounds were characterized using ¹H NMR, ¹³C, mass, IR and UV spectroscopic methods.
Chapter 2

Scheme 2.4. Synthesis of receptor 38: Reagents and conditions (i) 2-chloro ethanol, K2CO3 in dry CH3CN, (ii) 2-N-pivaloylamino-6-bromomethylpyridine, NaH in dry THF; (iii) 4N KOH in aqueous-ethanol, reflux, (iv) Isophthaloyl dichloride, dry CH2Cl2, Et3N.

Scheme 2.5. Synthesis of receptor 39: Reagents and conditions (i) 2-chloro ethanol, K2CO3 in dry CH3CN, (ii) 2-N-pivaloylamino-6-bromomethylpyridine, NaH in dry THF; (iii) 4N KOH in aqueous-ethanol, reflux; (iv) Isophthaloyl dichloride, dry CH2Cl2, Et3N.

2.4.2. Complexation studies

The sensitivity and selectivity of the receptors 38 and 39 were evaluated by observing the changes in 1H NMR, UV-vis and fluorescence emission in CHCl3.
Chapter 2

UV-vis studies

Initially, the photophysical behaviors of the receptors 38 and 39 were noticed in solvents of different polarities. In the ground state, the absorption peak at 289 nm for quinoline of 38 and at 290 nm for naphthalene of 39 in CHCl₃ are considerably affected in intensities as well as positions (red shift; ~18 nm) as the solvent polarity is varied (see Figures 2.32 and 2.33).

The absorption spectra of 38 and its 1:1 complexes with citric, D-(-)-tartaric, D-(-)-gluconic, succinic, glutaric and terephthalic acids in CHCl₃ were recorded to investigate the interactions in the ground state. Chloroform solutions of the 1:1 complexes were diluted gradually with chloroform and the change in intensity, as a function of the complex concentration, was linear in each case. Figure 2.34, for example, shows the effect of dilution on the UV spectra of 1:1 complex of citric acid with 38. In 1:1 complex of 38, the absorption at 290 nm is significantly reduced. The change in absorbance with complex concentration is found to be linear (Figure 2.35). Figures 2.36 and 2.37 indicate the case of 38 with D-(-)-tartaric acid, where similar nature of interaction is attributed. These changes in the UV-vis spectra were used conveniently to study the binding (Table 2.2). Citric acid, a tricarboxylic acid with more number of hydrogen bonding groups, shows higher binding constant and the value is higher than D-(-)-gluconic and D-(-)-tartaric acids. D-(-)-Gluconic acid with more number of OH groups in the backbone, exhibits a value of $1.55 \times 10^5$ M$^{-1}$ which is slightly less than tartaric and citric acids. The non-hydroxy dicarboxylic acids such as succinic, glutaric, terephthalic acids bind weakly
Chapter 2

compared to the hydroxy acids. Besides the dilution method we also followed a continuous variation method where the absorbance of receptor 38 was monitored as a function of guest concentration.\[1\] In order to do so the receptor was dissolved in CHCl$_3$ and the carboxylic acid guest, dissolved in CHCl$_3$ containing 0.8% DMSO, was gradually added to the receptor solution. The corresponding change in absorption of the receptor was noted after each addition. The binding constant values determined by this method are found to be less (Table 2.3) due to the presence of DMSO, a competitive hydrogen-bonding partner which reduces the binding affinity.

Interestingly, as we move from receptor 38 to receptor 39, the binding constant value is also reduced due to less number of hydrogen bonds formed during complexation and this occurs owing to the replacement of quinoline moiety in 38 by naphthalene, which does not take part in complexation. To ascertain the binding potencies, UV titrations of the receptor 39 in presence of the same guests were carried out in CHCl$_3$. The change in absorbance of the complexes of 38 with the acid guests on dilution with CHCl$_3$ was linear in each case. Figures 2.38 – 2.41, for example, demonstrate the changes in absorbance of the 1:1 complexes of citric acid and D-(-)-tartaric acid with receptor 39, respectively. The binding constant values are shown in Table 2.3.

![Figure 2.34](image1)

**Figure 2.34.** UV spectra of the complex of 38 with citric acid ($c = 1.67 \times 10^{-3}$ M) and its change of absorbance on dilution.

![Figure 2.35](image2)

**Figure 2.35.** Plot of absorbance vs. concentration of the complex of citric acid with 38.
Chapter 2

Figure 2.36. UV spectra of the complex of 38 with D(-)-tartaric acid ($c = 1.67 \times 10^{-5}$ M) and its change of absorbance on dilution.

Figure 2.37. Plot of absorbance vs. concentration of the complex of D(-)-tartaric acid with 38.

Figure 2.38. UV spectra of the complex of 39 with citric acid ($c = 1.67 \times 10^{-5}$ M) and its change of absorbance on dilution.

Figure 2.39. Plot of absorbance vs. concentration of the complex of citric acid with 39.

Figure 2.40. UV spectra of the complex of 39 with D(-)-tartaric acid ($c = 1.67 \times 10^{-5}$ M) and its change of absorbance on dilution.

Figure 2.41. Plot of absorbance vs. concentration of the complex of D(-)-tartaric acid with 39.
Table 2.2 Association constants of 38 by UV method

<table>
<thead>
<tr>
<th>Guest acid</th>
<th>$K_a$ in $\text{M}^{-1}\text{c}$</th>
<th>$K_a$ in $\text{M}^{-1}\text{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>$3.01 \times 10^5$</td>
<td>$2.98 \times 10^4$</td>
</tr>
<tr>
<td>D-(-)-tartaric acid</td>
<td>$2.78 \times 10^5$</td>
<td>$2.12 \times 10^4$</td>
</tr>
<tr>
<td>D-(-)-gluconic acid</td>
<td>$1.55 \times 10^5$</td>
<td>$1.67 \times 10^4$</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>$4.45 \times 10^4$</td>
<td>$3.29 \times 10^3$</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>$7.34 \times 10^4$</td>
<td>$5.04 \times 10^3$</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>$7.26 \times 10^4$</td>
<td>$9.23 \times 10^3$</td>
</tr>
<tr>
<td>Sebacic acid</td>
<td>$2.98 \times 10^3$</td>
<td>$1.96 \times 10^3$</td>
</tr>
<tr>
<td>Terephthalic acid</td>
<td>$2.00 \times 10^4$</td>
<td>$4.33 \times 10^3$</td>
</tr>
</tbody>
</table>

$^a$ Determined in dry CHCl$_3$ by dilution method (at wavelength 290 nm), $^b$ Determined in dry CHCl$_3$ by adding guests dissolved in CHCl$_3$ containing 0.8% DMSO (at wavelength 290 nm) c Errors in $K_a$ were $\leq 6\%$

Table 2.3 Association constants of 39 by UV method.

<table>
<thead>
<tr>
<th>Guest acid</th>
<th>$K_a$ in $\text{M}^{-1}\text{c}$</th>
<th>$K_a$ in $\text{M}^{-1}\text{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>$2.64 \times 10^4$</td>
<td>$4.86 \times 10^3$</td>
</tr>
<tr>
<td>D-(-)-tartaric acid</td>
<td>$3.36 \times 10^4$</td>
<td>$7.70 \times 10^3$</td>
</tr>
<tr>
<td>D-(-)-gluconic acid</td>
<td>$1.55 \times 10^4$</td>
<td>$5.28 \times 10^3$</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>$1.41 \times 10^4$</td>
<td>$7.78 \times 10^3$</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>$1.66 \times 10^4$</td>
<td>$1.54 \times 10^3$</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>$1.99 \times 10^4$</td>
<td>$8.11 \times 10^3$</td>
</tr>
<tr>
<td>Sebacic acid</td>
<td>$1.02 \times 10^4$</td>
<td>$2.83 \times 10^3$</td>
</tr>
<tr>
<td>Terephthalic acid</td>
<td>$1.09 \times 10^4$</td>
<td>$7.77 \times 10^3$</td>
</tr>
</tbody>
</table>

$^a$ Determined in dry CHCl$_3$ by dilution method (at wavelength 290 nm), $^b$ Determined in dry CHCl$_3$ by adding guests dissolved in CHCl$_3$ containing 0.8% DMSO (at wavelength 290 nm) c. Errors in $K_a$ were $\leq 6\%$

Fluorescence studies

To ascertain the excited state properties, fluorescence spectra of the receptor 38 were recorded in CHCl$_3$ both in presence and in absence of the guest acids. Figure 2.42 shows the fluorescence spectra of receptor 38 and its 1:1 complexes with citric, D-(-)-tartaric, D-(-)-gluconic, succinic, glutaric, adipic, sebacic and terephthalic acids. On complexation, fluorescence quenching of the monomer emission occurs significantly with simultaneous generation of new peak at longer wavelength, presumably due to formation of excimer. The degree of quenching and the appearance of excimer are dependent on the
nature of the acids. The nonhydroxy dicarboxylic acids of different chain lengths are less efficient in forming strong excimer than hydroxy di- and tricarboxylic acids. Terephthalic acid, an example of aromatic dicarboxylic acid, in contrary, did not produce any excimer upon complexation. This is attributed to the rigidity and steric feature of the aromatic diacid that presumably plays a key role not to bring the pendant quinolines close for excimer formation. We also tested the possibility of formation of excimer in presence of different aliphatic dicarboxylic acids of different chain lengths. The excimer emission resulted from the intramolecular excimer, rather than intermolecularly, as indicated by the dilution experiments at different concentrations in which the intensities of the ratio of excimer to monomer emission changed gradually (Figure 2.43). The formation of strong excimers in the presence of hydroxy dicarboxylic acids could be attributed to the guest acid templated hydrogen bond induced organization of the pendant quinoline moieties that are linked to the pyridine motifs through flexible ether chains. This was further confirmed by control experiment using propanoic acid. Propanoic acid as monocarboxylic acid is preferentially complexed into the pyridine amide sites and the lower rim of 38 remains non-interacting giving no excimer emission. It is mentionable that this excimer formation is dependent on the strength of binding. Upon addition of guests (dissolved in CHCl₃ containing 0.8% DMSO) to the CHCl₃ solution of 38 showed weak excimer only in presence of excess concentration of guest acids. In this aspect, change in fluorescence intensity of 38 in presence of excess concentration of citric and D-
(-)-tartaric acids, dissolved in CHCl₃ containing 0.8% DMSO, is represented by Figures 2.44 and 2.45, respectively.

Interestingly, under similar condition, the receptor 39 showed weak interaction with the same guests and did not produce strong excimer upon complexation. As shown in Figure 2.46, the initially present less intense peak at higher wavelength (~ 500 nm) for weak excimer in 39 is marginally perturbed in the 1:1 complexes with the respective guests. Figure 2.47 indicates the change in fluorescence of 39 in CHCl₃ upon gradual addition of
citric acid, dissolved in CHCl₃ containing 0.8% DMSO. It is of note that the change is insignificant compared to the case of the receptor 38 (see Figure 2.42). These observations prove the key role of quinoline in strong complexation of carboxylic acids, especially hydroxy acids.

**1H NMR study**

To identify the possible hydrogen bonding sites and also to realize the conformational behavior of both 38 and 39, ¹H NMR spectra were recorded in CDCl₃. To the receptor solutions in CDCl₃, diacids as considered in the present study were added in excess and the solutions were thoroughly sonicated for 10 min. The insoluble particles were removed by filtration and clear solutions were used to record the ¹H NMR spectra. In all cases the complexes were of 1:1 stoichiometries, confirmed from the integration ratio of the receptor to the guest signals in ¹H NMR. The receptor 38, in CDCl₃, showed a sharp peak at 9.45 ppm for the amide protons, which underwent a considerable downfield shift (Δδ = 0.21 - 1.08 ppm) upon addition of 1 molar equiv. of the diacids studied, suggesting that aminopyridyl moieties serve as potential binding sites for carboxylic acids. The CH₂ protons of the ethers, perfectly aligned into the cavity, also moved significantly to the downfield direction upon complexation. Among the three types of CH₂ protons (a, b and c, see the structure 38), a and c types moved more downfield (Δδ = 0.07 - 0.20) indicating a clear-cut case of H-bonding. This significant downfield shift of the CH₂ protons (a, c types) of the ethers in presence of the guest carboxylic acids (except terephthalic acid in Table 2.2), led us to suggest that the lower rim of the receptor 38 is actively involved in complexation for which there is a substantial conformational change of the receptor 38. As can be seen from Figure 2.48, a and c type protons of the ether chains undergo downfield shift upon complexation with citric acid. This subtle change via H-bonding presumably influences the quinoline groups to be close enough for formation of excimer. This is also evidenced from a change in the chemical shift values of the quinoline ring protons (Figure 2.48) during complexation with citric acid. The quinoline ring protons (marked as asterisk in Figure 2.48) suffer downfield shift upon complexation and led us to presume a weak edge to face type π-stacking interaction between the pendant quinolines.
Chapter 2

2.4.3. Theoretical calculations on receptors and selected complexes

In order to understand the flexible nature as well as modes of binding of the receptors 38 and 39 with the guest molecules studied in the present case, electronic structure calculations were carried out. Geometries of all compounds involved were subject to optimization at AM1 level.

It is evident from the optimized geometry of the complex of 38 in Figure 2.49a that citric acid is strongly complexed in the cleft involving a large number of hydrogen bonding interactions. Both the amides as well as the isophthaloyl peri proton form hydrogen bonds with one of the carboxylic acid groups. The other terminal carboxylic acid forms bifurcated hydrogen bonds at the lower rim with the quinoline ring nitrogens and this is further stabilized by the adjacent three hydrogen bonds, formed from the participation of the ether oxygen of one arm, -OCH₂- of another arm and one pendant quinoline ring hydrogen. The -CO₂H group, attached to the carbon to which -OH group is present, is found uncomplexed. The -OH at the middle carbon forms a single hydrogen bond with the ether oxygen of one arm. Surprisingly, the methylene hydrogens of citric acids also
Chapter 2

In comparison, this weak $\pi$-stacking interaction between the quinolines is no longer found in the complex of 38 with terephthalic acid (Figure 2.49b). In the complex, the pendant quinolines are separated enough and in the cleft carboxylic acid is complexed involving pyridine amide. The other carboxylic acid is single bonded to the one of the pendant quinolines via ring hydrogen. One of the phenyl hydrogens of the guest terephthalic acid is bonded to the quinoline ring nitrogen. The hydrogen bond distances associated with this complex are listed in Figure 2.49b. We also did the same calculations on the complex of receptor 39 with citric acid. As can be seen from Figure 2.49c, citric acid is complexed into the cleft with a number of hydrogen bonds and the pendant naphthalenes are separated by a large distance without showing any $\pi$-stacking.
Conclusion

Thus it is concluded that the conformationally flexible receptor 38 is able to distinguish hydroxy dicarboxylic acids from their non-hydroxy analogues and also aliphatic dicarboxylic acids from aromatic diacids by showing characteristic excimer emission, which is moderate and convenient. The findings have been adequately explained by theoretical results. In the design 38, quinoline ring nitrogen played a key role in the binding process like receptors 28 and 30 and was established by doing the control experiments on the receptor 39. The receptor 39 was found less effective than 38 in the binding and selection of the guest carboxylic acids.
2.5. Design and synthesis of Quinoline-based tripodal shaped receptor for citric acid

Citric acid is a tricarboxylic acid that plays an important role in the Krebs cycle to provide the vast majority of energy used by aerobic cells, e.g. in human beings. Several groups have reported the recognition of the citrate ion using various receptors. They have been discussed briefly in Section 2.2. Reviews of literature shows that the receptors reported so far are for citrate ion recognition. Receptor for citric acid recognition is rare in the literature. In this section, we report our endeavor on pyridine based tripodal fluororeceptors 46 and 47 that show a significant ability to bind citric acid in the less polar solvent CHCl₃.

2.5.1. Synthesis of the receptors 46 and 47

The receptors 46 and 47 were synthesized from 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene by reaction with fluorophore labeled 2-aminopyridines 32 and 34, respectively, in presence of K₂CO₃ in a dry CH₃CN and THF solvent mixture (Schemes 2.6 and 2.7). Compounds 46 and 47 were obtained in 30% and 33% yields, respectively, and were characterized by ¹H NMR, ¹³C and mass analyses.
2.5.2. Molecular modeling

These tripodal receptors 46 and 47 can adopt a folded conformation with the fluorophore...
Chapter 2

(naphthalene, quinoline) moieties upwards and downwards around the benzene core. Molecular modeling shows that the orientation of the hydrogen bonding groups around the central benzene core is in a tripodal fashion in both 46 and 47 (Figure 2.50).

2.5.3. Complexation studies on receptors 46 and 47

The binding abilities of the tripodal receptors 46 and 47 for citric and other carboxylic acids such as rac-malic and D-(-)-tartaric acids were investigated using $^1$H NMR, UV-vis and fluorescence methods.

$^1$H NMR study

To gain an insight about the binding interactions with citric acid, $^1$H NMR spectra of 46 and 47 were taken in CDCl$_3$ ($c = 3.08 \times 10^{-3}$ M and $3.31 \times 10^{-3}$ M, respectively). The amine protons in 46 and 47 appeared at 4.32 and 4.43 ppm respectively, and were too broad to detect accurately upon addition of citric acid (dissolved in CDCl$_3$ containing 4% DMSO-d$_6$). Even the amine protons in 46, which appeared at 4.32 ppm, were also broadened after addition of powdered citric acid to the dry CDCl$_3$ solution of 46 followed by sonication. The clear dissolution of citric acid was evident from the appearance of new peaks at 2.85 and 2.66 ppm for the –CH$_2$– groups of the citric acid (Figure 2.51). All the peaks in the aromatic region of 46 were resolved and no other new peaks were noticed. When dry HCl was passed into the chloroform solution of 46, immediate precipitation occurred to give an insoluble product. The $^1$H NMR of this insoluble product was recorded in DMSO–d$_6$. All the signals were broad and new peaks were observed in the regions 9.29, 7.90 and 7.27 ppm, presumably for the protonated quinoline, pyridine and ammonium cations, respectively. The absence of such new peaks at 9.29, 7.90 and 7.27 ppm during the complexation of citric, rac-malic and D-(-)-tartaric acids with both 46 and 47 thus confirmed that the tripodal receptors 46 and 47 were involved in complexation with the carboxylic acid guests in CDCl$_3$, mainly through H-bonding instead of ion pair binding via proton transfer.
UV-vis and fluorescence studies
Once the nature of the interactions had been established, both 46 and 47 were studied by UV-vis and fluorescence to establish their selectivities and sensitivities towards citric, rac-malic and D(-)-tartaric acids. Initially, the photophysical properties of 46 were determined in solvents of different polarities to gain an insight about its solvatochromic behavior. The absorption spectra of 46 in dry CHCl₃ exhibited a structureless absorption band at 310 nm, characteristic of quinoline. The position of this peak was unaltered in dry THF, CH₃OH and CH₃CN. A slight red shift in dry DMSO was observed (Figure 2.52a). In the presence of citric acid, the absorption peak at 310 nm for quinoline in 46 showed a large red shift ($\Delta\lambda = 21$ nm) only in CHCl₃ (Figure 2.52b). This large red shift of the absorption peak in CHCl₃ illustrated a strong hydrogen bond interaction between citric
acid and the receptor 46. Similar findings were observed with receptor 47 (Figures 2.53a and 2.53b).

A chloroform solution of the 1:1 complexes of citric, rac- malic and D-(-)- tartaric acids with the receptor 46 were diluted gradually with chloroform and the change in intensity, as a function of concentration was linear in each case. Figure 2.54, for example, shows the effect of dilution on the UV-vis spectra of the 1:1 complex of 46 with citric acid and the change in absorbance of the complex was found to be linear with complex concentration (Figure 2.55). This change in the UV-vis spectra was used to determine the
binding constant values (Table 2.4). Citric acid having more hydrogen bonding groups shows a higher binding constant in comparison to the others.

Similar control experiments on 47 were performed. For example, the effect of dilution on the UV spectra of the 1:1 complex of citric acid with 47 in CHCl3 Figure 2.56 and Figure 2.57 shows the change in intensity with complex concentration. The binding constant values determined by Benesi–Hildebrand plots and are displayed in Table 2.4 and it is found that binding constant values are less compared to receptor 46.
Chapter 2

Table 2.4. Binding constant values of 46 by UV method

<table>
<thead>
<tr>
<th>Guest acids</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>$K_b$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>$6.36 \times 10^3$</td>
<td>$5.75 \times 10^4$</td>
</tr>
<tr>
<td>rac-Malic acid</td>
<td>$7.25 \times 10^4$</td>
<td>$7.49 \times 10^3$</td>
</tr>
<tr>
<td>D-(-)-Tartaric acid</td>
<td>$7.62 \times 10^4$</td>
<td>$2.90 \times 10^4$</td>
</tr>
</tbody>
</table>

$a$ Determined in pure dry CHCl$_3$; $b$ Determined in dry CHCl$_3$ containing 0.7% DMSO. c. Errors in $K_b$ were $\leq 10\%$.

Table 2.5. Binding constant values of 47 by UV method.

<table>
<thead>
<tr>
<th>Guest acids</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>$K_b$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>$8.82 \times 10^4$</td>
<td>$2.28 \times 10^4$</td>
</tr>
<tr>
<td>rac-Malic acid</td>
<td>$1.02 \times 10^4$</td>
<td>$1.62 \times 10^4$</td>
</tr>
<tr>
<td>D-(-)-Tartaric acid</td>
<td>$1.81 \times 10^4$</td>
<td>$1.30 \times 10^4$</td>
</tr>
</tbody>
</table>

$a$ Determined in pure dry CHCl$_3$; $b$ Determined in dry CHCl$_3$ containing 0.7% DMSO. Errors in $K_b$ were $\leq 10\%$.

This underlines the fact that quinoline ring nitrogen in 46 has a key role in hydrogen bonding to increase the binding of the carboxylic acids.

The chemosensor behavior was simultaneously investigated by steady state fluorescence. As shown in Figure 2.58, the emission of quinoline in 46 varies with the polarity of the solvents when excited at 290 nm. Compound 46 displays a structure less monomer emission at 382 nm when irradiated at 290 nm in CHCl$_3$. The addition of citric, rac-malic and D-(-)-tartaric acids to a CHCl$_3$ solution of 46 in 1:1 stoichiometry gave a decrease in the fluorescence emission of the quinoline, along with a simultaneous generation of the excimer bands at 456, 444 and 464 nm respectively (Figure 2.59). As shown in Figure 2.59, the intensity of the excimer band varies with the nature of the carboxylic acid, and is found to be significant in case of citric acid. This excimer emission of 46 in presence of citric acid showed a sensitive dependence on the polarity of the solvent, being much less important in more polar solvents such as CH$_3$OH, THF and DMSO except for CH$_3$CN where a weak excimer band at 475 nm was observed (Figure 2.60).

We suggest that this excimer emission results from the guest-induced hydrogen bond-mediated upward folding of the quinoline moieties that occurs with citric acid due to its bigger size and strong hydrogen bonding interaction. This was proved by doing similar control experiments using 47 with the same carboxylic acids in CHCl$_3$. Interestingly, 47 in presence of the same acids, gave rise to a decrease in the monomer emission of the naphthyl moiety to different extents without showing any peak at 456 nm for excimer (Figure 2.61). Even upon addition of the tetrabutylammonium salt of citric acid to the
chloroform solution of 46, no measurable change in the fluorescence was observed
(Figure 2.62). These observations support the conclusion that the nitrogen of the
quinoline ring is indeed an important factor in the guest induced, hydrogen bond-
mediated, substantial conformational change of 46 which brings the quinoline moieties
close for the formation of the excimer. The excimer emission resulted from the
intramolecular excimer, rather than intermolecularly, as indicated by dilution experiments
at different concentrations in which the intensities of the ratio of excimer to monomer
emission changed gradually (Figure 2.63).
Chapter 2

**Figure 2.62.** Fluorescence change of 46 ($c = 2.20 \times 10^{-5}$ M) upon addition tetrabutylammonium citrate ($\lambda_{ex} = 290$ nm).

**Figure 2.63.** Plot of the ratio of excimer to monomer emission vs concentration of the complex of 46 with citric acid.

In order to find out the influence of the polar solvent in the binding of citric and other acids, fluorescence titrations on both 46 and 47 were conducted by taking the guest acids in CHCl₃ containing 0.7% DMSO. It is of note that the excimer was absent when the guest acids, dissolved in CHCl₃ containing 0.7% DMSO, were gradually added to the CHCl₃ solution of 46 ($c = 1.32 \times 10^{-5}$ M). Figure 2.64 shows the change in fluorescence of 46 upon addition of citric acid. The corresponding change in absorption in CHCl₃ on

**Figure 2.64.** Change in fluorescence of 46 ($c = 1.32 \times 10^{-5}$ M) in CHCl₃ upon addition of citric acid, dissolved in CHCl₃ containing 0.7% DMSO.

**Figure 2.65.** Change in absorbance of 46 in CHCl₃ ($c = 1.32 \times 10^{-5}$ M) upon addition of citric acid, dissolve in CHCl₃ containing 0.7% DMSO.
gradual addition of citric acid, dissolved in CHCl₃ containing 0.7% DMSO is also worth noting (Figure 2.65). The isosbestic point at 315 nm confirms the 1:1 stoichiometry of the complex. The stoichiometry was further ascertained from the break of both the fluorescence and UV titration curves at \([G]/[H] = 1\) (Figures 2.66 and 2.67 respectively).

However, the presence of DMSO, a competitive hydrogen bonding partner, reduces the binding affinity of 46 with the guest acids (Table 2.4, the binding is approximately 10 times less than the values in pure CHCl₃)⁴⁳ so that upward folding of the quinoline moieties are presumably less efficient than the cases in pure CHCl₃. The binding constant values, as shown in Table 2.4, indicate that the receptor 46 is an efficient binder of citric acid in CHCl₃ and even is more significant than receptor 47.

The selective detection of citric acid by simple tripod shaped receptors 46 and 47 inspired us to identify the critical role of pyridine amine binding sites in 46 and 47. For this, we attached the 8-aminoquinoline motif directly to the tripod center to have the structure 48.

![Figure 2.66. Fluorescence titration curves for 46 with citric acid, dissolved in CHCl₃ containing 0.7% DMSO](image)

![Figure 2.67. UV-Vis titration curves for 46 with citric acid, dissolved in CHCl₃ containing 0.7% DMSO.](image)
Chapter 2

The receptor 48 was synthesized according to the Scheme 2.8. The reaction of 8-aminouquinoline with 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene in the presence of K$_2$CO$_3$ in dry CH$_3$CN afforded the compound 48 as brownish white solid in 31% yield.

\[ \text{Reagents and conditions} \]

(i) 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene, K$_2$CO$_3$/dry CH$_3$CN, THF, 24 h

Energy optimized geometry of 48 is presented in Figure 2.68. It is evident from Figure 2.68 that the quinolone motifs are little disorganized around the central benzene ring.

\[ \text{Figure 2.68. Energy minimized structures of 48 [E = 108 25 kcal/mol]} \]

To evaluate the binding potential of 48 we performed $^1$H NMR, UV-vis and fluorescence titration experiments. Initially, we tested the interaction of 48 $^1$H NMR with the same guests as taken for the receptors 46 and 47 in CDCl$_3$. The signal at 5.99 ppm assigned for amine protons did not exhibit any characteristic change in chemical shift upon
complexation. Only the sharp signal at 5.99 ppm became broad. Figure 2.69, for example, corroborates the change in $^1$H NMR of 48 in the presence of equivalent amount of citric acid in CDCl$_3$. The spectrum of 48 in the presence of citric acid was recorded after thorough sonication. The signals for the methylene protons (-CH$_2$-) of citric acid were not found. This indicates that the receptor 48 is not efficient in complexation of the carboxylic acid guests like 46 and 47.

![Figure 2.69. $^1$H NMR (400 MHz, CDCl$_3$) spectra of receptor 48 (bottom) and the 1:1 complex with citric acid (top)](image)

Due to negligible change in $^1$H NMR of 48 upon addition of citric and the other acids as were taken for 46, we did not proceed further to investigate the photo physical properties of 48.

**Conclusion**

In conclusion, a simple modular approach has been used to synthesize a quinoline-based tripod receptor ensemble, displaying a marked citric acid binding in less polar solvent chloroform. This is solely relying on weak non-covalent interactions. The hydrogen
bond-mediated complexation of citric acid by our quinoline-based sensor 46 is followed by excimer emission. This excimer emission in 46 upon complexation is moderate and convenient to detect and distinguish citric acid from tartaric and rac-malic acids. The direct linking of 8-amino quinoline to the tripodal core introduces the structure 48, which was not at all capable of sensing citric acid in organic solvent.

2.6. Experimental Section

General:
All the solvents were dried by usual procedures prior to use. All the reactions were carried out under nitrogen atmosphere. FT-IR and UV spectra were recorded on Perkin Elmer model L120-00A and Lambda-25 respectively. Fluorescence was recorded by Perkin-Elmer LS-50B instrument. For $^1$H and $^{13}$C NMR spectra Bruker 300, 400 and 500 MHz were used. Elemental analyses were performed on Perkin-Elmer 2400CHN Elemental analyzer. Melting points were recorded in open capillaries and are uncorrected.

N-(6-((Quinolin-8-yloxy)methyl)pyridin-2-yl)pivalamide (31):

To a solution of 8-hydoxy quinoline (0.5 g, 3.44 mmol) in dry DMF (10 mL) taken in a 50 mL round bottom flask, 2-N-pivaloylamino-6-bromomethyl pyridine (0.933 g, 3.44 mmol), catalytic amount of potassium iodide and tetrabutylammonium bromide were added and the solution was refluxed under nitrogen atmosphere for 14 h. The reaction mixture was cooled to room temperature and 30 mL water was added to it. The resulting mixture was extracted with 3 x 50 mL CHCl$_3$ and dried over anhydrous Na$_2$SO$_4$. The solvent was removed under vacuum; the residue was purified by column chromatography.
Compound 31 (0.25 g, 0.74 mmol) was dissolved in 20 mL 4N ethanolic KOH solution and refluxed for 16 h. After completion of reaction, the volume of the reaction mixture was reduced by evaporation of ethanol. The reaction mixture was cooled and extracted with 3 x 50 mL CHCl₃ and dried over anhydrous Na₂SO₄. After removal of the solvent, the crude product was purified by column chromatography using 4% CH₃OH in CHCl₃ to give amine 32 (0.149 g, yield: 80%). The purified amine 32 was subsequently used in the next step without characterization.

**6-((Quinolin-8-yloxy)methyl)pyridin-2-amine (32):**

![Diagram of 6-((Quinolin-8-yloxy)methyl)pyridin-2-amine (32)]

Compound 31 (0.25 g, 0.74 mmol) was dissolved in 20 mL 4N ethanolic KOH solution and refluxed for 16 h. After completion of reaction, the volume of the reaction mixture was reduced by evaporation of ethanol. The reaction mixture was cooled and extracted with 3 x 50 mL CHCl₃ and dried over anhydrous Na₂SO₄. After removal of the solvent, the crude product was purified by column chromatography using 4% CH₃OH in CHCl₃ to give amine 32 (0.149 g, yield: 80%). The purified amine 32 was subsequently used in the next step without characterization.
To a solution of 5-octyloxy isophthaloyl dichloride (0.15 g, 0.45 mmol) in dry DCM (10 mL), amine 32 (0.227 g, 0.9 mmol) containing Et$_3$N (0.02 mL) was added in dry DCM (10 mL) dropwise with stirring. Stirring was allowed to continue overnight. After completion of the reaction, solvent was evaporated and saturated NaHCO$_3$ solution (20 mL) added to the reaction mixture. The aqueous layer was extracted with 3 x 50 mL CHCl$_3$, dried over anhydrous Na$_2$SO$_4$, concentrated under vacuo and the crude product was purified by column chromatography using 4% MeOH in CHCl$_3$ as eluent to give 28 (0.378 g, yield: 55%); mp 110-111 °C.

$^1$H NMR (400 MHz, CDC$_3$): $\delta$: 8.96 (s, 2H, -NHCO-), 8.95 (d, 2H, $J = 8$ Hz), 8.26 (d, 2H, $J = 8$ Hz), 8.19 (s, 1H), 8.12 (d, 2H, $J = 8$ Hz), 7.71 (t, 4H, $J = 8$ Hz), 7.42 (m, 2H), 7.37-7.33 (m, 6H), 6.95 (d, 2H, $J = 6$ Hz), 5.32 (s, 4H), 4.08 (t, 2H, $J = 8$ Hz), 1.85-1.80 (m, 2H), 1.46 (m, 2H), 1.33-1.15 (m, 8H), 0.86 (t, 3H, $J = 6$ Hz).

$^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$: 165 2, 160.3, 155.4, 154.3, 151.6, 149.7, 140.7, 139.5, 136.4, 136.1, 129 8, 126.9, 122.1, 120.5, 118.1, 118.0, 117.9, 113.7, 109.9, 71.1, 69.1, 32.2, 29 7, 29 6, 29.5, 26.3, 23.4, 14.5.

FT-IR: $\nu$ cm$^{-1}$ (KBr): 3300, 2924, 2853, 1674, 1597, 1577, 1457, 1109.

HRMS calcd for C$_{46}$H$_{44}$N$_6$O$_5$: 760.3363  Found: 760.3382.
Compound 33 (0.4 g, 1.19 mmol) was refluxed in 20 mL 4N ethanolic KOH solution for 12 h and the progress of the reaction was followed by TLC monitoring. After completion of reaction, the volume of the reaction mixture was reduced by evaporation of ethanol. The reaction mixture was cooled and extracted with 3 x 50 mL CHCl₃ and dried over anhydrous Na₂SO₄. The solvent was removed under vacuo and the crude product was purified by column chromatography (5% ethyl acetate in petroleum ether) to give thick gummy liquid 33 (1.21 g, yield: 75%).

**FT-IR: v cm⁻¹ (KBr):** 3436, 2958, 1686, 1579, 1521, 1455, 1152.

6 -((Naphthalen-1-yloxy)methyl)pyridin-2-amine (34):

Compound 33 (0.4 g, 1.19 mmol) was refluxed in 20 mL 4N ethanolic KOH solution for 12 h and the progress of the reaction was followed by TLC monitoring. After completion of reaction, the volume of the reaction mixture was reduced by evaporation of ethanol. The reaction mixture was cooled and extracted with 3 x 50 mL CHCl₃ and dried over...
anhydrous Na$_2$SO$_4$. After removal of the solvent, the crude product was purified by column chromatography using 4% CH$_3$OH in CHCl$_3$ to give amine 34 as brownish white solid (0.235 g, yield: 82%); mp 96-97 °C.

$^1$H NMR (400 MHz, CDCl$_3$): δ: 8.38 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 1.6$ Hz), 7.80 (d, 1H, $J = 8$ Hz), 7.50 - 7.42 (m, 4H), 7.34 (t, 1H, $J = 8$ Hz), 6.98 (d, 1H, $J = 8$ Hz), 6.84 (d, 1H, $J = 8$ Hz), 6.44 (d, 1H, $J = 8$ Hz), 5.20 (s, 2H), 4.46 (br s, 2H, -NH$_2$).

FT-IR: v cm$^{-1}$ (KBr): 3468, 3372, 2899, 1621, 1576, 1466, 1269, 1108.

$N^1,N^2$-Bis(6-((naphthalen-1-yl)oxy)methyl)pyridin-2-yl)isophthalimide (29)

$N^1,N^2$-Bis(6-((naphthalen-1-yl)oxy)methyl)pyridin-2-yl)isophthalimide (29)

Amine 34 (0.236 g, 0.98 mmol) containing Et$_3$N (0.02 mL) in dry DCM (10 mL) was added drop wise to a solution of isophthaloyl dichloride (0.1 g, 0.49 mmol) in dry DCM (10 mL) taken in a 50 mL r.b. The reaction mixture was stirred overnight under nitrogen atmosphere. After the completion of reaction, DCM was evaporated and 25 mL saturated NaHCO$_3$ solution was added to the reaction mixture. The aqueous layer was extracted with 3 x 50 mL CHCl$_3$. The organic layer was dried over anhydrous Na$_2$SO$_4$, evaporated under vacuo and crude product was purified by column chromatography using 2% MeOH in CHCl$_3$ as eluent to give a white solid product 29 (0.384 g, yield: 62%); mp 174 °C.

$^1$H NMR (400 MHz, one drop d$_6$-DMSO in CDCl$_3$): δ: 9 71 (s, 2H, -NHCO-), 8.62 (s, 1H), 8.35 (d, 2H, $J = 8$ Hz), 8.31 (d, 2H, $J = 8$ Hz), 8.20 (dd, 2H, $J_1 = 8$ Hz, $J_2 = 1.6$ Hz), 7.82 - 7.76 (m, 4H), 7.64 (t, 1H, $J = 8$ Hz), 7.50 - 7.41 (m, 6H), 7.36 (d, 2H, $J = 8$ Hz), 7.31 (t, 2H, $J = 8$ Hz), 6.78 (d, 2H, $J = 8$ Hz), 5.22 (s, 4H).
Chapter 2

$^{13}$C NMR (100 MHz, few drops d$_6$-DMSO in CDCl$_3$): δ: 165.1, 155.1, 153.3, 151.6, 138.9, 133.8, 133.6, 131.5, 128.6, 127.2, 126.7, 126.2, 125.7, 125.1, 124.7, 121.3, 120.2, 117.1, 113.2, 105.3, 69.8.

FT-IR: v cm$^{-1}$ (KBr): 3339, 3008, 2924, 1682, 1642, 1579, 1451, 1355, 1107.

Mass (EI): 631 (M$^+$ + H), 574.7, 387.1.

$N^1$-(6-((Naphthalen-1-yl)oxy)methyl)pyridin-2-yl)-$N^3$-(6-((quinolin-8-yloxy)methyl)pyridin2-yl)isophthalimide (30)

![Diagram of 30]

In a two-necked flask (250 mL), isophthaloyl dichloride (0.200 g, 0.98 mmol) was added to dry THF (30 mL), and stirred for 30 min. Amine 32 (0.247 g, 0.98 mmol) containing Et$_3$N (0.02 mL) and amine 34 (0.246 g, 0.98 mmol) containing Et$_3$N (0.02 mL) were dissolved separately in dry THF (50 mL). Then they were added dropwise from two different dropping funnels. Addition was continued for 3 h with stirring under nitrogen atmosphere. The reaction was prolonged to stir for 12 h. After that THF was evaporated and 50 mL saturated NaHCO$_3$ solution was added to it. The aqueous layer was extracted with 3 x 50 mL CHCl$_3$ and dried over anhydrous Na$_2$SO$_4$ to give the crude product which was purified by column chromatography using 2% CH$_3$OH in CHCl$_3$ as eluent to give 30 as white solid (0.198 g, yield. 32%), mp 138-140 °C.

$^1$H NMR (400 MHz, CDCl$_3$): δ: 8.94 (s, 1H, -NHCO-), 8.93 (s, 1H, -NHCO-), 8.65 (s, 1H), 8.37 (d, 1H, $J$ = 8 Hz), 8.34 (d, 1H, $J$ = 8 Hz), 8.29 (d, 1H, $J$ = 8 Hz), 8.20 (d, 2H, $J$ = 8 Hz), 8.09 (d, 1H, $J$ = 8 Hz), 7.84 - 7.76 (m, 2H), 7.74 (t, 1H, $J$ = 8 Hz), 7.68 (t, 1H, $J$
Chapter 2

$^1$H NMR (400 MHz, CDCl$_3$): 8.84 (d, 1H, $J = 2.4$ Hz), 8.15 (d, 1H, $J = 8$ Hz), 7.47 - 7.40 (m, 3H), 7.11 (d, 1H, $J = 8$ Hz), 5.54 (br s, 1H, -OH), 4.28 (t, 2H, $J = 4$ Hz), 4.08 (t, 2H, $J = 4$ Hz).

FTIR ($\nu$ cm$^{-1}$, KBr): 3400, 3157, 2922, 1577, 1380, 1116.

Mass (ESI$^+$): 212.1 (M+Na)$^+$, 190.1 (M+H)$^+$. 
Chapter 2

N-(6-((2-Quinolin-8-yloxy)ethoxy)methyl)pyridine-2-yl)pivalamide (41)

To a solution of 40 (0.5 g, 2.60 mmol) in dry THF, NaH (0.063 g, 2.60 mmol) was added and stirred under nitrogen atmosphere for 2 h. Then the solution of 2-(N-pivalylamino)-6-bromomethylpyridine (0.72 g, 2.60 mmol) dissolved in 10 mL THF was added and stirring was continued for overnight. After the completion of reaction the reaction mixture was concentrated under vacuo and 30 mL water was added to the reaction mixture. The aqueous layer was extracted with 3 x 100 mL CHCl₃ and dried over anhydrous Na₂SO₄. The solvent was evaporated and the crude product was purified by column chromatography using 20% ethyl acetate in petroleum ether as eluent to give compound 41 (0.702 g, yield: 70%) brownish gummy product.

\[ \text{H NMR (400 MHz, CDCl}_3\]: \delta 8.93 (dd, 1H, \text{J}_1 = 1.6 Hz, \text{J}_2 = 1.6 Hz), 8.29 (s, 1H, \text{-CONH-}), 8.15 - 8.11 (m, 2H), 7.65 (t, 1H, \text{J} = 8 \text{ Hz}), 7.46 - 7.38 (m, 3H), 7.16 (d, 1H, \text{J} = 8 \text{ Hz}), 7.10 (d, 1H, \text{J} = 7 \text{ Hz}), 4.66 (s, 2H), 4.46 (t, 2H, \text{J} = 4 \text{ Hz}), 4.11 (t, 2H, \text{J} = 4 \text{ Hz}), 1.32 (s, 9H).

\[ \text{FTIR (v cm}^{-1}, \text{KBr): 3411, 1689, 1452, 1107.}\]

\[ \text{Mass (ESI}: [M+ Na}^+ = 402.3, \text{M + H}^+ = 380.2. \]

N¹,N³-Bis(6-((2-Quinolin-8-yloxy)ethoxy)methyl)pyridin-2-yl)isophthalimide (38):

\[ \text{NMR (400 MHz, CDCl}_3\]: \delta 8.93 (dd, 1H, \text{J}_1 = 1.6 Hz, \text{J}_2 = 1.6 Hz), 8.29 (s, 1H, \text{-CONH-}), 8.15 - 8.11 (m, 2H), 7.65 (t, 1H, \text{J} = 8 \text{ Hz}), 7.46 - 7.38 (m, 3H), 7.16 (d, 1H, \text{J} = 8 \text{ Hz}), 7.10 (d, 1H, \text{J} = 7 \text{ Hz}), 4.66 (s, 2H), 4.46 (t, 2H, \text{J} = 4 \text{ Hz}), 4.11 (t, 2H, \text{J} = 4 \text{ Hz}), 1.32 (s, 9H).\]
To a solution of amide 41 (0.3 g, 0.79 mmol) in 20 mL ethanol, 10 mL 4N KOH solution was added and the reaction mixture was refluxed for 12 h. After the completion of reaction, monitored by TLC, the ethanol was evaporated and 20 mL water was further added to the reaction mixture. The aqueous layer was extracted with 3 x 100 mL CHCl₃ and dried over anhydrous Na₂SO₄. The solvent was evaporated and the crude product was purified by column chromatography using 4% methanol in chloroform to give the corresponding amine 42 (0.175 g, yield: 75%). Without characterization, the amine 42 was directly used in the next step. Compound 42 (0.1 g, 0.30 mmol) was dissolved in dry CH₂Cl₂ and 0.15 mL of Et₃N was added to it. Then this amine solution was added dropwise to a solution of isophthaloyl dichloride (0.034 g, 0.16 mmol) in dry CH₂Cl₂ and the reaction mixture was stirred for overnight at room temperature. The solvent was evaporated and saturated NaHCO₃ solution (30 mL) was added to the reaction mixture. The aqueous layer was extracted with 3 x 50 mL CHCl₃ and dried over anhydrous Na₂SO₄. The solvent was evaporated off and the crude product was purified by column chromatography using ethyl acetate as eluent to give 38 (0.146 g, yield: 60%); mp 60 °C.

¹H NMR (400 MHz, CDCl₃): δ 9.45 (s, 2H, -CONH-), 8.87 (d, 2H, J = 4 Hz), 8.70 (s, 1H), 8.30 (d, 2H, J = 8.24 Hz), 8.25 (d, 2H, J = 7.64 Hz), 8.08 (d, 2H, J = 8.28 Hz), 7.73 (t, 2H, J = 8 Hz), 7.65 (t, 1H, J = 7.76 Hz), 7.44 – 7.33 (m, 6H), 7.18 (d, 2H, J = 7.40 Hz), 7.07 (d, 2H, J = 7.36 Hz), 4.64 (s, 4H), 4.43 (t, 4H, J = 4 Hz), 4.08 (t, 4H, J = 4 Hz).

¹³C (100 MHz, CDCl₃): 164.8, 155.9, 154.4, 151.4, 148.9, 139.8, 139.1, 136.1, 134.5, 131.5, 129.4, 129.3, 126.6, 125.8, 121.6, 119.8, 117.6, 113.2, 108.7, 73.3, 69.1, 67.8.

FTIR (ν cm⁻¹, KBr): 3385, 1677, 1455, 1318, 1105.

Mass (ESI⁺): 743.3 (M+ Na)⁺, 721.4 (M + H), 361.3, 296.2.

2-(Naphthalene-1-yl oxy)ethanol (43)
To a solution of 43 (0.500 g, 2.6 mmol) in dry THF, NaH (0.063 g, 2.6 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 2 h. Then the solution of 2-(N-pivalylamino)-6-bromomethylpyridine (0.72 g, 2.6 mmol) dissolved in 10 mL THF was added and stirring was continued for overnight. After the completion of reaction, the reaction mixture was concentrated under vacuo and 30 mL water was added to the reaction mixture. The aqueous layer was extracted with 3 x 100 mL CHCl₃ and dried over Na₂SO₄. After the evaporation of solvent the crude product was purified by column chromatography using 3% ethyl acetate in petroleum ether as eluent to give a deep brown gummy product 43 (0.587 g, yield: 45%).

**1H NMR (400 MHz, CDCl₃):** δ 8.26 (d, 1H, J = 7.2 Hz), 7.80 (t, 1H, J = 6.8 Hz), 7.51-7.44 (m, 3H), 7.36 (t, 1H, J = 8 Hz), 6.83 (d, 1H, J = 7.6 Hz), 4.27 (t, 2H, J = 4.4 Hz), 4.10 (t, 2H, J = 4 Hz), 2.10 (br s, 1H, OH).

**FTIR (ν cm⁻¹, KBr):** 2933, 1579, 2922, 1400, 1269.

**Mass (ESI⁺):** 211.1 (M+ Na)⁺, 189.2 (M + H)⁺.

N-(6-((2-(Naphthalene-1-yloxy)ethoxy)methyl)pyridine-2-yl)pivalamide (44)

To a mixture of 1-naphthol (1 g, 6.90 mmol) and potassium carbonate (0.96 g, 6.90 mmol) in dry CH₃CN, 2-chloroethanol (1.12 g, 13.9 mmol) in 5 mL CH₃CN was added dropwise and the reaction mixture was refluxed for 60 h. After completion the reaction mixture was concentrated under vacuo and 30 mL water was added to the mixture. The aqueous layer was extracted with 3 x 100 mL CHCl₃ and dried over Na₂SO₄. After the evaporation of solvent the crude product was purified by column chromatography using 3% ethyl acetate in petroleum ether as eluent to give a deep brown gummy product 43 (0.587 g, yield: 45%).

**N-(6-((2-(Naphthalene-1-yloxy)ethoxy)methyl)pyridine-2-yl)pivalamide (44)**

To a solution of 43 (0.500 g, 2.6 mmol) in dry THF, NaH (0.063 g, 2.6 mmol) was added and the reaction mixture was stirred under nitrogen atmosphere for 2 h. Then the solution of 2-(N-pivalylamino)-6-bromomethylpyridine (0.72 g, 2.6 mmol) dissolved in 10 mL THF, was added and stirring was continued for overnight. After the completion of reaction, the reaction mixture was concentrated under vacuo and 30 mL water was added to the reaction mixture. The aqueous layer was extracted with 3 x 100 mL CHCl₃ and dried over Na₂SO₄. The solvent was evaporated and the crude product was purified by column chromatography using 10% ethyl acetate in petroleum ether as eluent to give compound 44 as deep brown gummy product (0.744 g, yield 74%).
**Chapter 2**

\[^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3)\]: 8.28 (d, 1H, J = 8 Hz), 8.19 (s, 1H, -CONH-), 8.16 (d, 1H, J = 8.40 Hz), 7.78 (d, 1H, J = 8 Hz), 7.68 (t, 1H, J = 8 Hz), 7.48-7.42 (m, 3H,), 7.35 (t, 1H, J = 8 Hz), 7.22 (d, 1H, J = 8 Hz), 6.82 (d, 1H, J = 8 Hz) 4.69 (s, 2H), 4.36 (t, 2H, J = 4.80 Hz), 4.06 (t, 2H, J = 4.80 Hz), 1.31 (s, 9H).

**FTIR (ν cm\(^{-1}\), KBr):** 3425, 2924, 1685, 1454, 1152.

**Mass (ESI\(^{+}\)):** 401.5 (M+ Na\(^{+}\)), 379.2 (M + H\(^{+}\))

\(N^1,N^3\text{-bis(6-((2-(naphthalen-1-yloxy)ethoxy)methyl)pyridin-2-yl)isophthalimide (39):}\)

![Diagram](image)

To a solution of amide 44 (0.4 g, 1.05 mmol) in ethanol (20 mL), 10 mL 4N KOH solution was added and the reaction mixture was refluxed for 18 h. After the completion of reaction, monitored by TLC, ethanol was evaporated and 20 mL water was added to the reaction mixture. The aqueous layer was extracted with 3 x 100 mL CHCl\(_3\) and dried over anhydrous Na\(_2\)SO\(_4\). The solvent was evaporated and the crude product was purified by column chromatography using 30% ethyl acetate in petroleum ether as eluent to give the corresponding amine 45 (0.243 g, yield: 78%). To a solution of amine 45 (0.15 g, 0.5 mmol) in dry CH\(_2\)Cl\(_2\), Et\(_3\)N (0.20 mL) was added and this solution was next added to the solution of isophthaloyl dichloride (0.05 g, 0.25 mmol) in dry CH\(_2\)Cl\(_2\) with stirring. The stirring was continued for overnight. After the completion of reaction solvent was evaporated and saturated NaHCO\(_3\) solution (30 mL) added to the reaction mixture. The aqueous layer was extracted with 3 x 50 mL CHCl\(_3\) and dried over anhydrous Na\(_2\)SO\(_4\).
Chapter 2

The solvent was evaporated and the crude product was purified by column chromatography using ethyl acetate as eluent to give 39 (0.190 g, yield: 52%); mp: 58 °C. 

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.94 (s, 2H, -CONH-), 8.53 (s, 1H), 8.25 (d, 4H, \(J = 8\) Hz), 8.15 (d, 2H, \(J = 8\) Hz), 7.78-7.74 (m, 4H), 7.64 (t, 1H, \(J = 8\) Hz), 7.46-7.39 (m, 6H), 7.33 (t, 2H, \(J = 8\) Hz), 7.27 (d, 2H, \(J = 8\) Hz), 6.80 (d, 2H, \(J = 8\) Hz), 4.70 (s, 4H), 4.36 (t, 4H, \(J = 4.4\) Hz), 4.05 (t, 4H, \(J = 4\) Hz).

\(^1\)C (100 MHz, CDCl\(_3\)): 165.4, 156.3, 154.1, 151.5, 138.6, 134.2, 133.9, 131.6, 128.9, 127.2, 126.2, 126, 125.6, 125.3, 124.9, 121.7, 120.3, 117.3, 113.2, 104.6, 73.5, 69.1, 67.6.

FTIR (v cm\(^{-1}\), KBr): 3405, 1690, 1451, 1267, 1133.

Mass (ESI\(^+\)): 719.3 (M + H\(^+\)), 361.

\(N,N',N''-(2,4,6\text{-trimethylcyclohexa-1,3-diene-1,3,5-triy})\text{tris(methylene)}\text{tris(6-(quinolin-8-yloxy)methyl)pyridin-2-amine) (46)}\):

![Image](image.png)

To a solution of 1,3,5-tris(bromomethyl)-2,4,6-trimethyl benzene (0.079 g, 0.197 mmol) and K\(_2\)CO\(_3\) (0.082 g, 0.594 mmol) in CH\(_3\)CN/THF (1:1 v/v, 20 mL) was added dropwise in a CH\(_3\)CN (5 mL) solution of 32 (0.150 g, 0.597 mmol). The mixture was stirred at rt for 72 h. After the filtration and evaporation of the solvent the crude product was purified by preparative TLC using 4% CH\(_3\)OH in CHCl\(_3\) to afford 46 (0.179 g, yield: 30%); mp: 100°C (decomposition).

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.98 (dd, 3H, \(J = 2.5\) Hz, 2 Hz), 8.13 (dd, 3H, \(J = 6.5\) Hz, 2 Hz), 7.45 - 7.41 (m, 6 H), 7.37 (d, 6H, \(J = 4.5\) Hz), 7.10 (t, 3H, \(J = 4.5\) Hz), 6.91
Chapter 2

(d, 3H, J = 7 Hz), 6.37 (d, 3H, J = 8 Hz), 5.41 (s, 6H), 4.51 (d, 6H, J = 4 Hz), 4.32 (br t, 3H, J = 4 Hz), 2.49 (s, 9H).

\(^{13}\)C NMR (125 MHz, CDCl\(_3\)): 153.0, 150.5, 149.3, 144.3, 135.4, 133.2, 131.9, 130.9, 128.8, 124.4, 121.6, 116.6, 114.8, 105.3, 104.9, 100.7, 66.6, 36.6, 24.7.

FTIR (v cm\(^{-1}\), KBr): 3386, 2922, 2852, 1602, 1571, 1499, 1465, 1377, 1317.

HRMS (TOF MS ES\(^{+}\)) calcd for C\(_{57}\)H\(_{51}\)N\(_{9}\)O\(_{3}\) 909.4115 Found: 910.4181 (M + 1), 932.4004 (M+ Na)

\(N,N',N''-(2,4,6\text{-trimethylcyclohexa-1,3-diene-1,3,5-triy})\text{tris(methylene)}\text{tris(6-((naphthalen-1-yloxy)methyl)pyridin-2-amine)}\) (47)

To a solution of 1,3,5-tris(bromomethyl)-2,4,6-trimethyl benzene (0.111 g, 0.278 mmol) and K\(_2\)CO\(_3\) (0.115 g, 0.833 mmol) in CH\(_3\)CN/THF (1:1 v/v, 20 mL) was added dropwise in a CH\(_3\)CN (5 mL) solution of 34 (0.2 g, 0.833 mmol). The mixture was stirred at rt for 48 h. After the filtration and evaporation of the solvent the crude product was purified by column chromatography using 40% ethyl acetate in petroleum ether as eluent to afford 47 (0.249 g, yield: 33%): mp 80 °C.

\(^{1}\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\): 8.40 (dd, 3H, J = 8 Hz, 2 Hz), 7.80 (dd, 3H, J = 8 Hz, 2 Hz), 7.52 – 7.47 (m, 9H), 7.42 (d, 3H, J = 8 Hz), 7.34 (t, 3H, J = 8 Hz), 6.96 (d, 3H, J = 8 Hz), 6.88 (d, 3H, J = 8 Hz), 6.60 (d, 3H, J = 8 Hz), 5.24 (s, 6H), 4.51 (s, 6H), 4.43 (br s, 3H), 2.47 (s, 9H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 157.9, 155.8, 154.3, 138 2, 136.9, 135.1, 134.5, 133.8, 127.4, 126.3, 125.8, 125 2, 122.1, 120.4, 110.0, 105.6, 105.4, 70.8, 41.6, 26.3.

FTIR (v cm\(^{-1}\), KBr): 3412, 2922, 1598, 1574, 1508, 1462, 1396, 1357

Mass (ESI\(^{+}\)): 907.0 (M + H), 761.1, 454.2.
To a solution of 1,3,5-tris(bromomethyl)-2,4,6-trimethyl benzene (0.092 g, 0.23 mmol) and K₂CO₃ (0.095 g, 0.687 mmol) in CH₃CN/THF (1:1 v/v, 20 mL) was added dropwise in a CH₃CN (5 mL) solution of 8-amino quinoline (0.099 g, 0.687 mmol). The mixture was stirred at rt for overnight. After the filtration and evaporation of the solvent the crude product was purified by column chromatography using 50% chloroform in petroleum ether as eluent to afford 48 (0.133 g, yield: 31%); mp: 120°C.

¹H NMR (400 MHz, CDCl₃): 8: 8.67 (d, 3H, J = 4 Hz), 8.02 (d, 3H, J = 8 Hz), 7.43 (t, 3H, J = 8 Hz), 7.35 (t, 3H, J = 8 Hz), 7.07 (d, 3H, J = 8 Hz), 6.82 (d, 3H, J = 8 Hz), 5.99 (br t, 3H), 4.46 (d, 6H, J = 4 Hz), 2.50 (s, 9H).

¹³C NMR (100 MHz, CDCl₃): 146.7, 144.7, 137.9, 137.3, 135.7, 133.4, 128.4, 127.6, 121.2, 113.8, 104.2, 42.9, 15.7.

FTIR (v cm⁻¹, KBr): 3401, 2869, 1573, 1515, 1475, 1379, 1121.

Mass (ESI⁺): 589.2 (M + H), 437.3, 301.3.

Methods for the determination of binding constant (Kₐ) values by UV titration

a) Binding constant determination by dilution method:

General method: Receptor was dissolved in 50 mL dry UV grade CHCl₃. From this solution 25 mL was taken in stoppered volumetric flask and to this carboxylic acid guest was added and sonicated for 10 minutes. The mixture was filtered to remove any insoluble particle. Different solutions of varied compositions of receptor-carboxylic acid complex solution were prepared from this 25 mL stock solution of receptor-carboxylic acid complex by diluting with UV-grade CHCl₃ maintaining the total volume 10 mL. The
different compositions (by volume) of receptor-carboxylic acid solution: CHCl₃ were 10:0, 7:3, 5:5, 3:7, 2:8, 1:9. UV-vis spectra were recorded for receptor itself, receptor-carboxylic acid complex solution and different solutions of varied compositions of receptor-carboxylic acid solution. From the spectral data the binding constants were calculated for the carboxylic acids.

Working formula. \[ \frac{d}{A_o} = \left( \frac{1}{K_A \varepsilon_c} \right)^{1/2} \frac{1}{(A_o)^{1/2} + 1/\varepsilon_c} \]

Where \( d \), \( A_o \), \( \varepsilon_c \) refer to the concentration, absorbance, molar extinction coefficient terms for the receptor-carboxylic acid complex.

The change in absorbance with different concentrations of the complex showed almost a linear dependence. This indicated the 1:1 stoichiometry of the complex.

b) Binding constant determination by continuous variation method:

General method: Binding constants were determined by UV-vis titration methods. Initially the receptor was dissolved in dry UV grade chloroform and taken in the cuvette. Then carboxylic acid guests, dissolved in dry CHCl₃ containing 0.8% DMSO, 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 constants were determined by using the expression

\[ \frac{A_q}{A - A_q} = \frac{[\varepsilon_m(\varepsilon_m - \varepsilon_c)](K_A C_g^{-1} + 1)}{\varepsilon_m} \]

where \( \varepsilon_m \) and \( \varepsilon_c \) are molar extinction coefficient for receptor and the hydrogen-bonding complex, respectively at selected wavelength, \( A_o \) denotes the absorbance of the free receptor at the specific wavelength and \( C_g \) is the concentration of the carboxylic acid guest. The measured absorbance \( A_q/A - A_q \) as a function of the inverse of the carboxylic acid guest concentration fits a linear relationship, indicating 1:1 stoichiometry of the receptor-carboxylic acid complex. The ratio of the intercept to the slope was used to determine the binding constant \( K_A \).

2.7 References and notes


10 "Alpha Hydroxy Acids for Skin Care". *Cosmetic Dermatology, Supplement:* 1-6. October 19.


Chapter 2

1051.


Chapter 2


40. MM2 calculations were performed using CS Chem 3D version 6.0.


42. The quenching efficiency was determined using the equation: $\phi_Q = \frac{(I_{\text{host}} - I_{\text{complex}})}{I_{\text{host}}}$, where $I_{\text{host}}$ and $I_{\text{complex}}$ are the fluorescence intensities (377 nm) of 28 and its complex, respectively.


Chapter 2


47. Energy minimization was carried out using MMX (PC Model Serena Software 1993). Molecular modeling was performed using standard constants, and the dielectric constant was maintained at 1.5.