CHAPTER 4

Design, Synthesis and Characterization of Cystine-based Bis-naphthalimides and Bis-urea Receptors for Fluoride Recognition

ABSTRACT

The first part of this chapter describes the synthesis and characterization of two cystine-derived bis-naphthalimide gelators (L1, L2). The UV-visible and fluorescence spectra of both the ligands displayed similar features in solvents such as acetonitrile and DMF. The fluorescence spectra of both the compounds featured a distinct monomer and long-wavelength excimer emissions in the aforementioned solvents. It was found that the excimer emissions for the two compounds could be preferentially quenched by triethylamine, and subsequently restored with hydrofluoric acid. The stimuli-responsive nature of the excimer emissions was demonstrated using anion stimuli in solution and in the gel phase. Thus, the excimer emission for L1 (or L2) could be switched ‘off’ using fluoride anions, and subsequently re-activated using tetrafluoroborate anions as the chemical stimulus. The various intermolecular interactions involved in the gelation process were investigated through FTIR and NMR studies. Second part of this chapter describes synthesis and characterisation of two cystine based bis-urea ligands. (3U-cys and 4U-cys). Interactions of 3U-cys and 4U-cys with fluoride anion were investigated using UV-visible, fluorescence and NMR studies.
Part A: Design and Synthesis of Cystine-based Bis-naphthalimides for Fluoride recognition

4.1 Introduction

The versatile luminescent characteristics of naphthalimide derivatives have evoked immense interest, evidenced by the frequent use of this fluorophore in optoelectronic materials, as ionophoric sensors/probes and in stimuli responsive systems.[158,159]

Bis-naphthalimide systems, where two naphthalimide motifs are present in close proximity, can generate monomer and excimer emissions, depending on the solvent, molecular environment and distance separating the fluorophores.[160,161] An excimer is a dimeric or hetero-dimeric molecule, formed only in the excited state, when one fluorophore in the excited state interacts with another fluorophore molecule in the ground state via weak $\pi-\pi^*$ stacking interactions.[162] Excimers have short life-time and its formation is diagnosed by red shifted emissions in the fluorescence spectrum, in comparison to the monomer emission.[163] Rigid, planar molecules such as anthracene, pyrene, and fluorene are known to form excimers in concentrated or aggregated states through a large $\pi-\pi$ overlap.[164-166]

In case of bis-naphthalimides derived from 1,ω-diaminoalkanes, the formation of intramolecular excimers was dependent on the spacer length.[129,160,161] In fact, for such bis-naphthalimide derivatives, with increasing spacer length, the excimer quantum yields increased (upto six methylene groups) while the emission maxima were progressively blue-shifted.[129,160,161,168]
The presence of amino-containing spacers often led to substantial quenching of naphthalimide emissions, apparently due to photo-induced electron transfer (PET). Subsequent exposure to protons cause disruption of the PET process, and leads to increase in the emission intensities. For bis-naphthalimides connected through amino-spacers (e.g. spermidine), the addition of protons (or even protic solvents) could lead to the emergence of a broad red-shifted emissions, with substantially enhanced lifetimes and emission quantum yields.[127]

Studies have shown that the monomer/excimer emissions of bis-naphthalimide derivatives can also be modulated by the coordination of metal ions and due to self-aggregation.[159,167] For instance, intramolecular dimerisation and excimer emission was observed for a N-benzocrown-1,8-naphthalimide ligand, following the coordination of Ba^{2+} ions.[159]

Scheme 4.1 Complexation of 81 with Zn^{2+} cation in 1:3 stoichiometry, triggering intramolecular dimerisation of the naphthalimide fluorophores.
The effect of metal ion binding on excimer fluorescence was illustrated by the of a naphthalimide imine ligand (81) with Zn\(^{2+}\) ions. In this example, the formation of 3:1 complex between naphthalimide–imine ligand, 81, and Zn(OTf)\(_2\) was accompanied by enhanced intramolecular excimer emissions at 470 nm.[167] Again, this excimer emission could be switched, by changing the metal/ligand stoichiometry (Scheme 4.1).

![Scheme 4.1 Proposed mechanism of stepwise complexation of 82 with Cu\(^{2+}\), and facilitating excimer formation](image)

In another instance, a piperazine bridged bis-naphthalimide ligand, 82, was shown to switch between the monomer and excimer fluorescence upon coordination of Cu\(^{2+}\) ion. [126,167] In the metal free environment, 82 produced dual emission bands at 450 nm and 550 nm, attributed to the naphthalimide monomer and excimer emission respectively. The monomer emissions at 450 nm could be enhanced by the addition of
excess Cu$^{2+}$, wherein addition of first equivalent of Cu$^{2+}$ caused no changes in the emission properties of 82 (Scheme 4.2).

However, compared to metal ions, the interactions of bis-naphthalimides with halide anions, particularly fluoride, and the effect of such interactions on their monomer/excimer emissions remain relatively elusive.[132,152,161] In this connection, we sought to examine the prospect of anion-induced switching of excimer and monomer emissions of the bis-naphthalimides. An important challenge in this regard is to develop a stimuli-responsive system, which could respond to fluoride anions through changes in monomer/excimer emission of bis-naphthalimides.
4.2 Results and Discussion

We synthesized and characterised two L-cystine-based bis-naphthalimides, \textbf{L1} and \textbf{L2} (Scheme 4.3) in two steps from 1, 8-naphthalic dianhydride and the corresponding 3-aminopropionic and 4-aminobutanoic acids.

![chemical structure](image)

Scheme 4.3 Synthesis of the ligands \textbf{L1} and \textbf{L2}

In a typical experiment, \textbf{ND1}, was synthesised by condensing naphthalene-1,8-dicarboanhydride (1, 8-NDA) and 3-aminopropanoic acid, and then it was coupled to L-cystine methyl ester using EDC–HOBT coupling strategies (Scheme 4.3); the desired bis-naphthalimide \textbf{L1} (or \textbf{L2}) was obtained as a solid, and characterised using $^1$H/$^{13}$C NMR and mass spectroscopy.

Since \textbf{L1} and \textbf{L2} had two 1,8-naphthalimide motifs linked by a disulfide spacer, we speculated that the molecules could generate monomer and eximer emission depending on the nature of aggregation. Accordingly, we undertook spectroscopic studies of \textbf{L1} and \textbf{L2} in different solvent systems, varying from CHCl$_3$, CH$_3$CN to DMF.
4.2.1 UV-visible and fluorescence studies

As shown in Figures 4.1a and 4.2a, the concentration dependent UV-visible spectra of L1 were L2 markedly similar. Both exhibited distinct absorptions at 334 nm with a shoulder at 346 nm. The fluorescence spectra of the two compounds also displayed similar features. For instance, when irradiated at 340 nm in acetonitrile, L1 produced two emissions at 381 nm and 465 nm (Figure 4.1b); of these, the emission at 381 nm was characteristic of the naphthalimide monomer, while the broad emission at 465 nm was attributed to the intramolecular dimerisation of the naphthalimide motifs. Similarly, the irradiation of L2 at 340 nm in acetonitrile produced two emissions at 382 nm and 467 nm, which corresponded to the naphthalimide monomer and excimer emissions.

![Figure 4.1 Concentration dependent (a) UV-visible and (b) fluorescence spectra of L1 in MeCN](image)

The formation of intramolecular naphthalimide dimers (i.e. excimers) could be recognised from the relative intensities of the monomer-dimer emissions. For instance,
the ratio of monomer and excimer emissions of \( \text{L1} \) at 381 nm and 465 nm was almost constant (\( I_{381/465} = 1.16 \pm 0.15 \)) for concentrations up to 0.015 mM.

Notably, the formation of intramolecular excimers between the two naphthalimide motifs for \( \text{L1} \) and \( \text{L2} \) (> 12 C–C bonds) was comparable to the previous reports for bis-naphthalimides linked by oligoethylene spacers.[161,169] The formation of intramolecular naphthalimide dimers (i.e. \( \text{L1} \) and \( \text{L2} \)) in the ground state was in agreement with the fact that the excitation spectra obtained in both the cases were similar. From these spectral features, we inferred that excimer emissions for \( \text{L1} \) and \( \text{L2} \) originate from intramolecular dimerisation of the naphthalimide moieties in the ground state (Scheme 4.4). The formation of such excimers was possibly favoured by intramolecular hydrogen bonds invoking the amide NH groups, the hydrophobic interactions between the naphthalimide groups, given the conformational flexibility of the cystine disulfide group.
Scheme 4.4 Possible formation of intramolecular dimers of the bisnaphthalimide motifs (L1) depicting the influence of intramolecular N–H···O interactions, and cystine S-S bridges

4.2.2 Effects of acid-base stimuli on L1 and L2

A stimuli-responsive system should be able to recognize an external stimulus (e.g. physical or chemical input), evaluate the inputs, and then respond through changes in its physical and/or chemical characteristics.[158,159,171] Among the various stimuli-responsive systems identified to date there has been a considerable interest in developing new types of luminescent system because of their potential applications as functional materials [172, 173].

In a related study, we noted that the addition of a 30-amine (i.e. triethylamine) to bis-naphthalimides L1 (or L2) in acetonitrile triggered a reduction in their emission profiles, particularly the long wavelength excimer emission. As shown in Figure 4.3, the addition of triethylamine to L1 in acetonitrile led to the substantial quenching of the 465 nm emission, compared to the monomer emission at 381 nm. This result is understandable because the amino-compounds have been known to induce the quenching of long-wavelength emissions via PET, as reported earlier for naphthalimide(-spermidine) derivatives.[174,175]
Figure 4.3  Effect of triethylamine (1.5 mM) on the emission of \textbf{L1} (0.001 mM.), in acetonitrile (inset: plots of $I/I_0$ vs. [triethylamine] for the emissions occurring at 381 nm and 465 nm).

Figure 4.4 Changes in the fluorescence spectra of the \textbf{L1}–triethylamine mixture following the addition of hydrofluoric acid (aq., 3.0 mM) ; (inset: plots of $I/I_0$ vs. [HF], for the emissions occurring at 381 nm and 465 nm).
Following this, we examined the influence of acid on the L1–triethylamine mixture, anticipating that the protonation of the 30-amine would block the incipient PET process, and hence restore the excimer fluorescence at 465 nm. Accordingly, the L1–triethylamine solution was titrated using hydrofluoric acid (aq. HF, 3.0 mM); the emission spectra obtained during the acid titration clearly revealed that the acid stimulus could switch ‘on’ the excimer emissions at 465 nm (Figure 4.4). Moreover, using the same amine–acid combination as stimuli, we could induce multiple cycles of reversible quenching and restoration of the 467 nm emissions of L2 in acetonitrile.

The amine-induced quenching of the excimer emissions of L1 and L2 could also be observed in solvents such as DMF and DMF-water. This result is in accordance with the suggestion that the fluorescence behaviour of substituted naphthalimides can be influenced by protons, and/or by changes in pH.[158,151] These features were complementary to two-step fluorogenic switches, demonstrated for 20-amine derived, intramolecularly quenched bis-naphthalimide systems, which could be activated using protons as inputs [170, 172].

4.2.3 Effects of fluoride anion on the fluorescence responses of L1 and L2

Notwithstanding the acid/base or proton-dependent switching of naphthalimide fluorescence in L1 and L2, we were curious to examine the effects of fluoride (i.e. TBAF) as anion stimuli on the monomer/excimer fluorescence of the bis-naphthalimide derivatives, and hence compare the affect of halide anions, including BF4- anions. We have already mentioned how amide NH group behave as potent hydrogen bond donor to strong acceptors such as fluoride anions.[23,30,45] Accordingly, we performed
fluorescence titrations of bis-naphthalimide L1 with fluoride anions (i.e. TBAF) in acetonitrile. As shown in Figure 4.5a, the incremental addition of fluoride (up to 20 equiv.) to L1 caused the emission intensity at 465 nm to gradually diminish, whereas the monomer emission at 381 nm increased 2-fold. Notably, the changes in fluorescence at 381 nm and 465 nm for L1 with increasing fluoride concentration were such that $I_{381/465}$ increased to 18.3.

![Figure 4.5 Variations in the fluorescence emissions at 381 nm and 465 nm for L1 (0.0005 mM; acetonitrile) (a) upon the addition of TBAF (inset shows relative changes in emissions, $I/I_0$ vs. [TBAF], at 381 nm and 465 nm); (b) following the addition of BF$_4^-$ anions to the L1–TBAF mixture (inset shows the relative changes in fluorescence, $I/I_0$ vs. [NaBF$_4$], at 381 nm and 465 nm).](image)

Subsequently, we noted that the effects of other halide anions on the excimer fluorescence were minor. Surprisingly, the addition of BF$_4^-$ anion (approx. 50 equiv.) to L1/TBAF produced an enhancement in both the monomer and excimer emissions (as seen in Figure 4.5b). This enhancement of excimer fluorescence is unusual because it
appeared to override the quenching effects of the fluoride anions, due to which the 465 nm emission was ‘switched-off’. Furthermore, the changes in the fluorescence output of \( \text{L1} \) brought about by the addition of fluoride anions, and subsequent addition of \( \text{BF}_4^- \) anion could be monitored visually under 365 nm illuminations (Figure 4.6).

Figure 4.6 Changes in the emissions of \( \text{L1} \) upon successive addition of TBAF, and NaBF\(_4\) in DMF, as viewed under UV-illumination (365 nm).

![Figure 4.6](image)

Figure 4.7 (a) Gradual disappearance of the excimer emission of \( \text{L2} \) (0.0004 mM; acetonitrile) at 467 nm upon addition of TBAF; (b) excimer emission at 467 nm ‘restored’ following the addition of \( \text{BF}_4^- \) anions to the \( \text{L2–TBAF} \) solution; (inset: rel.
changes in emissions, $I/I_0$ vs. [TBAF], and then [NaBF₄] monitored at 382 nm and 467 nm).

Similarly, we monitored the fluorescence responses of L₂, in acetonitrile, subsequent to the addition of fluoride anions. As shown in Figure 4.7, the incremental addition of the fluoride anion to L₂ was accompanied by a gradual disappearance of the excimer emission at 467 nm, while the monomer emission at 382 nm increased 1.5 times, with saturation at 25 equiv. of the anion. Furthermore, the addition of BF₄⁻ anions (approx. 55 equiv.) to the solution of L₂–TBAF caused the excimer emission at 467 nm to be restored, analogous to L₁.

Figure 4.8 Fluorescence profiles of L₂ (1) following successive addition of TBAF (2) and NaBF₄ (3) in acetonitrile, as observed under UV-illumination (365 nm) in DMF.

The abovementioned results, viz., quenching of the excimer emissions of L₁ and L₂ in the presence of fluoride anions, could be analysed as follows: First, the interaction of fluoride anions with the amide NH groups (of L₁ and L₂) could disrupt the formation of intramolecular excimers between the naphthalimide motifs. Indeed, the quenching of the excimer fluorescence in naphthalimides through fluoride-induced disruption of intramolecular hydrogen bonds has been reported earlier [175]. Alternatively, the anion–π interactions of the proximal fluoride with the naphthalimide motif could
facilitate a partial PET process, thereby leading to the quenching of the excimer fluorescence. Second, the quenching effects of fluoride anions were rather suppressed in polar solvents such as aqueous DMF and in the presence of MeOH. This indicates that in protic solvents, such as MeOH or aqueous DMF, the electronegative fluoride anions would preferably be hydrated, than the hydrogen bond to the amide NH group of the bis-naphthalimide.

In comparison, the effects of chloride, bromide and acetate anions on the bis-naphthalimide fluorescence were relatively minor, although acetate anions produced minor quenching of excimer emissions under identical conditions.

Moreover, the enhancement of monomer emissions in both the cases, L1 and L2, following the addition of fluoride and BF$_4^-$ anions was notable. Again, compared to the highly electronegative fluoride anion, the effects of BF$_4^-$ anions were expected to be relatively soft and unlikely to form strong hydrogen bonds. Although the precise nature of the interactions of BF$_4^-$ with the bis-naphthalimide was not clear, we inferred that the BF$_4^-$ anion could assist the dimerisation of the naphthalimide motifs and impede the fluoride-induced PET process. This proposition seems reasonable given the results obtained from the FTIR and $^1$H NMR investigations of the L2/NaBF$_4$ system.

As shown in Figure 4.9a, the addition of NaBF$_4$ to L2 in DMSO-d$_6$ caused perceptible complexation-induced upfield shifts for the naphthalimide CH resonances. However, in acetonitrile-d$_3$ as solvent, the interaction studies of L2 with NaBF$_4$ were inconclusive, apparently due to rapid gel formation, and limited solubility of the salt. Nevertheless, these results were consistent with previous reports that BF$_4^-$ and PF$_6^-$
anions facilitate anion–π interactions with hetero-aromatics, including electron deficient systems.[176]

- a)

![Naphthalimide CH resonance](image)

- b)

![Chemical-shift changes](image)

- c)

Figure 4.9 (a) Partial $^1$H NMR spectra of L2 (3mg in DMSO-d$_6$) following addition of BF$_4^-$ anions (as NaBF$_4$); (b), (c) The chemical-shift changes for the naphthalimide CH resonances were noteworthy, which were indicative of anion–π interactions between the BF$_4^-$ anions and the naphthalimide motif.
Figure 4.10 FTIR spectra of (a) L2/acetonitrile gel, and (b) upon addition of BF$_4^-$ anions (L2/NaBF$_4$). Changes in the amide NH absorption and the aromatic region are noteworthy.

The effect of the fluoride anion on the emissions of L1 (or L2) was also illustrated using NaF; despite its low solubility, the addition of NaF to bis-naphthalimide induced quenching of the excimer emission, albeit minor compared to TBAF. This quenching of the excimer vis-a`-vis monomer emissions by NaF could subsequently be reversed upon the addition of BF$_4^-$ anions.

### 4.2.4 Gel formation and its characterization

Interestingly, both bis-naphthalimides L1 and L2 produced fluorescent gel phases in DMSO and DMF; in case of DMSO, the critical gelation concentrations (CGC) were found to be 3mg/mL for L1 and 5mg/mL for L2. Figures 4.11a and 4.11b show the scanning electron microscopic (SEM) images of the gels obtained from L1 and L2 in DMSO, which indicated formation of fibrous networks involving the gelator molecules.
Similar self-assembled entangled networks could be visualised in case of L2/DMF gel (Figure 4.11c, d), and L2/acetonitrile gel, which on close inspection revealed fibres with dimensions > 2 μm.

![Figure 4.11 SEM images of the dried organo gel obtained from (a) L1 in DMSO; (b) L2 in DMSO; (c), (d) L2/DMF gel under different magnifications](image)

The critical gelation concentrations for L1 and L2 in DMF were found to be 3mg/mL and 6mg/mL respectively. Moreover, both L1 and L2 produced thermo-reversible gels in chloroform and acetonitrile that could also be triggered by ultra-sonic treatment.
However L1 organogel obtained from DMF shows different features. As shown in Figure 4.12, the particles were initially formed due to spontaneous aggregation of the L1 gelator (as tiny nucleation sites) while the formation of fibres was relatively slow (ageing 2-3 days). Such features were not observed for L2 in DMF, except at high concentration (>12mg/mL). It appears that the gelation of acetonitrile and DMF by L1 and L2 occurs due to hydrogen bonding between the amide NH groups and the solvent (immobilisation of the solvent molecules), with concomitant hydrophobic packing (stacking interactions) of the bis-naphthalimide motifs, which result in the formation of networks of self assembled fibres.

Figure 4.12 (a, b) SEM images of the dried organo gel obtained from L1 in DMF (5mg/mL) under different magnifications

We proposed that the gelation process involving bis-naphthalimide L2 (or L1) could be driven by multiple supramolecular interactions, viz., intermolecular hydrogen bonding involving the amide and naphthalimide groups and π–π interactions between naphthalimide motifs. Preliminary insights into the nature of the intermolecular interactions in gel phase vis-a-vis solution could be obtained from fluorescence experiments. For instance, the L2/DMF organogel was characterised by emissions at
399 nm and 468 nm respectively, whereas, the corresponding emissions for \textbf{L2} (0.004mM) in dilute DMF solution occurred at 382 nm and 460 nm (Figure 4.13). Such red-shifting of the fluorescence emissions were evident for both \textbf{L1} and \textbf{L2} in the gel phases, as compared to solution. In particular, the red-shift observed in the emission spectra for \textbf{L2} in the gel state were consistent with weak aromatic-\pi interactions of the naphthalimide motifs. Given that \textbf{L1} and \textbf{L2} exhibit red-shifted excimer emissions, particularly in the gel state, also provided support to the possible intramolecular dimerisation of the pendent naphthalimide groups.

Figure 4.13 Fluorescence spectra of \textbf{L2} under various solvent conditions (\(\lambda_{\text{ex}} 340\text{nm}\)).

FT-IR studies provided valuable insights into the driving forces for gelation process. Figure 4.14 represents a series of FTIR spectra recorded for \textbf{L2} under various condition from which the effect of hydrogen bonding interactions (intra and intermolecular) in the gelation process could be illustrated. In dilute solution, the FT-IR spectra of \textbf{L2} (0.3mg/mL) indicated a distinct amide NH absorption at 3303 cm\(^{-1}\); the absorption at 3303 cm\(^{-1}\) remained relatively unaffected within the gelator [\textbf{L2}] =1.0mg/mL, and emanated from intramolecular hydrogen bonds involving the amide
NH groups. However, at this point, a broad absorption appeared at 3470 cm$^{-1}$ in chloroform/ acetonitrile, which was shifted to 3440 cm$^{-1}$ with substantial broadening upon gel formation $[\text{L2}] = 2.0\text{mg/mL}$ that could be ascribed to the formation of extensive hydrogen bonded network.

![FT-IR spectra comparison](image)

Figure 4.14  Comparative study of FT-IR spectra of L2 under various conditions: (a) 0.1mg/mL in chloroform; (b) 1.0mg/mL in chloroform; (c) dried L2/acetonitrile gel, after removal of solvent; (d) L2/acetonitrile gel, when suspended in chloroform; the absorptions at 3303 cm$^{-1}$ in dilute solutions and in the aromatic region were distinct, of which the latter was apparently affected by changes in gelator concentration.
Figure 4.15 Comparative study of FT-IR spectra of L1 under various conditions: (a) L1/DMF gel after removal of solvent; (b) L1/DMF, when suspended in chloroform; (c) L1 when suspended in acetonitrile; the absorptions at 3446 cm\(^{-1}\) in dilute solutions was affected by changes in gelator concentration.

The crucial role of hydrogen bonding during the gelation process was also investigated using \(^1\)H NMR in chloroform-\(d\) and acetonitrile-\(d_3\). As indicated by \(^1\)H NMR, increasing the L2 concentration from 0.3mg/mL to 2mg/mL in chloroform-\(d\) did not affect either the naphthalimide CH or amide NH resonances significantly (Figure 4.16). The amide NH resonance at 7.078 ppm in dilute solutions, i.e. \([L2] < 2mg/mL\) remains relatively unaffected by changes in gelator concentration. Beyond the critical gelation concentration of 6mg/mL, the amide NH resonances shifted downfield, to 7.082 ppm, such that \(\Delta \delta = 0.02\) ppm, indicating that hydrogen bonding interactions were not dominant. However, the onset of gelation did cause the naphthalimide CH
resonances to shift upfield by ~ 0.01ppm; for instance, from 7.723 (i.e. spectra ‘a’) to 7.714 ppm (i.e. spectra ‘f’), and from 8.17 ppm to 8.16 ppm.

Figure 4.16  Concentration dependent $^1$H NMR spectra of L2 in CDCl$_3$ (a) 0.3mg/mL; (b) 1.0 mg/mL; (c) 2.0 mg/mL; (d) 4.0 mg/mL; (e) 6.0 mg/mL, with formation of partial gel; and (f) after the gelation process is complete.

Given that the weak hydrogen bonding ability of chloroform under these conditions, we anticipated intra-molecular hydrogen bonds between the amide NH groups to persist. These features were consistent with the observations made in the FT-IR spectra for L2 in chloroform. So, a clear correlation could be seen in the behaviour of the amide NH groups, with regard to intra-molecular hydrogen bonding, both from FT-IR and $^1$H NMR studies. Therefore, we inferred that formation of L2/chloroform gel was driven by aromatic-π stacking interactions, assisted by hydrogen bonding interactions between the amide NH groups.
Figure 4.17 Partial $^1$H NMR spectra of L2 (5mg/mL) showing the temperature dependent variations of the naphthalimide CH and amide NH resonances in acetonitrile-d$_3$: (a) 50°C; (b) 45°C; (c) 35°C; (d) 25°C; (e) 25°C after 12h; (The two sets of naphthalimide CH resonances could be attributed to the inequivalence of the naphthalimide motifs in the solution/sol state, designated as ‘●’, vis-a-vis the gel phase, indicated as ‘■’.

Following this, we sought to examine the effect of gelation on the naphthalimide CH and amide NH groups of the L2/acetonitrile gel using $^1$H NMR spectroscopy. Because L2 caused the rapid gelation of acetonitrile, it seemed pertinent to carry out temperature-dependent $^1$H NMR analysis of the L2–acetonitrile-d$_3$ system. As shown in Figure 4.17, the solution of L2 in acetonitrile-d$_3$ at 50°C produced a distinct set of resonances for the naphthalimide CH protons at 7.70, 8.20 and 8.42 ppm along with a minor set of resonances at 7.82, 8.34 and 8.54 ppm respectively; the integrated ratios for the major and minor signals were found to be ~9:1. At 50°C, the minor resonances were attributed to L2 molecules in the incipient gel, while the major resonances at 7.70,
8.20 and 8.42 ppm, originated from the solvated $\text{L}_2$ molecules. In other words, the two sets of resonances produced by the naphthalimide CH groups in acetonitrile (cf. in chloroform the signals were minor), were reminiscent of the inequivalence of the naphthalimide motifs in the solution/sol state vis-a-vis the gel phase [177]. With lowering of temperature, the set of naphthalimide CH resonances at 7.82, 8.34 and 8.54 ppm gradually gained prominence. Thus, the intensities of the two sets resonances became almost comparable at 25°C, and concomitantly gelation was initiated. The gradual changes observed in the naphthalimide CH resonances as function of temperature (Figure 4.17) reflects the importance of aromatic-π interactions during the gelation process.

It was noted that gel formation was accompanied by the appearance of an additional signal due to the aliphatic CH groups connected to the naphthalimide motif (Figure 4.18). However, the effect of gelation on the amide NH resonances of $\text{L}_2$ was minor, with a gradual shift from 7.07 ppm in solution (50 °C) to 7.12 ppm in the gel phase. With an increase in temperature, the amide NH resonance (of $\text{L}_2$) was shifted upfield; such changes could be explained by the breaking of the intermolecular hydrogen bonds associated with the amide NH group, viz. with the solvent and neighbouring gelator molecules.

As illustrated in Scheme 4.5, the emergence of downfield shifted resonances for the naphthalimide CH protons at 7.82, 8.34 and 8.54 ppm following gel formation was illuminating because it corroborated the presence of $\text{L}_2$ both as solvated species and in the gel phase. Moreover, noteworthy was the splitting/broadening of these resonances due to the cys-methyl ester group, which could be correlated to the proximal
interactions of this residue with the naphthalimide motifs groups during the formation of the L2/acetonitrile organogel (Figure 4.18).

![Image of NMR spectra](image)

**Figure 4.18** Partial $^1$H NMR spectra of L2 showing the temperature-dependent variations of the naphthalimide CH and amide NH resonances in acetonitrile-d$_3$: (a) 50°C; (b) 45°C; (c) 35°C; (d) 25°C; (e) 25°C after 12h. Splitting/broadening of the resonance due to the ester group (Cys) also indicated multiple interactions of this residue during the gel forming process.

The temperature-dependent self-assembling process of L2 in acetonitrile clearly reflected the importance of the aromatic–π stacking interactions in its gelation behaviour, which was supplemented by hydrogen bonds between the gelator molecules. Based on the results of the $^1$H NMR experiments, and given the red-shifted emissions observed for L2 in the gel phase, it seems reasonable that the naphthalimide...
motifs interact in a head-to-tail manner in the gel phase [168, 178].

Scheme 4.5  Formation of intramolecular hydrogen bonds for L2 in solvents such as acetonitrile, showing the plausible interaction of the F⁻ anions (i.e. TBAF) with the bis-
naphthalimide; The importance of aromatic–π and hydrogen bonding interactions between the gelator molecules during gel formation and fluoride-induced gel-to-sol transformation have been illustrated (inset: photographic images of the \( \text{L}_2/\text{acetonitrile} \) gel and solution \([\text{L}_2]=5\text{mgmL}^{-1}\)).

Moreover, as monitored by \(^1\text{H} \text{NMR}\), the onset of gelation for \( \text{L}_2/\text{acetonitrile-d}_3 \) (or chloroform-d) was marked by lower intensity signals, compared to those in solution. This is understandable because the formation of the \( \text{L}_2/\text{acetonitrile} \) gel restricted a larger proportion of \( \text{L}_2 \) gelator molecules to enter the gel structure, thereby reducing the thermal motions of the gelator molecules. Such features have been noted earlier in aromatic systems following aggregate-formation [179].

On the basis of these observations, it is inferred that the self assembly of the gelator molecules of \( \text{L}_2 \), in acetonitrile or chloroform is driven by aromatic–π stacking between the naphthalimide motifs and supplemented by hydrogen bonds involving the amide NH groups (Scheme 4.5). As mentioned earlier, the FTIR analysis indicated the presence of intramolecular hydrogen bonds in \( \text{L}_2 \), which in turn could explain the observations of excimer emission in the fluorescence spectra. This intramolecular nature of the incipient hydrogen bonds was also reflected in the \(^1\text{H} \text{NMR}\) spectra, with the amide NH resonances showing only minor downfield shifts. Considering these aspects, we inferred that hydrogen bonding between the amide NH groups were favoured when the gelator molecules adopted bent conformations. Previous studies have shown the importance of intramolecular hydrogen bonding interactions during the formation of self-assembled gels, and how such interactions were favored when the gelator molecules adopted bent conformations [177]. Again, these polar interactions facilitated the
hydrophobic packing of the naphthalimide groups (Scheme 4), such that the gelator molecules slowly self-assembled into entangled fibrous networks. In fact, the influence of the hydrophobic aromatic–π interactions between the naphthalimide groups and segregation of hydrophilic interactions during the gel formation process was recently reported [176, 179].

4.2.5 Gel-to-Sol transformation

Our studies have revealed that the luminescent properties of L1 and L2 can be tuned by acid/base stimuli. Again, we observed that L1 and L2 could serve as efficient organogelators. At this point, we speculated whether L1 and L2 gels could also exhibit stimuli-responsive behaviour towards anions such as fluoride. Subsequently, we found that the addition of fluoride anion to L1 gel in acetonitrile triggered collapse of the gel.

We reasoned that the gel-to-sol transformations emanated from the disruption of the hydrogen bonded network involving the gelator molecules, apparently initiated by the coordination of the fluoride anion to the amide NH groups. This effect of TBAF on the hydrogen bonding network of the L2–chloroform system could be identified by the disappearance of the absorption at 3304 cm$^{-1}$ in the FTIR spectra (Figure 4.19).

Further support for the effects of TBAF on the gel-to-sol transformation and the stimuli-responsive nature of the bis-naphthalimides, L1 and L2, could be obtained from $^1$H NMR studies. As evident from the $^1$H NMR spectra, the addition of TBAF (1.0 equiv.) to the L2/acetonitrile gel caused the shifting of the amide NH resonance from 7.15 to 6.87 ppm, along with splitting (figure 4.20). The addition of 10 equiv. of TBAF
was accompanied by a gradual dissolution and collapse of the organogel network (Scheme 4.6).

Figure 4.19 FTIR spectra of (a) $L_2$ (1.0mg/mL in chloroform); (b) $L_2$/acetonitrile gel, after removal of solvent, and (c) upon addition of fluoride anions (as TBAF, ~ 20uL of 1mmol stock); Changes in the amide NH absorption at 3304cm$^{-1}$ and the aromatic region are noteworthy

Further support to the hydrogen bonding situation of amide NH groups could be obtained by gradual addition of TBAF (upto 3 equiv.) to the $L_2$/chloroform gel, which caused downfield shift of the amide NH resonance from 7.09 to 7.23 ppm (Figure 4.21) with partial dissolution of the gel
Figure 4.20. (a) Partial $^1$H NMR of L2/acetonitrile-d$_3$ gel at room temperature; and (b) disruption of hydrogen bonds involving the amide NH groups, induced by the addition of 1.0 equiv. of fluoride anions.

Figure 4.21 Partial 1H NMR spectra showing the effect of TBAF on L2 (3.0 mg in 0.5mL chloroform-d), during the gel-to-sol transformation; (a) Partially formed
Scheme 4.6 Plausible hydrogen bonding interactions of L2 with fluoride anions, i.e. TBAF, in chloroform-d
Part B: Design and synthesis of L-cystine based bis-urea system

4.3 Introduction

On the basis of the properties displayed by the cystine based bis-naphthalimide gelator with fluoride anions, we envisaged to examine the nature of interaction of fluoride with an analogous bis-urea system. It is known that the urea group can serve as efficient hydrogen bond donors for binding anionic guests. In an early example, Hamilton et.al has described the design of a bis-urea ligand that was capable of binding dicarboxylates with high affinities \([109b]\). Subsequent studies also suggested that binding of anionic guests to urea receptor is dependent on the rigidity of the receptor framework \([180,181]\). Rigid cyclic bis-urea derivatives were found potentially useful for binding of anions with good selectivity \([182]\).

The chelating tendencies of bis-urea receptors have been increased by the introduction of rigid spacer group. ‘Cholapods’ represent an example of such molecule, wherein two urea subunits have been connected to the cholic acid skeleton, form stable complexes with chloride and bromide anions\([183]\).

Another example of rigid bis-urea framework is \(83\), which works as a efficient receptor for halide anion. On protonation of the pyridine N atom stable salt was formed and subsequently, spectrophotometric titration experiments with halides were studied in acetonitrile by monitoring a new red shifted band at 422 nm. 1:1 complex formation were noticed with high association constant (Cl\(^-\), \(\text{log}K = 4.6\); Br\(^-\), 4.8; I\(^-\), 4.9.) Crystal
structure of the chloride complex of the salt showed that chloride was attached to five hydrogen bond, four from the urea N-H fragment and one from the pyridinium N-H fragment, which accounted for its stability.

Macrocyclic urea hosts are known to show interesting anion binding ability, the design of flexible receptors can help us understand important aspects related to sequestering effects in anion binding and molecular adaptation through hydrogen bonding interactions.

A class of macrocycles 84 and 85 were reported in which three urea subunits were linked by xanthene and diphenyl ether spacers. Notwithstanding the same nature of the spacer, receptor 84 was firmly rigid, while 85 exhibited high degree of flexibility. Spectrophotometric studies revealed that a 1:1 Cl⁻ complex was formed with flexible macrocycle 85 which was 25-fold more stable than the Cl⁻ complexes of rigid macrocycle 84, logK = 6.0 ±0.1 and 4.6 ± 0.2, respectively, [Bu₄N]⁺ salts). These results attributed to the ability of the flexible receptor to place the N-H fragments in most favourable positions for the formation of hydrogen bonds with chloride. Further it was observed that addition of excess chloride led to the formation of 1:2 complexes with receptor 85, but not with receptor 84. This also attributed to the flexible nature of
receptor 85, due to which it could rearrange the urea subunits in order to interact with two chloride ions. [185]

Some flexible bis-urea systems have the advantage of selectively bind with anions with different coordination modes, because binding of anions triggered conformational changes in such molecules.

For instance, a tetradeutate bis(urea) ligand 86 was reported, which underwent conformational changes with respect to binding of different anions. Upon addition of the Cl⁻ anion, the ligand preferred a Z-shaped conformation, wherein Cl⁻ was hydrogen
bonded to both the NH protons of one urea group and a CH proton from a pyridine unit through three hydrogen bonds. On the other hand, upon replacement of the Cl\textsuperscript{−} anion by the HSO\textsubscript{4}\textsuperscript{−} the ligand adopted the U- or S-shaped conformation, where only NH hydrogen bonds were observed [186].

Thus, bis-urea ligands help to understand the role of intra-molecular interactions in conformationally mobile systems, compared to systems in which the bis-urea motif was restricted to a rigid platform. In this connection, we hypothesised that bis-urea derivatives with Cystine Bridge could serve as good anion receptors capable of responding to anion stimuli through changes in the molecular orientation. For that we have developed cystine based isomeric bis-urea system 3U-cys and 4U-cys and studied their interactions with fluoride anion.

4.4 Results and Discussions

The receptors were synthesised in one step from the corresponding cystine methyl ester.

![Scheme 4.7 Synthesis of 3U-cys and 4U-cys](image)

As shown in Scheme 4.7, the reaction of 3-urea carboxylic acid (UREA 2) and 4-urea carboxylic acid (UREA 3) with cystine methyl ester provided the respective bis-urea
receptors 3U-cys and 4U-cys. The receptors were characterised using $^1$H, $^{13}$C and mass spectrometry.

We envisage that urea based cystine ligands (3U-cys and 4U-cys) could adopt either an open or a closed conformation in response to the binding of anion, (Scheme 4.8)

```
\[ \text{Open} \quad \text{Closed} \]
```

Scheme 4.8 Conformational changes proposed for U-cys ligands

Such changes in the orientation of the urea motifs will be possible due to the flexibility of the S-S bond, and could help us examine how urea-anion interactions lead to changes in molecular conformation.

### 4.4.1 Spectroscopic investigations

Because of the poor solubility of ligands 3U-cys and 4U-cys in solvent like acetonitrile, methanol and THF etc, we did the spectroscopic studies in DMSO. The absorption spectra of the two isomer were markedly different. Ligand 3U-cys was characterised by a weak absorption at 260 nm, whereas 4U-cys produced a broad absorption at 280 nm (Figure 4.21a).
Similarly their fluorescence emission spectra also displayed different features. When excited at 300 nm in DMSO 3U-cys produced a sharp emission at 352 nm whereas 4U-cys produced a broad emission at 425 nm with a shoulder at 345 nm (Figure 4.21b).

We anticipated that the difference observed in the absorption and emission behaviour of 3U-cys and 4U-cys may originated from the extended conjugation for 4U-cys as shown in Scheme 4.9

Scheme 4.9 Extended conjugation shown for 4U-cys
4.4.2 Fluoride recognition studies

At this point, we were curious to examine the effect of fluoride on the UV-visible and fluorescence properties of 3U-cys and 4U-cys and to understand whether fluoride binding could trigger any change in the molecular orientation of the ligands.

As shown in Figure 4.22a, addition of 0.5 equivalent of fluoride to 3U-cys, increased its absorption at 462 nm wherein further addition of fluoride caused saturation. Similar changes were observed in the UV-visible spectra of 4U-cys, following the addition of fluoride (Figure 4.22b).

![Figure 4.22](image)

Figure 4.22 Changes observed in the UV-visible spectra of (a) 3U-cys and (b) 4U-cys (5×10⁻⁵ mmol) following the addition of TBAF.

Figure 4.23 shows the fluorescence titration spectra of 3U-cys (5×10⁻⁵ mmol) with TBAF in DMSO. Initial addition of 10 equivalent of fluoride diminished its original emission at 355nm, but further addition of fluoride led to the appearance of a new red shifted emission at 483 nm. Appearance of red-shifted emission indicated
intramolecular charge transfer originating from fluoride induced deprotonation of the urea NH at high concentration.

![Figure 4.23](image)

**Figure 4.23** (a) Changes observed in the fluorescence spectra of 3U-cys (5×10⁻⁵ mmol) following the addition of fluoride (upto 40 equiv.) (b) Variation in the emission intensity of 3U-cys with respect to equivalence of fluoride at 355nm and 483 nm.

In comparison to 3U-cys, changes observed in the emission spectra of 4U-cys following the addition of fluoride are completely different. As shown in figure 4.24, emission of 4U-cys decreased initially up to addition of 12 equivalent of fluoride. Further addition of fluoride enhanced the emission again at a new wavelength of 396 nm, which is blue shifted to its original emission at 425 nm. A ON-OFF-ON fluorescent nature of 4U-cys following the addition of fluoride indicated the formation of 1:2 urea/fluoride complex at high fluoride concentration.

NMR titration studies gave valuable informations about the nature of urea-fluoride interactions in 3U-cys and 4U-cys. As shown in figure 4.25, in the absence of anions, urea NH of 3U-cys resonate at around 9 ppm. Addition of incremental amount
of TBAF, led to downfield shifting and broadening for the urea NH signals. However deprotonation was not observed up to addition of 4 equiv of fluoride. This result is consistent with the fluorescence spectral changes observed for 3U-cys following the addition of fluoride, which shows the appearance of red shifted emission only at high concentration of fluoride.

Figure 4.24 (a) Changes observed in the fluorescence spectra of 4U-cys (5×10⁻⁵ mmol) following the addition of fluoride (upto 40 equiv.) (b) Variation in the emission intensity of 4u-cys with respect to equivalence of fluoride at 396 nm and 430 nm.

NMR titration spectra of 4U-cys with TBAF revealed fluoride induced deprotonation of urea NH upon addition of 1.5 equiv. of fluoride which was indicated by the disappearance of the urea-NH signals (Figure 4.26). At the same time the overall intensity of the aromatic proton decreased. From the ¹H NMR titration analysis, we
inferred that the ease of deprotonation is relatively faster in 4U-cys, which was attributed to the higher acidity of the urea NH in 4U-cys than that of in 3U-cys.

Figure 4.25 ¹H NMR spectra of 3U-cys (2 mg in 0.4mL) in DMSO-d₆ following the addition of TBAF (0-4 equiv.); Changes in the urea NH resonance is shown.
Figure 4.26 $^1$H NMR spectra of 4U-cys (2 mg in 0.6mL) in DMSO-d6 following the addition of TBAF; Deprotonation was observed for Urea NH signals at 8.78ppm and 9.17 ppm.

As shown in scheme 4.10, in case of 4U-cys, delocalization stabilized adduct is formed due to fluoride caused deprotonation of urea NH. However, in 3U-cys deprotonation produced only minor stabilization in the system.
Scheme 4.10 Possible interactions of [(a)3U-cys and (b) 4U-cys with TBAF in DMSO

Delocalization stabilized adduct

Partially delocalized
4.5 Conclusions

We have synthesised and characterised conformationally flexible cystine based bis-naphthalimides which could show stimuli responsive behavior with regard to acid/base and anionic stimuli. Remarkably, both bis-naphthalimides exhibit excimer emission in polar solvents. Fluoride-induced quenching and \( \text{BF}_4^- \) induced restoration of the excimer emissions could be visualized under 365 nm UV-illumination. Similar, ‘off/on’ switching of excimer emission for the bis-naphthalimides could also be achieved by the successive addition of \( \text{3}^\circ \)-amine and hydrofluoric acid. Both the ligands produced fluorescent gel, in acetonitrile, DMF and DMSO, which exhibited gel-to-sol transformation upon fluoride addition. \(^1\)H NMR studies indicated that the incipient gelation process was driven by hydrophobic and aromatic–\( \pi \) interactions between the naphthalimide motifs, supplemented by hydrogen bonding between the amide NH groups. The addition of fluoride caused disruption of the hydrogen bonds involving the amide NH groups, which led to excimer to monomer switching of fluorescence and concomitant gel-to-sol transition. Thus, we have illustrated a simple strategy for the development of stimuli-responsive organogelator, which are capable of reversibly interacting with acid/base and anionic stimuli.

In addition, we have developed two bis-urea systems and investigated their fluoride recognition abilities and gelation properties. But unlike naphthalimides, cystine based bis-urea derivatives does not lead to gel formation and the fluoride recognition properties of the bis-urea derivatives vary with respect to the geometry of the ligands.
4.6 Experimental Section

4.6.1 Materials and methods

All the chemicals were commercially available from Sigma-Aldrich or Spectrochem (India) and were used as received. Solvents for spectroscopic experiments were distilled under nitrogen atmosphere before use. All the $^1$H and $^{13}$C NMR spectra were obtained on a 300 MHz Bruker spectrometer, and reported in δ per ppm. The electronic absorption spectra were recorded on a Shimadzu UV-vis spectrophotometer (Model UV-1800), and fluorescence spectra were recorded using a Hitachi F2500 fluorimeter.

4.6.2 Synthetic procedures

**ND1**: Naphthalene- 1, 8-dicarboanhydride (2.011 g, 10 mmol) was mixed with beta-alanine (1.063 g, 10 mmol) in a round bottom flask equipped with a magnetic needle. To this mixture, DMF (10 mL) was added and the resulting mixture was allowed to stir at 80°C for about 12 h. Initially, the colour of the mixture was brown, and after 12 h, a homogeneous red solution was obtained. Subsequently, the reaction mixture was concentrated and cooled to 0°C, which produced ND1 as pale yellow crystals (yield 69%). $^1$H NMR (300 MHz, DMSO-d$_6$/CDCl$_3$) δ$_H$ 8.48 (d, 2H, J = 6.9 Hz), 8.16 (d, 2H, J = 6.9 Hz), 7.67 (t, 2H, J = 7.5 Hz), 4.36 (t, 2H, J = 7.2 Hz), 2.6 (t, 2H); $^{13}$C NMR (75 MHz, DMSO-d$_6$/CDCl$_3$) δ 173.20, 163.81, 133.89, 131.42, 131.07, 127.98, 126.75, 122.35, 35.98, 32.32.
**Bis-naphthalimide, L1:** To a solution of ND1 (0.540 g, 2.0 mmol) in dichloromethane (10 mL), N-(3-dimethylaminopropyl)-ethylcarbodiimide hydrochloride (EDC-HCl) (0.400 g, 2.1 mmol) and 1-hydroxybenzotriazole (HOBT) (0.27 g, 2 mmol) were added and the mixture was allowed to stir at 0° C for 30 min. To this mixture, L-cystine methyl ester dihydrochloride (0.341 g, 1.0 mmol) and triethylamine (0.7 mL, 5.0 mmol) were added and the mixture was allowed to stir for 12 h. After the reaction was complete, the mixture was concentrated under vacuum, and a viscous material was obtained. The addition of water afforded a pale yellow solid, which was filtered, and rinsed with distilled water. The solid product was recrystallised from acetone, and dried in air. Yield 84%; 1H NMR (300 MHz, DMSO-d$_6$) δ 8.59 (2d, amide NH), 8.39 (4H, m), 7.82 (1H, t, J = 7.2 Hz), 4.55 (1H, bm), 4.22 (2H, s), 3.61 (3H, s), 3.336 (H$_2$O), 3.050 (3H, s), 2.960 (3H, s), 2.933 (2H, m), 2.489 (2H, s). 13C NMR (75 MHz, DMSO-d$_6$): 171.79, 171.06, 164.17, 135.12, 132.12, 131.52, 128.22, 128.03, 122.96, 53.07, 52.14, 37.21, 34.14; FT-IR (cm$^{-1}$): 3448 (amide NH), 3304, 1743 (ester C=O), 1701, 1649 (amide C=O), 1587; ES-MS: m/z 793.81, calc. for (M+Na$^+$).

**ND2:** Naphthalene-1,8-dicarboxanhydride (2.981 g, 15 mmol) was mixed with 4-aminobutyric acid (1.556 g, 15 mmol) in a round bottom flask equipped with a magnetic needle. To this mixture, DMF (30 mL) was added and the resulting mixture was allowed to stir at 80 1C for about 12 h. The colour of the mixture at the time of mixing was brown. After about 12 h, a homogeneous yellow-brown solution was obtained. The desired product, ND2, was crystallised after the solution was placed in an ice bath. The solid compound was filtered, and washed with EtOH–water to afford a yellow solid. Yield 74%; 1H NMR (300 MHz, DMSO-d$_6$/CDCl$_3$) δ 8.3 (d, 2H), 8.0 (d, 2H), 7.5–7.4
(t, 2H), 4.0–3.9 (t, 2H); 13C NMR (75 MHz, DMSO-d_{6}/CDCl_{3}) 174.53, 163.74, 133.75, 131.20, 130.80, 127.69, 126.62, 122.12, 40.51, 40.23, 39.95, 39.67, 39.39, 39.22, 39.11, 38.83, 31.44, 23.15.

**Bis-naphthalimide, L2:** as described for L1. The recrystallised product was obtained as a pale yellow solid. Yield 75%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta_H$ 8.54 (4H, d, J = 7.2 Hz), 8.15 (4H, d, J = 7.5 Hz), 7.71 (4H, t, J = 7.5 Hz), 7.06 (2H, amide NH), 4.90 (2H, m, J = 4.2 Hz), 4.25 (2H, m, J = 4.5 Hz), 3.75 (3H, s), 3.24 (4H, d, J = 5.1 Hz), 2.39 (4H, t, J = 4.8 Hz), 2.14 (4H, m), 1.65 (residual H$_2$O). 13C NMR (75 MHz, CDCl$_3$):
172.33, 171.0, 164.31, 133.97, 131.43, 131.31, 128.0, 126.89, 122.36, 77.41, 52.69, 51.72, 40.57, 39.50, 33.66, 24.14. FT-IR (cm$^{-1}$): 3310 (amide NH), 1740 (ester C=O), 1693 (amide C=O), 1657, 1587; ES-MS: m/z 821.85, calc. for (M +Na$^+$)

**Gel formation with L1 and L2.** In a typical gelation experiment, a known amount of the bis-naphthalimide gelator (3 mg mL$^{-1}$ for L1 and 5 mg mL$^{-1}$ for L2) and solvent (1.00 mL) were mixed in a screw-capped glass vial and maintained at 80°C for 2 h. In the process a clear solution was obtained, which upon cooling to room temperature over a period of time (≈2 h) led to gel formation. Moreover, the gelation of L2 in chloroform, acetonitrile, DMF and DMSO could be triggered by ultra-sonic irradiation for 2–3 minutes. The stability of the organogel was tested using the “inversion” method.
Figure 4.27: $^1$H spectra of ND1 in DMSO-d$_6$/CDCl$_3$

Figure 4.28: $^{13}$C NMR spectra of ND1 in DMSO-d$_6$/CDCl$_3$
Figure 4.29: $^1$H NMR spectra of L1 in DMSO-$d_6$

Figure 4.30: $^{13}$C NMR spectra of L1 in DMSO-$d_6$
Figure 4.31: $^1$H NMR spectra of ND2 in DMSO-d$_6$/CDCl$_3$

Figure 4.32: $^{13}$C NMR spectra of ND2 in DMSO-d$_6$/CDCl$_3$
Figure 4.33 $^1$H NMR spectra of L2 in CDCl$_3$

Figure 4.34 $^{13}$C NMR spectra of L2 in CDC
Figure 4.35. $^1$H NMR spectra of 3u-cys in DMSO-d$_6$

Figure 4.36. $^{13}$C NMR spectra of 3U-cys in DMS
Figure 4.37 $^1$H NMR spectra of 4U-cys in DMSO

Figure 4.38 $^{13}$C NMR spectra of 4U-cys in DMSO
Figure 4.39 ES-MS of L1 in MeCN (m/z = 793.81, calc. for M+Na+)

Figure 4.40 ES-MS of L2 (m/z = 821.85, calc. for M+N)
Figure 4.41 ES-MS of 3U-cys (m/z = 745.20, calc. for M+H^+)

Figure 4.42 ES-MS of 4U-cys (m/z = 745.21 calc. for M+H)

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