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Naphthyridine-based symmetrical and unsymmetrical pyridinium amides in sensing of biotin salt

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Two naphthyridine-based receptors have been designed and synthesised for biotin salt. The compounds serve as good hosts for the detection of biotin carboxylate rather than biotin ester. The correct dispositions of the binding groups under an isophthaloyl spacer enable the receptors to bind both the cyclic urea and the carboxylate ends simultaneously with moderate binding constant values. The receptors are effective for the binding of tetrabutylammonium salt of biotin with a concomitant increase in the fluorescence of naphthyridine and show appreciable binding of biotin salt in CH_3CN containing 1.2% DMSO. The binding was monitored in CH_3CN containing 1.2% DMSO and DMSO using ^1H NMR, UV–vis and fluorescence spectroscopic methods.

Keywords: naphthyridine-based receptors; fluorimetric detection; biotin salt; 3-aminopyridinium salt

Introduction

Design and synthesis of fluorescent receptors with less complicated structures for substrates of biological significance are very appealing in the area of molecular recognition research (*1*). In this context, suitable heterocycles with a number of hydrogen-bonding atoms are considered to be useful for providing a set of hydrogen bonds for complexation of complementary guest molecules. Naphthyridine motif in this capacity is a well-known heterocycle, which has been used as a supramolecular synthon in the area of supramolecular chemistry (*2*). In our earlier report, we have shown its use in fluorimetric detection of tartaric acid, citric acid, etc. (*3*). Goswami and Mukherjee (*4*) devised a dinaphthyridine receptor, which was excellent in solubilising urea in the less polar solvent chloroform. Zimmerman and co-workers (*5*) reported its enormous uses in different contexts in supramolecular chemistry. The relative background of this simple heterocycle synthon was thus encouraging and we thought it would be interesting to design a receptor for sensing and selective recognition of biotin and its derivatives.

Biotin (referred to vitamin H in humans) is an essential cofactor for a number of enzymes that have diverse metabolic functions (*6*). It is involved in carbon dioxide uptake, transfer and removal reactions (*7*). Structurally, it consists of a pentanoic acid side chain and a *cis*-fused bicyclic moiety with a sulphur atom in the ring. Crystallographic studies have confirmed the relative stereochemistry at the asymmetric carbon (*8*). The crystal structure of biotin shows that the carboxyl group of one biotin molecule is intermolecularly hydrogen bonded to the urea linkage of the other biotin molecule. The valeryl chain is severely twisted from the maximally extended all *trans*-conformation. To bind and sense this interesting biological substrate of defined stereochemistry, considerable effort was directed with a limited number of designed receptors. In this aspect, the use of Troger's base receptor, as reported by Wilcox and co-workers (*9*), was noteworthy. Herranz et al. (*10*) reported the recognition of biotin methyl ester. Goswami and Dey (*11*) designed pyridine-based simple receptors for complexation of biotin itself. In their approach, both the carboxylic acid functional group and the cyclic urea part of the bicyclic unit were simultaneously complexed by pyridine amide motifs. In relation to this, the fluorometric detection of biotin carboxylate anion is unknown in the literature. As part of our ongoing research in molecular recognition of carboxylic acids and carboxylates by artificial synthetic receptors (*12*), here we report two new bis-amide salts **1** and **2** for detection and sensing of biotin salt in solvents such as DMSO and CH_3CN containing 1.2% DMSO.

Results and discussion

Synthesis

Receptors **1** and **2** were synthesised by following the procedures given in Schemes 1 and 2. High dilution coupling of 3-aminopyridine and *n*-propylamine with isophthaloyl diacid chloride in the dry CH_2Cl_2 solvent gave the hetero-bis-amide receptor **4**, which was further reacted with 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine (*13*) in CH_3CN under refluxing condition to afford the bromide

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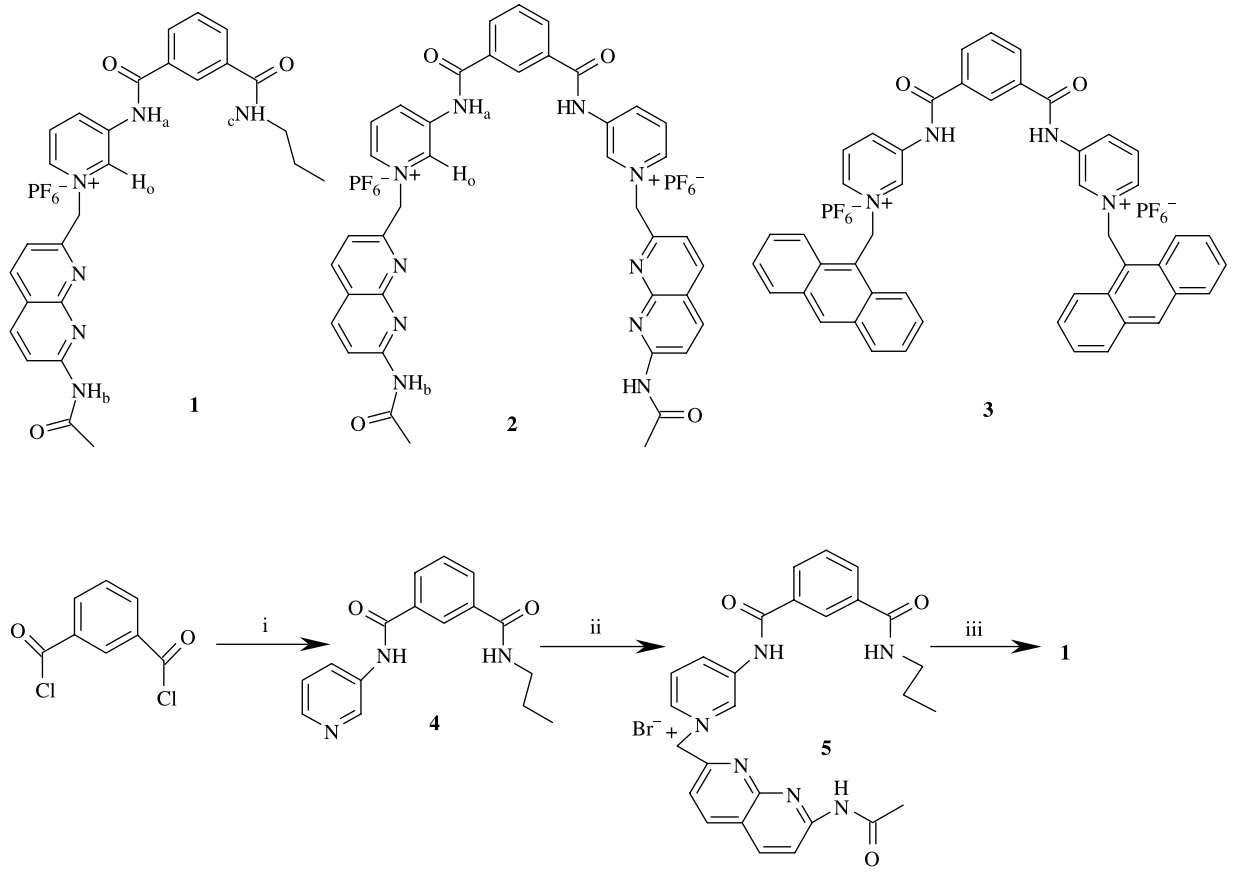
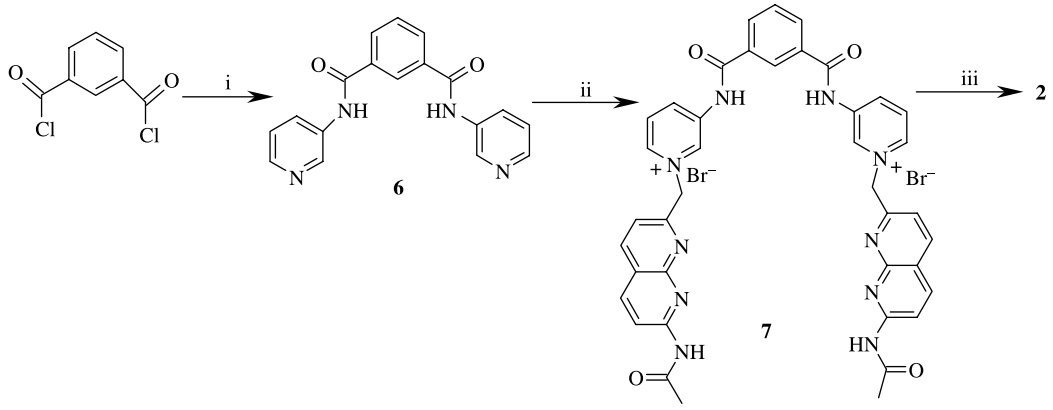


Scheme 1. (i) Addition of *n*-propylamine and 3-aminopyridine at high dilution condition, Et_3N, dry DCM; (ii) 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine in dry CH_3CN, heating with stirring for 48 h and (iii) NH_4PF_6–MeOH.

salt **5**. Anion exchange of the bromide salt **5** using NH_4PF_6 yielded the desired salt **1** in 67% yield. Similarly, the symmetrical bis-amide **6**, obtained from the reaction between 3-aminopyridine and isophthaloyl diacid chloride in dry CH_2Cl_2 according to Scheme 2, was reacted with 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine in dry CH_3CN to give compound **7**. Subsequent replacement of the Br^- ions by PF_6^- ions in **7** afforded the salt **2** in 13% yield.

To gain an insight into the binding and sensing of tetrabutylammonium salt of biotin by the designed synthetic receptors **1** and **2**, UV–vis, fluorescence and ^1H NMR spectroscopic techniques were employed. In this study, biotin methyl ester and tetrabutylammonium acetate were also considered to establish the mode of binding of carboxylate ion of biotin salt into the open clefts of the receptors.



Scheme 2. (i) 3-Aminopyridine, triethylamine in dry DCM; (ii) 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine in dry CH_3CN, heating with stirring for 48 h and (iii) NH_4PF_6 in MeOH.

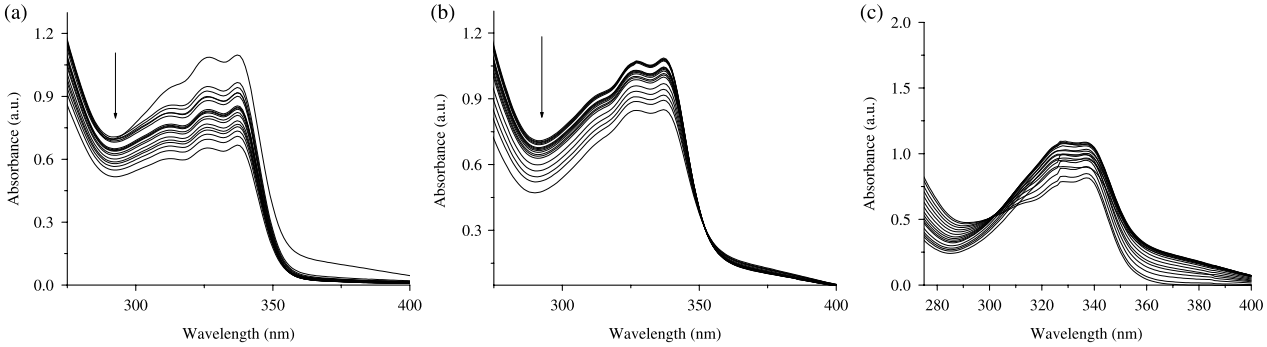


Figure 1. Changes in UV–vis spectra for (a) **1** ($c = 6.37 \times 10^{-5}$ M) in DMSO upon addition of tetrabutylammonium salt of biotin carboxylate, (b) **1** ($c = 6.54 \times 10^{-5}$ M) in DMSO upon addition of biotin methyl ester and (c) **1** ($c = 6.54 \times 10^{-5}$ M) in DMSO upon addition of tetrabutylammonium acetate.

UV–vis experiment

The UV–vis experiments on both receptors **1** and **2** were performed in solvents of different polarities (DMSO and CH_3CN containing 1.2% DMSO). Initially, the characteristic UV–vis changes were investigated upon addition of tetrabutylammonium salt of biotin to a DMSO solution of **1**. Upon addition of biotin salt, the intensities of the absorption peaks at 312, 325 and 337 nm for naphthyridine were decreased in a regular fashion (Figure 1(a)). There was no red or blue shift of the absorption peaks during complexation. UV–vis titration of receptor **1** was also carried out in DMSO on addition of varying amounts of biotin methyl ester. As can be seen from Figure 1(b), the change in absorbance of **1** is less compared to the case of **1** with biotin salt. The change in absorbance of **1** was further tested with tetrabutylammonium acetate (Figure 1(c)) to establish the binding feature of biotin salt. In this aspect, it is seen that the change in absorbance at 312, 325 and 337 nm for naphthyridine is less and irregular in nature compared to the case of biotin salt in Figure 1(a).

To get more hydrogen-bonding arrangements around the functionalities of biotin salt, the symmetrical bis-amide **2** was interesting. Upon successive addition of biotin salt to a DMSO solution of receptor **2**, the change in absorbance of the peaks for naphthyridine was more significant and comparable to receptor **1**. In this aspect, Figure 2(a) demonstrates the UV–vis titration spectra of **2** in the presence of increasing amounts of biotin salt. In contrast to this, the titration spectra of **2** in the presence of biotin methyl ester and tetrabutylammonium acetate are indicated in Figure 2(b) and (c), respectively. During titration with biotin salt, biotin methyl ester and tetrabutylammonium acetate, there was no red or blue shift of the absorption peaks of receptor **2**.

Continuous variation methods were used to determine the stoichiometric ratios of the receptors and the guests (*14*). Figures 3 and 4 show the Job plots of the product of the concentration of the host and the relative change in absorbance due to complexation with the mole fraction of the guests for a series of solutions in which the total concentration of the receptor and the guest, in each case, was constant. In all the cases, the crossing of the curves at 0.5 illustrates that both the receptors formed 1:1 complexes with the biotin salt as well as biotin methyl ester. The spectral features of **1** and **2** in UV–vis were also collected in CH_3CN containing 1.2% DMSO. To have stronger hydrogen-bonding interactions of receptors **1** and **2**

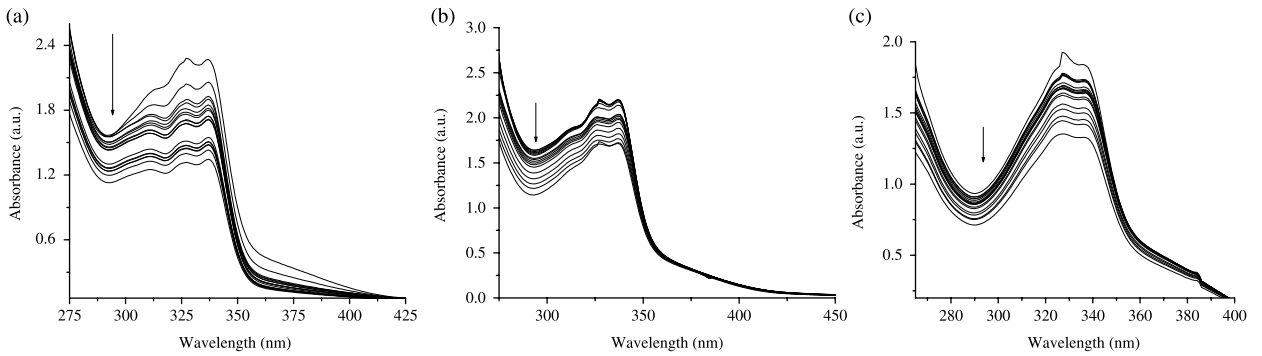


Figure 2. Changes in UV–vis spectra for **2** ($c = 6.54 \times 10^{-5}$ M) in DMSO upon addition of (a) tetrabutylammonium salt of biotin carboxylate, (b) biotin methyl ester and (c) tetrabutylammonium acetate.

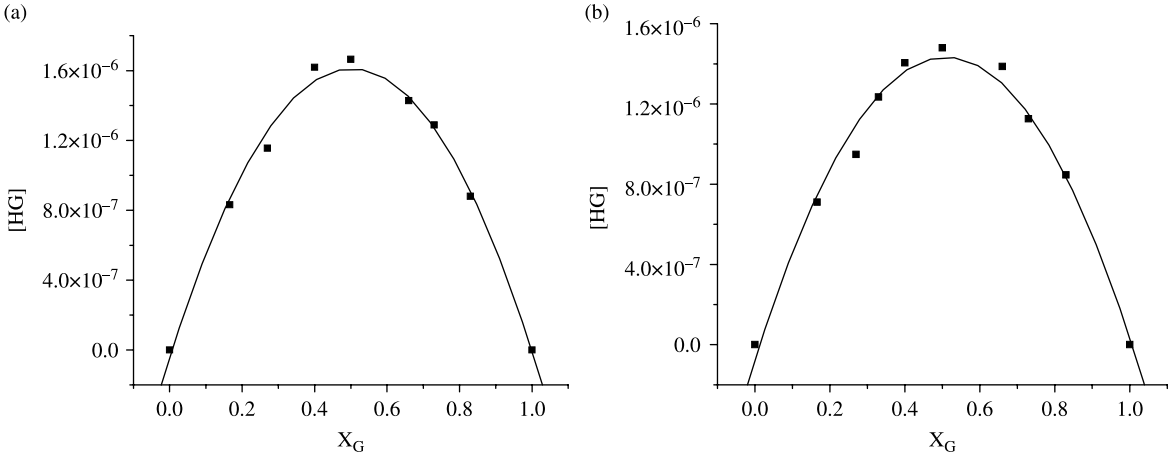


Figure 3. (a) Job plot of receptor **1** (at 337 nm) with tetrabutylammonium salt of biotin; the total concentration of the receptor and the guest is 6.37×10^{-6} M. (b) Job plot of receptor **1** (at 337 nm) with biotin methyl ester; the total concentration of the receptor and the guest is 6.37×10^{-6} M.

with the substrates, the polarity of the medium was tuned. DMSO is a competitive hydrogen-bonding partner that reduces the desired host–guest interactions to a significant extent. Considering the solubility of the receptors, we took CH_3CN containing 1.2% DMSO as a medium where the hydrogen-bonding interactions between the receptors and substrates were expected to be favourable and little stronger than the case in pure DMSO. In this connection, the changes in absorbances of **1** and **2** in the presence of tetrabutylammonium acetate, tetrabutylammonium salt of biotin and biotin methyl ester were monitored in the CH_3CN containing 1.2% DMSO solvent.

Figure 5 corroborates the UV–vis spectral changes of both **1** and **2** in the presence of biotin salt and biotin methyl ester in CH_3CN containing 1.2% DMSO. The change in absorbance of **2** is considerable than **1**.

Fluorescence experiment

Simultaneous fluorescence study of **1** and **2** both in the presence and absence of biotin salt was performed in the solvents DMSO and CH_3CN containing 1.2% DMSO. For example, upon addition of tetrabutylammonium salt of biotin to the DMSO solution of **1**, the emission at 353 nm for naphthyridine was increased gradually (Figure 6(a)), whereas the emission of **1** was marginally perturbed in the presence of biotin methyl ester (Figure 6(b)). Figure 6(c) shows the comparison of emission of **1** in the presence of 10 equiv. amounts of biotin salt and biotin methyl ester. Similar experiment on receptor **2** was carried out on excitation of the receptor solution at 315 nm. The emission spectrum of **2** in DMSO showed characteristic peaks at 371 and 453 nm (Figure 7). The peak at longer wavelength is presumably due to the complex formed at the excited state

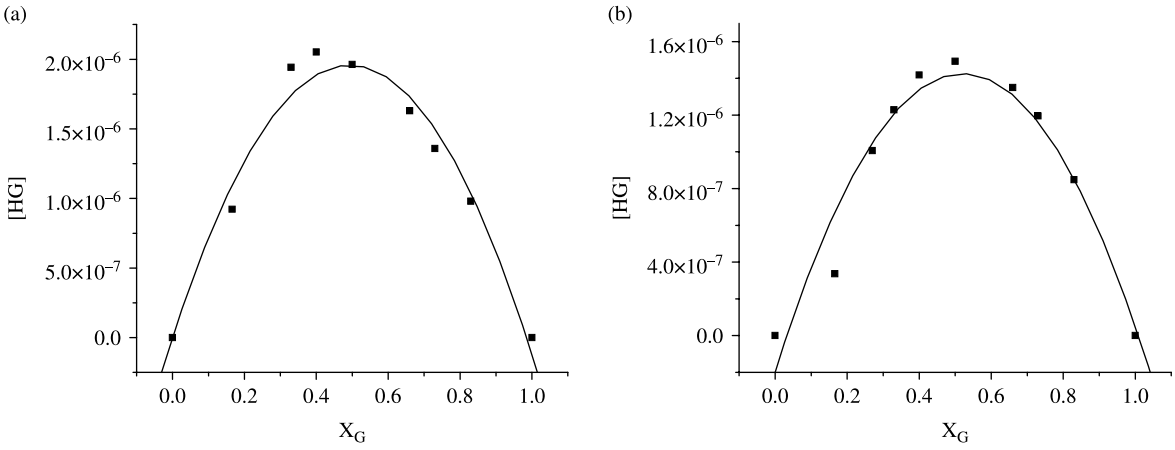


Figure 4. (a) Job plot of receptor **2** (at 337 nm) with tetrabutylammonium salt of biotin; the total concentration of the receptor and the guest is 6.37×10^{-6} M. (b) Job plot of receptor **2** (at 337 nm) with biotin methyl ester; the total concentration of the receptor and the guest is 6.37×10^{-6} M.

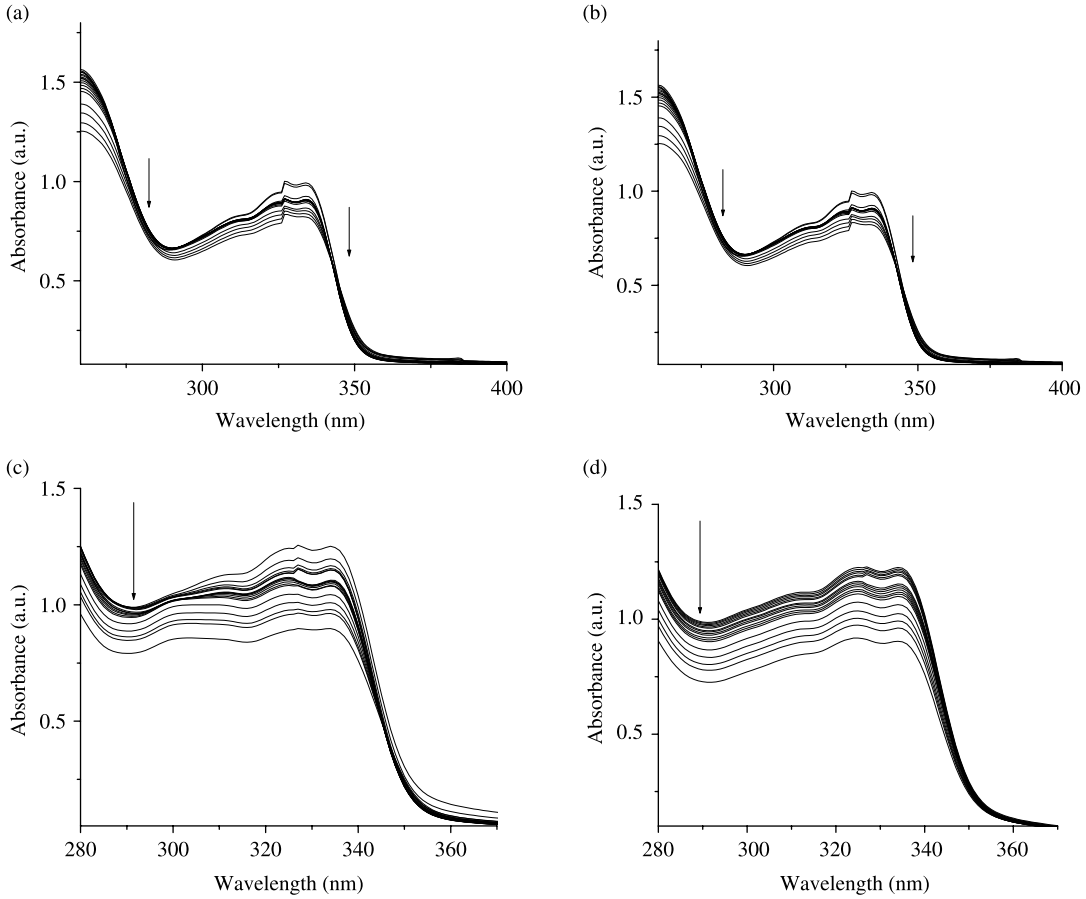


Figure 5. Changes in UV–vis spectra for (a) **1** with biotin salt, (b) **1** with biotin methyl ester, (c) **2** with biotin salt and (d) **2** with biotin methyl ester in CH_3CN containing 1.2% DMSO.

either between the pendant naphthyridines (excimer) or between naphthyridine and pyridinium moieties (exciplex). In the presence of biotin salt, the monomer emission intensity at 371 nm is greatly increased compared to the emission at 453 nm (Figure 7(a)). The gradual increase in intensity of the monomer emission of the naphthyridine motif in both **1** and **2** during complexation is attributed to the hydrogen bond-mediated perturbation of the excited $n\pi^*$ state in a destabilising manner for which the $\pi\pi^*$ state becomes the lowest energy singlet excited state (*1c*, *15*). However, the change in monomer emission of **2** is considerable and distinguishable from **1**. In comparison, the change in emission of **2**, in the presence of biotin methyl ester, is insignificant (Figure 7(c)). In an effort to understand the nature of the peak at 453 nm in **2**, we thoroughly investigated its interaction with all the guests (tetrabutylammonium acetate, tetrabutylammonium salt of biotin and biotin methyl ester) in the solvents such as MeOH and CH_3CN containing 1.2% DMSO. The results were compared with the findings obtained in pure DMSO. Figure 8(a) shows the change in fluorescence of **2** in different solvents. With the change in polarity of the solvents, the monomer emission peak shifts to either direction (Figure 8(a)). But under identical condition, the position of absorption peaks of **2** was almost unaltered (Figure 8(b)). It is mentionable that, while the peak for excimer at longer wavelength due to closely spaced naphthyridines in **2** was noticed in both DMSO and CH_3CN containing 1.2% DMSO, the same was not found in pure MeOH. We, therefore, investigated the change in monomer versus excimer emission of **2** with guest concentration in the DMSO and CH_3CN containing 1.2% DMSO solvents only.

It is mentionable that the emission characteristic of **2** in CH_3CN containing 1.2% DMSO is concentration dependent (Figure 8(c)). Almost negligible monomer emission of **2** in concentrated solution indicated its strong self-association.

In CH_3CN containing 1.2% DMSO, the excimer in **2** is exclusively destroyed at high concentration of biotin salt (Figure 9(a)) and the monomer emission is smoothly increased to a considerable extent. In comparison, the emission of **2** in the same solvent is hardly changed in the presence of biotin methyl ester (Figure 9(b)). But the change in emission of **2** is quite appreciable in the presence

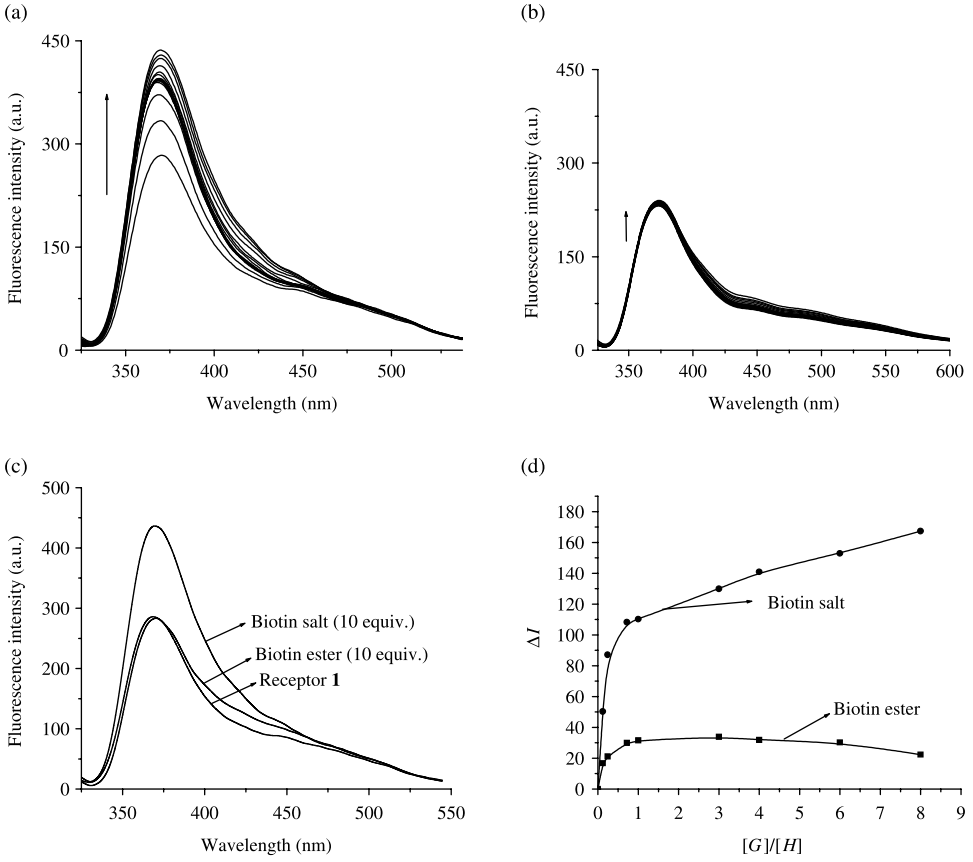


Figure 6. (a) Changes in fluorescence spectra for **1** ($c = 6.37 \times 10^{-5}$ M) in DMSO upon addition of tetrabutylammonium salt of biotin. (b) Changes in fluorescence spectra for **1** ($c = 6.37 \times 10^{-5}$ M) in DMSO upon addition of biotin methyl ester. (c) Comparison in emission of **1** in the presence of 10 equiv. biotin salt and biotin methyl ester. (d) Titration curves for biotin salt and biotin methyl ester with **1**.

of acetate (Figure 9(c)). In this connection, Figure 10 describes the plot of I_M/I_{Ex} (I_M and I_{Ex} indicate the intensities of the monomer and the excimer, respectively) versus guest concentration in CH_3CN containing 1.2% DMSO (Figure 10(a)) and DMSO (Figure 10(b)). In pure DMSO, while the emissions of both the monomer and the excimer in **2** are increased considerably in the presence of biotin salt (Figure 10(b)), the same are decreased in the presence of acetate and the excimer still persisted. These findings thus clearly underline the fact that, in DMSO, the isophthalamide core of **2** is effectively involved in complexation of the carboxylate end and the naphthyridine sites are hardly bonded to the cyclic urea motif of biotin salt.

If the naphthyridine sites were really involved, the excimer at 453 nm would be destroyed in principle. But this has not happened. On the contrary, the gradual disappearance of the excimer in the presence of biotin salt in CH_3CN containing 1.2% DMSO indicates that both the isophthaloyl diamide core and the naphthyridine sites of **2** are simultaneously involved in complexation of biotin salt. This is insignificant in the case of biotin methyl ester due to high steric demand and electroneutrality of the ester group (Figure 9(b)). The simultaneous involvement of both the naphthyridine site and the diamide core of **2** in complexation of biotin salt was proved by the titration experiment of **2** in CH_3CN containing 1.2% DMSO in the presence of acetate where the excimer emission was almost unperturbed (Figure 9(c)).

Based on these, we suggested the possible modes of binding of biotin salt by receptors **1** and **2** (Figure 11). In the suggested modes, the carboxylate ion is bonded in the cleft under the influence of both hydrogen-bonding and charge–charge interactions and the bicyclic urea motif is adhered to the pendant naphthyridine sites via hydrogen bonds (**1A/2A**). The other equilibrium forms **1B**, **2B** and **2C** are also possible in solution where only the carboxylate ion is hydrogen bonded, leaving the cyclic urea motif uncomplexed. In our earlier publication, we reported that the anthracene-based pyridinium salt **3** under an isophthaloyl spacer is able to bind carboxylate, especially aliphatic carboxylate anion, in the cleft involving both hydrogen-bonding and charge–charge interactions (*16*). This finding, additionally, enable us to suggest that the carboxylate part of biotin carboxylate salt is particularly involved in binding in the isophthaloyl diamide core according to the modes suggested in Figure 11. Based on the results, it was thus

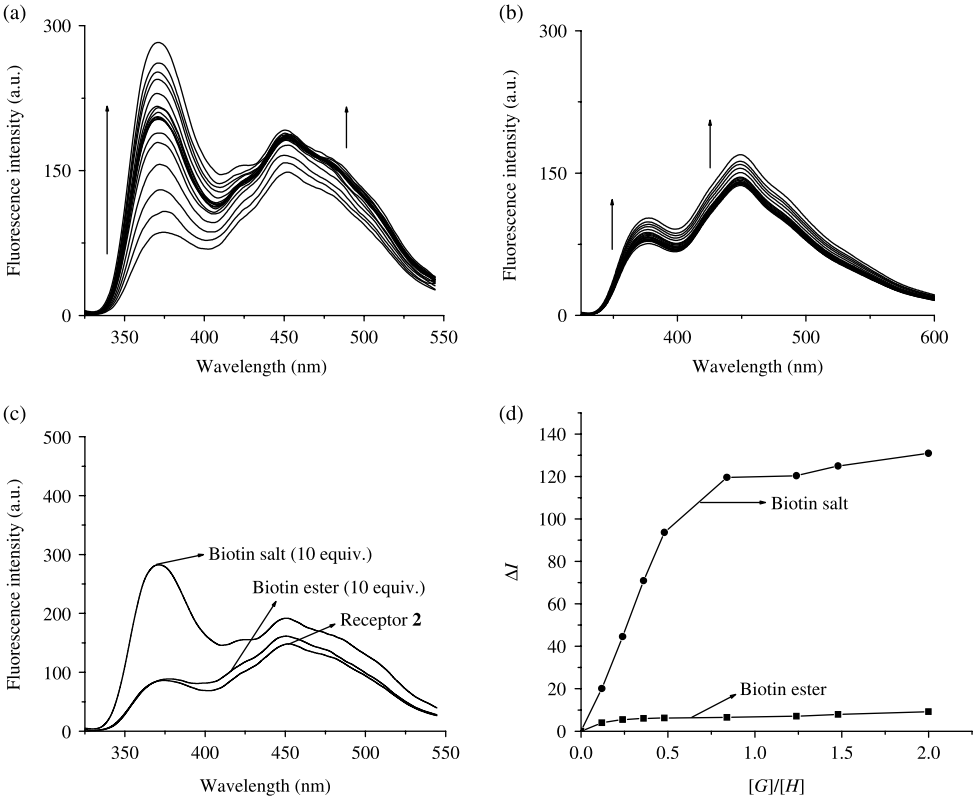


Figure 7. (a) Changes in fluorescence spectra for **2** ($c = 6.54 \times 10^{-5}$ M) in DMSO upon addition of tetrabutylammonium salt of biotin. (b) Changes in fluorescence spectra for **2** ($c = 6.54 \times 10^{-5}$ M) in DMSO upon addition of biotin methyl ester. (c) Comparison in emission of **2** in the presence of 10 equiv. biotin salt and biotin methyl ester. (d) Titration curves for biotin salt and biotin methyl ester with **2**.

concluded that receptor **1** binds both the ends of biotin salt in either the DMSO or CH_3CN containing 1.2% DMSO solvent. On the contrary, receptor **2** favours synergic binding (Figure 11, **2A**) only in CH_3CN containing 1.2% DMSO of low polarity over the case of DMSO itself, where the binding of the cyclic urea motif at the naphthyridine site is hardly possible either due to locking of the lower rim for the formation of a strong excimer or due to disposition of the naphthyridines at the distal positions.

^{1}H NMR study

For further confirmation, ^{1}H NMR of both receptors **1** and **2** in DMSO-d_6 was recorded both in the presence and absence of the tetrabutylammonium salt of biotin as well as biotin methyl ester. In ^{1}H NMR of **1**, the amide protons H_a and H_c moved less downfield ($\Delta\delta_{NH_a} = +0.18$ ppm, $\Delta\delta_{NH_c} = +0.03$ ppm) in the presence of equivalent amount of tetrabutylammonium salt of biotin carboxylic acid. The same was true for symmetrical bis-amide receptor **2**

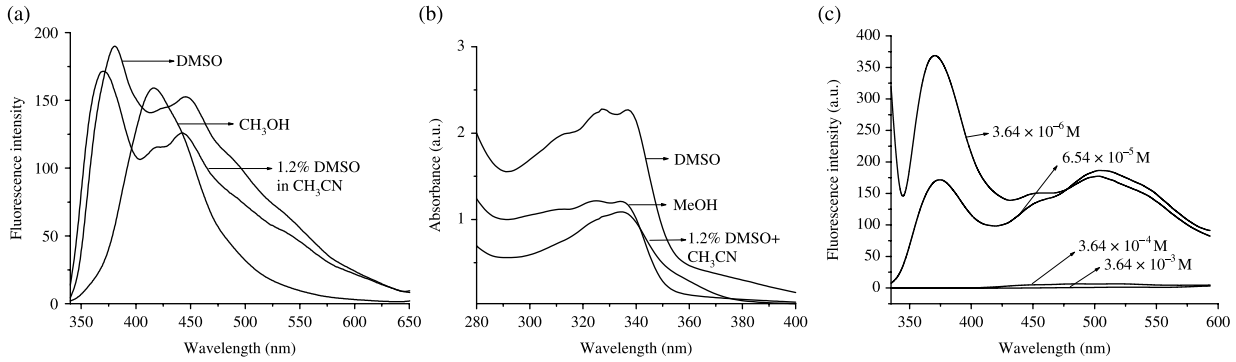


Figure 8. (a) Changes in fluorescence spectra of **2** ($c = 6.54 \times 10^{-5}$ M) in different solvents, (b) changes in absorbance of **2** ($c = 6.54 \times 10^{-5}$ M) in different solvents and (c) change in emission of **2** at different concentrations in the solvent CH_3CN containing 1.2% DMSO.

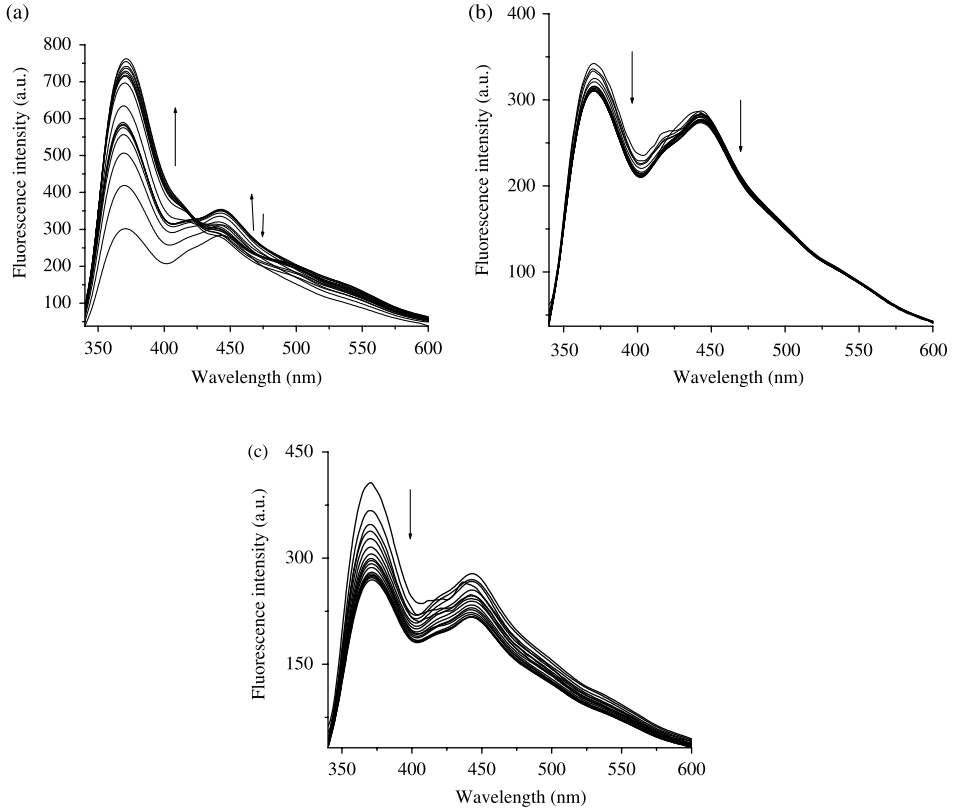


Figure 9. Changes in fluorescence spectra for **2** ($c = 6.54 \times 10^{-5}$ M) in CH_3CN containing 1.2% DMSO: (a) upon addition of tetrabutylammonium salt of biotin, (b) biotin methyl ester and (c) tetrabutylammonium acetate.

($\Delta\delta_{NH_a} = +0.16$ ppm, $\Delta\delta_{NH_b} = 0$ ppm). The small change in chemical shift of the amide protons during complexation was due to the interference of DMSO, which is known as a competitive binding partner. The binding of DMSO in the core of isophthaloyl diamide is well established and has been shown by Kovalchuk et al. (*17*). DMSO was used to perform experiments due to partial solubility of receptors **1** and **2** in solvents such as $CHCl_3$ and CH_3CN. Nevertheless, in both **1** and **2**, the pyridyl H_o protons underwent a significant downfield shift upon complexation ($\Delta\delta_{H_o} = +0.07$ ppm for **1**, $\Delta\delta_{H_o} = +0.09$ ppm for **2**). More importantly, the change in chemical shift of the key

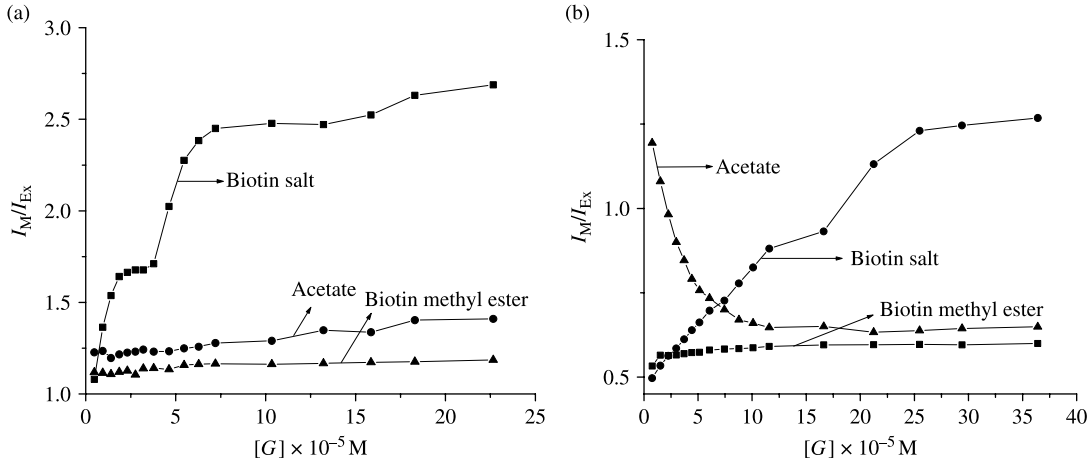


Figure 10. Plot of I_M/I_{Ex} versus guest concentration for receptor **2** with: (a) biotin salt, biotin methyl ester and acetate in CH_3CN containing 1.2% DMSO and (b) biotin salt, biotin methyl ester and acetate in DMSO.

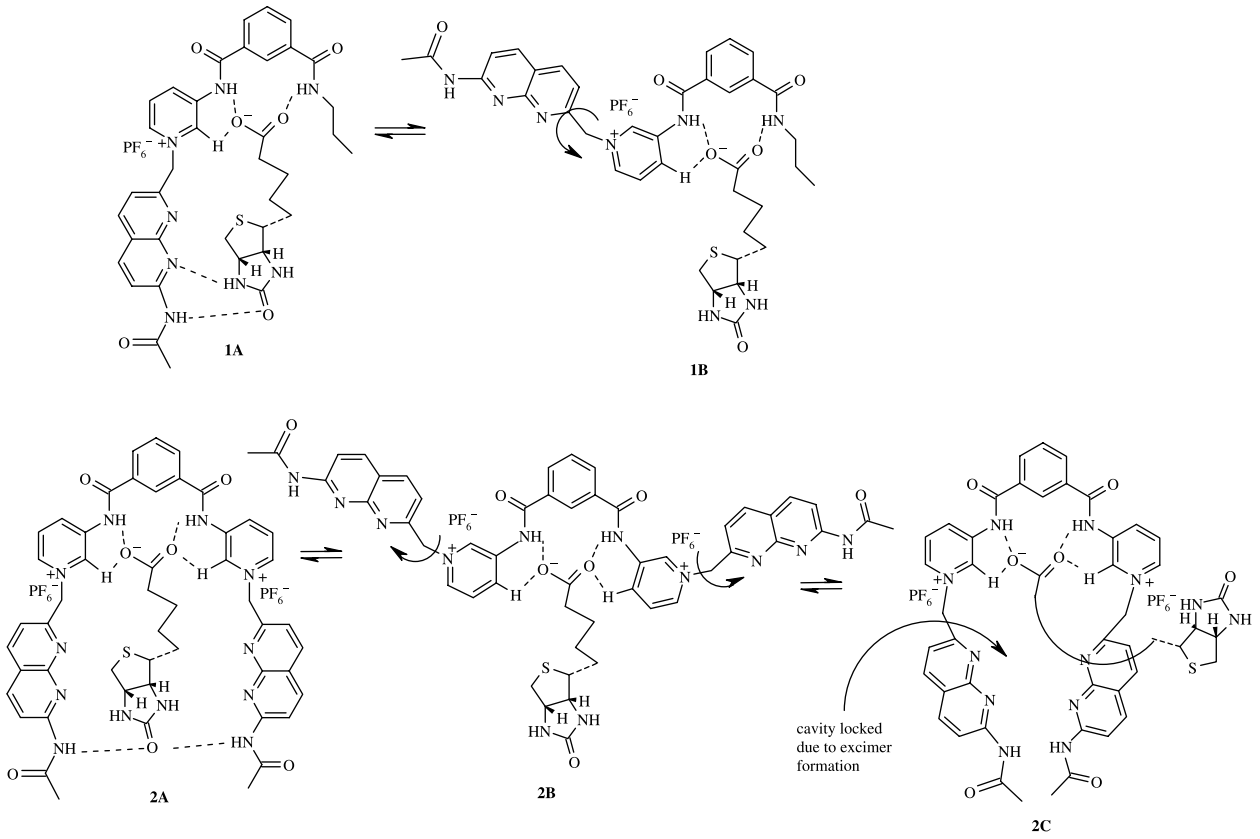


Figure 11. Hydrogen-bonded complex of biotin carboxylate with receptors **1** and **2**.

amide protons of both **1** and **2** in the presence of biotin methyl ester was insignificant. Even the pyridyl H_o protons in both **1** and **2** did not exhibit any measurable change in chemical shift similar to that of **1** and **2** with biotin salt. This was explained to be due to the steric nature of the ester group for which the ester carbonyl was refused in the binding process into the isophthaloyl diamide core. To realise the effect of DMSO in the binding process, we further studied the hydrogen-bonding interactions of both **1** and **2** with biotin salt and biotin methyl ester by ^{1}H NMR in CD_3CN containing 1.2% DMSO-d_6. Interestingly, during titration of **1** with biotin salt, the amide protons H_a, H_b and H_c of **1** underwent significant downfield chemical shifts. In the presence of biotin methyl ester, the changes in chemical shift values of H_a, H_b and H_c were relatively less than the case with biotin salt. Figure 12, in this aspect, shows the spectral changes during titration of **1** with biotin salt. In comparison to **1**, receptor **2** under similar condition showed weak interactions. During titration with biotin salt, the amide proton H_a of **2** exhibited relatively greater change than the amide proton H_b (Figure 13). In both **1** and **2**, the amide proton H_b appeared as a broad singlet, which upon successive addition of biotin salt became too broad to detect. This is, indeed, much pronounced in the case of receptor **2**. A closer look into the chemical shift values of urea protons of biotin salt reveals that during complexation with **1**, significant downfield shifts of the urea signals take place. In contrast to this, urea protons of biotin salt moves upfield on complexation with **2**, although the amide proton H_b showed a downfield shift. This underlines the fact that the bicyclic urea motif of biotin salt interacts with the pendant naphthyridines of **2** not in a plane which contains naphthyridine. This is possibly due to steric feature exerted by the two naphthyridines. In the case of **1**, the cyclic urea motif interacts in a common plane so that urea protons come in hydrogen bond distances with the naphthyridine ring nitrogens. On the other hand, a simultaneous downfield shift of H_p upon complexation was appreciable. This may be either due to the participation of H_p in the formation of C—H···O hydrogen bonds with carboxylate ion that stabilise the complex via the mode **B** or a closer approach of the amide carbonyl oxygen to H_p upon complexation via mode **A** (Figure 11). Between **A** and **B**, the form **A** is more probable in solution due to a greater number of hydrogen bonds in the complex. Titration curves in Figures 12(b) and 13(b) indicate the change in chemical shifts of the interacting protons and

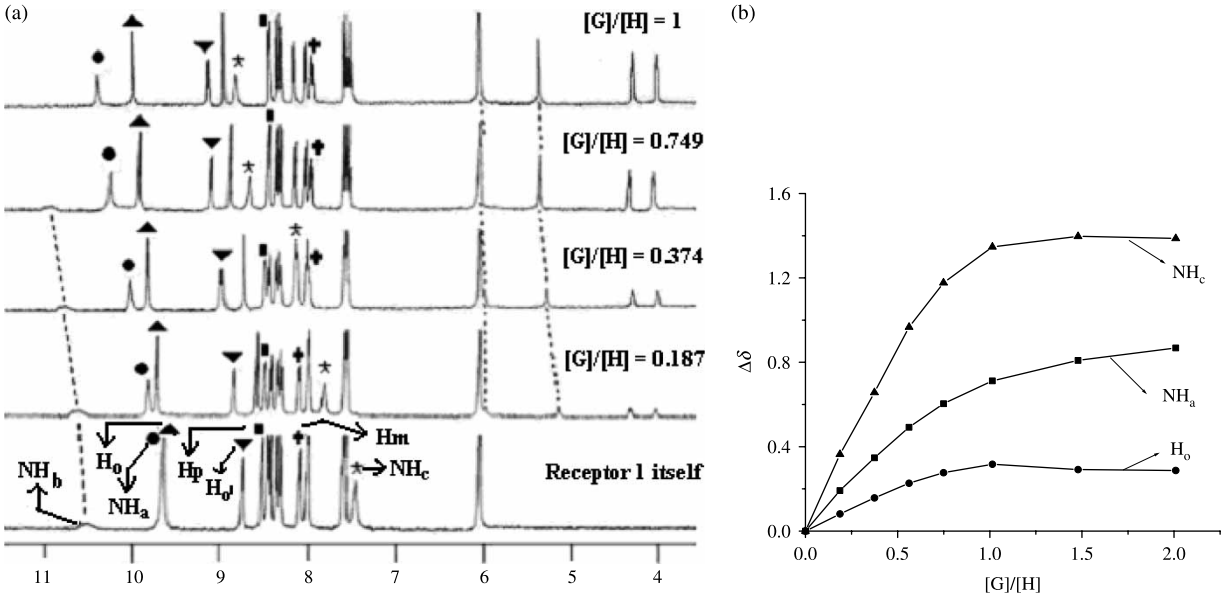


Figure 12. (a) Partial ^{1}H NMR spectra (CD_3CN containing 1.2% DMSO-d_6, 400 MHz) of **1** upon addition of tetrabutylammonium salt of biotin and (b) titration curves for **1** ($c = 3.64 \times 10^{-3}$ M) with tetrabutylammonium salt of biotin.

also present the stoichiometries attained by both **1** and **2** in the binding process. The breaks of the titration curves at $[G]/[H] = 1$ indicated 1:1 stoichiometries of the complexes. These notable findings thus prove that biotin salt is comfortably complexed by **1** in the mode **1A**. In contrast, symmetrical diamide **2** is less efficient in complexation of biotin salt and follows a binding structure **2A** where the naphthyridine amide hydrogens are involved in weak hydrogen bonding, leaving urea protons uncomplexed. In both the cases, the carboxylate end of biotin salt is strongly complexed in the diamide cores involving hydrogen-bonding and charge–charge interactions.

In the case of biotin methyl ester, all the amide and H_o protons of **1** and **2** underwent a negligible downfield shift, suggesting poor interactions. These detailed experiments in ^{1}H NMR thus fully support our proposition on the binding structures (Figure 11). Receptor **1** efficiently interacts with biotin salt and follows the hydrogen-bonding structure **1A** as shown in Figure 11. Similarly, receptor **2** binds the same via the

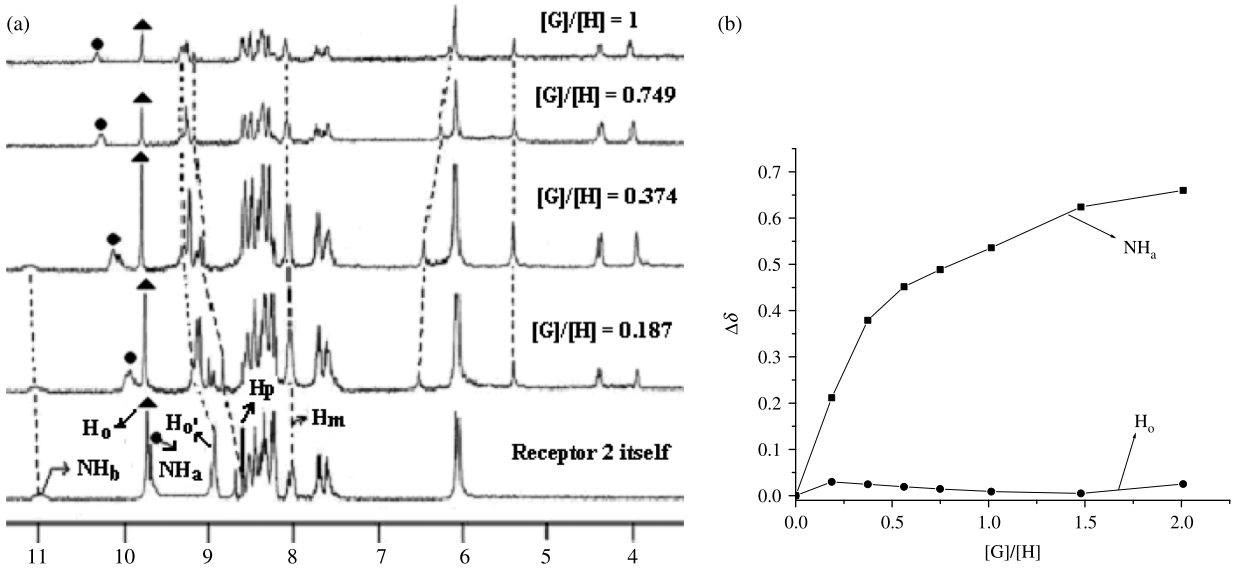


Figure 13. (a) Partial ^{1}H NMR spectra (CD_3CN containing 1.2% DMSO-d_6, 400 MHz) of **2** upon addition of tetrabutylammonium salt of biotin and (b) titration curves for **2** ($c = 3.64 \times 10^{-3}$ M) with tetrabutylammonium salt of biotin.

Table 1. Binding constants (K_a) of receptor **1**.

Guests	K_a in M^{-1} by the fluorescence method		K_a in M^{-1} by the UV method		K_a in M^{-1} by ^1H NMR
	DMSO	CH_3CN containing 1.2% DMSO	DMSO	CH_3CN containing 1.2% DMSO	CD_3CN containing 1.2% DMSO-d_6
Biotin salt	1.89×10^4	5.12×10^4	2.57×10^4	1.89×10^4	2.72×10^4
Biotin methyl ester	6.33×10^3	5.60×10^3	3.69×10^3	8.89×10^3	[a]
Acetate	1.19×10^4	5.14×10^4	1.44×10^4	1.74×10^4	

[a] Not determined due to negligible change in ^1H NMR.

Table 2. Binding constants (K_a) of receptor **2**.

Guests	K_a in M^{-1} by the fluorescence method		K_a in M^{-1} by the UV method		K_a in M^{-1} by ^1H NMR
	DMSO	CH_3CN containing 1.2% DMSO	DMSO	CH_3CN containing 1.2% DMSO	CD_3CN containing 1.2% DMSO-d_6
Biotin salt	9.70×10^3	4.22×10^4	2.85×10^4	1.11×10^4	6.16×10^2
Biotin methyl ester	4.17×10^3	7.05×10^2	2.59×10^3	7.54×10^3	[a]
Acetate	1.95×10^4	1.91×10^4	6.94×10^3	1.14×10^4	

[a] Not determined due to negligible change in ^1H NMR.

mode **2A**, although the involvement of naphthyridines is, indeed, less due to their closeness.

Binding study

Complexation-induced change in chemical shift values of the key amide protons of both **1** and **2** in CD_3CN containing 1.2% DMSO-d_6 was used to determine the binding constant values (Tables 1 and 2). To realise the binding potencies of both **1** and **2**, the binding constants were also determined from the UV–vis and fluorescence titrations and the stoichiometries of the complexes were confirmed from the break of the titration curves [see the inserts of Figures 6(d), 7(d), 12(b) and 13(b)] as well as from the UV Job plots (Figures 3 and 4). In all the cases, receptors **1** and **2** were found to maintain a 1:1 stoichiometry with biotin salt and biotin methyl ester. For a complex of 1:1 stoichiometry, the binding constant value was determined by using the data obtained from both the fluorescence and UV titrations (Tables 1 and 2) (*18*). As can be seen from Tables 1 and 2, receptors **1** and **2** are efficient binder of tetrabutylammonium salt of biotin rather than biotin methyl ester and especially in the CH_3CN containing 1.2% DMSO solvent. DMSO being a competitive hydrogen bond acceptor interacts with the isophthaloyl diamide cores of **1** and **2** and reduces the binding constant values (*17*). The presence of CH_3CN containing 1.2% DMSO has also a marked effect on the binding event for which the binding constant values reported in Tables 1 and 2 are close in magnitude for each guest. The degree of self-association of the biotin salt as well as biotin ester, which may vary to different extents in solvents of different polarities, cannot be ignored during interaction with receptors **1** and **2**. Due to the more polar character of the excited state, the binding constant values determined by the fluorescence method are presumably marginally different from the values obtained by the UV method. The binding constant value for **1** with biotin salt by the NMR method (*19*) is in accordance with the values obtained by the fluorescence and UV methods. In the case of **2**, the binding constant value obtained for biotin salt by the NMR method is found to be significantly less than the values determined by the fluorescence and UV methods. This is due to less involvement of the naphthyridine for effective hydrogen bonding of the urea motif of biotin salt like **1**. Additionally, we suggest that there is an effective self-association of the closely spaced pendant naphthyridines in **2** in the NMR concentration range used ($c = 3.64 \times 10^{-3}$ M). This is evidenced from the emission spectra of **2** in different concentrations [Figure 8(c)]. With a decrease in concentration of **2** in CH_3CN containing 1.2% DMSO, the monomer emission of naphthyridine was found to increase followed by a change in emission for excimer or exciplex at higher wavelength. At higher concentration, the monomer emission of **2** was almost level of and, in turn, the emission was observed only in the excimer region. This simple experiment suggests that at higher concentration, receptor **2** exhibits self-association for which the hetero-association with biotin salt is less in magnitude. The hetero-association is significant at lower concentrations, used for recording the UV and fluorescence spectra. The biotin salt is comfortably complexed in the open cleft of **1** with a higher value than that of receptor **2**.

Conclusions

In summary, naphthyridine-based symmetrical and unsymmetrical pyridinium amides **1** and **2** bearing multiple hydrogen-bonding sites were synthesised. All the receptors form 1:1 complexes with tetrabutylammonium salt of biotin as well as biotin methyl ester and are found to be effective in sensing biotin salt rather than biotin methyl ester. The simultaneous binding of carboxylate ion and urea motif is possible into the open clefts of receptors **1** and **2**. Receptor **1** binds both the carboxylate end and the bicyclic urea motif of biotin salt involving both the isophthaloyl hetero-bis-amide core and naphthyridine site together. On the contrary, receptor **2** follows this mode preferably in CH_3CN containing 1.2% DMSO than pure DMSO where the involvement of the naphthyridine site in complexation is less. The carboxylate end of biotin salt is strongly complexed in the isophthaloyl diamide core by N—H···O, C—H···O hydrogen bonds and charge–charge interactions and the binding of biotin salt is well reported by a concomitant change in fluorescence of the appended naphthyridine. Thus, in spite of not using additional fluorophore in **1** and **2**, both of them act as promising fluorescent chemosensors for biotin salt rather than biotin ester. To our knowledge, such fluorometric detection of biotin salt by exploiting fluorescence property of naphthyridine is a first-time approach in the literature.

Experimental section

General

Solvents were dried and distilled prior to use. Dichloromethane was distilled from anhydrous $CaCl_2$. Triethylamine was distilled from calcium hydride. Acetonitrile was dried over CaH_2. All other commercially available reagents were used without further purification. Melting points were recorded in open capillaries and are uncorrected. FT-IR spectra were recorded on a Perkin-Elmer model L120-00A. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 and 500 MHz. UV–vis spectra were taken with a Lambda-25 spectrophotometer. Fluorescence spectra were obtained with Perkin-Elmer LS-50B and LS-55 spectrofluorimeters.

N1-Propyl-N3-(3-pyridyl)isophthalamide (4)

The unsymmetrical hetero-bis-amide **4** was obtained by dropwise addition of *n*-propylamine (0.291 g, 4.92 mmol) and 3-aminopyridine (0.463 g, 4.92 mmol) to isophthaloyl diacid chloride (1 g, 4.92 mmol) in dry dichloromethane (DCM) at high dilution condition under nitrogen atmosphere. Triethylamine (4.92 mmol) was added in each amine solution during the reaction. After completion of addition of the amines, the reaction mixture was stirred overnight. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated and the residual mass was extracted with $CHCl_3$–CH_3OH mixture (3× 20 ml). The organic extract was next washed with $NaHCO_3$ solution (3× 15 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residual mass was purified by silica gel column chromatography using ethyl acetate–petroleum ether (4:1) as the eluent to afford compound **4** (300 mg, 21% yield, mp 152°C).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.6 (s, 1H, amide NH), 8.95 (s, 1H), 8.64 (brt, 1H), 8.43 (s, 1H), 8.32 (d, $J = 4$ Hz, 1H), 8.20 (d, $J = 8$ Hz, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 8.05 (d, $J = 7.6$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.42–7.39 (t, $J = 8$ Hz, 1H), 3.25 (q, $J = 8$ Hz, 2H), 1.58–1.53 (m, 2H), 0.91 (t, $J = 8$ Hz, 3H) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.4 (two amide carbonyl carbons overlapped), 145.5, 142.8, 136.6, 135.9, 135.4, 131.2, 131.0, 129.3, 128.2, 127.5, 124.4, 41.9, 23.2, 12.3 ppm. FT-IR (KBr) ν: 3324, 3314, 2967, 2926, 1670, 1636, 1544, 1419 cm^{-1}. Mass (ESI): 284.2 $[M+H]^+$, 225.2.

N1-(7-[3-(3-[(Propylamino)carbonyl]benzoylamino)-1 pyridiniumyl]methyl[1,8]naphthyridin-2-yl)acetamide hexafluorophosphate (1)

Compound **4** (0.07 g, 0.25 mmol) was treated with 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine (0.1 g, 0.38 mmol) in dry CH_3CN and the reaction mixture was refluxed for 48 h to afford the bromide salt **5** (60 mg, 43.2% yield). Compound **5** (0.04 g, 0.07 mmol) in MeOH was subsequently treated with aqueous NH_4PF_6 solution to carry out the anion exchange reaction. After heating with stirring of the solution for 20 min, a precipitate appeared. Filtration of the precipitate followed by thorough washing with ether afforded receptor **1** in 67% yield (31 mg, mp 174°C).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.37 (s, 1H, amide NH), 11.09 (s, 1H, amide NH), 9.63 (s, 1H), 8.91 (brs, 1H), 8.79 (d, $J = 8$ Hz, 1H), 8.64 (brt, 1H), 8.52–8.45 (m, 3H), 8.39 (d, $J = 8$ Hz, 1H), 8.24 (brt, 1H, amide NH), 8.12–8.08 (m, 2H), 7.72–7.67 (m, 2H), 6.34 (s, 2H), 3.27 (m, 2H), 2.10 (s, 3H), 1.57–1.52 (m, 2H), 0.89 (t, $J = 4$ Hz, 3H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.1, 166.0, 165.3, 157.1, 154.6, 153.8, 141.0, 139.5, 138.3, 137.1, 135.9, 135.1, 133.2, 130.7, 130.3, 128.7, 127.8, 126.9, 119.4, 119.0, 115.0, 64.4, 41.0, 23.9, 22.2, 11.3 ppm. FT-IR (KBr) ν: 3428, 3309, 3067, 2967, 2876, 1686, 1639, 1459 cm^{-1}. Mass (ESI): 483.3 $[M - PF_6 + 1]^+$, 441.4, 284.3.

N1,N3-Di(3-pyridyl)isophthalamide (6)

The symmetrical bis-amide **6** was obtained by coupling of 3-aminopyridine (1.2 g, 12.8 mmol) with isophthaloyl

diacid chloride (1.3 g, 6.4 mmol) in dry DCM followed by addition of triethylamine (1.4 mmol) under nitrogen atmosphere. The reaction mixture was allowed to stirring for overnight. After completion of the reaction, the solvent was removed under vacuum. The residual mass was extracted with $CHCl_3$–CH_3OH mixture (3× 20 ml). The organic layer was washed with $NaHCO_3$ solution (3× 15 ml) and dried over anhydrous Na_2SO_4. The solvent was removed and the residual mass was purified by column chromatography over silica gel using chloroform–methanol (4:1) as the eluent to afford the desired compound **6** (730 mg, 36% yield, mp 210°C).

^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.69 (s, 2H, amide NH), 8.96 (d, $J = 2$ Hz, 2H), 8.59 (s, 1H), 8.33 (d, $J = 4$ Hz, 2H), 8.23–8.18 (m, 4H), 7.74 (t, $J = 8$ Hz, 1H), 7.43 (d, $J = 8$ Hz, 1H), 7.42 (d, $J = 8$ Hz, 1H) ppm. ^{13}C NMR (DMSO-d_6, 125 MHz): δ 166.2, 145.6, 142.9, 136.5, 135.5, 131.8, 129.6, 128.2, 128.0, 124.4 ppm. FT-IR (KBr) ν: 3418, 3054, 1676, 1613, 1585, 1556, 1481, 1429 cm^{-1}. Mass (ESI): 319.1 $[M+H]^+$, 225.2, 130.2.

N1-[7-(3-[(3-[(1-[7-(Acetylamino)[1,8]naphthyridin-2-yl]methyl-3-pyridiniumyl)amino] carbonylbenzoyl)amino]-1-pyridiniumylmethyl) [1,8]naphthyridin-2-yl]acetamide dihexafluorophosphate (2)

Compound **6** (0.05 g, 0.16 mmol) was treated with 2-*N*-acetyl-7-bromomethyl-1,8-naphthyridine (0.13 g, 0.47) in dry CH_3CN and the reaction mixture was refluxed for 48 h to afford the salt **7** (0.023 g, 0.14 mmol), which was used in the next step for reaction with NH_4PF_6 in MeOH for anion exchange. The reaction yielded the desired compound **2** in 13% yield (30 mg, mp 194°C) as a grey solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.45 (s, 2H, amide NH), 11.08 (s, 2H, amide NH), 9.61 (s, 2H), 8.94 (brs, 2H), 8.79 (brd, 2H), 8.63 (brt, 2H), 8.51 (d, $J = 8$ Hz, 2H), 8.46 (d, $J = 8$ Hz, 2H), 8.39 (d, $J = 8$ Hz, 2H), 8.26 (brs, 3H), 7.78 (brs, 1H), 7.71 (d, $J = 8$ Hz, 2H), 6.33 (s, 4H), 2.10 (s, 6H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 170.1, 165.9, 157.1, 154.6, 153.8, 139.5, 138.3, 137.3, 136.0, 131.7, 131.6, 129.0, 127.8, 127.6, 119.3, 119.0, 115.0, 64.4, 23.9 ppm (two carbons in the aromatic region are missing due to overlapping). FT-IR (KBr) ν: 3747, 3435, 1688, 1608, 1552, 1504, 1437 cm^{-1}. Mass (ESI): 863 $[M-PF_6-1]^+$, 717 $[M-2PF_6-1]$, 518.2, 476.2, 359.3.

Binding studies

The studies on binding properties of **1** and **2** were carried out in different solvents such as DMSO and CH_3CN containing 1.2% DMSO. The UV–vis titrations were carried out with **1** and **2** by adding different amounts of guests. The absorbance value during each addition was noted. Then, for the complexes of receptors **1** and **2** with guests, $[A_0/(A - A_0)]$ as a function of the inverse of guest concentration was plotted. The plot fits a linear relationship, indicating the 1:1 stoichiometry of the receptor–guest complex. The ratio for the intercept versus slope gives the association or binding constant (K_a) for the receptor–guest complex shown in Tables 1 and 2. A similar experiment was done with **1** and **2** in the fluorescence and the emission intensities were recorded to evaluate the binding constant values. In NMR, $\Delta\delta/[G]$ as a function of $\Delta\delta$ was plotted. The plot fits a linear relationship and the slope of the curve gives the binding constant value. Stoichiometries of the complexes were determined from the break of the titration curves which were obtained from the plot of $\Delta\delta$ versus $[G]/[H]$.

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