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SHORT COMMUNICATION

Naphthyridine amide–urea conjugate: a case toward selective fluorometric sensing of *N*-acetyl proline carboxylate

Kumaresh Ghosh · Tanmay Sarkar

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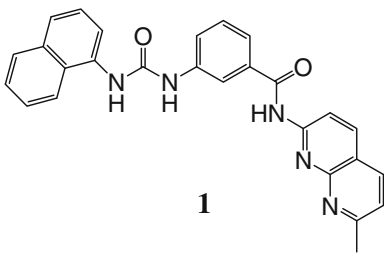
Abstract A simple neutral naphthyridine-based chemosensor **1**, which selectively recognizes the tetrabutylammonium salt of *N*-acetyl-L-proline over the other *N*-acetyl-L-amino acid salts studied in $CHCl_3$ containing 0.1% DMSO, has been designed and synthesized. Moreover, the complexation-induced change in emission characteristics of **1** distinguishes the amino acid salts examined from their conjugate acids. Interaction studies were performed by UV–vis, fluorescence and NMR spectroscopic methods.

Keywords Naphthyridine · Urea binding site · Proline salt recognition · Fluorometric distinction · DFT calculation

Design and synthesis of artificial receptors for a specific function is of utmost interest in the area of supramolecular chemistry [1–3]. During last decades, a great deal of attention has been focused on the recognition of neutral [4], cationic [5, 6] and anionic substrates [7] by abiotic receptors. Of the substrates, anions are important because of their significant roles in biology and environment [8–11]. Among the different kinds of anions, carboxylates are important as target species in molecular recognition as they serve important functions in biological systems [12]. Carboxylic acid functional group is present in amino acids, keto and hydroxy acids etc. and therefore, recognition of these substrates is a field of research in supramolecular chemistry [13–17]. Conversion of such acids into their carboxylate salts and their recognition by designed synthetic receptors is of continued interest in our laboratory [18–21]. In relation to this, we wish to report here a simple naphthyridine-based fluororeceptor **1**, which shows a preference for the tetrabutylammonium salt of *N*-acetyl-L-proline over other *N*-acetyl-L-amino acid salts examined by exhibiting complexation-induced greater change in emission.



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The receptor **1** was obtained according to the Scheme 1. Initially, methyl *m*-aminobenzoate was coupled with 1-naphthylamine in the form of urea linkage to give compound **2** using triphosgene as the reagent that converts amine into isocyanate functional group in situ. The ester group of **2** was next hydrolysed in the presence of LiOH in aqueous methanol to give the acid **3**. DCC coupling of 2-amino-7-methyl-1,8-naphthyridine with the acid **3** finally afforded the desired compound **1** in 56% yield.

All the compounds were unequivocally characterized by ^{1}H NMR, ^{13}C, FTIR and mass analysis.

Naphthyridine is a unique motif that plays important role in molecular recognition of various biologically important substrates [22–24]. Use of this motif as hydrogen bonding building block is well established in the literature.

Electronic supplementary material The online version of this article (doi:10.1007/s10847-011-9929-2) contains supplementary material, which is available to authorized users.

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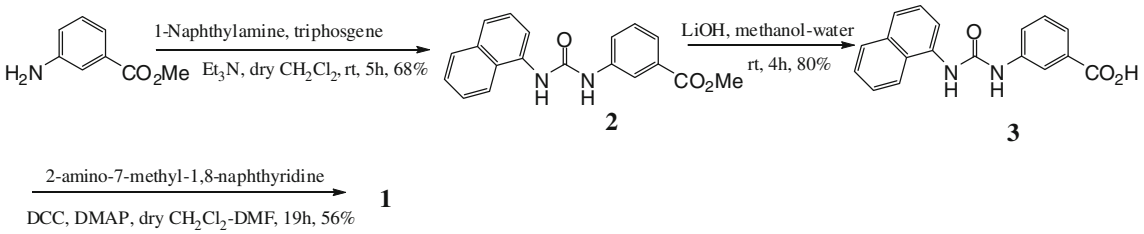

Scheme 1 Synthesis of receptor **1**

During the course of our work in molecular recognition, we used this motif in the sensing of hydroxy acid such as citric acid [25]. Inspiring results prompted us to take further this motif in the construction of new synthetic molecular architecture **1** for sensing and distinguishing the amino acid derivatives in organic solvent. In the design, naphthyridine amide has been considered as (i) hydrogen bonding site for the binding of carboxylic acid of amino acids and also (ii) fluorophore. In the present design it is connected to the naphthalene-based urea motif, which also provides both hydrogen bond donors and fluorophore unit. However, they are well disposed in a concave face to provide the recognition surface for tetrabutylammonium salts of amino acids.

In order to understand the recognition behavior of **1** towards the tetrabutylammonium salts of *N*-acetyl-L-amino acids such as valine, alanine, proline, phenyl glycine, UV–vis, fluorescence and ^{1}H NMR studies were performed. In addition, the hydrogen bonding behavior of **1** with pyruvic acid and its salts was studied under similar condition.

The receptor solution of **1** in $CHCl_3$ containing 0.1% DMSO gave emissions at 362 and 452 nm when excited at 290 nm. The emission at 362 nm is attributed to the naphthalene chromophore and emission at 452 nm is assigned to the naphthyridine motif. These two emissions are well resolved and distinct to interpret. Upon addition of the anionic guests to the receptor solution of **1**, the emission changed to the different extents. Figure 1 shows the change in emission of **1** upon addition of 1 equivalent amount of a particular anionic species. In comparison, the emission of **1** was merely perturbed when corresponding acid analogue of each carboxylate guest was added (Fig. 1) to the receptor solution. Among all the guests, salt of *N*-acetyl proline showed a marked change in emission. The complexation induced decrease in emission of **1** in the presence of *N*-acetyl-L-proline salt is represented in Fig. 2. The monomer emissions of both naphthyl and naphthyridine motifs underwent significant change suggesting the involvement of the bonding sites in complexation of the guest. The corresponding *N*-acetyl-L-proline weakly perturbed these two emissions although the receptor binding sites have the complementarities with the guest acid

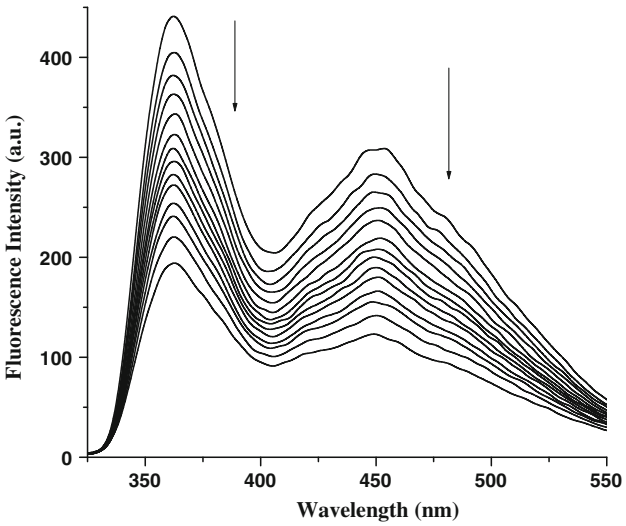

Fig. 2 Change in emission of **1** ($c = 4.83 \times 10^{-5}$ M) upon gradual addition of tetrabutylammonium salt of *N*-acetyl-L-proline

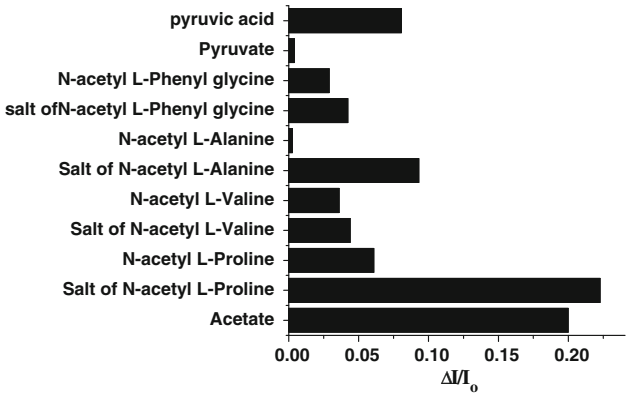

Fig. 1 Change in fluorescence ratio of **1** upon addition of one equivalent of anions ($c = 4.83 \times 10^{-5}$ M) at 362 nm

(supporting information in Supplementary material). Interestingly, while the change in emission of **1** at 452 nm was greater upon addition of *N*-acetyl α-amino carboxylic acids, the change in emission at 362 nm was much significant upon adding *N*-acetyl α-amino carboxylates. This observation probably indicated the relative preference of these two different guests towards the binding domains

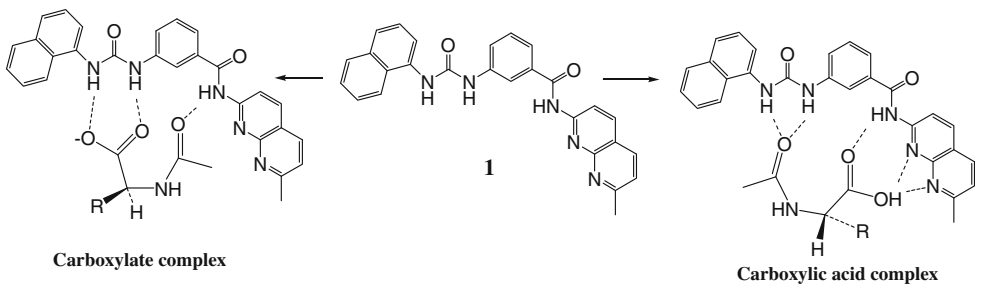

Fig. 3 Different modes of binding of *N*-acetyl amino carboxylic acids and *N*-acetyl amino carboxylate

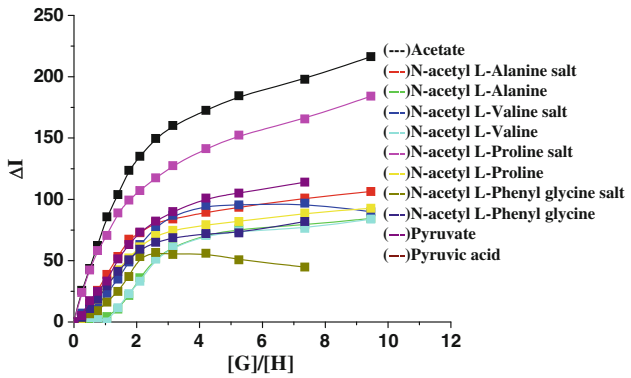

Fig. 4 Fluorescence titration curves for **1** ($c = 4.83 \times 10^{-5}$ M) with the guests in $CHCl_3$ containing 0.1% DMSO at 450 nm

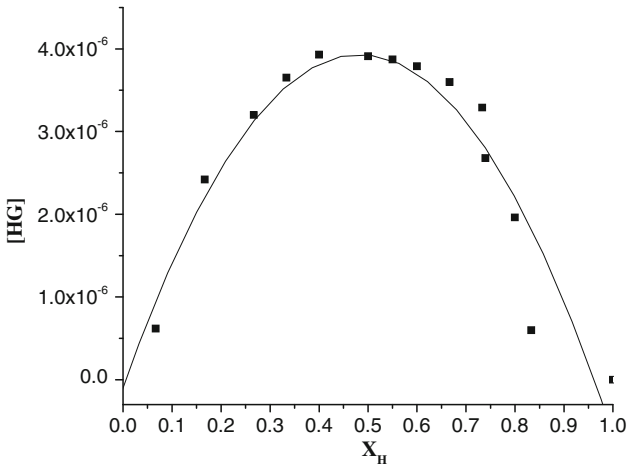

Fig. 5 Fluorescence Job plot for **1** with *N*-acetyl-L-proline salt in $CHCl_3$ containing 0.1% DMSO ([H] = [G] = 2.66×10^{-5} M)

(urea and naphthyridine amide) of **1**. Thus the different modes of binding of **1** for α-amino acids and its salt are demonstrated in Fig. 3.

The greater nucleophilic character of anionic guests over their neutral analogues has a clear-cut reflection on this aspect. The change in emission of **1** was also noted upon addition of tetrabutylammonium salts of pyruvic and acetic acids. Between the two, acetate ion interacted strongly. We believe that this is presumably due to more basic character and less steric nature of acetate ion.

However, the stoichiometries of the complexes were determined from the titration curves as well as Job plots. As can be seen from Fig. 4, receptor **1** shows greater change in emission with *N*-acetyl-L-proline salt and acetate. Other guests indicated small change in emission upon complexation. For the receptor **1**, there is a chance of formation of 1:1 and 2:1 (guest:host) complexes depending on the nature of guest as well as the relative orientation of the urea and amide functional groups of **1**. The receptor **1** can initially form 1:1 complex, which in the presence of excess concentration of guests, may be disrupted to attain a 2:1 (guest to host) stoichiometry. In the present case, the stoichiometry of the complexes of **1** with the anionic guests except pyruvate in the excited state was determined as 1:1. This was confirmed by fluorescence Job plot [26] (supporting information in Supplementary material). Figure 5, for example shows the Job plot for **1** with *N*-acetyl-L-proline salt. The Job plots for *N*-acetyl valine salt and acetate also showed 1:1 stoichiometries (supporting information in Supplementary material). For the carboxylic acids, emission of **1** was barely affected up to the addition of 1 equivalent amount; further addition caused change in emission although weakly.

Analysis of the emission titration results allowed us to determine the binding constant values. Non-linear curve fitting method [27] using Origin 6.1 gave the binding constant values. The results are summarized in Table 1, and demonstrate that anionic guests bind with moderate values. Among the anions, salt of *N*-acetyl-L-proline exhibited higher binding value compared to others. For pyruvate, 1:1 model did not fit well. However, 2:1 model fits properly. This situation happens due to the cooperative/non-cooperative action of the amide and urea moieties in the binding process. Structural aspect of the guest species may have the contribution in this regard. To our opinion, initial binding of *N*-acetyl amino acid salt to the urea motif (mode **A**) presumably activates the amide in further bonding either in the mode **B** (cooperative mode) or in mode **C** (non-cooperative mode) (Fig. 6). Mode **B** will lead to higher binding over the modes **A** and **C**. Actually which mode will be dominated in solution that will depend upon nature of the guest. All the anionic guests except pyruvate salt (supporting information in Supplementary material),

Table 1 Binding constant values (K_a) determined by fluorescence method in $CHCl_3$ containing 0.1% DMSO

Guest	K (M^{-1})
N-acetyl-L-proline carboxylate[a]	$K = (2.13 \pm 0.178) \times 10^4$; R = 0.998
N-acetyl-L-valine carboxylate[a]	$K = (3.37 \pm 1.04) \times 10^3$; R = 0.974
N-acetyl-L-alanine carboxylate[a]	$K = (5.54 \pm 0.944) \times 10^3$; R = 0.985
N-acetyl-L-phenyl glycine carboxylate[a]	$K = (8.41 \pm 0.455) \times 10^3$; R = 0.980
Pyruvate[a]	$K_{1:1}$ = nd, $K_{2:1} = (1.46 \pm 0.23) \times 10^4$; R = 0.989
Acetate[a]	$K = (8.53 \pm 1.18) \times 10^3$; R = 0.989
Pyruvic acid	$K = (6.45 \pm 1.40) \times 10^3$; R = 0.980

[a] Tetrabutylammonium salts were taken

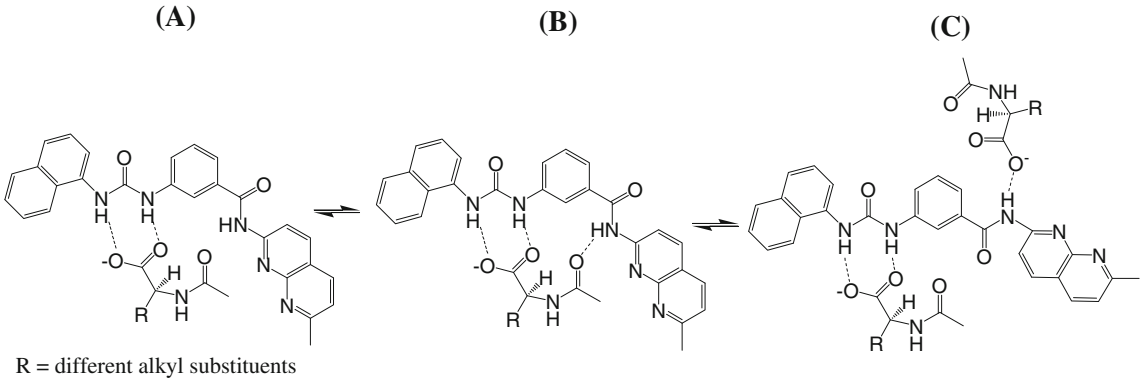

Fig. 6 Possible modes of binding of *N*-acetyl amino acid salt

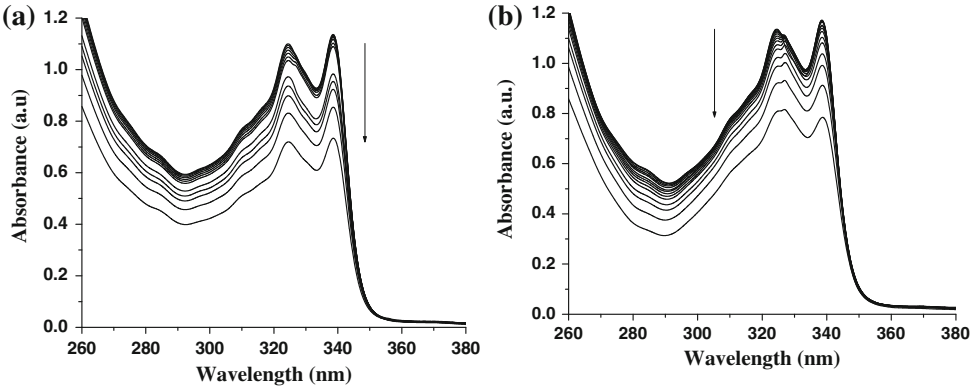

Fig. 7 **a** Change in absorbance of **1** ($c = 4.83 \times 10^{-5}$ M) upon gradual addition of *N*-acetyl-L-proline and **b** upon gradual addition of tetrabutylammonium salt of *N*-acetyl-L-proline in $CHCl_3$ containing 0.1% DMSO

follow 1:1 binding model giving emphasis on the mode **B**. For all the acids except pyruvic acid, the binding constant determination by fluorescence method was difficult due to minor change in emission titration spectra (supporting information in Supplementary material). Thus the receptor **1** fluorometrically distinguishes salts of *N*-acetyl α-amino acid from their conjugate acids showing marked change in emission titration spectra as well as binding constant values.

The interaction of **1** with the same guests in the ground state was also realized by UV–vis titration experiments. In all cases, the absorption of **1** decreased to the small extents when guest solutions were progressively added to the receptor solution. This was also true for the acid analogues. For example, the change in absorption of **1** upon titration with *N*-acetyl-L-proline and its salt is represented in Fig. 7. The stoichiometry of the complexes in the ground state was also ascertained from the Job plot and analysis of the results revealed 1:1 (guest:host) stoichiometry in each case [26] (supporting information in Supplementary material). However, we did not measure the binding potencies of **1** with the guests in the ground state due to minor change in UV–vis titration spectra.

The involvement of the binding sites of **1** in complexation of the anionic and neutral guests was established by ^1H NMR in $CDCl_3$ containing 3% d_6-DMSO. In the presence of equivalent amount of salt of *N*-acetyl-L-proline the urea and amide protons altogether underwent downfield chemical shift and thereby suggested their involvement in complexation through hydrogen bonding more preferably through the mode **B**. The similar experiment was done in the presence of the equivalent amount of the other salts.

Comparison of the results corroborated greater downfield shifting of the interacting protons in **1** only in the presence of the salt of *N*-acetyl-L-proline. The changes in chemical shift of the urea and amide protons of **1** in the presence of equivalent amount of the acid analogues were small in comparison to their salts and thus indicated weak interactions. Figure 8, represents the ^{1}H NMR changes of **1** upon addition of equivalent amount of the guests such as *N*-acetyl-L-valine, *N*-acetyl -L-valine salt and *N*-acetyl-L-proline salt.

The receptor **1** and its 1:1 complex with the salt of *N*-acetyl proline in the mode **B** as shown in Fig. 6 was optimized by DFT method using 6-31G* basis set [28] and the popular b3LYP functional [29, 30]. Figure 9 represents the DFT optimized geometries of the receptor **1** and its complex with *N*-acetyl-L-proline salt. Mulliken charges on the different atoms of the binding sites have been shown in Fig. 9. In the complex, all the hydrogen bond donors are intimately involved in the formation of hydrogen bonds and one hydrogen of $-CH_3$ group forms bifurcated hydrogen bonds with the naphthyridine ring nitrogens. Furthermore, MM2 calculations [31] on the complexes of **1** with *N*-acetyl-L-valine, alanine, phenylglycine salts were performed and the hydrogen bonding schemes were realized (supporting information in Supplementary material).

In conclusion, we have designed and synthesized an easy-to-make naphthyridine-based fluororeceptor **1**, which selectively recognizes the tetrabutylammonium salt of *N*-acetyl-L-proline over the other amino acid salts examined in the present study. Inspite of hydrogen bonding

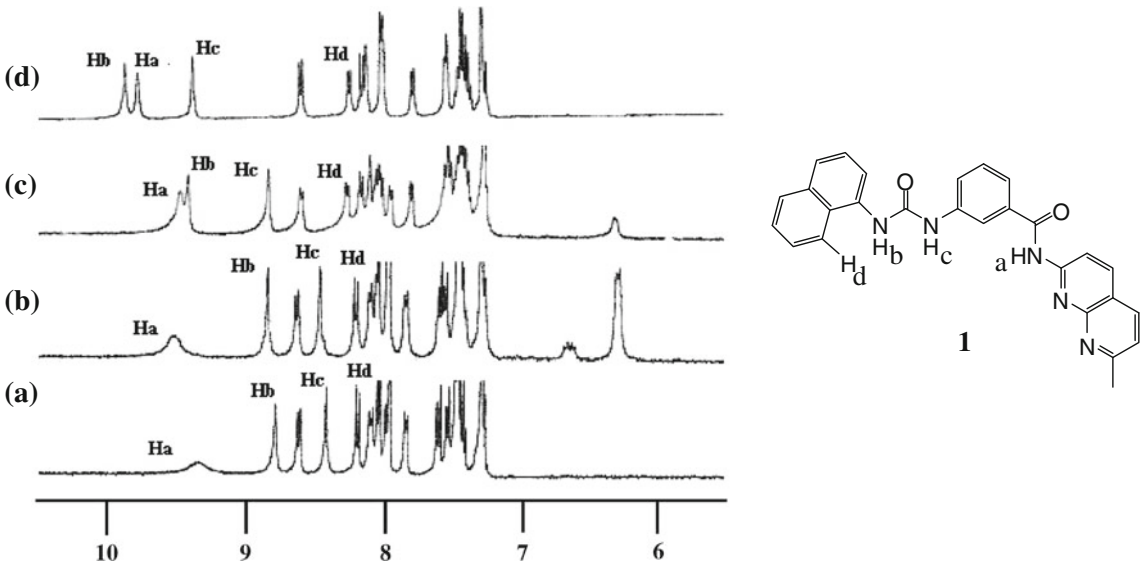

Fig. 8 Changes in 1H NMR of (*a*) **1** (400 MHz, CDCl3 containing 3% d6-DMSO; $c = 5.16 \times 10^{-3}$ M) and in the presence of equivalent amount of (*b*) L-*N*-acetyl valine (*c*) L-*N*-acetyl valine salt (*d*) L-*N*-acetyl proline salts

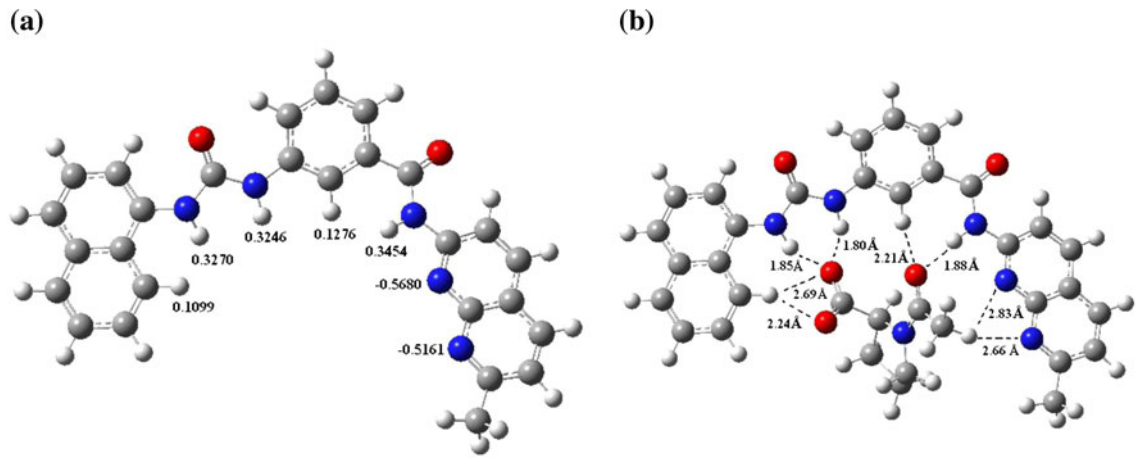

Fig. 9 DFT optimized geometries of (a) receptor **1** (HF = −1465.808 au, Dipole moment = 0.9121 D) and its complex with *N*-acetyl-L-proline salt (HF = −2019.149 au, Dipole moment = −5.0401 D)

complementarity of naphthyridine in **1** with carboxylic acid of *N*-acetyl amino acid, receptor **1** exhibits its much preference for carboxylates by showing significant change in emission titration spectra. Experimental findings reveal that our naphthyridine-based simple receptor can be useful in the fluorometric distinction of *N*-acetyl amino acid salt over their conjugate acids. This has a strong relevance with the function of active site of an enzyme. Among the anionic guests examined in the present study, *N*-acetyl-L-proline salt shows preference toward the open cleft of **1** involving cooperative hydrogen bonding interactions with the urea and amide motifs in a compact manner. Calculation performed at the DFT level of theory explained the cooperativity in the binding process. The greater quenching of emission and higher binding constant value as determined altogether underline the selective sensing of *N*-acetyl-L-proline salt. Further study along this direction is in progress in our laboratory.

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