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A benzthiazole-based simple receptor in fluorescence sensing of biotin ester and urea

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ABSTRACT

A benzthiazole-based receptor **1** has been designed and synthesized for recognition of biotin ester and urea in $CHCl_3$ containing 1% CH_3CN. The receptor binds biotin methyl ester and urea with moderate binding constant values and shows significant increase in emission of benzthiazole motif. The emission characteristics of **1** upon complexation clearly distinguishes biotin methyl ester and urea from thiourea and *N*,*N*′-dimethylurea. Characterization and sensing properties of the receptor **1** were evaluated by ^1H NMR, UV–vis, and fluorescence spectroscopic methods.

The design and synthesis of artificial receptors to study the hydrogen bonding interaction with biologically relevant molecules are important in the area of molecular recognition.[1,2] It is worth mentioning that the recognition and detection of bioactive substrates such as urea,[3] biotin,[4] carbohydrates,[5] carboxylic acids,[6] and amino acids[7] have gained considerable success. Among the substrates biotin (referred to as vitamin H in human) is an essential cofactor for a number of enzymes that have diverse metabolic functions.[8] Structurally it consists of a pentanoic acid side chain and a cis-fused bicyclic moiety with sulfur atom in the ring. Crystallographic studies have confirmed the relative stereochemistry at the asymmetric carbon.[9,10] 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.[11] 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 efforts were directed at a limited number of designed receptors. In this aspect, the use of Troger's base receptor as reported by Wilcox and co-workers, was noteworthy.[12] Claramunt and co-workers have reported the recognition of biotin methyl ester.[13] Pyridine amide-based simple receptor is also known to bind biotin itself involving both the carboxylic acid and the cyclic urea part of the bicyclic unit of biotin.[4] Similarly urea is also of particular importance as it is toxic, pollutant, and causes serious biological disorders.[14,15] To the best of our knowledge, very few fluorescent receptors for urea recognition are known in the literature. Goswami et al. have reported the fluorometric detection of urea by a macrocyclic receptor.[16] A 2,6-bis(2-benzimidazole)pyridine receptor as developed by Iyer and co-workers shows urea binding with concomitant change in fluorescence.[17] Non-fluorescent urea receptors with considerable binding ability are also known. Crown ether-based receptors as developed by Pedersen[3] and carboxylic acid containing crown ether receptor of Reinhoudt[18] are known to bind urea. Reinhoudt and co-workers have also explored the concept of using an electrophilic center to bind urea in the cavity of crown ether.[19] Bell et al. have reported the synthesis of naphthyridine-fused polyaza heterocycles that were potentially effective for urea recognition.[20] An investigation of hydrogen bonding interaction of benzimidazole-based receptors with urea and barbiturate in polar solvent is reported.[21] Goswami and Mukherjee reported the solubilization and recognition of urea using a simple dinaphthyridine receptor.[22] Recently we have shown that a pyridine amide-based macrocyclic receptor can make a strong inclusion complex with urea.[23] In contrast to the binding of small molecule urea, simple bis amide receptor is sometimes very efficient in sequence-specific binding of high molar mass copolyimides.[24]

Our ongoing interest in the selective binding of biologically relevant species by our synthetic receptor inspired us to work on simple system which is easy to make for fluorometric detection of both biotin methyl ester and urea in common organic solvent. In relation to this, we report here the design, synthesis, and host-guest interaction of benzthiazole-based receptor **1** which shows significant increase in emission upon complexation of biotin methyl ester and urea in $CHCl_3$ containing 1% CH_3CN.

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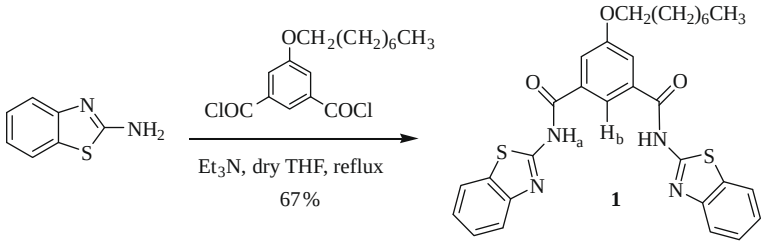

Scheme 1. Synthesis of receptor **1**.

Receptor **1**[25] was synthesized according to Scheme 1. The reaction of 2-aminobenzthiazole with 5-octyloxyisophthaloyl dichloride (prepared by etherification of diethyl 5-hydroxyisophthalate with octyl bromide in dry acetone using K_2CO_3 and hydrolysis of the esters followed by reaction with oxalyl chloride) in the presence of triethyl amine in dry THF afforded the desired compound in 67% yield.

Receptor **1** can adopt different conformations in solution. The *syn–syn* conformation with nitrogen atoms into the cavity was optimized with the guests containing urea moiety. Figure 1 shows the MM2 optimized geometries[26] of **1** with biotin methyl ester, urea, and thiourea. In all the structures the urea motif is complexed into the cleft with all possible hydrogen bonds. Thiourea is weakly complexed with less number of longer hydrogen bonds due to its more steric nature than urea.

In order to understand the recognition properties of **1** with biotin methyl ester, urea, thiourea, and *N,N′*-dimethylurea, ^{1}H NMR, fluorescence, and UV titrations were performed in $CHCl_3$ containing 1% CH_3CN. Receptor **1** ($c = 6.17 \times 10^{-5}$ M) showed the broad emission at 426 nm when excited at 308 nm in $CHCl_3$ containing 1% CH_3CN. Upon gradual addition of biotin methyl ester, urea, thiourea, and *N,N′*-dimethylurea to the solution of receptor **1** in $CHCl_3$ containing 1% CH_3CN, the emission of benzthiazole was increased to different extents. Among the guests examined, receptor **1** displayed strong fluorescence enhancement effect with biotin methyl ester. Figure 2 shows the comparison of change in emission of **1** in the presence of 3 equiv amounts of a particular guest in $CHCl_3$ containing 1% CH_3CN. It is evident from Figure 2 that receptor **1** shows a clear-cut selectivity with biotin methyl ester. To investigate the binding abilities of receptor **1** toward the guests examined, titration studies were performed. Figures 3 and 4, for example, show the change in emission of **1** in the presence of increasing amounts

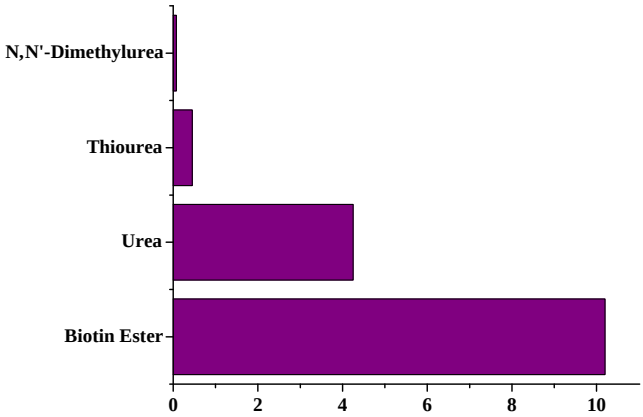

Figure 2. Fluorescence ratio ($I_0 - I/I_0$) of receptor **1** ($c = 6.17 \times 10^{-5}$ M) at 426 nm upon addition of 3 equiv of a particular guest in $CHCl_3$ containing 1% CH_3CN.

of biotin methyl ester and urea, respectively, in $CHCl_3$ containing 1% CH_3CN. Upon complexation of both biotin ester and urea the emission peak at 426 nm of **1** underwent a red shift of 15 nm, suggesting a strong hydrogen bonding interaction. During titration no other spectral changes were observed in the emission spectra, that is, there was no evidence of either exciplex or excimer emission. Figure 5 represents the fluorescence titration curves for biotin methyl ester, urea, thiourea, and *N,N′*-dimethylurea. The linear nature of the curves for thiourea and *N,N′*-dimethylurea indicated their weak interactions with receptor **1**. Simultaneous UV–vis titrations of **1** with the same guests were carried out in $CHCl_3$ containing 1% CH_3CN. The changes in absorbance of **1** upon complexation

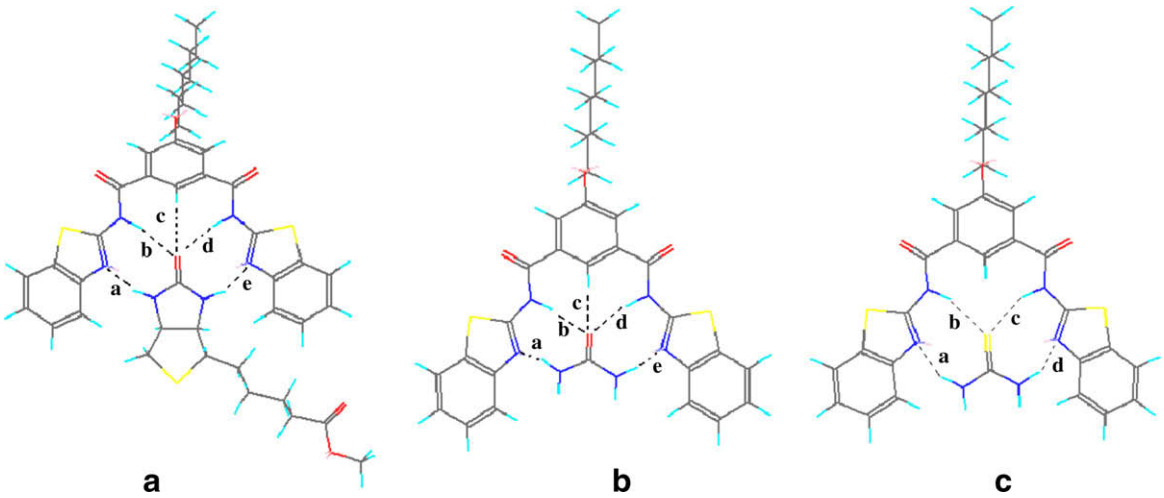

Figure 1. MM2 optimized geometries of (a) **1** with biotin methyl ester [$a = 2.25$ Å, $b = 2.04$ Å, $c = 2.42$ Å, $d = 2.04$ Å, $e = 2.17$ Å, $E = 13.79$ kcal/mol]. (b) **1** with urea [$a = 2.21$ Å, $b = 2.07$ Å, $c = 2.52$ Å, $d = 2.06$ Å, $e = 2.08$ Å, $E = -11.35$ kcal/mol] and (c) **1** with thiourea [$a = 2.25$ Å, $b = 2.81$ Å, $c = 2.85$ Å, $d = 2.34$ Å, $E = 3.65$ kcal/mol].

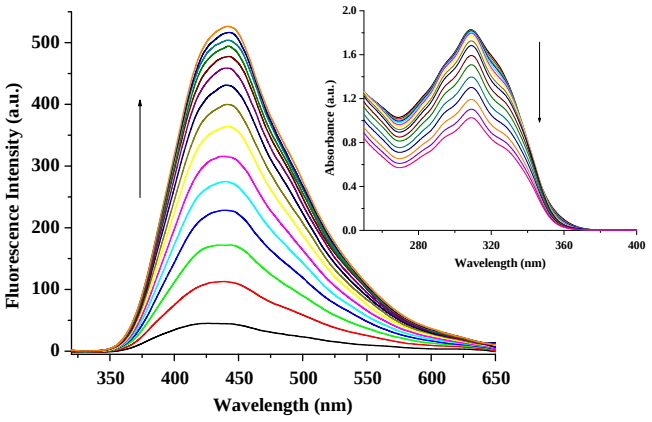

Figure 3. Change in emission of **1** ($c = 6.17 \times 10^{-5}$ M) upon gradual addition of biotin methyl ester in $CHCl_3$ containing 1% CH_3CN; inset: change in absorbance of **1** ($c = 6.17 \times 10^{-5}$ M) upon gradual addition of biotin methyl ester in $CHCl_3$ containing 1% CH_3CN.

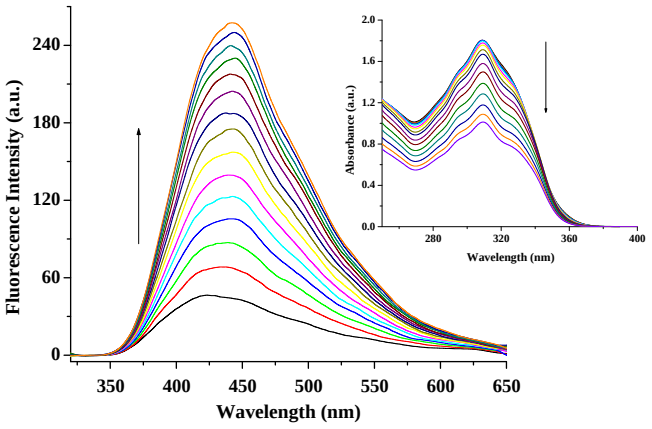

Figure 4. Change in emission of **1** ($c = 6.17 \times 10^{-5}$ M) upon gradual addition of urea in $CHCl_3$ containing 1% CH_3CN; inset: change in absorbance of **1** ($c = 6.17 \times 10^{-5}$ M) upon gradual addition of urea in $CHCl_3$ containing 1% CH_3CN.

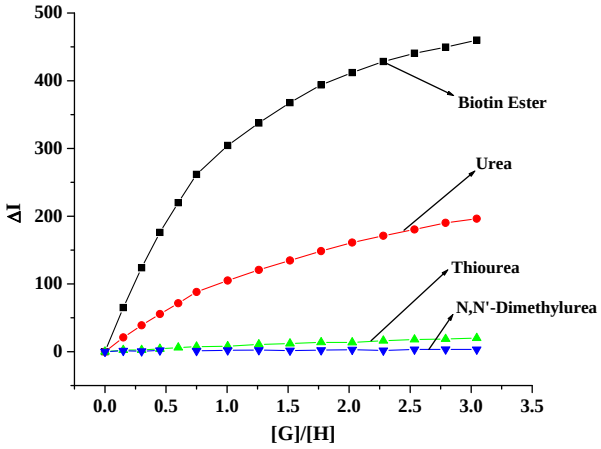

Figure 5. Plot of change in emission of **1** at 426 nm versus the ratio of guest to host concentration in $CHCl_3$ containing 1% CH_3CN.

of biotin methyl ester and urea are represented in the inset of Figures 3 and 4, respectively. During complexation in the ground state there was no shifting of the absorption band in either direction. The sharp break of the fluorescence titration curves at $[G]/[H] = 1$ for both biotin methyl ester and urea confirmed the 1:1 stoichiometries of the complexes (see Fig. 5).

Fluorescence titration data were used to determine the binding constant values.[27] As can be seen from Table 1, the simple receptor **1**, shows selectivity for biotin methyl ester. Urea being smaller in size is preferentially complexed than thiourea (based on the mode as shown in Fig. 1) in the excited state. Weak binding of thiourea is attributed to the bigger size of the sulfur atom that poorly fits into the cavity than urea. In this context, some characterization data of the C═S···HO and C═O···HO hydrogen bonds as reported by Kryachko et al.[28] suggested that polarizability of the sulfur atom and charge transfer phenomena are also responsible for the weaker hydrogen bond interaction of sulfur. This cannot be ruled out in the present study also. *N,N′*-Dimethylurea is weakly complexed into the open cleft due to its bulky nature. It is mentionable that CH_3CN being polar solvent, reduces the binding constant values to some extent. To realize the influence of polarity of CH_3CN in the binding process the titration experiments were also conducted in dry CH_3CN and the binding constant values are tabulated in Table 1. The binding constant values in CH_3CN are significantly less compared to the values determined in $CHCl_3$ containing 1% CH_3CN. It is important to note that the change in fluorescence ratio $(I_0-I)/I_0$ upon addition of particular guest is also significantly reduced (Fig. 6). The 1:1 stoichiometry of the complexes in CH_3CN was also confirmed from UV job plot. In this context, Figure 7 shows the job plot for biotin methyl ester and urea with **1**.

However, to substantiate the mode of binding as shown in Figure 1, ^1H NMR of **1** in the presence of the equivalent amount of guests was recorded in dry $CDCl_3$ containing 1% CD_3CN. Figure 8, for example, shows the change in ^1H NMR of **1** in the presence of biotin methyl ester. During complexation the amide protons

Table 1
Binding constant values for **1** from fluorescence titration

Guests	K_a (M^{-1})[a] in $CHCl_3$ containing 1% CH_3CN	K_a (M^{-1})[b] in CH_3CN
Biotin methyl ester	9.79×10^3	4.94×10^2
Urea	6.42×10^3	3.63×10^2
Thiourea	7.26×10^2	[c]
N,N′-Dimethylurea	[c]	2.11×10^2

[a] Binding constant values were determined at wavelength 426 nm.
[b] Binding constant values were determined at wavelength 455 nm.
[c] Binding constant values were not determined due to minor change.

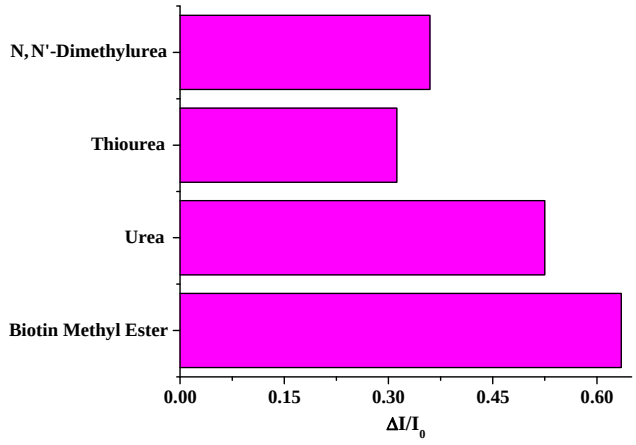

Figure 6. Fluorescence ratio (I_0-I/I_0) of receptor **1** ($c = 8.24 \times 10^{-5}$ M) at 455 nm upon addition of 30 equiv of a particular guest in CH_3CN ($\lambda_{ex} = 305$ nm).

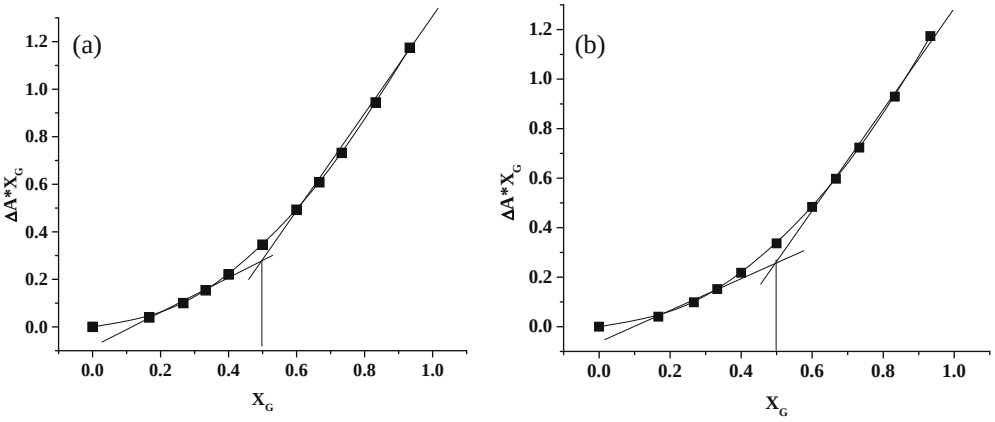

Figure 7. Job plots of **1** with biotin methyl ester (a) and urea (b) from UV method in CH_3CN.

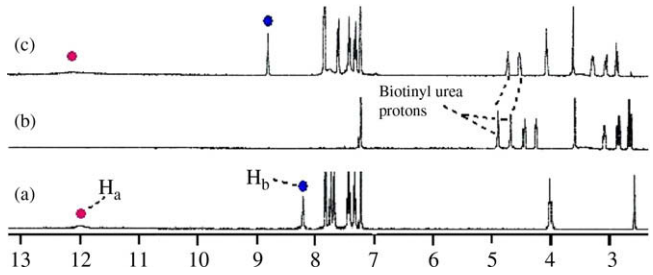

Figure 8. Partial ^1H NMR spectra of (a) receptor **1** ($c = 4.8 \times 10^{-3}$ M), (b) biotin methyl ester and (c) 1:1 complex of **1** with biotin methyl ester in $CDCl_3$ containing 1% CD_3CN.

appearing at δ 12.02 ppm moved downfield ($\Delta\delta$ = 0.18 ppm) to the lesser extent and became broad. Furthermore, the isophthaloyl peri proton H_b underwent significant downfield shift ($\Delta\delta$ = 0.6 ppm) upon complexation. In presence of equivalent amount of urea, thiourea, and *N,N′*-dimethylurea this peri proton of **1** exhibited the downfield chemical shifts of 0.50, 0.29, 0.16 ppm, respectively. This clearly indicated the participation of the peri proton in the formation of hydrogen bond with the urea carbonyl oxygen of the guests. Negligible changes in chemical shift of H_b of **1** in the presence of thiourea and *N,N′*-dimethylurea corroborated their weak interaction compared to urea (for urea see supplementary data). It is also of note that in the 1:1 complex of **1** with biotin methyl ester, the cyclic urea protons of biotin ester exhibited an upfield chemical shift with respect to the guest (biotin ester) itself. Moreover, the signals for aromatic protons of benzthiazole moiety shifted drastically, demonstrating probably a subtle conformational change of benzthiazole moiety in **1** during the course of binding of biotin methyl ester in solution phase. This experiment thus confirmed that biotin ester forms a hydrogen-bonded complex in the mode as shown in Figure 1a, where the cyclic urea part instead of more bulkier ester group of biotin is involved in the complexation into the isophthaloyl diamide core.

In conclusion, we have shown that receptor **1** which is simple and easy-to-make, can bind biotin ester and urea with moderate binding constant values. The detection of these biologically relevant guests is possible by fluorescence. The significant change in emission of **1** in the presence of biotin methyl ester and urea clearly distinguishes them from thiourea and substituted *N,N'*-dimethylurea. The distinction is best possible in less polar solvent and in the present case $CHCl_3$ containing 1% CH_3CN is the excellent choice as solvent in monitoring the host–guest interaction effectively. Further work in this direction is underway in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data (binding constant curves for **1** with biotin methyl ester and urea in $CHCl_3$ containing 1% CH_3CN, change in emission of **1** in the presence of biotin methyl ester and urea in CH_3CN, and change in ^1H NMR of **1** in the presence of equivalent amount of urea in $CDCl_3$ containing 1% CD_3CN are available) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.117.

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25. Mp 198 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 12.18 (br s, 2H, amide NH), 8.20 (s, 1H), 7.82 (d, 2H, J = 7.6 Hz), 7.73 (s, 2H), 7.67 (d, 2H, J = 7.6 Hz), 7.43 (t, 2H, J = 7.6 Hz), 7.33 (t, 2H, J = 7.6 Hz), 3.99 (t, 2H, J = 6 Hz), 1.80–1.77 (m, 2H), 1.46–

1.43 (m, 2H), 1.32–1.34 (m, 8H), 0.8 (t, 3H, J = 6.8 Hz); ^{13}C ($CDCl_3$, 125 MHz) δ 179.6, 164.8, 160.1, 146.7, 133.6, 131.5, 126.3, 124.1, 121.5, 119.9, 118.9, 117.9, 68.7, 31.8, 29.3, 29.2, 29.0, 25.9, 22.6, 21.7; FTIR (KBr, ν in cm^{-1}) 3334, 2924, 2855, 1672, 1597, 1545, 1445; Mass (EI positive ionization mode, m/z) 559.0 $(M+H)^+$, 581.1 $(M+Na)^+$.

26. Energy optimization was done using CS Chem 3D version 7.0.
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