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Design and Synthesis of a Neutral Fluorescent Macrocyclic Receptor for the Recognition of Urea in Chloroform

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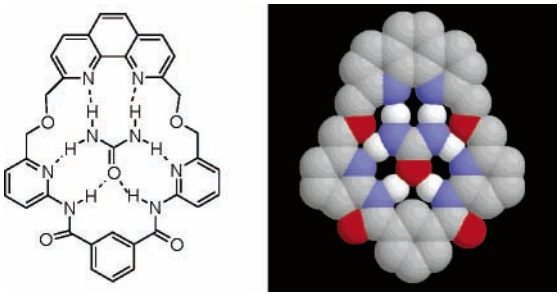
ABSTRACT



An artificial macrocyclic fluorescent receptor 1 has been designed and synthesized for the recognition of urea. 1 shows significant fluorescence quenching on complexation with urea and thiourea in chloroform and thus may be used as a synthetic fluorescent molecular sensor for their determination in a nondegradative way.

Urea is the chief nitrogenous end product of the metabolic breakdown of proteins in all mammals and some fishes and not only occurs in the urine of all mammals but also in their blood, bile, milk, and perspiration. The design and synthesis of an artificial receptor for urea for possible use as a fluorescent sensor[1] is important in the clinical identification of diabetes and kidney and thyroid diseases or to extract excess blood urea into a nonpolar solvent. Potentiometric sensors, based on gel-immobilized enzymes, and calorimetric sensors decompose urea into CO_3^{2-} and NH_4^+ ions in the former case and CO_2 and NH_3 in the later.[2] A neutral nonenzymatic sensor would be preferred to determine urea in a nondegrative way. This communication presents such a neutral receptor that shows appreciable fluorescence quenching upon binding of urea.

We have designed[3] a novel neutral fluorescent macrocyclic receptor **1** having two pyridylamide moieties and a 1,10-phenanthroline moiety as hydrogen bonding (HB) ligands to encircle urea by combined hydrogen bonding to urea carbonyl (HB acceptor) as well as its four NH atoms (HB donor) and also possible sideways hydrogen bonds by ether oxygens (and for possible secondary electrostatic interactions).[4] The incorporation of the fluorescent chromophore phenanthrolene in receptor **1** is aimed to allow possible hydrogen bonding (as a hydrogen bond acceptor) with the two anti hydrogens of urea carbonyl as well as the other two separate pyridine amides with isophthaloyl spacer, where pyridine nitrogens act as hydrogen bond acceptors for the remaining two hydrogens of urea and the two amide NH bonds of **1** act as two hydrogen bond donors to carbonyl

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(1) (a) De Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (b) Fabbrizzi, L. *Acc. Chem. Res.* **1999**, *32*, 846. For recent works, see: (c) Mello, J. V.; Finney, N. *Angew. Chem., Int. Ed.* **2001**, *40*, 1536. (d) Miyaji, H.; Sato, W.; Sessler, J. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 1777. (e) Pugh, V. J.; Hu, Q.-S.; Pu, L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3638. (f) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. L.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963. (g) Hartley, J. H.; James, T. D.; Ward, C. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3155. (h) Aoki, I.; Harada, T.; Sakaki, T.; Kawahara, Y.; Shinki, S. *J. Chem. Soc., Chem. Commun.* **1992**, 1341. (i) Lakowicz, J. R.; *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983; pp 257−301.

(2) Cattarall, R. W. *Chemical Sensors*; Oxford University Press: New York, 1997; pp 23, 50−53.

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oxygen of urea. Thus, it can bind urea strongly to form a stable and less polar complex compared to the individual components[5] engaging all the possible polar hydrogen bonding groups[3f] to solubilize urea in chloroform.

The suggested mode of complexation of urea with receptor **1** and the CPK model of the energy-minimized structure of the urea complex are shown in Figure 1, which shows the

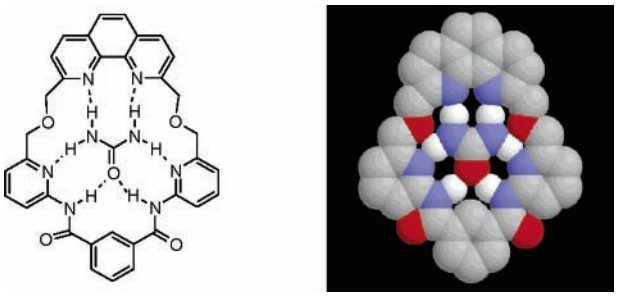
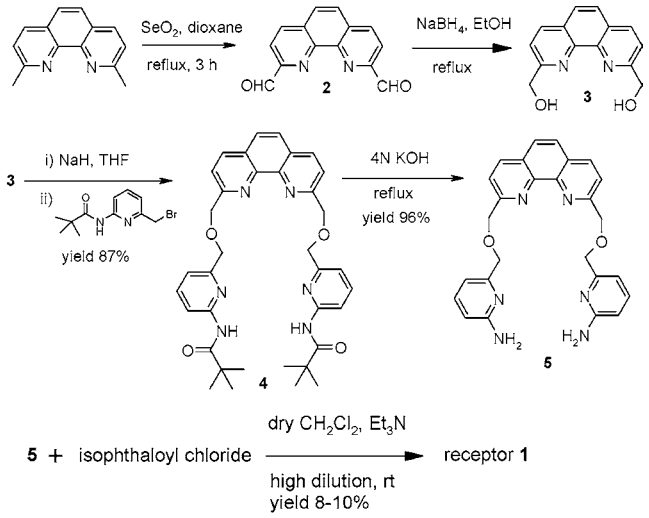


Figure 1. Structure of receptor **1** and the proposed urea complex (left) and a CPK model of the energy-minimized structure of the complex (right).

complementarity of urea with receptor **1** to form a stable complex. The synthesis of receptor **1** is delineated in Scheme 1. 1,10-Phenanthroline-dimethanol **3**[6] is coupled with 2-piv-

Scheme 1. Synthesis of Receptor **1**



aloylamino-6-bromomethylpyridine[7] (obtained by NBS bromination of 2-pivaloylamino-6-methylpyridine) by sodium hydride to give **4**, which on hydrolysis forms the diamine **5**. High-dilution coupling of **5** with isophthaloyl chloride affords the desired macrocyclic receptor **1** in 8−10% yield.

The ^{1}H NMR experiment in $CDCl_3$ reveals that the receptor **1** forms a strong 1:1 complex to solubilize urea, which is evident from the appearance of four urea protons at δ 6.36 (Figure 2). The downfield shift of amide protons

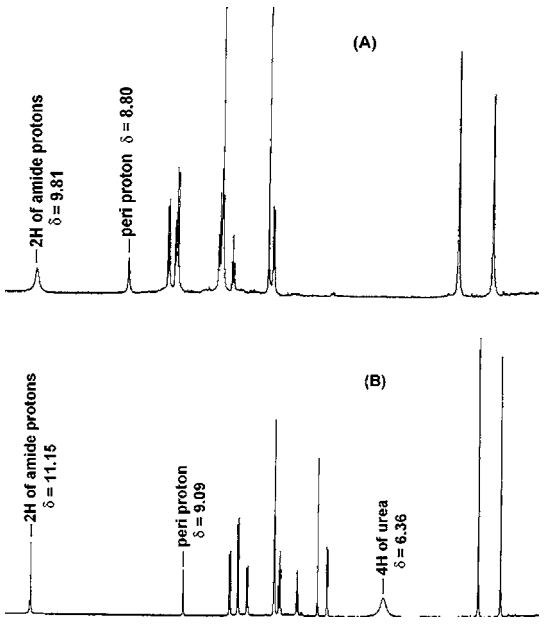


Figure 2. (A) ^{1}H NMR of receptor **1**. (B) ^{1}H NMR of urea complex with **1**.

occurs in the 1:1 complex from δ 9.81 to 11.15 ppm ($\Delta\delta$ = 1.34 ppm), and also the *peri* proton of isophthaloyl (C2H) moiety shifts downfield from δ 8.80 to 9.09 ppm ($\Delta\delta$ = 0.29 ppm) possibly due to the close approach of the urea carbonyl oxygen for strong complexation as suggested in Figure 1. The strong complexation is also evident from the better resolution of the peaks in the NMR spectrum. Two overlapping doublets at δ 8.4 appear as two separate doublets with the middle peaks merging on complexation, which reduces free rotation compared to the uncomplexed receptor. During the study of binding constant measurement by the dilution method, it was observed that on dilution of the 1:1 complex, there is no shift of the amide and urea protons in

(3) (a) Hegde, V.; Hung, C.-Y.; Madhukar, P.; Cunningham, R.; Höpfner, T.; Thummel, R. P. *J. Am. Chem. Soc.* **1993**, *115*, 872. (b) Hegde, V.; Madhukar, P.; Madura, J. D.; Thummel, R. P. *J. Am. Chem. Soc.* **1990**, *112*, 4549. (c) Crego, M.; Marugán, J. J.; Raposo, C.; Sanz, M. J.; Alcázar, V.; Caballero, M. C.; Morán, J. R. *Tetrahedron Lett.* **1991**, *32*, 4185. (d) Bell, T. W.; Beckles, D. L.; CraggLiu, P. J.; Maioriello, J.; Papoulis, A. T.; Santora, V. J. In *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society Books: Washington, DC, 1993; pp 85−103. (e) Bell, T. W.; Liu, J. *J. Am. Chem. Soc.* **1988**, *110*, 3673. (f) Bell, T. W.; Hou, Z. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1536. (g) Goswami, S. P.; Mukherjee, R. *Tetrahedron Lett.* **1997**, *38*, 1619. (h) van Veggel, F. C. J. M.; Reinhoudt, D. N. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: New York, 1991; pp 83−108. (i) van Doorn, A. R.; Schaafstra, R.; Bos, M.; Harkema, S.; van Eerden, J.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1991**, *56*, 6083. (j) van Staveren, C. J.; Aarts, V. M. L. J.; Grootenhuis, P. D. J.; Droppers, W. J. H.; Van Eerden, J.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1988**, *110*, 8134. (k) van Staveren, C. J.; van Eerden, J.; van Veggel, F. C. J. M.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1988**, *110*, 4994.

(4) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008.

(5) Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024.

(6) Chandler, C. J.; Deady, L. W.; Reiss, J. A. *J. Heterocycl. Chem.* **1981**, *18*, 599.

(7) Hansen, D. W., Jr.; Adelstein, G. W. (Searle, G. D., and Co.). *Chem. Abstr.* **1991**, *115*, 136387g.

the NMR spectra, which implies the formation of a strong urea complex.

The fluorescence spectra of receptor **1** and its urea and thiourea complexes in chloroform at 298 K are shown in Figure 3, using 10^{-6} M concentration. Receptor **1**, when excited at 272 nm (λ_{max} of **1** in chloroform at 298 K), gives emission maxima around 365 and 384 nm. On complexation with urea and thiourea, significant fluorescence quenching takes place in both cases as shown in Figure 3, where the fluorescence maximum (λ_F) of the urea complex is slightly blue shifted and the thiourea complex is slightly red shifted with respect to **1**. To study the effect of free urea concentration in solution, we recorded different fluorescence spectra of urea complex solutions having increasing urea concentrations, and we observed about 50% quenching in all cases with respect to **1** [Figure 3]. There was no change in the quenching and shift of λ_F when the fluorescence spectra of **1** in chloroform at 298 K were recorded in the presence of excess urea or in ethyl alcohol, dilute HCl, or water. This implies that quenching was due to the urea complex of **1**. To validate this implication, excitation and absorption spectra of the complex were also compared. So by quenching, shift of λ_F, and also by comparison of absorption and excitation spectra, fluorescence response by urea binding was detected. Though the overall binding of urea with **1** is greater than that of thiourea, the quenching by thiourea complex of **1** is greater than that of the urea complex, which may be due to the greater tendency of thiourea for ground-state complex formation through hydrogen bond interaction with the fluorophore, i.e., the phenanthroline[9] part of **1**. Thus, though thiourea makes a weaker hydrogen bond with sulfur compared to oxygen, interaction of thiourea with the fluorophore, i.e., the phenanthroline[9] part of **1**, may be greater due to the fact that thiourea NH is more acidic[8] than urea. It may also be noted that $S_1 \rightarrow T_1$ intersystem crossing in the phenanthroline moiety will be more efficient in the presence of the S atom of thiourea due to large spin−orbit coupling.

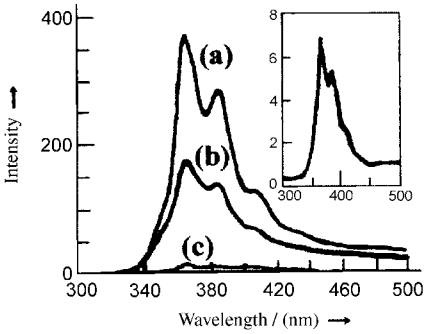


Figure 3. Fluorescence spectra in $CHCl_3$ (6.64×10^{-6} M/L in each case) at 298 K for (a) receptor **1**, (b) the urea complex of **1**, and (c) the thiourea complex of **1**. The emission spectrum for c is depicted in the inset.

The absorbance effects at different dilutions of the urea complex with receptor **1** at λ_{max} 272 and 239 nm, respectively, were shown to give straight lines. To determine the approximate binding constant (K_a) of **1** with urea by the fluorescence technique,[10] at first a 10^{-6} M 1:1 urea complex solution was prepared by dilution of the solution, used in NMR showing a 1:1 complex. Different compositions (by volume) of the urea complex of **1** in chloroform were then prepared by dilution of the above solution and also by dilution of the residue obtained by evaporating the NMR solution of the 1:1 complex. The solubility of urea in chloroform is so limited that an accurate value of K_a cannot be determined. Here, the binding of receptor **1** is so tight (as also suggested from the fact that there is no change in the NMR spectrum of the 1:1 complex on dilution) that significant dissociation does not occur as a result of the minor change in concentration, and thus the emission intensity should decrease linearly as a function of concentration, which is actually the case here. However, the fluorescence experiments suggest that K_a for urea cannot be less than the order of 10^5. For thiourea, K_a ($\approx 10^3$) is much less than that for urea. This suggests that receptor **1** has a stronger affinity for urea than for thiourea.

We have thus developed a nonenzymatic neutral fluorescent synthetic probe for notoriously insoluble urea in chloroform. The 1,10-phenanthroline moiety of **1** possibly lies in the binding zone to influence the fluorescence property during complexation, showing the potential applicability of **1** as a fluorescent sensor for urea and thiourea in a nondegradative way.

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Supporting Information Available: Experimental details and spectral data (^{1}H and ^{13}C NMR and mass spectra) for **1** and its urea complex (^{1}H NMR), **4**, figure for fluorescence spectra of receptor **1** in chloroform with varying amounts of urea and their one representative calculation for approximate K_a determination for the urea complex of **1**, the linear regression analysis, and also the corresponding change of fluorescence spectra on dilution. This material is available free of charge via the Internet at http://pubs.acs.org.

OL050034H

(8) (a) Bordell, F. G.; Algrim, D. G.; Harrelson, J. A., Jr., *J. Am. Chem. Soc.* **1990**, *112*, 2008. (b) Fan, E.; van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369.

(9) (a) Alpha, B.; Lehn, J.-M.; Mathis, G. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 266. (b) Kalyansundaram, K. *Photochemistry of Polypyridines and Porphyrin Complexes*; Academic Press: London, 1992; p 92.

(10) (a) Colquhoun, H. M.; Goodings, E. P.; Maud, J. M.; Stoddart, J. F.; Wolstenholme, J. B.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 2* **1985**, 607. (b) Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; John Wiley and Sons: New York, 1987; p 340. (c) Roy, J.; Bhattacharya, S.; Ghosh, S.; Mazumder, D.; Bhattacharyya, S. P. *Chem. Phys.* **1997**, *222*, 161. (d) Ghosh, P.; Bharadwaj, P. K.; Roy, J.; Ghosh, S. *J. Am. Chem. Soc.* **1997**, *119*, 11903.