Selective Recognition of Tryptophan through Inhibition of Intramolecular Charge-Transfer Interactions in an Aqueous Medium

ARTICLE *in* ORGANIC LETTERS · MARCH 2007

Impact Factor: 6.36 · DOI: 10.1021/ol062636m · Source: PubMed

3 AUTHORS, INCLUDING:

Mahesh Hariharan
Indian Institute Of Science Education and Re…
45 PUBLICATIONS **843** CITATIONS
SEE PROFILE

Suneesh C Karunakaran
Georgia Institute of Technology
8 PUBLICATIONS **66** CITATIONS
SEE PROFILE

ORGANIC LETTERS

2007
Vol. 9, No. 3
417−420

Selective Recognition of Tryptophan through Inhibition of Intramolecular Charge-Transfer Interactions in an Aqueous Medium

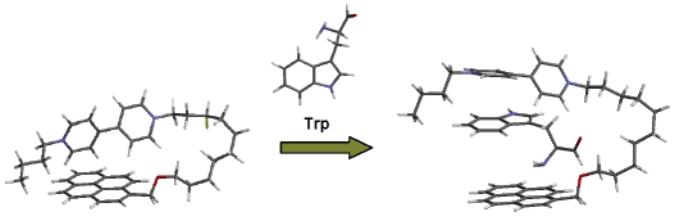
Mahesh Hariharan, Suneesh C. Karunakaran, and Danaboyina Ramaiah*

Photosciences and Photonics, Chemical Sciences and Technology Division, Regional Research Laboratory (CSIR), Trivandrum 695019, India

rama@csrrltrd.ren.nic.in; d_ramaiah@rediffmail.com

Received October 27, 2006

ABSTRACT



A novel donor–acceptor conjugate 1 was synthesized, and its interactions with various amino acids have been investigated as compared to the model system 2. The conjugate 1 unusually forms an intramolecular charge-transfer complex in the aqueous medium and undergoes selective binding interactions with tryptophan. The uniqueness of this system is that it selectively recognizes tryptophan among all other amino acids and involves synergistic effects of π-stacking, electrostatic, and donor–acceptor interactions.

Selective recognition of amino acids has great significance as they assemble together to yield proteins, enzymes, structural elements, and many macromolecules of biological function.[1−3] Of all the amino acids, the recognition of tryptophan (Trp) is vital as it forms the precursor for the secretion of the sleep inducing hormone, melatonin,[4] and has also been implicated as a possible cause of Schizophrenia due to its improper metabolism under in vivo conditions.[5,6] Most known receptors for amino acids employ the complementary hydrogen bonding for their recognition.[7] However, such recognition in an aqueous medium would be limited due to the competitive hydrogen bonding of the solvent.[8] The selective recognition involving electrostatic interactions would also be limited due to the dipolar nature of all the natural amino acids.[9] There are a few reports in the literature,[10] which employ autofluorescence for the detection and quantification of Trp. However, such a method would be limited because other aromatic amino acids such as tyrosine (Tyr) and phenylalanine (Phe), which exhibit similar

(1) (a) Folmer-Anderson, J. F.; Kitamura, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2006**, *128*, 5652. (b) Shinoda, S.; Okazaki, T.; Player, T. N.; Misaki, H.; Hori, K.; Tsukubu, H. *J. Org. Chem.* **2005**, *70*, 1835. (c) Comeau, A. B.; Seto, C. T. *Org. Lett.* **2004**, *6*, 83. (d) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208. (e) Sirish, M.; Schneider, H.-J. *Chem. Commun.* **1999**, 907.

(2) (a) Ros-Lis, J. V.; Garcia, B.; Jimenez, D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Gonzalvo, F.; Valldecabres, M. C. *J. Am. Chem. Soc.* **2004**, *126*, 4064. (b) Hortala, M. A.; Fabbrizzi, L.; Macrotte, N.; Stomeo, F.; Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20.

(3) (a) Hamilton, A. D.; Lehn, J.-M.; Sessler, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 5158. (b) Liu, Y.; Han, B.; Li, B.; Zhang, Y.; Zhao, P.; Chen, Y.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1998**, *63*, 1444.

(4) (a) Horacek, J.; Zavesicka, L.; Tintera, J.; Dockery, C.; Platilova, V.; Kopecek, M.; Spaniel, F.; Bubenikova, V.; Hoschl, C. *Physiol. Res.* **2005**, *54*, 235. (b) Bao, Z.; Sun, S.; Li, J.; Chen, X.; Dong, S.; Ma, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 6723.

(5) Rosse, R. B.; Schwartz, B. L.; Zlotolow, S.; Bancy-Schwartz, M.; Trinidad, A. C.; Peace, T. D.; Deutsch, S. I. *Clin. Neuropharmocol.* **1992**, *15*, 129.

(6) Williamson, B. L.; Klarskov, K.; Tomlinsion, A. J.; Gleich, G. J.; Naylor, S. *Nat. Med.* **1998**, *4*, 983.

(7) (a) Grawe, T.; Schrader, T.; Finocchiaro, P.; Consiglio, G.; Failla, S. *Org. Lett.* **2001**, *3*, 1597. (b) Liu, S. Y.; Fang, L.; He, Y. B.; Chan, W. H.; Yeung, K. T.; Cheng, Y. K.; Yang, R. H. *Org. Lett.* **2005**, *7*, 5825.

(8) (a) Kim, S.-G.; Kim, K.-H.; Shin, S. K.; Ahn, K. H. *J. Am. Chem. Soc.* **2003**, *125*, 13819. (b) Huang, X.; Fujioka, N.; Pescitelli, G.; Koehn, F. E.; Williamson, R. T.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **2002**, *124*, 10320.

10.1021/ol062636m CCC: $37.00
Published on Web 01/10/2007

absorption and fluorescence emissions, albeit in low yields,[11] would interfere with the recognition. Progress in this area would require new strategies for the recognition of Trp in the aqueous medium and subsequent signaling of the event through optical changes.[12]

In this context, we designed a novel donor−acceptor conjugate **1**, for the selective recognition of Trp by making use of its beneficial electron-donating, electrostatic, and π-stacking properties (Figure 1 and Supporting Information

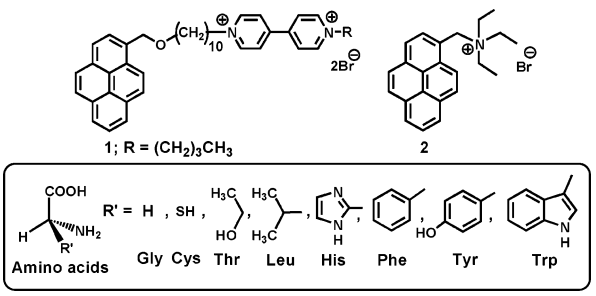


Figure 1. Structures of the viologen-linked pyrene conjugate **1**, the model compound **2**, and selected amino acids.

Figures S1−S4). Interestingly, our results indicate that **1** forms a fluorescent intramolecular charge-transfer (ICT) complex in the aqueous medium and exhibits unusual selectivity for Trp. Uniquely, this system is devoid of hydrogen bonding but recognizes Trp selectively through inhibition of the ICT complex involving π-stacking, electrostatic, and donor−acceptor interactions and signals the event through changes in steady-state and time-resolved fluorescence, ^{1}H NMR, and cyclic voltammetric (CV) techniques.

Figure 2 shows the absorption spectra of the viologen-linked pyrene conjugate **1** and the model compound **2** in an aqueous medium. In addition to the absorption corresponding to the pyrene chromophore at 344 nm, the viologen-linked pyrene derivative **1** shows a new and diffused band in the long wavelength region that extends up to 440 nm. Similarly, the fluorescence spectrum of **1**, on the other hand, showed a broad and red-shifted emission centered at 475 nm, in addition to the characteristic pyrene chromophore emission at 400 nm (inset of Figure 2). In contrast, the model compound **2** exhibited only the absorption and fluorescence spectra that are characteristic of the pyrene chromophore.

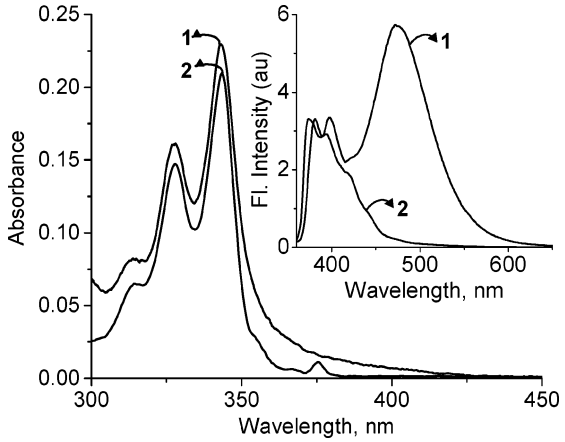


Figure 2. Absorption spectra of **1** (8.5 μM) and the model compound **2** (8.15 μM) in an aqueous medium. Inset shows the corresponding emission spectra. Excitation wavelength = 340 nm.

The picosecond time-resolved fluorescence studies of **1** in an aqueous medium showed a biexponential decay with lifetimes of 4.3 (22%) and 121 ns (78%), when monitored at 400 nm (Figure 3). However, when the emission was

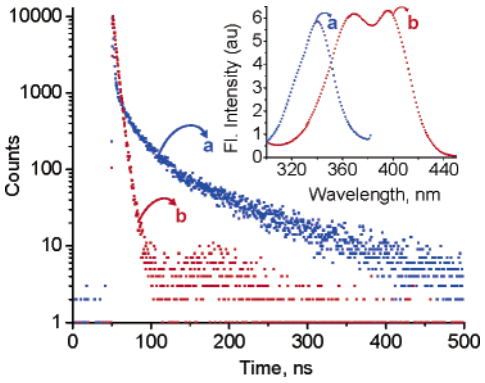


Figure 3. Picosecond time-resolved fluorescence spectra of **1** (13 μM) in an aqueous medium. Emission collected at (a) 400 and (b) 475 nm. Excitation wavelength = 335 nm. Inset shows excitation spectra of **1** (8.5 μM) in an aqueous medium. Emission monitored at (a) 400 and (b) 475 nm.

monitored at 475 nm, we observed only monoexponential decay with a lifetime of 4.3 ns (100%).

To have a better understanding of the species that exhibited long wavelength emission at 475 nm in the case of derivative **1**, we have recorded the fluorescence excitation spectra, following the emission at different wavelengths (inset of Figure 3). When monitored at 400 nm, the fluorescence excitation spectrum of **1** is found to be characteristic of the pyrene moiety. In contrast, when monitored at 475 nm, the excitation spectrum showed a peak around 400 nm that extended up to 440 nm. This band is different from the absorption of the pyrene chromophore (344 nm).

(9) (a) Folmer-Anderson, J. F.; Kitamura, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986. (b) Schmuck, C.; Bickert, V. *Org. Lett.* **2003**, *5*, 4579. (c) Sasaki, S.; Hashizume, A.; Citterio, D.; Fujii, E.; Suzuki, K. *Tetrahedron Lett.* **2002**, *43*, 7243. (d) Sirish, M.; Chertkov, V. A.; Schneider, H.-J. *Chem.−Eur. J.* **2002**, *8*, 1181.

(10) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. In *Molecular Biology of the Cell*, 4th ed.; Garland Science: New York, 2002.

(11) (a) Galian, R. E.; Pastor-Perez, L.; Miranda, M. A.; Perez-Prieto, J. *Chem.−Eur. J.* **2005**, *11*, 3443. (b) Wang, X.; Nau, W. M. *J. Am. Chem. Soc.* **2004**, *126*, 808.

(12) 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.

The nature of the unusual species observed in the case of the viologen-linked pyrene conjugate **1** was further investigated through the effects of concentration, solvent, and temperature (Supporting Information Figures S5−S8). With the increase in concentration of the conjugate **1** in the aqueous medium, we observed an increase in emission intensity at 475 nm as well as at 400 nm corresponding to the pyrene chromophore. However, the relative change in fluorescence intensity (I_{475}/I_{400}) with an increase in concentration of **1** showed a sigmoidal nature. On the other hand, the fluorescence spectra of **1** in methanol and phosphate buffer (pH 7.4; 10 mM) showed only the emission that is characteristic of the pyrene chromophore. The broad and structureless emission at 475 nm was not observed under these conditions, indicating its sensitivity to the nature of the medium.[13] As the temperature increases, the emission at 475 nm showed a decrease in intensity with a concomitant increase in the intensity of the pyrene chromophore at 400 nm. However, the emission at 475 nm could be observed even at 75 °C, indicating the stability of this species at these temperatures.[14]

The new broad absorption band observed (370−440 nm) in the case of the conjugate **1** could be attributed to the formation of the fluorescent intramolecular charge-transfer (ICT) complex between the pyrene chromophore and the viologen moiety. This assignment is based on the intermolecular fluorescence quenching of the pyrene chromophore by methylviologen, characterization of the pyrene radical cation and reduced viologen by laser flash photolysis studies,[15] and the theoretically calculated favorable change in free energy ($\Delta G = -1.6$ eV)[16] for such an electron transfer between pyrene and viologen moieties. Further evidence for the involvement of a fluorescent ICT complex is obtained from the negligible results with the model compound **2**, which lacks the viologen unit. In addition, we observed both folded-sandwich and extended conformers through AM1 calculations, wherein the former one is expected to undergo effective ICT interactions.

Our next objective was to evaluate and employ the beneficial properties of the viologen-linked pyrene **1** for the recognition of amino acids because this conjugate forms a novel fluorescent intramolecular charge-transfer (ICT) complex in the aqueous medium. We selected a few naturally occurring important aromatic and, for comparison, aliphatic L-amino acids (Figure 1) and have investigated their interactions with the conjugate **1** through photophysical, ^{1}H NMR, and CV techniques. Figure 4 shows the changes in the fluorescence spectra of the conjugate **1** with an increase in concentration of Trp in the aqueous medium. We observed a regular decrease in the fluorescence intensity at 475 nm along with a concomitant increase in intensity at 400 nm, corresponding to the ICT complex and the pyrene chromophore, respectively. The Benesi−Hildebrand analysis of the fluorescence changes observed at 475 nm gave a 1:1 stoichiometry for the complex formed between the conjugate **1** and Trp with an association constant of $K_{ass} = 1300 \pm 29$ M^{-1} and the change in free energy of −17.7 kJ mol^{-1}. This value is in good agreement with the association constant ($K_{ass} = 1261 \pm 33$ M^{-1}) calculated using the fluorescence changes observed but excited at 400 nm (Supporting Information Figure S9). When we titrated the conjugate **1** with Phe and Tyr, we obtained significantly lower association constants of 73 and 52 M^{-1}, respectively, whereas other amino acids such as Gly, Cys, Thr, Leu, and His under similar conditions exhibited negligible changes in both the absorption and the fluorescence properties of **1** (Supporting Information Figure S10). These results indicate that the conjugate **1** interacts selectively with Trp through altering the ICT complex formation, whereas all other amino acids exhibit negligible interactions (Supporting Information Figure S11). Interestingly, the selectivity of the conjugate **1** toward Trp was observed even in the presence of equimolar amounts of other amino acids.

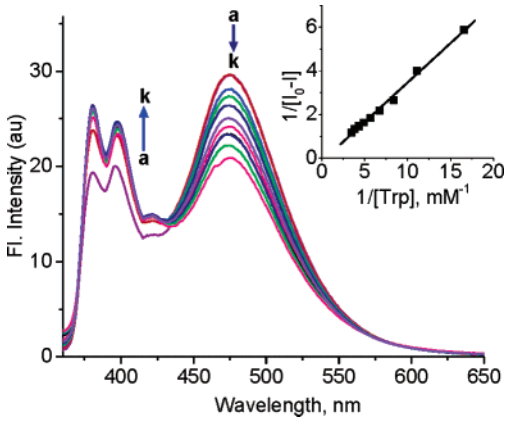


Figure 4. Change in the fluorescence spectrum of **1** (13 μM) in an aqueous medium with an increase in the addition of Trp. [Trp] (a) 2.9 and (k) 27.0 × 10^{-4} M. Inset shows the Benesi−Hildebrand analysis. Excitation wavelength = 340 nm.

The complex formation between the conjugate **1** and Trp was further demonstrated using picosecond time-resolved fluorescence, CV, and ^{1}H NMR techniques. In CV, conjugate **1** exhibited two reversible one-electron reduction processes centered at −0.46 and −0.74 V, characteristic of the viologen moiety (Figure 5). However, in the presence of Trp, we observed a significant decrease in current intensity of 7.2 μA (32%) and 13.5 μA (33%), indicating the formation of a strong complex[17] between **1** and Trp. The time-resolved decay analysis indicated that **1** alone exhibits a monoexponential decay with a lifetime of 4.3 ns when monitored at

(13) (a) Schneider, H.-J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442. (b) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417.

(14) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley-Interscience: New York, 1970; Chapter 9.

(15) (a) Hariharan, M.; Joseph, J.; Ramaiah, D. *J. Phys. Chem. B* **2006**, *110*, 24678. (b) Joseph, J.; Eldho, N. V.; Ramaiah, D. *Chem.−Eur. J.* **2003**, *9*, 5926. (c) Joseph, J.; Eldho, N. V.; Ramaiah, D. *J. Phys. Chem. B* **2003**, *107*, 4444.

(16) Murov, S. L.; Carmichael, I.; Hug, G. L. In *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker, Inc.: New York, 1993.

(17) (a) Neelakandan, P. P.; Hariharan, M.; Ramaiah, D. *Org. Lett.* **2005**, *7*, 5765. (b) Neelakandan, P. P.; Hariharan, M.; Ramaiah, D. *J. Am. Chem. Soc.* **2006**, *128*, 11334. (c) Jisha, V. S.; Arun, K. T.; Hariharan, M.; Ramaiah, D. *J. Am. Chem. Soc.* **2006**, *128*, 6204.

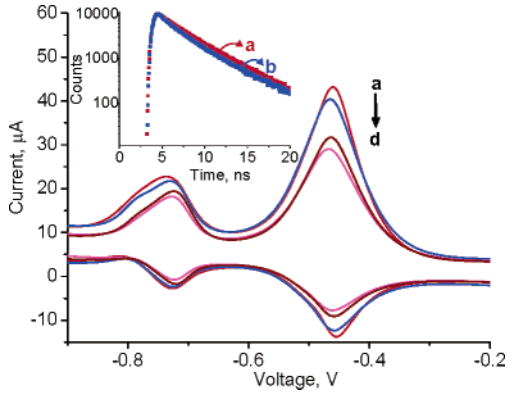


Figure 5. Square wave voltammograms of **1** (0.28 mM) with an increase in the addition of Trp. [Trp] (a) 0, (b) 1.02, (c) 2.0, and (d) 2.95 mM in water and a scan rate of 100 mV/s. Inset shows the picosecond time-resolved fluorescence spectra of **1** (13 μM) in an aqueous medium in the absence (a) and presence (b) of Trp (27×10^{-4} M). Emission collected at 475 nm and an excitation wavelength of 335 nm.

475 nm, whereas a significantly decreased lifetime of 3 ns was observed in the presence of Trp (inset of Figure 5). The successive additions of Trp to a solution of **1** in D_2O resulted in broadening and an upfield shift ($\Delta\delta$ 0.1 ppm) of protons corresponding to the pyrene and viologen moieties in the ^{1}H NMR spectra (Supporting Information Figure S12).[18] The Benesi−Hildebrand analysis of the corresponding changes in chemical shift values gave an association constant of 1337 $\pm$ 32 M^{-1}. This is in good agreement with the values obtained from the fluorescence data and the changes in chemical shift of the protons corresponding to Trp (downfield shift; $\Delta\delta$ 0.1 ppm) observed with the increase in addition of the conjugate **1** (Supporting Information Figure S13, K_{ass} = 1257 $\pm$ 19 M^{-1}).

The interesting observation is that the conjugate **1** exhibits unusually high selectivity for Trp among all other amino acids through inhibition of the ICT complex. This could be attributed to the enhanced π-electron cloud of the indole moiety of Trp, which promotes better π-stacking interactions with the pyrene and viologen moieties,[19] followed by the electronic interactions between the carboxyl group of Trp and the viologen moiety. These interactions lead to the formation of a stable complex between **1** and Trp. In contrast, other aromatic amino acids such as Tyr and Phe and aliphatic amino acids Gly, Cys, Thr, and Leu showed negligible interactions due to their lower π-electron density and hence are incapable of forming stable complexes with the conjugate **1**.

We further carried out the minimum energy AM1 calculations to understand the nature of complexation between the conjugate **1** and Trp. As shown in Figure 6, these calculations

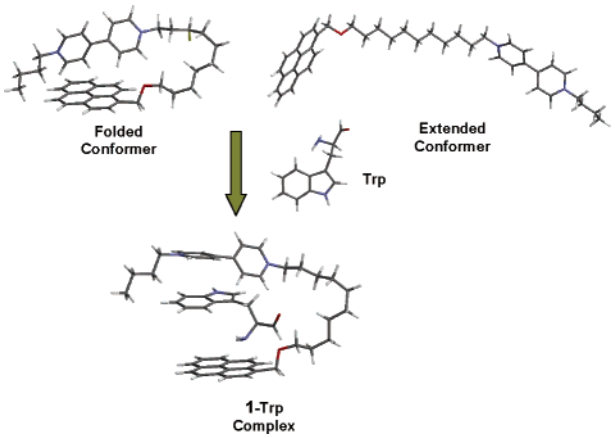


Figure 6. Minimum energy conformer of **1** in the absence and presence of Trp obtained through AM1 calculations.

revealed a 1:1 stoichiometry for the complex, wherein the indole unit of Trp is located in close proximity to the folded conformer of **1**. As a consequence of the complexation, the folded conformer of **1** transforms to a relatively stable, expanded, tweezerlike structure. The evidence for such a transformation is obtained from the time-resolved fluorescence, CV, and ^{1}H NMR studies. We observed a significantly reduced lifetime and intensity of the species corresponding to the ICT complex in the picosecond time-resolved studies. Further, the decrease in current intensity and broadening and shielding of protons of the viologen moiety due to the interactions with the indole moiety confirm the formation of a stable complex between Trp and the conjugate **1**.

In summary, we have developed a novel donor−acceptor conjugate which can form a fluorescent intramolecular charge-transfer complex in the aqueous medium. Results indicate that the formation of the ICT complex is favored through the cooperativity effect arising from hydrophobic, π-stacking, and electrostatic interactions of the units present in the system. Interestingly, this conjugate forms a novel molecular recognition system that can discriminate Trp from other natural amino acids involving synergistic effects of both π-stacking and electrostatic interactions and signals the event through changes in the fluorescence intensity. Further studies are in progress to understand the effect of a spacer on the ICT complex formation and the nature of interactions involved in the recognition of amino acids.

Acknowledgment. This work was supported by CSIR and DST, Government of India. This is contribution no. PPD-235 from RRL, Trivandrum, India.

Supporting Information Available: Details of synthesis and calculations, Figures S1−S13 showing fluorescence, and ^{1}H NMR spectra of the conjugate **1** under various conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

OL062636M

(18) NMR titrations of Trp using different concentrations of the conjugate **1** (3−13 μM) afforded similar association constants (±2%).

(19) (a) Bush, M. E.; Bouley, M. D.; Urbach, A. R. *J. Am. Chem. Soc.* **2005**, *127*, 14511. (b) Werner, F.; Schneider, H.-J. *Helv. Chim. Acta* **2000**, *83*, 465. (c) Malta, L. F. B.; Senra, J. D.; Medeiros, M. E.; Antunes, O. A. C. *Supramol. Chem.* **2006**, *18*, 327.